EVALUATION OF NATURAL RUBBER LATEX GLOVES AND VULCANIZATES WITH SPECIAL REFERENCE TO RESIDUAL ZINC DITHIOCARBAMATE ACCELERATORS

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A THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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DECLARATION

I, **Elizabeth K. Abraham**, hereby declare that I have personally carried out the work depicted in the thesis entitled **'Evaluation of natural rubber latex gloves and vulcanizates with special reference to residual zinc dithiocarbamate accelerators'** under the direct supervision of Dr. P. Ramesh, Scientist, Polymer Processing Laboratory, Biomedical Technology wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram, Kerala.

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This is to certify that the thesis entitled **'Evaluation of natural rubber latex gloves and vulcanizates with special reference to residual zinc dithiocarbamate accelerators'** which is being submitted by Mrs. Elizabeth K. Abraham, for the award of the degree of Doctor of Philosophy to the Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram, is a record of an original research work carried out by her under my direct supervision and guidance. The thesis has fulfilled all the requirements as per the regulations of this institute. The results embodied in this thesis have not been submitted to any other University for the award of any degree or diploma.

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Elizabeth K. Abraham

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ABBREVIATIONS

AIDS	Acquired immuno deficiency syndrome
ALARP	"as low as reasonably practicable"
ANOVA	Analysis of variance
ASTM	American Society for Testing and Materials
BAEP	Brainstem auditory evoked potential
BgVV	Bundesinstitut für Gesundheitlichen Verbraucherschutz und Veterinärmedizin (Federal Institute for Health Protection of Consumers and Veterinary Medicine, Germany)
BIS	Bureau of Indian Standards
CBEC	Copper dibenzyldithiocarbamate
CBS	*N*-Cyclohexyl-2-benzothiazolesulfenamide
CDBC	Copper dibutyldithiocarbamate
CDCP	Center for Disease Control and Prevention
CDEC	Copper diethyldithiocarbamate
CDNC	Copper diisononyldithiocarbamate
CFR	Code of federal regulations
CGSB	Canadian General Standards Board
CIBC	Copper diisobutyldithiocarbamate
CPPD	*N*-Cyclohexyl *N*-phenyl-*p*-phenylenediamine
CSO	Cotton seed oil
DCBS	Benzothiazyl-2-dicyclohexyl sulfenamide
DEHP	Di(2-ethylhexyl) phthalate
DETU	Diethyl thiourea
DL	Detection limit
DMDC	Dimethyl dithiocarbamate
DMDTCs	Dimethyldithiocarbamates
DPG	Diphenylguanidine
DPNRL	Deproteinized natural rubber latex
DPPD	*N*,*N*-Diphenyl-*p*-phenylenediamine
DPTT	Dipentamethylene thiuramdisulfide
EB	Elongation at break

EBDTCs	Ethylene bisdithiocarbamates
EEC	European Economic Community
ETU	Ethylene thiourea
FDA	Food and Drug Administration
GC	Gas chromatography
GHS	Globally harmonized system
HBV	Hepatitis B virus
HIV	Human immunodeficiency virus
HPLC	High performance liquid chromatography
IC_{50}	Median inhibitory concentration
IgE	Immunoglobulin E
IPPD	*N*-Isopropyl-*N*- phenyl-*p*-phenylenediamine
ISO	International Organization for Standardization
KAN	Kommission Arbeitsschutz Normung (Commission for Occupational Health and Safety and Standardization, Germany)
LC-APCI-MS	Liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry
LD_{50}	Median lethal oral dose
MBSS	4-Morpholinyl-2-benzothiazyl disulfide
MBT	2-Mercaptobenzothiazole
MBTS	Bis(2,2'-benzothiazolyl disulfide)
MDA	Medical Device Agency, UK
MMBI	4-Methyl-2-mercaptobenzimidazole
MMBT	4-Morpholinyl-2-benzothiazyl disulfide
MMDTCs	Monomethyldithiocarbamates
MRLs	Maximum residue levels
NBSL	National Biological Standards Laboratory
NR	Natural rubber
NRL	Natural rubber latex
OBTS	*N*-Oxydiethylene-2-benzothiazole sulfenamide
OECD	Organization for Economic Co-operation and Development
OSHA	Occupational Safety and Health Administration
OTOS	*N*-Oxydiethylene thiocarbamyl-*N*-oxydiethylene sulfenamide
p.p.h.r	Parts of additive per 100 parts of rubber

PPVL	Peroxide prevulcanized natural rubber latex
PVC	Polyvinyl chloride
RQL	Reliable quantitation limit
RVNRL	Radiation vulcanized natural rubber latex
SCMPMD	Scientific Committee on Medicinal Products and Medical Devices
SDS PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SR	Synthetic rubber
SRPP	Small rubber particle protein
TBBS	*N-tert*-Butyl-2-benzothiazolesulfenamide
TBTD	Tetra butylthiuramdisulfide
TB_ZTD	Tetra benzylthiuramdisulfide
TETD	Tetra ethylthiuramdisulfide
TLC	Thin layer chromatography
TMTD	Tetra methylthiuramdisulfide
TMTM	Tetra methylthiurammonosulfide
TS	Tensile strength
TTCA	2-Thiothiazolidine-4-carboxylic acid
UDS	Unscheduled DNA synthesis
UHMWPE	Ultra high molecular weight polyethylene
USP	United States Pharmacopeia
UV-VIS	Ultra violet-visible
VFA	Volatile fatty acid
ZBEC	Zinc dibenzyldithiocarbamate
ZDBC	Zinc dibutyldithiocarbamate
ZDEC	Zinc diethyldithiocarbamate
ZDMC	Zinc dimethyldithiocarbamate
ZDNC	Zinc diisononyldithiocarbamate
ZEPC	Zinc ethylphenyldithiocarbamate
ZIBC	Zinc diisobutyldithiocarbamate
ZMBT	Zinc mercaptobenzothiazole
ZPC	Zinc pentamethylenedithiocarbamate

NOTATION

SD	Standard deviation
$\bar{x}$	Mean
SE	Standard error
V_r	Volume fraction of rubber in the swollen material
D_p	Diffusion coefficient
M_t	Amount of additive migrated at time 't'
M_0	Amount of migrant present in the polymer initially
χ	Rubber-solvent interaction parameter
ρ_r	Density of rubber
ρ_s	Density of solvent
CoV	Coefficient of variation
HL CA	*In vitro* cytogenicity tests using human lymphocytes
ML MUT	Mouse lymphoma cell mutation assay
Mn	Mouse bone marrow micronucleus test
SA	Surface area
V_s	Molar volume of solvent (toluene)
ρ_c	Crosslink density

ABSTRACT

The thesis deals with the release of allergologically relevant, bioavailable residual zinc dithiocarbamates, a commonly used vulcanization accelerator, from natural rubber latex (NRL) medical gloves and NRL vulcanizates and with the subsequent exposure of users to dithiocarbamates. The release studies from gloves were carried out under real-use (employing human subjects) and simulated-use (employing artificial sweat) conditions. Since the commercially available medical gloves varied in their composition, NRL vulcanizates of known composition were prepared using different zinc dithiocarbamate accelerators and dithiocarbamate-sulphur ratios. These vulcanizates were extracted in artificial sweat, a physiologically simulated medium under simulated-use conditions to study the effect of various factors on the extent of dithiocarbamate-release into artificial sweat. It was also the intention to develop some NRL formulations having adequate mechanical properties as well as low residual dithiocarbamate content suitable for medical glove manufacture.

The results of the present study indicated that the quality of latex medical gloves marketed in India is a matter of concern with regard to the cytotoxicity and water-extractable proteins. Suitable analytical techniques such as ultraviolet-visible (UV-VIS) spectroscopy, thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) were employed to identify and quantify the various zinc dithiocarbamate accelerators. The results indicated that the extraction of gloves in artificial sweat may be used for the determination of the extent of ZDEC-release, which in turn could be used to estimate the 'anticipated exposure' of users to ZDEC from gloves. It was found that the rubber solubility of zinc dithiocarbamates controls the extent of dithiocarbamate-release from latex vulcanizates into artificial sweat. Factors such as storage time, amount of residual ZDEC content and nature of the leaching medium were found to affect the extent of ZDEC-release into artificial sweat. It was found that both the amount of residual dithiocarbamates and the mechanical properties varied with respect to the dithiocarbamate-sulphur ratio. Two latex formulations containing ZDEC, the widely used accelerator in the Indian latex industry, suitable for medical glove manufacture were developed.

CHAPTER 1

INTRODUCTION

Over the past two decades, exposure to natural rubber latex (NRL) medical products especially gloves caused a multitude of adverse allergic reactions among both the healthcare personnel and the general public (Dillard, 1997; Taylor and Leow, 2000). Allergic reactions to NRL are classified into three types and they include irritant contact dermatitis, allergic contact dermatitis (delayed-type hypersensitivity), and immediate-type hypersensitivity (latex allergy). Irritant contact dermatitis and allergic contact dermatitis, which account for over 80% of the allergic incidents, are induced by residual chemicals such as thiurams, dithiocarbamates, thiazoles, antioxidants, and their decomposition products (Nakamura *et al.,* 1990; Heese *et al.,* 1991; Conde-Salazar *et al.,* 1993; Knudsen *et al.,* 1993; Marks *et al.,* 2000; Horwitz *et al.,* 2002; Nettis *et al.,* 2002; Geier *et al.,* 2003). A number of other toxicological effects have also been reported due to chemicals used in the rubber industry (Graham *et al.,* 1983; Straif *et al.,* 1999; Debbarh *et al.,* 2002; Li and Yu, 2002; Nicolaysen *et al.,* 2004). The immediate-type hypersensitivity, on the other hand, is caused by proteins that are inherently present in NRL.

A number of alternatives such as radiation/peroxide vulcanized natural rubber latices, and synthetic latex products were introduced into the health care market to address the problems posed by NRL medical devices. Although radiation/peroxide vulcanized latices are free from sensitizing accelerators, they suffer from a number of limitations such as poor aging resistance, and low tactile

sensitivity (Gazeley and Pendle, 1989; Davies and Gazeley, 1993; Sekhar, 2005). Synthetic latex products, though free from allergenic proteins, suffer from serious disadvantages which include (i) higher modulus than NRL products, (ii) poor barrier integrity resulting in the seepage of deadly viruses during medical procedures, (iii) carcinogenic monomers which pose a problem to both industrial workers and health care personnel, and (iv) release of toxic gases during disposal by incineration (Korniewicz *et al.*, 1990; SCMPMD, 2000; Delzell *et al.*, 2001; Hasma *et al.*, 2003). Moreover, the European Commission's scientific committee on medicinal products and medical devices (SCMPMD) cautions that most synthetic rubber products containing sensitizing chemicals pose a similar risk of sensitization as that of NRL medical products (SCMPMD, 2000). It is by no means clear that the overall health risks imparted by the alternatives are less than those caused by NRL products.

Natural rubber latex, therefore, remains the material of choice for barrier products such as gloves and condoms owing to the unique characteristics such as superior barrier integrity, elasticity, tear resistance, durability, excellent tactile sensitivity and comfort. In the wake of more people getting sensitized to residual chemicals, the European Commission guidelines on medical devices recommend that wherever possible, for each hazardous chemical used or generated during NRL processing, the technical documentation needs to include an estimate of the anticipated exposure to patients and users, identification of tolerable intake and quality control measures (EEC/93/42, 2004). However, a systematic study on the release and the subsequent exposure to allergologically relevant, bioavailable residual accelerators and their determination in latex health care products is not yet available (SCMPMD, 2000; EEC/93/42, 2004).

In this scenario, it would be of great significance to assess the release of dithiocarbamates, a commonly used vulcanization accelerator, from NRL medical gloves. It has been well established that dithiocarbamates show a higher degree of allergenicity and cytotoxicity when compared to other rubber additives (Nakamura *et al.*, 1990; De Jong *et al.*, 2002). So far, there is no standard by American Society for Testing and Materials (ASTM) or International Organization for Standardization (ISO) for the quantification of leachable dithiocarbamates in latex medical products (Bader, 2004). Therefore, it was attempted to develop suitable analytical methods to

dithiocarbamate-sulphur ratios are elaborated in the next chapter. As the use of medical devices normally entails their direct or indirect contact with patients, it is necessary to establish the safety of these products before they are marketed. Medical device safety evaluation assesses the risk of adverse health effects from a device using a battery of tests provided by ISO 10993. As ZDEC is widely used as accelerator in the Indian latex industry, two formulations containing ZDEC were subjected to biological tests such as *in vitro* cell culture test, intracutaneous irritation test and sensitization test and the results are presented in chapter 9. The thesis concludes with a summary, conclusions and future prospects which are detailed in chapter 10.

CHAPTER 2

LITERATURE SURVEY

2.1. INTRODUCTION

The use of natural rubber (NR) in the medical field dates back to the discovery of the vulcanization process in the middle of the 19th century. The unique characteristics of NR such as superior barrier protection, elasticity, tear resistance, durability, excellent tactile sensitivity and comfort make it the material of choice for several medical applications. The majority of these applications are confined to skin and mucosal membrane contacting medical devices such as gloves, condoms, urinary catheters, face masks, tubes, diaphragms, syringes, dressings, tape, bandages etc. Over the past two decades, several dramatic changes have occurred in the latex medical device industry following reports of serious allergic reactions to natural rubber latex (NRL) products, especially gloves (Dillard, 1997). The desire for better safety and health has led to the rise of synthetic rubber (SR) products in the health care sector as well. The world elastomer consumption has been increasing with a total production of NR and SR at 8.42 and 11.92 million tons respectively for the year 2004 (IRSG, 2004). The total consumption of NRL is reported to be about 11% of the global NR production (van der Heijden *et al.,* 1997). Despite the commercial existence of SR, natural rubber remains a practically indispensable raw material in a great deal of rubber products, including medical devices.

2.2. NATURAL RUBBER LATEX

Natural rubber latex is a milky white fluid obtained from the rubber tree (Hevea brasiliensis) by the controlled wounding of the tree, commonly known as tapping. Being a natural product, the composition of the fresh NRL varies between wide limits. A typical composition is given in Table 2.1 (Blackley, 1997a; Pillay, 1980).

Table 2.1. Composition of fresh NRL

Constituent	Weight (% m/m)
Rubber hydrocarbon	30-40
Proteins	1.0-1.5
Resins	1.0-2.5
Fats	0.5-1.0
Sugars	0.5-1.0
Water	60-70
Ash	<1.0

The density of fresh NRL varies in the range 0.97-0.98 g cm^{-3}, pH 6.5-7.0, and surface energy 40-45 mJ m^{-2}. The substances present in the freshly tapped latex are distributed through three phases: rubber, aqueous, and lutoid. Rubber particles ranging from 0.02-3.0 μm in size are usually spherical but some times oval or pear shaped. The density of rubber particles is approximately 0.92 g cm^{-3}. They are strongly protected in suspension by a film of adsorbed proteins and phospholipids, which give the rubber particles a negative charge. The adsorbed proteins and phospholipids on the rubber phase impart colloidal stability to latex. The composition of the rubber phase is given in Table 2.2. (Blackley, 1997a).

Table 2.2. Composition of the rubber phase

Constituent	Weight (%m/m)
Rubber hydrocarbon	86
Water	10
Proteins	1
Phospholipids	3

The rubber hydrocarbon is predominantly cis-1,4 polyisoprene (Figure 2.1).

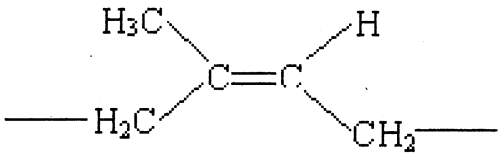


Figure 2.1. Chemical structure of isoprene unit

Trace metals, notably magnesium, potassium and copper are also associated with the rubber particles to an aggregate amount of approximately 0.05%. The lipids associated with rubber phase are composed of sterol and sterol esters (ca. 0.4% m/m), fats and waxes (ca. 0.6% m/m), and phospholipids (ca. 2% m/m). The aqueous phase of fresh NRL has a density of approximately 1.02 g cm^{-3}, and contains carbohydrates, electrolytes, proteins and amino acids. The lutoids contain mainly water. In addition, lutoids contain small amounts of proteins, and phospholipids. They are very labile and disappear when the latex is ammoniated.

The total protein content in fresh latex is approximately 1-1.5 wt%, of which 27% is adsorbed on the rubber particle, an approximately equal quantity (25%) in the bottom fraction (lutoid fraction or 'B'serum), and the remainder (about 48%) in the serum phase ('C'serum) (Hashim, 1993; Blackley, 1997a). The quantity of proteins adsorbed on the rubber particle is a clonal characteristic. The rubber-bound proteins are largely anionic in nature having an isoelectric point between 3.5 and 6.0. The isoelectric point of the rubber phase varies from 4.0-4.6 and this variation has been ascribed to the presence of more than one type of protein on the rubber particle. The principal proteins present in the aqueous phase are α-globulin and hevein. A major proportion of the water-extractable proteins are derived from "B" serum and "C" serum. They consist of both anionic and cationic proteins with the isoelectric point varying between 3.5 and 9.5, the majority being anionic proteins.

Significant advances have been made in the identification of latex proteins. About 250 proteins have been identified in fresh NRL; 50-60 are found to be allergenic in nature, of which 15 have been given allergen designation (Palosuo, 2002). Table 2.3 gives a list of allergenic proteins that have been identified in the

finished latex products, and the allergen designation is given by the International Union of Immunological Societies (www.expasy.org).

Table 2.3. List of allergenic proteins

Name	Allergen designation	Molecular weight (kDa)*	Length (AA)**
Rubber Elongation Factor	Hev b 1	14.6	137
β-1,3-gluconase	Hev b 2	36	-
Small rubber particle protein (SRPP)	Hev b 3	22.4	204
Microhelix component	Hev b 4	100, 110, 115	-
Acidic protein	Hev b 5	15.9	150
Hevein (hevein preprotein)	Hev b 6.01	20	204
Hevein (N-domain)	Hev b 6.02	5	-
Pro-hevein (C domain)	Hev b 6.03	14	-
Patatin	Hev b 7.01 Hev b 7.02	42 44	-
Profilin 1	Hev b 8.0101	14.19	131
Enolase 1	Hev b 9	51	445
Manganese superoxide dismutase	Hev b 10	26	-
Class I chitinase	Hev b 11	33	-
Lipid transfer protein	Hev b 12	9.3	-
Esterase	Hev b 13	42	391

* as determined by SDS PAGE analysis; ** length of the sequence of amino acids

Hevein, the second principal protein isolated from the bottom fraction of the latex, is synthesized as a 20 kDa hevein preprotein that is subsequently processed into a 5 kDa amino terminal hevein (N-domain) and a 14 kDa carboxyl terminal hevein (C-domain). The allergens Hev b 1, Hev b 3, Hev b 6 and Hev b 7 are proteins that are involved in the biosynthesis or coagulation of rubber. Proteins that are a part of the plant's defense system are represented by Hev b 2, Hev b 6, Hev b 7, and Hev b 11. Hev b 4, Hev b 5 and Hev b 8 are classified as structural proteins, while Hev b 9 and Hev b 10 are house keeping proteins (Niggemann and Breitender, 2000). Several

other proteins are present in hevea serum, but their characterization is yet to be completed. Lutoid particles, found in the bottom fraction of the latex, contain microfibrillar proteins. This protein, having a lower isoelectric point than hevein, comprises about 40 wt% of the total protein of the lutoid particles in young latex vessels, but is absent in older latex vessels.

2.3. NATURAL RUBBER PRODUCTS IN THE MEDICAL FIELD

A wide variety of consumer and health care products are made from NRL owing to its excellent barrier properties, and tear resistance. The major medical products include gloves, Foley catheters, vaginal diaphragms, tubes for IV drips, stethoscope-tubing, baby bottle teats, soothers, syringe plungers, vial stoppers, face masks, tourniquets, dental dam, tracheostomy tubes etc. Natural rubber accounts for most of all elastomers used in the manufacture of dipped medical devices; however its blood compatibility is poor compared to other elastomers (Ingles, 2000). The surface of the uncoated latex products is generally rough, and it sometimes leads to poor performance of urinary catheters *in vivo* (Axelsson *et al.,* 1977; Riley *et al.,* 1995). Hydrogel or silicone coated NRL catheters greatly improve the *in vivo* performance (Wironen *et al.,* 1997). Unfilled NR has a high resistance to the action of aqueous biological media; however, a particular problem associated with the material is allergy. With the advent of universal precautions against Acquired Immuno deficiency Syndrome (AIDS), hepatitis B, hepatitis C etc., many regulatory bodies such as the Center for Disease Control and Prevention (CDCP) and Occupational Safety and Health Administration (OSHA) advocated the use of gloves and condoms among both the health care workers and the general public. The wide spread use of gloves and other barrier products resulted in an increase in the incidence of allergic reactions among users. Various factors such as proteins, chemical additives used to impart specific properties of the final product, glove powder, the method of manufacturing (formulation and processing), the manufacturing environment etc. have been identified as sources of great concern. However, the benefits appear to outweigh the risks associated with the use of NRL products.

2.4. PRODUCTION OF NR BASED MEDICAL DEVICES

Both the latex and dry forms of NR are used in the manufacture of medical devices. The fresh NRL is centrifuged twice to remove the serum bound proteins to obtain the double centrifuged natural rubber latex. Further reduction in proteins to a level of about 0.008% is possible by the treatment of the latex with enzymes and/or surfactants, and the latex thus obtained is called the deproteinized natural rubber latex (DPNRL). The bulk of the latex medical products such as gloves, condoms, tubing, and catheters is produced from either double centrifuged or DPNR latices by a process called dipping. The dipping process consists essentially in the immersion of a former into a suitably formulated latex compound, followed by the slow withdrawal so as to form a uniform layer of latex on the former. The process is completed by drying, leaching, and vulcanizing the deposit. It is desirable to form a rolled bead at the neck of the article in order reinforce the thin latex deposit against tearing.

Condoms and gloves are produced in very large numbers on highly automated production lines. Coagulant dipping is used for the production of gloves, and straight dipping for thin walled articles like condoms. In contrast to the production of condoms and gloves, special medical products are produced on a much smaller and semi-automated scale. Often, product-related machinery is used. Thicker products such as balloon, ultrasound transducer covers, catheters etc are produced by coagulant dipping or heat-sensitive dipping processes. Latex tubes of length of 10 m are often produced by extrusion. Natural rubber in the dry form (pale creep) is used for the production of syringe plungers, vial stoppers, and injection ports on intravascular tubing mainly by extrusion or compression molding process.

2.5. ALLERGIC REACTIONS TO NRL PRODUCTS

The term allergy is described as an immune reaction resulting from the reaction between the antibody in the body and the antigen (allergen). It is the extent of the immune reaction, and its detrimental effects that make it a disease entity. The initial stage of allergy is sensitization, which involves the development of immunoglobulin E (IgE) antibody and/or T-cells to latex antigens. Once sensitized, subsequent exposure to antigen will lead to a series of immune reactions, triggering allergic reactions in the body. Exposure to latex antigens may occur either through

dermal contact (glove) or mucosal membrane contact (condoms, Foley catheters, balloons used in barium enema examinations etc.). The direct contact with tissue or intravascular exposure occurs during surgical procedures (gloves), or through containers of injectable materials or through tracheostomy tubes.

There are three distinctive types of allergic reactions to NRL that differ in their mechanisms of induction as well as clinical manifestations. They include irritant contact dermatitis, allergic contact dermatitis (delayed-type hypersensitivity), and immediate-type hypersensitivity (latex allergy) (www.cdc.gov; Taylor and Leow, 2000). Generally, irritant contact dermatitis and allergic contact dermatitis are together known as contact dermatitis which often involves hands, wrists and forearms, although any area can be affected. The contact dermatitis is often regarded as a career-threatening problem (Wilson *et al.,* 1991).

2.5.1. Irritant contact dermatitis

Irritant contact dermatitis is a non-immunogenic response. The symptoms consist of dry crusty hard bumps, and horizontal cracks on the skin appearing 48 to 72 h after contact. Lesions are localized to the areas of the skin in direct contact with the rubber product. The dermatitis may fade over if the exposure is discontinued. It is important to note that the irritant contact dermatitis affects many non-latex allergic individuals. Irritant contact dermatitis is found to be an important factor in inducing allergic contact dermatitis and latex allergy (Taylor and Praditsuwan, 1996).

Irritant contact dermatitis to rubber products is caused by residual chemical additives (thiazoles, thiurams and dithiocarbamates), glove powder, hand cleansers, and/or simple friction between the glove and the skin (Wrangsjo *et al.,* 1994). Typical irritant contact dermatitis to gloves is given in Figure 2.2.

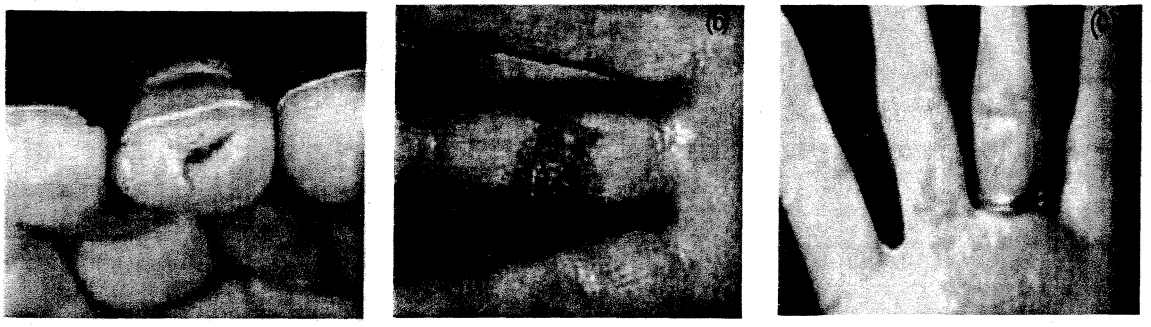


Figure 2.2. Typical irritant contact dermatitis to (a) and (b) gloves causing cracks; and (c) gloves causing rashes

2.5.2. Allergic contact dermatitis

Also called delayed-type hypersensitivity, allergic contact dermatitis is caused by the presence of residual chemicals such as accelerators (added during manufacturing to accelerate the vulcanization process), antioxidants (which increase the shelf life and durability of the latex goods), and their decomposition products. Allergic contact dermatitis is typically mediated by specifically sensitized T-lymphocytes. In the induction period, allergenic chemicals (usually with molecular weight < 400) permeate the skin, and bind with the Langherhans cells, which then direct the allergen to a regional lymph node. Interaction with the T-lymphocytes in the lymph node results in the replication of sensitized T-lymphocytes thus completing the induction phase. Sensitization can occur after just one single exposure, but requires a lag period of a few days to a couple of weeks for the induction to be complete. On re-exposure to the allergens (elicitation phase), the sensitized T-cells stimulate the local release of a number of inflammatory agents resulting in the elicitation of allergic symptoms. This normally takes from 12 to 96 h for a reaction to occur, but more usually 48-72 h after exposure. Clinically, a red, raised and palpable area at, and sometimes slightly beyond the area of contact with the glove is observed, accompanied by subjective symptoms such as itching, burning and tingling. Prolonged exposure to the causative agent can lead to chronic condition characterized by dry, cracked and scaly skin. Figure 2.3 shows some typical allergic contact dermatitis to gloves and face masks.

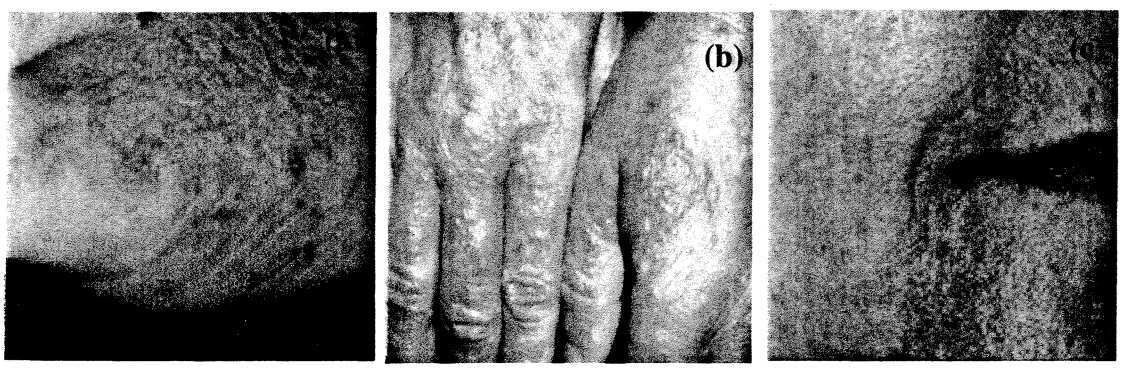


Figure 2.3. Typical allergic contact dermatitis to (a) and (b) gloves; and (c) face mask

Induction of allergic contact dermatitis is found to be dependant on the concentration of the allergen on the surface of the skin. One can become sensitized after a single contact with a high dose of an allergen, but frequent contacts with low

doses of allergen over a long period of time may also result in sensitization. The range of concentration of the allergen needed for sensitization is quite large. Although an approximately linear dose-response is observed in humans, there is wide individual variability in the elicitation threshold concentration of allergens and it can span at least a 100-fold range (Emmet *et al.,* 1994; Basketter *et al.,* 1997).

2.5.3. Immediate-type hypersensitivity

Immediate-type hypersensitivity, commonly referred as latex allergy, is caused primarily by the proteins present in the NRL. It is immediate in nature and believed to be mediated by IgE antibody (Leynadier *et al.,* 1989; Sussman *et al.,* 1991; Wilson *et al.,* 1991; Slater, 1992). The interaction of antigenic proteins and IgE antibody triggers the release of mediators such as histamine and arachidonic metabolites from the mast cells resulting in increased vascular permeability and tissue edema (Leynadier *et al.,* 1989; Slater, 1992). A late phase response caused by the recruitment of other inflammatory cells such as basophils, eosinophils and neutrophils can occur hours after the initial reaction. The reactions may range from local skin reactions such as wheal and flare reaction, hives, and eczema to life threatening anaphylactic reactions. Anaphylactic shock is caused by vasodilation and vascular leakage resulting from enhanced permeability of the post capillary venules in the vascular beds of visceral organs, skin, and mucosal membranes. Bronchial and gastrointestinal muscle spasms produce acute airway obstruction and colicky abdominal pain. Increased vascular permeability in the skin results in pruritis, angioedema etc. Signs on the skin surface include flushing of the skin especially at the face, hives, raised bumps on hands, contact rash which disappears on removing gloves, seeping thick and crusty sores on hands, itching etc. Symptoms involving the nose, throat, airway and eyes include sneezing, rhinitis, conjunctivitis, red watery itchy eyes, bronchospasm, wheezing, angioedema, difficulty in breathing, chest pain, cyanosis, faintness etc. The cardiovascular symptoms include hypotension, sinus tachycardia and anaphylaxis. On the other hand, gastrointestinal symptoms include nausea, vomiting, abdominal cramping and diarrhoea (Lawson, 2001). These clinical manifestations occur minutes after contact with the NRL goods either directly or from inhalation of airborne latex allergens. Typical immediate-type reactions are shown in Figure 2.4.

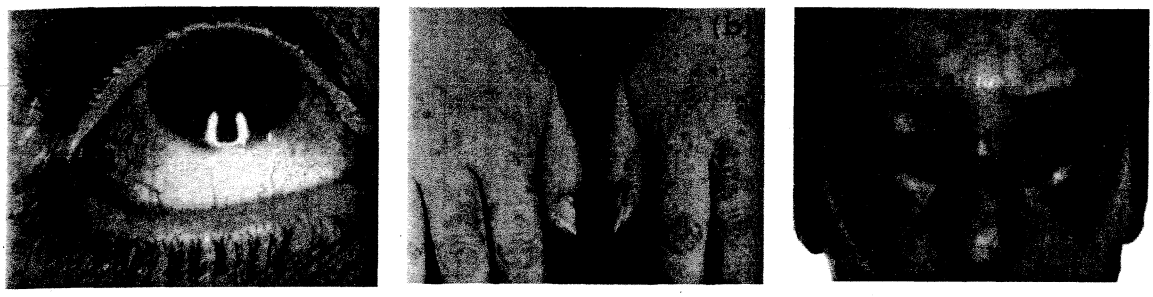


Figure 2.4. Immediate-type reactions: (a) conjunctivitis; (b) contact urticaria to hands; and (c) angioedema

2.5.4. Latex fruit syndrome

The association of latex allergy and allergy to plant-derived foods is referred to as latex fruit syndrome. The existence of a latex-fruit syndrome was proposed, based on the clinical observation of an unexpected but significantly high rate of cross reactivity of immediate fruit allergy with NRL allergy among a group of 25 patients (Blanco *et al.,* 1994). Approximately 30-50% of individuals who are allergic to NRL show an associated hypersensitivity to some plant-derived foods (Wagner and Breiteneder, 2002). Being natural in origin, several of the latex proteins show homology to other plant proteins. Hev b 1 has homology to papain from papaya; Hev b 5 to proteins in kiwi, potato, and sugar beet; Hev b 6 to hevein-like proteins and class I chitinases, which are widely distributed in plants; Hev b 7 to a potato protein called patatin; and Hev b 8 to profilins (Meade *et al.,* 2002). It appears that the hevein family of proteins (Hev b 6 and 11) are pan-allergens responsible for much of the cross-reactivity of latex with other plant and food allergens (Blanco *et al.,* 1999). The food types with cross reactivity to NRL include banana, kiwi, chestnut and avocados (Fernadez De Corres *et al.,* 1993; Rodriguez *et al.,* 1993). With an increasing population being prone to allergic reactions, the list is extended with the addition of hazelnut, peanut, celery, melon, potato, papaya, fig, passion fruit, tomato, grapes, pineapple, cherry, peaches, apricot, carrot and plum. The current understanding on latex fruit syndrome is reviewed in detail by Blanco (2003).

2.6. PREVALENCE OF ALLERGY TO NRL

Nutter (1979) first reported a case of immediate-type reaction caused by NRL gloves. However, the bulk of the allergic reactions to medical products containing NRL was reported from Europe and USA in the early 1990's. It is clearly evident that reports showing appreciable percentage of medical personnel getting sensitized to

NRL goods occurred during the period when 'universal precautions' against blood-borne infectious diseases were implemented world wide. The problem had reached such proportions that by August 1997, FDA (Food and Drug Administration) MedWatch reporting program had recorded more than 2500 significant allergic reactions to latex; 62% of these glove-related reports alleged the occurrence of adverse allergic reactions including anaphylaxis; 4% of cases alleged glove powder residue complaints, and the remaining 33% reports were primarily related to concerns regarding glove barrier integrity (Dillard, 1997). Certain groups such as health care workers, children with spina bifida, dentists etc are identified as high risk groups. Various reports suggest that the prevalence of sensitization among healthcare workers to latex varies; 3% in Spain (Esteve *et al.,* 2003), 12% in Italy (Verna *et al.,* 2003), 8.6% in Taiwan (Chen and Lan, 2002), 19% in Turkey (Ozkan and Gokdogan, 2003), and 10-17% in USA (Reed, 2003). Atopic children, especially those with food allergy, also belong to the high risk groups; pacifiers and balloons probably being the most important sensitizers in children (Novembre *et al.,* 1997; Porri, 1997; Niggemann *et al.,* 1998). Children with congenital malformations like spina bifida also belong to high-risk groups, and the prevalence of allergy is in the range 28-67% (Beaudouin *et al.,* 1994; Ylitalo *et al.,* 1997; Buck *et al.,* 2000; Martinez-Lage *et al.,* 2001; Dehlink *et al.,* 2004). Studies conducted among dental students showed a low prevalence of allergy at the beginning of their studies, but increased numbers of them become sensitized during the later stages of their study (Field, 1999). Other special groups who may be at high risk of sensitization to latex protein include patients who have undergone multiple surgeries, with genitourinary tract abnormalities, with food allergies, and individuals having an allergic predisposition (atopy). Prevalence of allergy to NRL among general population is found to be below 1% (Liss and Sussman, 1999; Charous *et al.,* 2002).

The incidence of anaphylaxis is reported to vary in the range 1.21-15.04% among the US population (Neugut *et al.,* 2001). Recent cases of anaphylaxis from latex have been reported from Japan (Sato *et al.,* 2003; Fujie *et al.,* 2004). Glove-related urticaria, rhinoconjunctivitis and/or asthma were reported among 24.6%, and glove-related hand dermatitis among 36.9% of the laboratory workers in Netherlands who regularly used gloves (de Groot *et al.,* 1998). A study conducted in an Italian hospital showed a high prevalence of rubber glove-induced allergic reactions among

the employees; 93.2% were having contact dermatitis, 4.4% had contact urticaria, and 2.4% experienced contact dermatitis associated with contact urticaria (Nettis *et al.,* 2002). Chen *et al.,* (2004) published the first statistically analyzed study of allergic contact dermatitis in Asia for the period 1986-2000, and reported that 4.4% of the patients had allergic contact dermatitis. These allergic manifestations are expected to increase as the general population is becoming more sensitive to a wide variety of chemicals, foods and other substances.

2.6.1. Causes of allergy

The factors contributing to allergic reactions to NRL products primarily fall into three categories:

1. Proteins that are inherently present in NRL.
2. Chemicals which are added to the latex formulations to improve the mechanical properties of NRL products.
3. Glove powder (corn starch) used for easy donning of gloves. Cotton fluff used for flocking of household and industrial gloves is also a cause of allergy.

The major route of exposure of humans to allergens is mostly by skin and mucosal membrane. It is argued that the perspiration solubilises the protein in the latex gloves leading to the dermal adsorption of protein (Beezhold *et al.,* 1994a). In the case of chemicals, they are released into hand sweat, become bioavailable, and induce irritation or allergic contact dermatitis (Knudsen *et al.,* 1993; SCMPMD, 2000). Another possible route of exposure is inhalation of the airborne latex proteins (Beezhold and Beck, 1992; Tomazic *et al.,* 1994; Baur, 2003).

Carrillo *et al.,* (1986) were the first to show that latex derived water soluble proteins are allergens, and demonstrate that a 30 kDa protein from the saline extracts of gloves showed allergenicity. Subsequently, latex proteins of various molecular weights were identified as allergens (Turjanmaa *et al.,* 1988; Morales *et al.,* 1989). Several researchers in their study have shown that proteins having molecular weight approximately 14 kDa and 30 kDa show significantly higher affinity towards IgE antibody. Czuppon *et al.,* (1993) reported that rubber elongation factor is a major allergen in latex. During the period 1996-'97, proteins such as rubber elongation factor, hevein preprotein, hevamine, glucanase and others have been identified as

allergenic proteins (Beezhold *et al.,* 1996; Chen *et al.,* 1996; Beezhold *et al.,* 1997). There is considerable heterogeneity in individual reactivity to specific latex proteins, and there appears to be preferential reactivity to certain proteins among different patient populations (Beezhold *et al.,* 1994b). It has been reported that Hev b 5, 6, and 7 are the common allergens in health care workers with occupational exposures (Sussman and Beezhold, 1997; Yip *et al.,* 2000). On the other hand, Hev b 1 and Hev b 3 have been found to be major determinants of latex allergy in children with spina bifida (Wagner *et al.,* 1999). Other latex antigens include: chitinases (I and II), lysozyme and proteasome subunit (Breitender and Scheiner, 1998).

Beezhold and Beck (1992) identified the interaction between latex proteins and cornstarch powder, and suggested that the cornstarch played a major role in the development of latex allergy. Later on, Tomazic *et al.,* (1994) proved that cornstarch binds the proteins, became aerosolized when the gloves were removed or donned. On inhalation of these aerosolized powder particles, a series of reactions such as rhinitis, asthma, anaphylaxis etc. manifest (Tarlo *et al.,* 1990; Edelstam *et al.,* 2002; Barbara *et al.,* 2004). Corn starch may also serve as an adsorbent/absorbent for the unbound chemicals that may act as sensitizers for the user upon contact (Heese *et al.,* 1991). Besides, starch contamination occurs with surgical procedures, and sometimes leads to granulomas, peritoneal adhesions or wound infections (De Lomas *et al.,* 1992; Ellis, 1994; Giercksky *et al.,* 1994; Hunt *et al.,* 1994; Swanson *et al.,* 1994; Luijendijk *et al.,* 1996; Duron *et al.,* 1997; Homdahl, 1997; Reddy *et al.,* 1999; Edlich *et al.,* 2001). Starch powder may also act as a vehicle for endotoxin, which gives rise to a multitude of reactions such as fever, pituitary and stress hormone release, tachycardia, hypotension, angiogenesis, disruption of the balance of coagulation and fibrinolysis (Williams and Halsey, 1997). Cotton fluffs bind allergenic proteins, which on inhalation may also contribute to the development of allergic reactions (Baur, 2003).

Although a diverse selection of chemicals are used, investigations have shown that the accelerators, mainly thiurams, dithiocarbamates and mercaptobenzothiazoles are the most common cause of type IV allergy (von Hintzenstern *et al.,* 1991). Not all thiurams or dithiocarbamates have the same propensity to elicit allergic responses due to differences in the chemical structure. The relative sensitizing potential of these compounds depends upon three factors: (i) the potential of the compound itself to act

as an allergen; (ii) the concentration of the compound present; (iii) the ability of the compound to permeate the skin which depends upon the physico-chemical characteristics of the compound, and also the condition of the skin. Degradation of accelerators during the process of vulcanization produces allergenic by-products. The thiurams breakdown during vulcanization to liberate dithiocarbamates and secondary amines, which again is a source of allergy. If the allergenic chemicals are structurally similar, they can demonstrate immunological cross reactivity (Knudsen and Menne, 1996a; Knudsen *et al.,* 2000b). Thiurams and dithiocarbamates cross-react and some mercapto compounds also show cross-reactive responses. Among antioxidants, sensitization is predominantly caused by the paraphenylene diamine compounds such as *N*-Isopropyl-*N*-phenyl-*p*-phenylenediamine (IPPD), *N,N*-Diphenyl-*p*-phenylenediamine (DPPD), or *N*-Phenyl-*N*-cyclohexyl-*p*-phenylenediamine (CPPD).

2.7. ISSUES RELATED TO RESIDUAL CHEMICALS

There is reliable scientific evidence that the chemicals present in NRL pose a risk of sensitization especially to an atopic population (Cronin, 1980; Estlander *et al.,* 1986; von Hintzenstern *et al.;* 1991; Conde-Salazar *et al.,* 1993; Wrangsjo and Meding, 1994; Durate *et al.,* 1998; Gottlober *et al.,* 2001). A variety of rubber products used in domestic, medical, and industrial fields cause allergy. The primary sensitizing substances in the domestic and medical fields include thiurams, dithiocarbamates, and mercapto compounds. The sensitizing substances in industrial rubber products such as masks, conveyer belts, rubber tyres, inner tubes, hoses and rubber boots are comparably stronger, and affect mainly men. In this case, sensitization is predominantly caused by the antioxidants of the paraphenylenediamine group such as IPPD, DPPD, or CPPD. Other chemicals such as mercapto compounds and diphenylguanidine (DPG) present in heavy duty black rubber products also cause allergy. Dermatologists in Finland and the United Kingdom reported that the most common type of chemicals inducing allergic contact dermatitis in the general population were the rubber chemicals (Kanerva *et al.,* 1995; Cherry *et al.,* 2000). With the increased general interest in the health and safety about materials used at work, there has been an upsurge in testing and investigation of these chemicals. Studies at allergy centres worldwide have shown that the frequency of

allergic reactions to rubber chemicals varies in the range 3-14% as shown in Table 2.4 (Fuchs, 1996).

Table 2.4. Prevalence of sensitization to rubber chemicals

Study period	Country	Positive reactions (%)
1965-1976	England (Cronin, 1980)	Thiuram mix - 2.3-5.4
1979	Hungary	14.1
1976-1979	Australia	10.2
1981-1984	France	7.9
1980-1982	Finland (Lammintausta and Kalimo, 1985)	Thiuram mix, carba mix, PPD mix and mercapto mix - 4.7
1996-1998	North America (Marks *et al.*, 2000)	Carba mix - 7.3 Thiuram mix - 6.9 *p*-phenylenediamine - 6.0
1985-1990	Germany (von Hintzenstern *et al.*, 1991)	Thiuram mix and carbamix - 3.8

Allergic reactions to synthetic gloves made of polyvinyl chloride (PVC), and nitrile rubber have also been reported (Estlander *et al.*, 1986; Aalto-Korte *et al.*, 2003; Horn and Aldridge, 2003). Estlander *et al.*, (1986) reported 68 cases of allergy caused by rubber or plastic gloves. Two patients had contact urticaria due to rubber gloves; 38 of them had positive reactions to rubber chemicals and glove material; 14 to glove material only, and 11 to rubber chemicals. Five cases of allergic eczema from plastic gloves were diagnosed, all due to PVC gloves. A ten year study among 7000 patients in Spain to rubber chemical mixes showed that the rubber additives, mainly accelerators and antioxidants, are a cause of occupational contact dermatitis and the prevalence was put at 14.7% (Conde-Salazar *et al.*, 1993). Studies showed that over 85% of the patients with glove intolerance had either irritant contact dermatitis or allergic contact dermatitis or both (Heese *et al.*, 1991; Finn and Rycroft, 1996; Nettis *et al.*, 2002; Horwitz *et al.*, 2002).

The scientific literature cites a number of adverse toxicological effects other than allergic contact dermatitis. The cytotoxic nature of NRL materials has been well-documented (Wilsnack, 1976; Ikarashi *et al.*, 1992; Talja *et al.*, 1993;

Pariente *et al.,* 2000; Drewa *et al.,* 2004). It has been reported that the cytotoxicity and tissue irritancy of latex medical devices are mainly due to zinc dialkyldithiocarbamate residues (Nakamura *et al.,* 1990; Ikarashi *et al.,* 1992; Nicolaysen *et al.,* 2004). A study by Ikarashi *et al.,* (1992) showed that the thickness of the inflammatory layer in the rabbit implantation test could be correlated with the amount of leachable chemicals from the rubber gloves. Their study indicated that the degree of inflammatory response is attributed to the amount, and toxic potential of residual chemicals as well as their rate of leaching into the tissue. Clinical complaints such as urethritis and urethral strictures from the use of Foley catheters made of NRL have been reported (Graham *et al.,* 1983; Wilksch *et al.,* 1983; Graham *et al.,* 1984; Talja *et al.,* 1986; Singh *et al.,* 1994; Woodward, 1997). Wilksch *et al.,* (1983) analyzed some of the urinary catheters that reportedly caused rapid strictures in patients, and reported an excellent correlation between the cytotoxic effects of soluble extracts from catheters and the degree of acute and chronic inflammation induced during the subcutaneous implantation in rats. Graham *et al.,* (1984) observed a good agreement between *in vitro* cell culture and rabbit implantation tests of urinary catheters and concluded that the tissue damage was apparently caused by leachable chemicals in the catheters.

Ikarashi *et al.,* (1992) demonstrated that mercaptobenzothiazole accelerators have lower cytotoxicity than dithiocarbamate type even though they are known to be contact allergens. 2-(2-hydroxyethylmercapto) benzothiazole, which formed during ethylene oxide sterilization of MBT cured rubber products, is reported to be highly toxic (Petersen *et al.,* 1981; Salmona *et al.,* 1984). Many nitrosamines, which are produced mainly from thiurams and dithiocarbamates, have shown to be carcinogenic in a number of animals and this led to the strong presumption of carcinogenicity in humans. A detailed review on the issues related to nitrosamines is given in the forthcoming section.

2.7.1. Assessment of the hazardous nature of rubber chemicals

In vitro cytotoxicity test is a kind of screening test which assesses the cytotoxicity potential of chemicals. There are three methods for sample application in the test (ASTM F 895, 1984; ISO 10993-5, 1999). They are: (i) direct contact test, (ii) test on extract and (iii) agar diffusion test. The direct contact test evaluates the

direct effect of cell-material interaction. The methods (ii) and (iii) are for evaluating the effect of toxic chemical(s) leaching from the material to the cells. Nakamura *et al.*, (1990) developed a new *in vitro* colony assay using V79 cells to assess the cytotoxicity potential of rubber chemicals, and expressed it in terms of IC_{50} value. The IC_{50} value of a substance is defined as the percentage extract concentration that will suppress colony formation to 50% of the control value (Table 2.5).

The short-term toxicity of any chemical is determined by animal feeding tests and skin irritation tests (ASTM E 1163, 1987; ISO 10993-1, 1999; ISO 10993-10, 1995; OECD-420, 2001). In animal feeding tests, the LD_{50} (median lethal oral dose) value is determined. LD_{50} is a statistically derived single dose of a substance that is expected to cause death in 50% of animals (usually rats) when administered by the oral route and is expressed as the weight of the substance administered per unit body weight of the experimental animal (mg/kg). The Organization for Economic Co-operation and Development (OECD) gives a guideline for testing of chemicals for the determination of acute oral toxicity by fixed dose method, which gives an idea about the hazardous properties and allows the substance to be ranked according to the Globally Harmonized System (GHS) (OECD-420, 2001). The test essentially involves stepwise dosing of animals using fixed doses of 5, 50, 300 and 2000 mg/kg (exceptionally an additional dose of 5000 mg/kg may be considered). The different classification by GHS includes category 1, 2, 3, 4, 5 and the unclassified. In cases where an animal tested at the lowest dose of 5 mg/kg dies, the substance is assigned to GHS Category 1.

The LD_{50} and IC_{50} values of some of the commonly used rubber chemicals are given in Table 2.5.

Table 2.5. LD_{50} and IC_{50} values of rubber chemicals

Name of the rubber chemical	LD_{50} (mg/kg) (BRMA, 1999)	IC_{50} (μg/ml) (Nakamura *et al.*, 1990)
Diphenylguanidine (DPG)	350	a
Bis(2,2'-benzothiazolyl disulfide (MBTS)	>5000	>200
2-Mercaptobenzothiazole (MBT)	3000	49
Tetra ethylthiuramdisulfide (TETD)	3400	a
Tetra methylthiuramdisulfide (TMTD)	560-1050	a
Zinc mercaptobenzothiazole (ZMBT)	540	a
Zinc ethylphenyldithiocarbamate (ZEPC)	>2,000	1.6
Zinc pentamethylenedithiocarbamate (ZPC)	a	0.41
Zinc dibenzyldithiocarbamate (ZBEC)	>16,000	a
Zinc dibutyldithiocarbamate (ZDBC)	>10,000	5.40
Zinc diethyldithiocarbamate (ZDEC)	3,500	0.40
Zinc dimethyldithiocarbamate (ZDMC)	500 - 1,400	0.24

a-not reported

Skin sensitization to chemicals is assessed by the skin patch tests or modified draize-95 test (ASTM D 6355, 1998; FDA, 1999; ISO 10993-10, 1995). The patch test is to determine whether a finished NRL product contains residual chemicals that might cause skin reaction in individuals who are already allergic to one or more of the following group of chemicals, namely thiazoles, thiurams and dithiocarbamates. The modified draize-95 test evaluates whether a finished NRL product may induce allergy from the chemicals in the unsensitized general population. The current methods to evaluate sensitivity to latex products relate to patch tests of various rubber chemical mixes and such tests are being done in numerous patch test clinics throughout the world. The North American Contact Dermatitis Group has studied the prevalence of sensitivity to rubber chemicals over a period of 29 years, and recommends that patch test be performed with rubber chemical mixes rather than single substances (Marks *et al.*, 1998; 2000). The commonly used chemical mixes are carba mix (DPG, ZDBC and ZDEC in equal quantities to get a final concentration of 0.25 mg/cm^2), thiuram mix (TMTM, TMTD, TETD, and DPTT mixed in equal quantities to get a final

concentration of 0.025 mg/cm^2) and mercapto mix (CBS, MBTS and MMBT mixed in equal quantities to get a final concentration of 0.075 mg/cm^2) dispersed in a gel vehicle.

With regards to the carcinogenicity, there are now half a dozen short-term cancer tests that have been shown to give useful results with a particular group of chemicals (Purchase, 1976). Using these tests, chemicals with mutagenic activity and carcinogenic activity can easily be found out. The best known method at present is the Ames test (Ames, 1973). Tinkler *et al.,* (1998) used five different tests to assess the mutagenicity potential of zinc dithiocarbamate accelerators. They were (i) bacterial mutagenicity test with salmonella typhimurium (Ames test) (ii) *in vitro* cytogenicity test using human lymphocytes (HL CA) (iii) mouse lymphoma cell mutation assays (ML MUT) (iv) mouse bone marrow micronucleus test (Mn), and (v) rat liver (*ex vivo*) unscheduled DNA synthesis (UDS) test.

The ultimate proof of the safety of the chemicals used in the industry is obtained by showing that the health of the workers in the industry is unaffected by the chemicals and this is usually done by an epidemiological survey. The survey includes tracing a population which entered a factory between set dates, and recording the eventual mortality patterns of the population. It is then compared with an equivalent local population outside the factory (cohort studies).

2.7.1.1. Epidemiological survey

The epidemiological surveys are essentially retrospective in nature in that a considerable number of years will usually elapse between exposure to the chemical under scrutiny and deaths from the disease. Cohort studies on many types of cancers among the workers of the rubber factory have been reported; many of them report an increased risk of cancer (Bovet and Lob, 1980; Negri *et al.,* 1989; Szeszenia-Dabrowska *et al.,* 1991; Ietri *et al.,* 1997; Sorahan *et al.,* 2000; Li and Yu, 2002; Veys, 2004). Significantly high risk of bladder cancer has been reported for the exposed workers for the period 1946-1995, but this reversed when the carcinogen (β-naphthylamine as contaminants in antioxidants) was removed from processing from October 1949 (Veys, 2004). An increased risk of lung cancer with duration of exposure among workers in a tyre-curing department was observed in another cohort

study by Li and Yu (2002). In India, a number of surveys among factory workers handling rubber chemicals have been reported (Kumar and Tandon, 1997; Tandon and Kumar, 1997; Yadav and Chhillar, 2001). The effects of factory environment on functional integrity of auditory pathway have been studied in forty rubber factory workers using Brainstem Auditory Evoked Potentials (BAEPs) technique to detect early subclinical impairments. The results indicated that 47% of the workers showed abnormalities in prolongations of either peak latencies or interpeak latencies when compared with age and sex matched control subjects not exposed to rubber factory environment (Kumar and Tandon, 1997). In another study, the genotoxic effect (mitotic index, chromosomal aberrations, and sister chromatid exchanges) on somatic chromosomes of human lymphocytes of 50 workers exposed to environmental pollutants generated in a rubber tyre industry was investigated. All the parameters showed a significant increase ($p<0.01$) in the exposed workers compared with the control groups indicating that the environmental pollutants generated in rubber industry were genotoxic (Yadav and Chhillar, 2001). It has been reported that the rubber factory environments affect the conduction processes in optical pathways from their origin in the retina to striate cortex in 51 % of the factory workers (Tandon and Kumar, 1997). A patch test study using Indian Standard Series and indigenous antigens among seventy five patients with clinically suspected contact dermatitis in Delhi showed that mercaptobenzothiazole was the cause in 6.6 % of the patients (Singhal and Reddy, 2000).

2.7.2. Dithiocarbamates

In industry, dithiocarbamates are used as vulcanization accelerators for rubber, slimicides in water-cooling systems, in sugar, pulp and paper manufacturing. They are also used as scavengers in waste water treatment owing to their ability to chelate heavy metals. They show antabuse effect, and hence are used to treat chronic alcoholism. Apart from this, they are used as fungicides, they being effective against a broad spectrum of plant diseases. Their widespread use, and the large quantities released into the environment, led to an increased exposure of humans to dithiocarbamates.

Based on their likelihood to generate a common type of toxic molecule or a reactive intermediate, or their ability to mimic a common biologically active molecule

that interferes with the normal homeostasis of the cell, dithiocarbamates are classified into three groups: ethylene bisdithiocarbamates (EBDTCs), dimethyldithiocarbamates (DMDTCs) and monomethyldithiocarbamates (MMDTCs) (Figure 2.5). Most of the dithiocarbamates shown in Figure 2.5 are used as fungicides. The dithiocarbamates used in latex industry are mainly the zinc salts, and are shown in Figure 2.6.

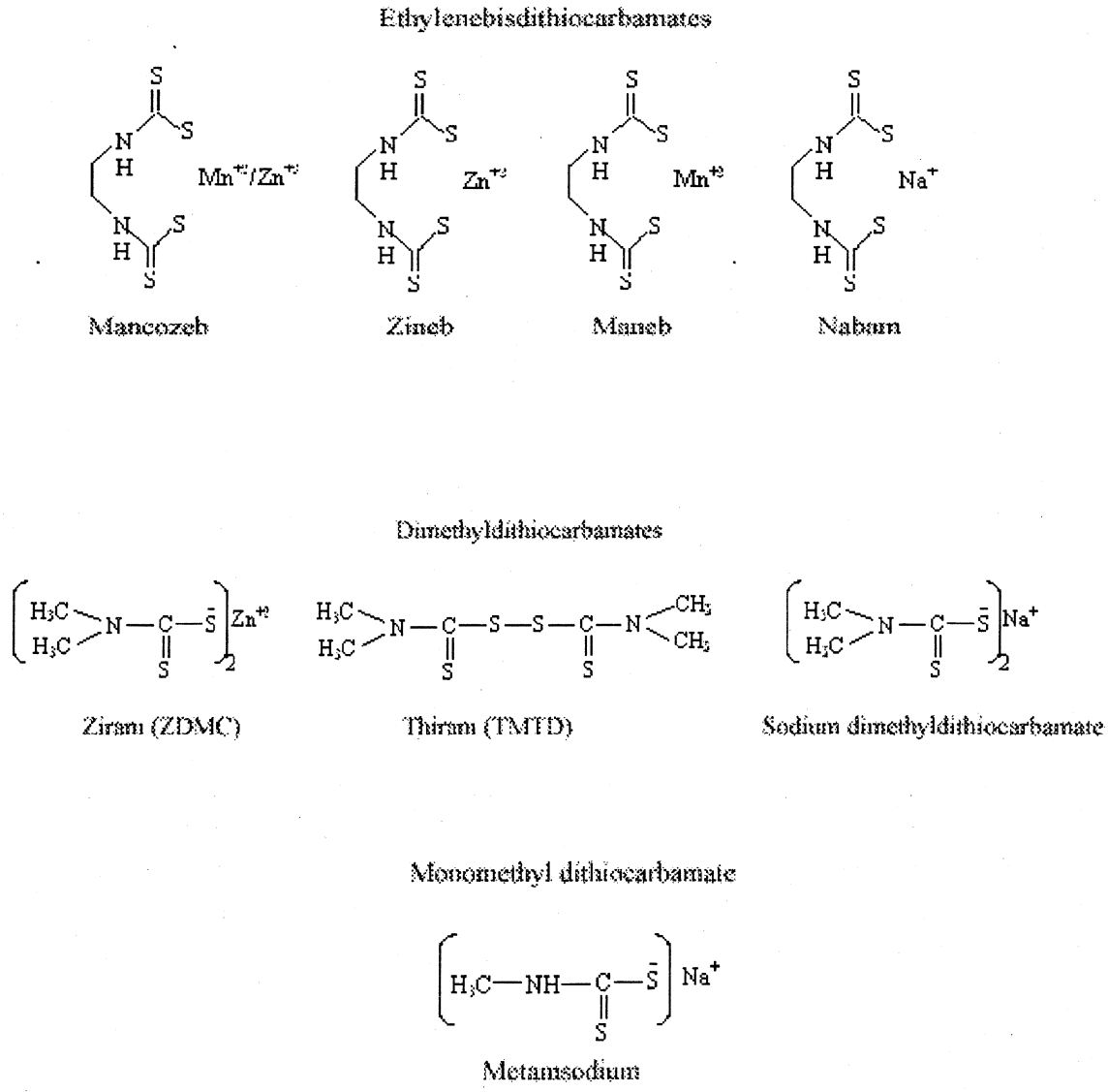


Figure 2.5. Examples of the different types of dithiocarbamates

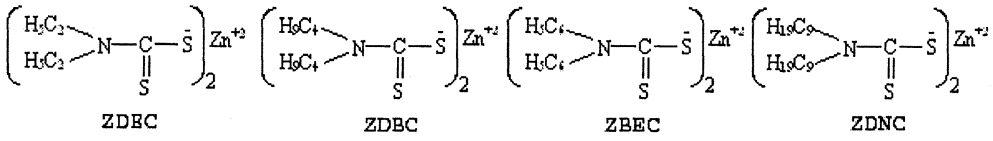


Figure 2.6. Different types of zinc dithiocarbamates used in the latex industry

Dithiocarbamates are absorbed by a number of routes such as skin, mucosal membrane, respiratory and gastrointestinal tracts which then undergo extensive biotransformation as evidenced from animal studies (Mulkey, 2001). The metal complexed alkylenebisdithiocarbamates are absorbed poorly both by the gastrointestinal tract and by the skin. The acute oral and dermal toxicities of various dithiocarbamates are generally low (WHO, 1988). Most products have low volatility and hence only limited information on inhalation toxicity is available. When dithiocarbamates are inhaled as dust, local irritation of the respiratory tract occurs. This dust can also induce eye and dermal irritation. Some are sensitizing agents, and the prevalence of allergic reactions is already detailed in section 2.7. Dimethyldithiocarbamate is reported to be positively associated with leukemia as indicated in a study among the workers employed for at least 1 year during 1943-1991 at any of the six plants that manufactured synthetic rubber (Delzell *et al.,* 2001).

Dithiocarbamates undergo extensive biotransformation with the release of carbon disulfide under acidic conditions existing in the stomach of the rats following oral ingestion (Truhaut *et al.,* 1973; Brocker and Schlatter, 1979; Johnson *et al.,* 1996). Other degradation products include sulphur, 6-Dihydro-3H-imidazole, ethylene thiourea (ETU), 2,4 Dithiazole-3-thione, hydrogen sulfide, 2-Thiothiazolidine-4-carboxylic acid (TTCA), and the parent amine (WHO, 1988). Figure 2.7 depicts the metabolic pathway (biotransformation) of thiram, a typical dimethyldithiocarbamate compound.

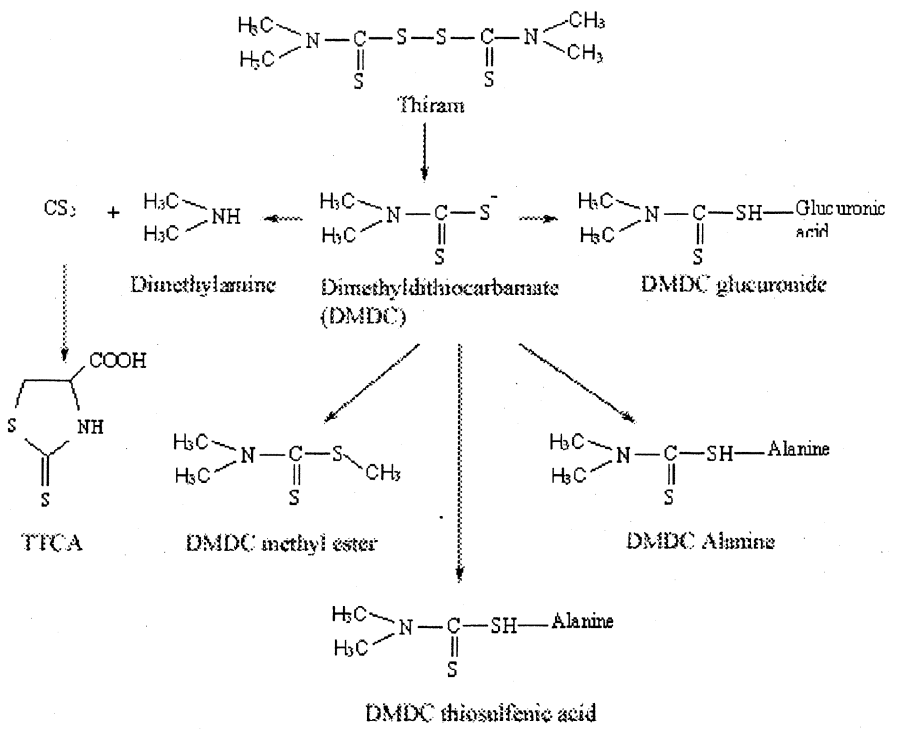


Figure 2.7. Biotransformation of thiram (adapted from Johnson *et al.,* 1996)

The rate of degradation depends on a number of factors such as pH, and the type of the compound. The EBDTCs are generally unstable in the presence of oxygen, moisture or biological systems. The dialkyldithiocarbamates undergo hydrolysis in water especially under acidic conditions. The half life of these compounds is pH dependent and the rate of decomposition increases with decreasing pH (Miller and Latimer, 1962; Aspila *et al.,* 1969; Van Leeuwen, 1986). For example, the ZDMC undergoes a pH dependent degradation in water and the half lives are given in Table 2.6 (Van Leeuwen, 1986).

Table 2.6. pH dependent degradation of ZDMC

pH of water	Half life (h)
3.8	0.25
5.7	9.1
7	121
8	433

Aspila *et al.,* (1969) reported that the dialkyldithiocarbamates decomposed rapidly in acidic solution into carbon disulfide and the respective dialkyl amine.

The decomposition half lives of dimethyl and diethyl dithiocarbamates were 15.5 and 7.3 sec at pH 2.2. The half lives increased to 36 and 13.9 sec respectively at a higher pH of 3.4. The degradation of dithiocarbamates in acid depends on the nature of the compound: the chelated compounds (eg. ziram) release carbon disulfide slowly compared to the non-chelated compounds (eg. sodium diethyldithiocarbamate) (Mulkey, 2001). They also undergo photo degradation and the half life of ZDBC is reported to be 0.691 h at 25 °C (Meylan and Howard, 1993).

The three modes of toxic action elicited by dithiocarbamates are due to (i) their capacity to generate carbon disulfide, (ii) the biotransformation to ETU, and (iii) the ability to chelate physiologically important ions such as copper. Ethylene thiourea, a major degradation product of the EBDTCs class, is of toxicological concern due to its carcinogenicity, teratogenicity, and antithyroid properties (WHO, 1988; Belpoggi *et al.,* 2002). The observed toxicological effects were found to be neuropathy, thyroid toxicity, central nervous system defects, and cholinesterase inhibition (Mulkey, 2001). Neuropathy and thyroid activity are the two common effects identified with dithiocarbamates. Dithiocarbamates induce neuropathy through carbon disulfide formed by the degradation *in vivo* (Jirmanova and Lukas, 1984; Schaumburg and Berger, 1992; Anthony *et al.,* 1996; Barlow *et al.,* 2003). Clinically, neuropathy involves degeneration and/or demyelination of sciatic or spinal nervous tissue. The experimental animals exposed to carbon disulfide become progressively weak, beginning at the hind limbs; and may experience weakness in more proximal muscle groups (Anthony *et al.,* 1996). In exposed humans, there is an initial stocking-and-glove distribution of sensory loss, which may progress to involve more proximal sensory and motor axons (Mulkey, 2001). Johnson *et al.,* (1996) showed that the dithiocarbamates had, on an average, comparable bioavailability as an equimolar dose of pure carbon disulfide administered orally to rats. Long-term exposure to dithiocarbamates has been associated with parkinsonism, and epidemiological studies have found an increased risk of neurocognitive impairment associated with long-term exposure to pesticides in general and to EBDTCs specifically (Debbarh *et al.,* 2002). Thyroid toxicity is mainly induced by ETU (Miller, 1982). The dithiocarbamates such as mancozeb, maneb and ziram induced thyroid toxicity in rats and mice. A 2-year long carcinogenicity study in rats with ziram provided evidence of an increased incidence of C-cell carcinomas (NTP Technical report, 1983). The developmental

toxicity studies revealed that mancozeb and thiram induced central nervous system defects in rats. Dithiocarbamates chelate with heavy metals such as copper, cadmium, nickel and lead, and hence are widely used for the treatment of metal intoxication. However, dithiocarbamate-chelates are lipophilic in nature, and lead to the redistribution of the heavy metals into the brain (Mulkey, 2001). In another instance, the dithiocarbamates alter the redistribution of cadmium within the organism, thereby increasing the cadmium content in the lungs of experimental rats, and the observed structural changes are more serious than those of cadmium exposure alone (Tatrai *et al.,* 2001).

2.7.2.1. Zinc dithiocarbamates

2.7.2.1.1. Toxicological aspects

Zinc dithiocarbamates are widely used as vulcanization accelerators as they possess a very active vulcanization time-temperature profile required in the manufacture of NRL products (Pendle, 1997). A comparative evaluation of the toxicity and allergenicity potential of some of these dithiocarbamate accelerators has been carried out by various researchers. The order of the cytotoxicity potential was ZDMC > ZDEC = ZPC > ZEPC > ZDBC (Nakamura *et al.,* 1990). Korhonen *et al.,* (1983) showed that dithiocarbamate type chemicals showed embryo toxicity in the order ZDEC > ZEPC > ZDBC. It has been shown that as little as 0.005% of zinc dithiocarbamate is enough to cause complete inhibition of the curing of the vinyl polysiloxane impression materials used in dentistry (Causton *et al.,* 1993). The intracutaneous irritation potential of ZDEC, ZDBC and ZBEC is found to be in the order ZDEC > ZDBC > ZBEC (Mohanan *et al.,* 1998). Zinc dimethyldithiocarbamate and other bis dithiocarbamates have been shown to be goitrogenic in laboratory animals and possibly in humans (Sen *et al.,* 1974). The allergenic potency of various dithiocarbamates is in the order ZDEC > ZPC > ZDMC > ZDBC as determined by local lymph node assay with *ex vivo* tritium thymidine labeling (De Jong *et al.,* 2002). According to Kaniwa *et al.,* (1986), the order of the allergenic potency was ZDMC >> ZDEC, ZEPC > ZDBC, ZPC based on the incidence of positive reactions during patch testing of patients. A study by National Biological Standards Laboratory (NBSL, Australia) showed that ZDEC was released from a particular brand of catheter which was a subject of clinical complaint in 1980 (Graham *et al.,* 1983). Other researchers

showed that the residual chemicals in urinary catheters caused tissue damage in animals and humans (Talja *et al.,* 1986; Singh *et al.,* 1994). Toxic effects of dithiocarbamates to liver, kidney, testis, and placenta in humans and animal models have also been reported (Koizumi *et al.,* 1979; Cereda *et al.,* 1989; Guven *et al.,* 1998; Delzell *et al.,* 2001; Debbarh *et al.,* 2002; Thompson *et al.,* 2002). Recently, it has been reported that ZDEC in concentrations of about 0.25% weight of the penrose drain may induce local toxicity and delayed wound healing in mice (Nicolaysen *et al.,* 2004).

Data concerning the mutagenicity of dithiocarbamate are generally incomplete and inconsistent. Zinc dimethyldithiocarbamate has been found to be mutagenic to bacteria, mammalian cells, and drosophila (Brooks *et al.,* 1983; Donner *et al.,* 1983; Haworth *et al.,* 1983; Moriya *et al.,* 1983; Rannug *et al.,* 1984; Tennant *et al.,* 1987; Hemavathy and Krishnamurthy, 1989; Tripathy *et al.,* 1989). A study on factory workers occupationally exposed to ZDMC showed evidence of chromosome damage in lymphocytes cultured from peripheral blood (Pilinskaya, 1970). Tinkler *et al.,* (1998) conducted mutagenicity studies of ZDMC, ZDEC, and ZDBC using Ames test, *in vitro* cytogenicity tests using human lymphocytes (HL CA), mouse lymphoma cell mutation assays (ML MUT), mouse bone marrow micronucleus tests (Mn), and rat liver (*ex vivo*) unscheduled DNA synthesis (UDS) tests. The study indicated that the ZDMC must be considered as genotoxin (and thus a probable carcinogen). On the other hand, ZDEC was found to be genotoxic *in vitro* but was not clearly genotoxic *in vivo* and is considered to have an intermediate activity between those of ZDMC and ZDBC. Zinc dibutyldithiocarbamate showed no evidence of mutagenicity in *in vitro* and *in vivo* mutagenicity testing. Figure 2.8 shows the mutagenicity profile of ZDMC, ZDEC and ZDBC.

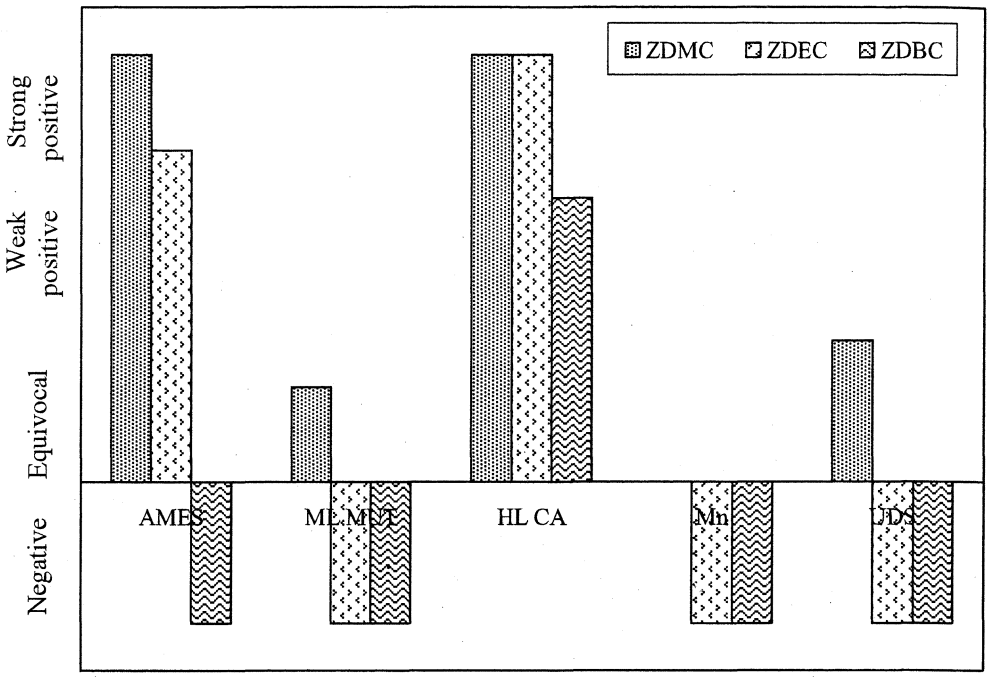


Figure 2.8. Mutagenicity profile of ZDMC, ZDEC and ZDBC (adapted from Tinkler *et al.*, 1998)

N-Nitrosamines, a degradation product of some of the accelerators, are of particular concern owing to their carcinogenicity. Nitrosamines are commonly found in rubber, metal, food, and leather industries. The rubber products that are of particular concern are baby bottle teats, soothers, gloves, condoms, potable water components and pharmaceutical products that are vulcanized by traditional accelerators such as dithiocarbamate, thiuram, and sulfenamides. Most of these accelerators produce nitrosamines whose level in the environment is regulated by many organizations. Table 2.7 lists some of the candidate accelerators that are capable of producing regulated and safe nitrosamines.

Table 2.7. List of accelerators that produce nitrosamines

Accelerator	Regulated nitrosamines	Safe nitrosamines
Dithiocarbamates	ZDMC, ZDEC, ZPC, ZDBC	ZDNC, ZBEC
Thiurams	TMTM, TMTD, TETD, TBTD, DPTT	TBzTD
Sulfenamides	CBS, TBBS, OBTS, MBSS, OTOS	DCBS
Thioureas	ETU, DETU	-

These accelerators decompose to produce secondary amines, which are then nitrosated to form nitrosamines (Figure 2.9). Amines can be nitrosated in air or in

solution under acidic, neutral or alkaline conditions. The nitrosating agents are nitrogen oxides (NO, NO_2, N_2O_4, N_2O_3), nitrite and nitrous acid (Pitts *et al.,* 1978; Challis *et al.,* 1978; Fine, 1979). The nitrosation reaction is catalyzed by thiocyanate, halide ions, metal ions, formaldehyde and ozone (Sander *et al.,* 1975; Challis *et al.,* 1978; Fine, 1979).

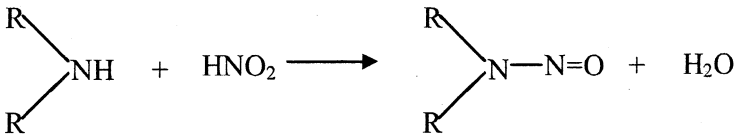


Figure 2.9. Formation of nitrosamine

Barnes and Magee (1954) showed that *N*-dimethylnitrosamine was a powerful carcinogen in experimental animals. The massive studies started thereafter identified over 250 nitrosamines as mutagens and carcinogens, of which 90% have exhibited carcinogenic activity in a wide range of animal species (Fiddler *et al.,* 1996). The target organs are liver, kidney, lungs, skin and eyes, as revealed from studies using experimental animals (Yahagi *et al.,* 1977; IARC, 1978; Edelman *et al.,* 1980; Castegnaro *et al.,* 1992). There are several studies which indicated that the users of rubber products and workers of the rubber factory are constantly exposed to the carcinogenic nitrosamines. The primary routes of exposure to nitrosamines are ingestion, inhalation, and dermal contact. It has been shown that ZDMC decompose under acidic conditions to dimethyl nitrosamine in the presence of nitrite (Mirvish, 1975). Exposure measurements to nitrosamines in general areas or personal breathing zones in 24 rubber manufacturing plants were taken for the period 1992 to 1995, and it has been found that the workers were exposed to five different nitrosamines of which *N*-nitrosodimethylamine is the most frequently encountered compound (Oury *et al.,* 1997). Another epidemiological study (study period 1976-1991) on mortality rates among female workers employed in a rubber tyre factory indicated excessive deaths from cirrhosis of the liver, which may be due to exposure to nitrosamines (Straif *et al.,* 1999). Persons with acidic perspiration who use latex gloves containing secondary amine accelerators are expected to be at a higher risk than others (Dillard, 1997). In the case of baby feeding bottle teats, the formation of nitrosamines is accelerated by boiling used for sterilization (Rowat, 2001). The nitrosamine level may be raised further inside the baby's body, when precursor amines are converted into

nitrosamines under acidic conditions prevailing in the stomach (Lijinsky and Epstein, 1970). Thiuram type accelerators get decomposed to dithiocarbamates in presence of excess of ammonia and zinc oxide present in the latex compound, and hence equally pose a threat as in the case of dithiocarbamates (Barnard and Lewis, 1988).

The risks posed by the chemical and their degradation products are a concern as far as the health of the users is concerned. The fact that chemicals used in the manufacture of NRL products are well known contact allergens is only a concern. It is the extent to which they are bioavailable that makes them a hazard to the health of the users.

2.7.2.1.2. Bioavailability

Bioavailability is defined as the state of being capable of absorbed and available to interact with the metabolic processes of an organism (EPA, 1992). Bioavailability encompasses several processes: the release of the chemical from the media, uptake of the chemical across biological membranes such as the skin, stomach, intestine, or lungs and the distribution of the chemical to specific organs or cells. Zinc ethylenebisdithiocarbamate (zineb) is reported to be absorbed through the gastrointestinal tract to a limited extent (Hayes, 1982). Permeation of chemicals through the skin occurs by a process called diffusion, which is governed by Fick's First Law (EPA, 1992). Skin thickness, condition of the skin, concentration of chemical available on the skin, and the physical/chemical properties of the chemical are the major determinants of the absorption rate of chemicals through the skin. Thus, it is not surprising that variations in skin thickness at various anatomical sites, particularly of the stratum corneum, can influence the rates of permeation of the substances. It has been found that absorption through skin in the genitals in man is about 12 times faster compared to forearm (Maibach *et al.*, 1971).

Tinkler *et al.*, (1998) demonstrated the possibility that the dithiocarbamates present in latex goods may be available for uptake by users. No information is available on the dermal permeation of these dithiocarbamate residues present in the latex goods. Reports of allergic reactions to dithiocarbamates imply limited permeation through the skin. When the gloves are worn, the hands perspire and sweat will be formed between the skin and the glove. This warm and moist environment is

an ideal condition to solubilise the residual chemicals present in the gloves. Once these chemicals are solubilised, a portion of the solubilised chemicals permeates the skin, sensitizing the user (Knudsen *et al.,* 1993). In addition, the sweat formed will not be able to escape from the occluded skin. This will result in the reabsorbtion of the sweat into the skin that may further increase the likelihood of dermal absorption of chemicals (Boeniger, 2002). Chemical uptake through mucosal membrane, as in the case of condoms, is likely to exceed that of an equal concentration through intact skin (Maibach *et al.,* 1971). It has been suggested that dithiocarbamates in condoms and catheters that are in contact with the mucosal membrane may be considered as completely available to the human body (Tinkler *et al.,* 1998).

Various researchers made attempts to study the bioavailability of residual chemicals using different extraction media such as phosphate buffer, acetone, human plasma and artificial sweat (Knudsen *et al.,* 1993, 2000a; Emmet *et al.,* 1994; Knudsen and Menne, 1996b). It has been reported that extraction with phosphate buffer and acetone may not fully reflect the bioavailable chemical residues (Knudsen *et al.,* 2000a). The human plasma has been shown to bind the residual chemicals giving erroneous results (Emmet *et al.,* 1994). In the case of extraction using artificial sweat, it has been observed that gloves releasing higher amounts of dithiocarbamates and/or thiurams into artificial sweat elicit a greater number of positive reactions during patch test than those releasing smaller amounts (Knudsen *et al.,* 1993). It, therefore, implied that the amount of residual accelerators released into artificial sweat may be a good indication of the allergenicity of the gloves. Knudsen *et al.,* (1993) suggested that the amount of residual chemicals released into the human sweat influences the quantity that permeates the skin i.e., the bioavailable amounts of chemicals. This implied that the determination of residual chemicals released into the human sweat may give an estimate of the bioavailability of these chemicals. However, the use of human subjects for routine tests is not a feasible method necessitating the development of an *in vitro* test for the determination of bioavailability. Although there are several methods that allow the identification and quantification of chemicals, *in vitro* tests that measure allergologically relevant bioavailable residues of chemicals in the products are not yet available (EEC/93/42, 2004).

2.7.2.1.3. Determination of dithiocarbamates

The toxicological evaluations of dithiocarbamates showed that they differ greatly in their degree of toxicological action and mechanisms (Nakamura *et al.,* 1990; Tinkler *et al.,* 1998; Mulkey, 2001; Van Och *et al.,* 2001). Determination of residual dithiocarbamates using a suitable extraction medium is, therefore, important to assess the safety of the products made from NRL. The extraction medium should swell the vulcanized product in order to extract the residual accelerators from the interior of the product. Also, the extraction medium or the conditions should not decompose chemical residues. It has been reported that some dithiocarbamates, for example, piperidine pentamethylenedithiocarbamate underwent complete decomposition when the vulcanizate containing the above compound was soxhlet extracted using acetone (Parker and Berriman, 1954). Other dithiocarbamates also undergo minor decomposition during hot acetone extraction, however, the effect is small compared to piperidine pentamethylenedithiocarbamate. Tetramethylthiuramdisulfide was found to get decomposed in cold acetone and it is recommended to use cold dichloromethane for its extraction (Parker and Berriman, 1954). The extraction of rubber products containing MMBT and CBS in acetone yielded new compounds suggesting that acetone and other ketones are not suitable for the quantification of such chemical residues (Hansson *et al.,* 1997).

Several methods are described in the literature for the determination of dithiocarbamate fungicide residues in vegetables and food stuffs. The predominant method for the determination of dithiocarbamates and its metabolites is based on their decomposition to carbon disulfide in an acid medium followed by iodometry (Clarke *et al.,* 1951), spectrometry (Dubey and Stan, 1998; Heise *et al.,* 2000; Caldas *et al.,* 2001), and head space chromatography (Ahmad *et al.,* 1996; Perz *et al.,* 2000). But these methods are time consuming and preclude the analysts from distinguishing different types of dithiocarbamates (Malik and Faubel, 1999; Blasco *et al.,* 2004). Dithiocarbamates have also been determined in vegetable foodstuffs using HPLC (Gustafsson and Falhgren, 1983), extraction voltammetry (Ulakhovich *et al.,* 1983), and titrimetry (Verma *et al.,* 1982). The estimation methods other than acid decomposition include iodometry in anhydrous solvents (Grand and Tamres, 1968; Clyde, 1983), indirect titration with ethylenediamine tetraacetic acid (Hyman, 1969),

and polarography (Halls *et al.,* 1968). However, all of these methods suffer from the following disadvantages: (i) methods other than gas chromatography (GC) are indirect, time-consuming and sensitivity is low; and (ii) gas chromatographic methods are sensitive, but suffer from a lack of selectivity since all dithiocarbamate pesticides evolve carbon disulfide on acid hydrolysis. Blasco *et al.,* (2004) described a quantitative solid phase dispersion and liquid chromatography-atmospheric pressure chemical ionization mass spectrometry (LC-APCI-MS) method for the simultaneous analysis of dazomet, disulfiram, thiram, ETU and propylene thiourea residues in fruits and vegetables.

With regard to the health care products made from NRL, a number of analytical methods based on HPLC and GC have been reported for the analysis of allergologically relevant accelerators (Kaniwa, 1987; Knudsen *et al.,* 1993; 2000a; Mathieu *et al.,* 2000; Depree *et al.,* 2004). However, the quantification of zinc dithiocarbamates by HPLC is associated with a number of problems. Ligand substitution reactions between the zinc dithiocarbamates and nickel from the stainless steel components of the chromatographic system lead to the formation of multiple peaks or the absence of any peaks altogether (Hutchins *et al.,*1982; Depree *et al.,* 2004). Attempts to minimize these exchange reactions with the use of glass or polytetrafluroethylene-lined columns or by deactivating the metal parts with an organosilane were made (Häring and Ballschmiter, 1980; Shih and Carr, 1981). However, both methods suffer from disadvantages such as loss of column efficiency and lengthy procedures respectively. Mathieu *et al.,* (2000) reported another method, which minimizes the ligand exchange reactions by using polyetheretherketone-lined chromatographic components coupled with saturation of system with zinc ions, but it is expensive. Although a number of estimation methods are available for the determination of zinc dithiocarbamates, there seems to be no agreement on the best applicable method for the analysis of residual chemicals in the latex products (EEC/93/42, 2004).

The formation of highly coloured metallic complexes by dithiocarbamates has been a subject of interest to many researchers. Metal salts of copper, cobalt, nickel, lead, and iron have been used for the detection of dithiocarbamates in the solvent extracts of vulcanized rubber (Parker and Berriman, 1954). Dithiocarbamate

compounds have sulphur atoms as electron donor in their structures and, uncharged chelates are formed when metal ions react with these bifunctional ligands. Also, the high molar absorptivities of metal dithiocarbamate complexes allow the estimation of these complexes by spectrophotometry (Tokalioglu *et al.,* 2002). The use of cobalt chloride in the determination of dithiocarbamates has been well-documented (Kaniwa, 1987; Tinkler *et al.,* 1998; Depree *et al.,* 2004). However, the determination of dithiocarbamates as their cobalt complexes is severely limited because of the poor peak shape, poor linear standard curves, and the presence of extra peaks when more than one type of accelerator is present (Kaniwa, 1987; Depree *et al.,* 2004).

2.8. POWDER FREE GLOVES AND OTHER ALTERNATIVES

The powder free gloves emerged as a result of adverse health effects from the use of powdered gloves. One method to make powder free gloves is chlorination. Chlorination of the NRL gloves is performed by immersing the gloves in a dilute chlorine solution (usually between 0.050-0.30 wt%) (Aziz, 1993). The chlorine reacts with the rubber surface to reduce the natural tackiness, thus eliminating the need to add a dusting powder to the glove. Chlorination suffers from a number of disadvantages such as deterioration of the physical properties, poor reproducibility in chlorination level, strong odour, presence of skin irritants, and over-chlorination (Khew and Ho, 1997; Aziz, 1993). Wood *et al.,* (1997) showed that chlorination process adversely affects shelf life, grip and in-use durability of the glove. An accelerated aging test of powder free gloves, all of them believed to be of chlorinated types, done at the FDA laboratory showed a dramatic decrease (70-90%) in tensile strength at 21 days of aging (Walsh *et al.,* 2001). Despite many disadvantages, chlorination remains the popular method of making powder free gloves.

The peroxide prevulcanized natural rubber latex (PPVL) and radiation vulcanized natural rubber latex (RVNRL) were introduced to address the problems posed by accelerator residues and nitrosamines. Davies and Gazeley (1993) showed that leached PPVL films have poor resistance to accelerated aging (loses about 80-90% of its tensile strength) while, unleached films show much better resistance. Although the RVNRL showed high tensile strength values together with low modulus and high elongation suitable for the preparation of coagulant films, the heat aging behaviour of RVNRL films was not good (Makuuchi *et al.,* 1988;

Gazeley and Pendle, 1989). The heat aging behaviour is improved by the addition of tris(nonylated phenyl) phosphate but it may cause sensitization on skin contact (Ciba-Geigy, 1992; Tay, 2001).

Another alternative to powdered NRL glove is a NRL glove having a synthetic polymer lining on its inner side, and the slippery inner surface facilitated easy donning on wet or dry hands (James *et al.,* 1985; Fisher *et al.,* 1996; Nile *et al.,* 1996). Synthetic polymer lining may be of a hydrogel, silicone or polyurethane. The NRL glove is dipped into a solution of the hydrogel prior to the final curing stage of glove manufacture. A commercially available hydrogel coated glove is Biogel® marketed by M/s. Regent Medical, coated with a terpolymer of polyhydroxyethyl methacrylate, polyacrylic acid and a crosslinking agent (Heese *et al.,* 1991). The latest trend in the clinical field is to use specific gloves for each application. A range of powder free gloves such as Biogel® A, Biogel® M, Biogel® Neotech® A, Biogel® Reveal™ A, Biogel® orthopedic™, Skinsense™, Biogel® indicator™ underglove etc. is commercially available from Regent Medical. The Biogel® indicator™ underglove acts as a part of a puncture indication system that can be worn in combination with other latex surgical gloves. When a barrier breach occurs in the outer glove, a fluid passes through the hole and a conspicuous green patch develops at the site of the hole. The Skinsense™ Biogel® coated examination glove is specifically marketed for the safe handling of cytotoxic drugs. The Biogel® orthopedic™ is about 30% thicker than the biogel surgical gloves and is designed for more rigorous medical procedures.

Other viable alternatives include the use of glove liners made of cotton, nylon, synthetic fibers (Kevlar®, Spectra® etc) or combination of stainless steel and fibers woven together, which are worn underneath the NRL gloves. These liners do not facilitate donning, but offer localized protection for fingers or hands against punctures, lacerations and abrasions. Gloving creams are sometimes used to facilitate donning, and at other times used to reduce skin irritation. However, such glove liners and creams will do nothing to eliminate the occurrence of airborne allergens when used with powdered gloves. Research on glove perforation and hand injury during usage has led to other alternatives too. Double gloving was recommended among surgeons in an effort to maintain at least one intact latex layer throughout the medical procedure (American Academy of Orthopedics, 1989; Gerberding *et al.,* 1990).

Gloves made from synthetic latices are introduced in an attempt to reduce the allergic reactions due to proteins. Even though the synthetic polymer latices are available in a wide range, only a few are used for the manufacture of dipped products. Those synthetic latices that have adequate wet gel strength can be used for the manufacture of dipped goods and they include nitrile, polychloroprene, styrene butadiene, styrene ethylene butadiene, polyurethane, silicone and vinyl latices (eg. PVC). The polychloroprene products are marketed under the names Duraprene, Dermaprene, Biogel® Neotech® A, Neolon etc.; the styrene butadiene rubbers are marketed under the trade names Elastyren and Allergard; and the styrene block polymers are marketed as Tactyl 1 and Synthesys. Gloves made of water based polyurethane formulations have been marketed. Silicones are becoming a preferred alternative to latex for its non allergenicity combined with its low initial modulus even though it is lower than that of latex.

2.9. COMPARISON OF NRL WITH SYNTHETIC MATERIALS

The period of World War II witnessed a quick rise of the synthetic rubber industry, and a fall of the NR production. Attempts to derive rubber from alternative crops failed, mostly because of disappointing yields. Although recovery of NR production took less time than expected, the synthetic rubber production continued to dominate.

Indisputably, NR offers a number of benefits over its synthetic counterparts. The cultivation of NR trees offers a clear ecological advantage. An ecosystem of 33-year-old Hevea trees annually produces 450 tons of biomass per hectare, compared to 475-664 tons/hectare in Malaysian rainforests and 295-475 tons/hectare in Brazilian and Thai rainforests (Kox, 2000). It is worth mentioning that a rubber plantation is hardly inferior to a primary tropical forest in terms of carbon fixing. In north-east India, the quality of soils that were depleted as a result of shifting cultivation has improved substantially following planting of rubber trees (Kox, 2000). The mature rubber trees continue to supply latex for 20 to 35 years, after which the wood is used in the furniture industry. Natural rubber is green and biodegradable but synthetic rubbers will decompose more slowly or not at all depending on the type (Tsuchii *et al.*, 1985; Tsuchii and Takeda, 1990; Low and Tan, 1992; Amir Hashim, 2002; Havinga, 2003). Replanting of rubber trees guarantees an endlessly renewable

source of production, in contrast to production of SR which depletes finite oil reserves (Havinga, 2001). Another difference between NR and SR is the much lower energy consumption involved in the the production of NR (Jones, 1994; Rahaman, 1994; IRRDB, 1998).

Although the synthetic rubbers are free from allergenic proteins, they have a number of serious disadvantages which often go unnoticed. Gloves made from synthetic latices are usually high in modulus and are difficult to don requiring the glove size to be slightly larger than the hand size, which often results in wrinkling or sagging of gloves. Natural rubber latex has low modulus, which enables the surgeon to use gloves slightly smaller than the hand size for an intimate yet comfortable fit.

One of the properties which make gloves and condoms useful in the health care field is their barrier integrity. The scientific literature suggests that the replacement of NRL gloves with synthetic gloves may introduce new risks like the seepage of deadly viruses during medical procedures (Dalgleish and Malkovsky, 1988; Korniewicz and Laughon, 1989; Korniewicz *et al.,* 1990; Jacobs and Czuba, 2000). Korniewicz *et al.,* (1990) studied the microbial penetration through vinyl and latex gloves using ϕX174 bacteriophage, which is smaller than human immunodeficiency virus (HIV) or hepatitis B virus (HBV), under simulated-use conditions. The overall virus leakage is found to be 8% for latex gloves and 23% for vinyl gloves, and is attributed to elastic resealing property of the NR gloves. As perforation frequencies over 80% occur during individual surgeries, the use of latex gloves with elastic resealing capability is preferred over the synthetic counterparts (Pate, 1990; Patel *et al.,* 2004).

The synthetic polymer production itself poses considerable danger to the workers as many of the monomers used in the manufacture of SR are either carcinogenic or toxic or both. For example, vinyl chloride for the production of PVC is a potent human carcinogen, and butadiene for the production of nitrile latex is associated with leukemia in factory workers (BRMA, 1999; Delzell *et al.,* 2001). Allergic reactions like eczema and urticaria to PVC gloves have been reported mainly due to the additives present in it (Estlander *et al.,* 1986). It has been found that di(2-ethylhexyl) phthalate (DEHP), diisononyl phthalate, di(2-ethylhexyl) adipate and

4-nonylphenol contained in PVC gloves migrated into both polar and nonpolar media indicating their migration into fatty foods (Kawamura *et al.,* 2000).

Moreover, SR products pose a grave threat to humans as they release carcinogenic or toxic gases during incineration, which is indispensable for the disposal of medical goods. Considering this aspect, the most hazardous glove material is PVC as it discharges acid hydrogen chloride, vinyl chloride monomer and carcinogenic dioxins to the environment. Among the other synthetic materials, nitrile and polyurethane can liberate hydrogen cyanide upon combustion and neoprene produces hydrogen chloride (http://www.lgm.gov.my/latex_allergy/framelpa.html). In the case of natural rubber, it releases sulphur dioxide due to presence of additives, which is often considered better compared to others. In terms of cost, the synthetic gloves are 5-10 times costlier compared to NRL gloves. To summarize, the risk of allergic contact dermatitis and other toxic effects is not confined to NRL products; the currently available synthetic alternatives also pose a similar risk, depending on the chemicals used for their production.

2.10. REGULATORY ASPECTS

Medical devices, containing NRL, are regulated by various regulatory agencies functioning in the respective countries, for example FDA in USA, German federal ministry of health in Germany, European Economic Community (EEC) in European Union etc. Natural rubber is listed in Title 21 Code of Federal Regulations (CFR) section 177.2600 for use as a component of any repeat-use, or food contact rubber article. Over the past few years, FDA has been reviewing the requests to ban powdered gloves. These requests have been based on a number of clinical and experimental reports that cornstarch (glove powder) can enhance the development of infection, cause granulomas and adhesion, act as a carrier for allergens/bacteria/endotoxin and serve as a potent source of occupational asthma. In 1999, FDA proposed regulations to reclassify all surgeons and patient examination gloves as class II medical devices because it believes that general controls are insufficient to produce reasonable assurance of safety and effectiveness in terms of: (i) barrier integrity concerns, (ii) shelf life considerations, (iii) contamination concerns and (iv) concern about exposure to NRL allergens and role of glove powder as a carrier of allergens and other substances. In order to enable the user to distinguish

between powdered and powder free gloves and to select the appropriate glove type to suit their needs, FDA proposes to reclassify the gloves into four categories: (i) powdered surgeons gloves, (ii) powder free surgeons gloves, (iii) powdered examination gloves, and (iv) powder free examination gloves.

The FDA relies upon valid scientific evidence in the classification process to determine the level of regulation for the device. Earlier the gloves were labeled as 'hypoallergenic' which means that the allergic reactions to any component of the device would be minimal. This labelling has been based on the modified human draize test. While this test may be appropriate for detecting sensitization to residual chemicals, the test does not detect sensitivity to proteins present in NRL. The FDA requires the gloves to meet special controls since the modified draize test gives no reasonable assurance that the allergic reactions to NRL products will be reduced for individuals who are sensitive to NRL proteins.

The proposed new special controls to classify gloves into class II devices include a proposed guidance document entitled 'Medical glove guidance manual', and a new label caution statement including protein and powder labelling requirements (FDA, 1999). The agency now requires that all medical devices containing NRL carry a warning for those who are allergic and also the term 'hypoallergenic' should not be used anymore. This regulation was issued in September, 1997 and went into effect on September 30, 1998 (21 CFR 801.437). The guidance document recommends that the manufacturers of powdered surgeon's and patient examination gloves limit the amount of powder to no more than 120 mg powder/glove as determined as per ASTM D 6124 (2001), regardless of the glove type. It also recommends that the amount of total trace (residual) powder on a powder free glove regardless of the glove type should be no more than 2 mg particulate weight per glove. As far as the level of proteins is concerned, the amount of water-extractable proteins based on ASTM D 5712 (1999) method should be no more than 1200 μg/glove for any of the four types of gloves mentioned above. FDA does not allow products to be labelled with total protein levels lower than 50 μg/g, which is the sensitivity limit of the modified Lowry test method used in ASTM D 5712 (1999). As regards the concentration of accelerators in rubber articles intended for repeated use, the total accelerators should not exceed 1.5% by weight of the rubber product and only approved accelerators

should be used (21 CFR 177.2600). The FDA is also proposing to add an expiry date on all the four types of gloves. Previously, it was not required to include the expiry date. In view of the quality concerns especially regarding the degradation of the barrier integrity over time, FDA stated that the expiry date is necessary to allow users to avoid gloves that may have degraded. The expiry date is decided based on the stability shelf life and sterility shelf life and the expiry date is the one, whichever is shorter. Upon successful completion of the accelerated aging study, a shelf life claim of 2 years can be made in the case of gloves. Real time aging studies could allow additional time to be added to the shelf life. The combination of microorganisms, starch and moisture may result in microbial growth sufficient to cause discoloration, and unpleasant odour. The FDA suggests only some process controls to control the bioburden, but American Society for Testing and Materials (ASTM), in June 2002, began work on developing standards for the measurement of bioburden in gloves (Lammerding and Day, 1978; Thomas, 2002). To summarize, the FDA is emphasizing the need of implementing quality controls to minimize (i) pinholes after accelerated or real time aging (ii) chemical residues and water-soluble proteins (iii) the amount of donning powder on powdered gloves to the lowest level needed for easy donning (iv) the bioburden during the production of medical gloves and (v) the bioburden and moisture content of finished medical gloves.

National Institute for Occupational Safety and Health (NIOSH) issued an alert to prevent allergy in work place (NIOSH, 1997). The CDCP is updating its guidelines for infection control in health care workers. The OSHA (1995) standard 29 CFR 1910.1030 contains a requirement that an employer must provide low-allergenic gloves or other alternatives to latex sensitive employees. ASTM standards for surgical and examination gloves recommend a level of <200 $\mu g/dm^2$ of proteins on all examination and surgical gloves. On the other hand, the recommended level of antigenic protein limit is 10 $\mu g/dm^2$ (ASTM D 3577, 2002; ASTM D 3578, 2002). The Canadian General Standards Board (CGSB) requires that a disposable powdered medical latex glove should have less than 250 $\mu g/g$ of proteins, and a powder free glove less than 50 $\mu g/g$ of proteins. In Germany, the Kommission Arbeitsschutz Normung (KAN) proposed the upper limit of protein level of 30 $\mu g/g$ in medical devices containing NRL.

In Germany, the rubber products for food contact applications are required to be unobjectionable from aspects of health, smell and taste. There is a BgVV Recommendations XXI, which define rubber articles into five categories, and are given Table 2.8.

Table 2.8. Categories defined by BgVV Recommendations XXI

	Definitions of use	Examples
Category I	Contact with foodstuffs during intended use for longer than 24 h up to several months	Storage containers, container linings, seals with large surface area, sealing rings for cans, bottles etc.
Category II	Contact with foodstuffs during intended use for a maximum of 24 h	Hose for food conveyance, bottle stoppers and caps, pressure cooker sealing rings, coffee machine tubes etc.
Category III	Contact with foodstuffs during intended use for a maximum of 10 min	Tea cups, milking machine hose, seals for milk processing machines, membranes, pistons fittings, pump stators, gloves, aprons.
Category IV	Contact with foodstuffs during intended use for only very short periods of time or only with a very small surface area	Conveyor belts, roller covers, suction and pressure hose e.g. for filling and emptying ship tanks, wagons etc.
Special category	Intended use in the consumption of food: toys that intentionally or in a predictable way are put into the mouth	Air balloons, toys, baby bottle teats and caps, biting rings, denture protectors.

The German health ministry also put forward some requirements with regard to the residual chemicals in the finished rubber products and they are given in Table 2.9. No requirements has been set for rubber products falling into category IV.

Table 2.9. Requirements for finished rubber products by the German health ministry

Requirements for finished articles	Category I	Category II	Category III	Special Category
Maximum level of zinc (%)	3	3	3	1.04
Maximum level of lead (%)	0.003	0.003	0.003	0.001
Migration of primary aryl and or secondary *N*-alkyl aryl amines	1 μg/ml*	1 μg/ml*	1 μg/ml*	0.5 μg/ml**
Content of aliphatic and cycloaliphatic amines	Not detectable	Not detectable	Not detectable	Not detectable
Migration of formaldehyde into water	3 μg/ml	3 μg/ml	3 μg/ml	3 μg/ml

* test duration at 40 °C: category I-10days; category II-24 h; category III-10 min; simulant: water, acetic acid 3%, and ethanol 10%
** test duration at 40 °C for 1h; stimulant-artificial saliva

The level of nitrosamine and nitrosatable amines is regulated in many countries. The European Union has adopted Directive 93/11/EEC to control the release of nitrosamines and *N*-nitrosatable substances from rubber teats and soothers. The maximum release levels of total nitrosamines and nitrosatables during extraction in saliva at 40 °C for 24 h should not exceed 0.01 and 0.1 mg/kg of rubber respectively in rubber teats and soothers. The nitrosamines are produced during vulcanization process and their levels in the air are also regulated in many countries. In Germany, the concentration of nitrosamines in the atmosphere is regulated to a maximum of 1 $\mu g/m^3$. In the US and Canada, the concentration of nitrosamines is not regulated till now; however, agencies are monitoring, documenting and reporting the level of nitrosamines in the rubber facilities to petition the governments for implementing restrictions on nitrosamine level in workplace atmosphere.

The European Union has already set the release limits for MBT, antioxidants, nitrosamines, and nitrosatable amines into artificial simulants from teats and soothers (EN 12868, 1999; EN 1400-3, 2002). According to the standard EN 1400-3 (2002), the release limit of MBT has been fixed at 8 mg/kg rubber. More stringent limits have been set for some antioxidants such as 2,6 Bis(1,1dimethylethyl)-4-methylphenol (60 $\mu g/dm^2$), and 2,2'Methylenebis(6-(1,1-dimethylethyl)-4-methylphenol (30$\mu g/dm^2$). Due to the widespread use of dithiocarbamates as fungicides, their residues are present in the environment. The residues present in the foods are analyzed in terms of carbon disulfide. The maximum residue levels (MRLs), expressed in terms of carbon disulphide, have been set in a number of fruits treated with dithiocarbamate fungicides. In the US, the permitted levels of ziram residues have been set at 7 ppm.

2.11. RISK MANAGEMENT

All medical procedures are associated with some degree of risk and it is inevitable that some risk must be accepted in the interests of improving the health of the patient. In practice, the elimination, or minimization of risk means that the risk can be considered to be so low that there is "no need to bother about it"; such risks are termed as "broadly acceptable" (ISO 14971, 2003). If it is impossible to implement controls that ensure the risk as broadly acceptable, the risk must be reduced to the "as low as reasonably practicable" (ALARP) region.

There are some standards which deal with the risk management in medical devices (EN 1441, 1997; ISO 14971, 2003). Hazard identification, exposure assessment, toxicity assessment, risk evaluation, risk control measures, and post-production information are indispensable components of the risk management. In the case of latex medical devices, factors such as proteins, chemicals, endotoxin, powder, nitrosamines etc. are identified as hazards, and they make significant contribution to the exposure and subsequent risk. Once the hazards are identified, the next step is exposure assessment. Exposure assessment is to identify and define the exposures that occur, or are anticipated to occur, in human populations (IPCS, 1993). Both ASTM and European standard give test methods that enable the exposure assessment (ASTM F 1313, 1990; ASTM D 5151, 1992; ASTM D 5712, 1999; EN-455-3(E), 1999; ASTM D 6124, 2001; EN-455-1, 2000; EN-455-2, 2000; ISO 10993-17, 2002). The bulk of the risk management has been concentrated on issues concerning proteins.

The risk of sensitization to NRL can be reduced by minimizing the amount of allergenic proteins. Once the subject is sensitized to NRL, subsequent exposure to latex may trigger an allergic reaction. It is not currently possible to establish a threshold level of exposure for sensitization. Studies have shown that latex gloves with a low leachable protein content elicit a lower percentage of positive responses in latex sensitized individuals than gloves with higher protein residues. Exposure assessment of particular protein causing allergy is, however, hampered by the lack of validated methods. Total water-extractable proteins are currently used as a surrogate for allergen exposure. Two methods, the modified Lowry (ASTM D 5712, 1999) and an amino acid analysis (EN-455-3(E), 1999), both measuring total extractable protein, have been described and standardized for some types of products. However, neither of these methods distinguishes between sensitizing and non-sensitizing proteins. Immunoassays based on the use of human IgE antibodies recognizing specific NRL allergens have been developed and used in a limited scale to measure total allergen contents of latex gloves and other NRL devices (ASTM D 6499, 2003). The major drawback in such assays is the limited availability of proper human sera and problems in standardization. Standardized techniques for the determination of the powder content of medical gloves are available (EN-455-3(E), 1999; ASTM D 6124, 2001). In the case of residual chemicals, there is no agreement on the best applicable method for the estimation of the bioavailable residues of rubber chemicals in the products (EEC/93/42, 2004).

Once the exposure assessment is over, the risk is estimated and evaluated. Acceptable levels of proteins and chemicals are still not identified as there is insufficient understanding of factors relevant to the safety assessment in this area (EEC/93/42, 2004). For example, the analytical methods available today do not differentiate between allergenic and non-allergenic proteins, but detect the amount of total protein instead. As a result, the risk of sensitization or elicitation arising from contact with NRL-containing products cannot be estimated with any confidence. A similar situation exists in the case of residual chemicals. Further methods to measure and control these allergenic components are currently under development. As far as the powder is concerned, its use does not increase the allergenicity of the gloves, but it can bind residual proteins, become air borne and may cause allergic reactions in sensitized individuals. This implied that controlling the powder content alone

provides no additional protection to sensitized individuals. With regard to the risk of adhesions or granuloma formation due to powder, its presence is regarded as a residual risk.

A number of risk control measures are considered necessary; including measures to ensure that exposure to allergenic proteins and chemicals is maintained below an acceptable level. The manufacturer has to determine what risk control measures can reasonably be adopted to achieve the optimum balance of risks and benefits. Any risk remaining after implementing all applicable risk control measures is termed the "residual risk". The residual risk must be outweighed by benefits. The manufacturer has a responsibility to communicate effectively with users to inform them about residual risks to allow them to manage these risks effectively. Therefore, it is necessary to include appropriate warnings in the documents accompanying the product. As risk control measures, EEC put forward some guidelines for manufacturers regarding medical devices containing NRL (EEC/93/42, 2004). As far as proteins are concerned, the technical documentation needs to contain an indication of allergen content, technical justification of measured allergen level etc. The product labeling needs to include: (i) a prominent indication that the device contains natural rubber latex; and (ii) a warning that the product may elicit allergic responses in individuals who are sensitized to latex. With regard to residual chemicals, the technical documentation needs to include: (i) identification of a tolerable intake (level of chemicals without risk of appreciable harm to health) determined on the basis of a toxicological risk analysis; (ii) an estimate of anticipated exposure to patients and users, to the extent necessary to verify that the tolerable intake will not be exceeded; (iii) identification of process control limits or quality control measures, sufficient to verify that exposure will not exceed the tolerable intake. However, exposure below the tolerable intake cannot be guaranteed where it is not feasible to manufacture products with sufficiently low residue levels. Moreover, for some chemicals (particularly sensitizers), it may not be possible to determine a tolerable intake. In these circumstances, exposure to chemicals must be reduced to a level as low as reasonably practicable, and the presence of the residue must be treated as a residual risk.

M/s. Ansell has their own risk management system who developed customized guidelines to effectively deal with issues on latex allergies, caring for latex-sensitive patients, creating latex-safe environments, aeroallergen transmissions, proper hand barrier protection and surgical gloves and electrosurgery (www.ansellhealthcare.com).

2.12. SCOPE AND OBJECTIVES OF THE PRESENT WORK

A number of alternatives such as radiation/peroxide vulcanized natural rubber latices, and synthetic latex products were introduced into the health care market to address the problems posed by NRL medical devices. Although radiation/peroxide vulcanized latices are free from sensitizing accelerators, they suffer from a number of limitations such as poor aging resistance, and low tactile sensitivity (Gazeley and Pendle, 1989; Davies and Gazeley, 1993; Sekhar, 2005). Synthetic latex products, though free from allergenic proteins, suffer from serious disadvantages which include (i) higher modulus than NRL products, (ii) poor barrier integrity resulting in the seepage of deadly viruses during medical procedures, (iii) carcinogenic monomers which pose problem to both industrial workers and health care personnel, and (iv) release of toxic gases during disposal by incineration (Korniewicz *et al.,* 1990; SCMPMD, 2000; Delzell *et al.,* 2001; Hasma *et al.,* 2003). Moreover, the European Commission's Scientific Committee on Medicinal Products and Medical Devices (SCMPMD) cautions that most synthetic rubber products containing sensitizing chemicals pose a similar risk of sensitization as that of NRL medical products (SCMPMD, 2000). It is by no means clear that the overall health risks imparted by the synthetic latex products are less than that caused by NRL products.

Natural rubber latex, therefore, remains the material of choice for barrier products such as gloves and condoms. However, the risks from various hazards such as proteins, glove powder, chemicals etc must be assessed and managed by risk management programs so that the anticipated benefits outweigh the risks. It is also required to identify the contributory factors, which add to the risks posed by the hazards (ISO 14971, 2003). Considerable progress has been made to reduce the risks from protein and glove powder, and the risk control measures include labeling the protein and powder level on the glove pack. On the other hand, little has been done to address the risks associated with residual chemicals probably due to the incomplete

and inconsistent data concerning different types of rubber chemicals. In the wake of more people getting sensitized to residual chemicals, the European Commission guidelines on medical devices recommend that wherever possible, for each hazardous chemical used or generated during NRL processing, the technical documentation needs to include an estimate of the anticipated exposure to patients and users, identification of tolerable intake and quality control measures (EEC/93/42, 2004). However, a systematic study on the release and the subsequent exposure to allergologically relevant, bioavailable residual accelerators and their determination in latex health care products is not yet available (SCMPMD, 2000; EEC/93/42, 2004).

In this scenario, it would be of great significance to assess the release of dithiocarbamates, a commonly used vulcanization accelerator, from NRL medical gloves. It has been well established that dithiocarbamates show a higher degree of allergenicity and cytotoxicity when compared to other rubber additives (Nakamura *et al.,* 1990; De Jong *et al.,* 2002). So far, there is no standard by American Society for Testing and Materials (ASTM) or International Organization for Standardization (ISO) for the quantification of leachable dithiocarbamates in latex medical products (Bader, 2004). Therefore, it was attempted to develop suitable analytical method to quantify the dithiocarbamates released into a simulated medium, namely, artificial sweat. The release studies in gloves were carried out under real-use (employing human subjects) and simulated-use (employing artificial sweat) conditions. Since the commercially available medical gloves varied in their composition, known NRL formulations were prepared using different dithiocarbamates and dithiocarbamate-sulphur ratios to study the dithiocarbamate-release. It was also the intention to develop some NRL formulations having adequate mechanical properties as well as low residual dithiocarbamate content suitable for medical glove manufacture. The present study addressed the following aspects:

1. Evaluation of medical gloves marketed in India

    (i) Evaluation of some of the commercially available surgical and examination NRL gloves for cytotoxicity, amount of dithiocarbamates released and water-extractable proteins.

(ii) Identification and quantification of dithiocarbamates present in the gloves by analytical techniques, namely ultraviolet-visible (UV-VIS) spectroscopy, thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). The test method was validated to determine the detection and quantitation limits.

2. Comparison of the extent of release of residual dithiocarbamates in gloves under real-use (employing human subjects) and simulated-use (employing artificial sweat) conditions. Effect of pH and sweat rate on the amount of dithiocarbamates released was studied. Assessment of the anticipated exposure of users to dithiocarbamates was performed.

3. The extent of release of different commercially available dithiocarbamates, namely zinc diethyldithiocarbamate (ZDEC), zinc dibutyldithiocarbamate (ZDBC), zinc diisobutyldithiocarbamate (ZIBC), zinc dibenzyldithiocarbamate (ZBEC), and zinc diisononyldithiocarbamate (ZDNC) into artificial sweat from NRL sheets.

4. Effect of various factors such as shelf time, ZDEC-sulphur ratio, and residual ZDEC content on the amount of ZDEC released into artificial sweat from NRL vulcanizates.

5. Effect of water and alkali leaching on the amount of ZDEC released into artificial sweat.

6. Evaluation of mechanical properties and residual dithiocarbamate content of NRL vulcanizates using different dithiocarbamate accelerators and dithiocarbamate-sulphur ratios. Effect of alkali leaching, and aging on the mechanical properties of selected latex formulations were also investigated.

7. Biological studies (*in vitro* cell culture cytotoxicity, intracutaneous irritation and sensitization) on two latex formulations containing ZDEC. As the use of medical devices normally entails their direct or indirect contact with patients, it is necessary to establish the safety of these products before they are marketed. Medical device safety evaluation assesses the risk of adverse health effects due to use of a device using a battery of tests provided by ISO 10993. The choice of test

program for a device depends on the nature and the duration of the contact. Latex sheets vulcanized with different accelerators were subjected to a preliminary screening test by *in vitro* cell culture test. As ZDEC is still widely used as accelerator in the Indian latex industry, two formulations containing ZDEC were further subjected to biological tests such as intracutaneous irritation test (to detect the local irritation by extracts from medical devices) and sensitization test (to assess the response of the organism's immune system to the device).

CHAPTER 3

MATERIALS AND METHODS

3.1. MATERIALS

Eleven brands of commercially available surgical/examination gloves made of NRL were collected from local sources and were code named in English alphabets. Table 3.1 gives the details of the gloves used for the present study.

Table 3.1. Details of the different brands of NRL gloves

Sl. No.	Sample codes	Glove type
1	A	Surgical, pre-powdered
2	B	Surgical, pre-powdered
3	C	Surgical, pre-powdered
4	D	Surgical, pre-powdered
5	E	Surgical, pre-powdered
6	F	Surgical, pre-powdered
7	G	Surgical, pre-powdered
8	H	Surgical, pre-powdered
9	I	Examination, powder free
10	J	Examination, powdered
11	K	Examination, powder free

Double centrifuged natural rubber latex (60%), conforming to the specifications of Bureau of Indian Standards (BIS) IS: 11001-1984, was supplied by M/s Meenachil Rubber Marketing Society Pvt. Ltd., Kerala, India. Table 3.2 gives the properties of the double centrifuged latex used in the present study.

Table 3.2 Properties of double centrifuged natural rubber latex

Properties	Value
Dry rubber content, % m/m	60.03
Non rubber solids, % m/m	1.45
Total alkalinity, % m/m	0.8
KOH Number	0.69
Mechanical stability time, sec	1050
Volatile fatty acid number (VFA)	0.025
Sludge content, % m/m	0.0017
Coagulum content, % m/m	0.0014
Copper, ppm	2.5
Manganese, ppm	Trace

Table 3.3 gives the various chemicals used in the present study and their sources.

Table 3.3. List of chemicals and their sources

Chemical	Source
Acetone, HPLC grade	s. d fine-chem Ltd., India
Chicken egg ovalbumin	Sigma Aldrich, USA
Cuprous sulphate pentahydrate	Merck, Germany
Cobaltous chloride hexahydrate	s. d fine-chem Ltd., India
Disodium hydrogen phosphate dihydrate	Merck, Germany
Dispersol F	Bayer India Ltd., Mumbai, India
Folin-Ciocalteu Phenol reagent	Merck, Germany
Lactic acid	Sigma Aldrich, U.S.A
4-Methyl-2-mercaptobenzimidazole (MMBI)	Bayer AG, Germany
Phosphotungstic acid	Sigma Aldrich, USA
Sodium carbonate, anhydrous	Merck, Germany
Sodium chloride	s. d fine-chem Ltd., India
Sodium deoxycholate	Sigma Aldrich, USA
Sodium dihydrogenphosphate	Merck, Germany
Sodium hydroxide	Merck, Germany
Sodium tartarate	Merck, Germany
Sulphur	Bayer India Ltd., Mumbai, India
Trichloroacetic acid	Sigma Aldrich, USA
Zinc diethyldithiocarbamate (ZDEC)	NOCIL, Mumbai, India
Zinc dibenzyldithiocarbamate (ZBEC)	Bayer AG, Germany
Zinc dibutyldithiocarbamate (ZDBC)	Bayer India Ltd., Mumbai, India
Zinc diisobutyldithiocarbamate (ZIBC)	R.T. Vanderbilt Company Inc., USA
Zinc diisononyldithiocarbamate (ZDNC)	Robinson Brothers Limited, England
Zinc oxide	Bayer India Ltd., Mumbai, India
Urea	s. d fine-chem Ltd., India
Vanox MBPC (2,2'-Methylenebis(6-*t*-butyl-4-methylphenol)	R.T. Vanderbilt Company Inc., USA
Vanox ZS (Hindered phenol)	R.T. Vanderbilt Company Inc., USA
Wingstay L (Reaction product of Butylated p-cresol and cyclopentadiene)	Plasti Chem Pvt India, India

Solvents such as dichloromethane, hexane and ether were procured from local sources. Silica gel 60 plates for thin layer chromatography were supplied by Merck, Germany and used without activation. Deionized water for HPLC was prepared in the laboratory and filtered through a 0.45 μm polyethersulfone filter (Pall Corporation, USA) before use.

3.2. EVALUATION OF COMMERCIALLY AVAILABLE NRL GLOVES

Exposure to NRL medical gloves caused a multitude of adverse allergic reactions among both the healthcare personnel and the general public in the last two decades (Dillard, 1997). Extensive research showed that the residual proteins and chemicals caused these adverse reactions. In this scenario, some of the commercially available medical gloves marketed in India were assessed for their cytotoxicity potential, extent of dithiocarbamate-release and water-extractable proteins.

3.2.1. *In vitro* cytotoxicity test

The gloves were subjected to *in vitro* cell culture cytotoxicity test by direct contact and test on extract as detailed in section 3.6.1 to evaluate their cytotoxicity potential.

3.2.2. Dithiocarbamate-release in artificial sweat

The gloves were extracted in artificial sweat, a physiologically simulated medium, to quantify the amount of dithiocarbamates released into artificial sweat. The amount of dithiocarbamates released into artificial sweat from latex products is expected to provide a good indication of the glove's cytotoxicity potential. Table 3.4 gives the composition of artificial sweat used for the present study (EN 1811(E), 1998).

Table 3.4 Composition of artificial sweat

Constituent	Weight (%)
Sodium chloride	0.5
Lactic acid	0.1
Urea	0.1
Deionized water	To make to 100

The pH of the artificial sweat was adjusted to 6.5 ± 0.1 by adding 1% aqueous ammonia solution. As the dithiocarbamates are unstable in highly acidic medium, the pH of artificial sweat was kept at 6.5 in all experiments unless otherwise specified (Aspila *et al.*, 1969; Tokalioglu *et al.*, 2002). The glove (2 g) was cut into pieces of approximately 2×0.5 cm^2 size and 100 ml of artificial sweat was added to it. The extraction was carried out at 37 ± 2 °C for a period of 24 h with constant mechanical shaking. After extraction, the artificial sweat was extracted with 40 ml of dichloromethane for five times. The solvent fraction in each extraction was collected and distilled off using a flash evaporator at 60 ± 2 °C to recover the residue. The residue was analyzed as detailed in section 3.2.4 to identify and quantify the amount of dithiocarbamates released into artificial sweat (sweat-extractable dithiocarbamates).

3.2.3. Dithiocarbamate-release in dichloromethane

The amount of free dithiocarbamates (residual dithiocarbamates) in the gloves was determined by extracting the gloves in dichloromethane. Dichloromethane has been employed as a solvent for the extraction of latex products in order to determine the level of residual accelerators (Report Number R 6536). As the extraction of rubber products in solvents at boiling temperatures may cause decomposition of dithiocarbamates, the latex products/vulcanizates were extracted in dichloromethane at room temperature (Parker and Berriman, 1954).

Extraction was carried out using 1 g of the glove cut into 2×0.5 cm^2 sized pieces in 50 ml dichloromethane at room temperature (28 ± 2 °C) for 24 h. After extraction, the solvent fraction was separated, and subjected to flash distillation at 60 ± 2 °C to recover the residue. The residue was subjected to further analysis as detailed in section 3.2.4 to quantify the amount of residual dithiocarbamates.

3.2.4. Identification and quantification of dithiocarbamates in latex gloves

Identification of the type of dithiocarbamate present in latex goods is required as they differ in their extent of contribution to the toxicological effects. The UV-VIS spectroscopy and TLC were used for this purpose. On the other hand, the quantification was carried out using HPLC.

3.2.4.1. Ultraviolet-visible (UV-VIS) spectroscopy

A preliminary idea about the nature of the residual chemicals released into artificial sweat and dichloromethane may be obtained from the UV-VIS spectroscopic data. Standard compounds of dithiocarbamates and some antioxidants were dissolved in dichloromethane and scanned in the range 200-500 nm using a UV-VIS spectrophotometer (Model UV-1601, Shimadzu, Japan) to obtain the absorption wavelengths. The residue obtained after sweat extraction of the gloves was dissolved in dichloromethane, scanned, and the absorption wavelengths were recorded, and compared with those of the standard compounds.

3.2.4.2. Thin layer chromatography (TLC)

Thin layer chromatography is a widely used technique for the qualitative analysis of compounds. The residues containing zinc dithiocarbamates obtained after sweat or dichloromethane extraction of latex gloves were subjected to TLC to determine if the residues contained single or mixtures of dithiocarbamates and, if present, to find the respective R_f values. It was found that the zones produced on the silica gel chromatographic plate by zinc dithiocarbamates were diffused. To obtain a good separation on the TLC plate, zinc dithiocarbamates were converted into their respective copper complexes by reacting them with copper(II) sulphate as detailed in the following section 3.2.4.2.1.

A solution of hexane and ether in the ratio 90:10 (v/v) was used as the mobile phase for TLC. Adequate time was given for the chamber to get saturated with solvent vapors. Using a micro syringe, 5 µl each of the above test samples and standards were applied on the TLC plate. The plate was dried and kept in a rectangular chamber containing the mobile phase. When the solvent front had migrated about 13 cm above the application line, the plate was removed and dried. They were then kept in another chamber saturated with iodine vapors for color development. The distance moved by the sample spot and the solvent front from the application line was noted. The respective R_f values (*ie.*, the ratio of the distance moved by the sample spot to that of the solvent front) were determined and compared with the standard.

3.2.4.2.1. Preparation of copper complexes of dithiocarbamates

The conversion into copper complexes was carried out using a 10 mmol solution of copper(II) sulphate prepared in a 1.6% ammonia solution. The procedure for the conversion of zinc dithiocarbamates into their respective copper complexes is as follows. The residue obtained after sweat/dichloromethane extraction was re-dissolved in 10 ml of dichloromethane. A 5 ml of this solution was pipetted out and mixed with 1.7 ml of ammoniacal copper(II) sulphate solution. The mixture was then vortexed for 2 min to obtain the copper-dithiocarbamate complex. The dichloromethane layer was separated out and the copper-dithiocarbamate complex was recovered as residue by evaporating the solvent. This residue was re-dissolved in 5 ml of HPLC grade acetone for further analysis. Known quantities of ZDEC, ZDBC, ZIBC, ZBEC and ZDNC were converted into their respective copper complexes, and served as standard solutions.

3.2.4.3. High performance liquid chromatography (HPLC)

High performance liquid chromatography was used to quantify the amount of zinc dithiocarbamates released into artificial sweat/dichloromethane using a variable-wavelength UV detector 486 set at 265 nm. The mobile phase used was dichloromethane-hexane (80:20, v/v) with a flow rate of 1.0 ml/min. However, the chromatogram was found to be complicated. Moreover, it has been reported that the zinc dithiocarbamates undergo substitution reaction with the metal parts of the HPLC column owing to the labile nature of dithiocarbamate ligands (Hutchins *et al.*, 1982). To avoid this, zinc dithiocarbamates were converted into more stable copper complexes. Bond and Wallace (1981) reported that the dithiocarbamates could be used for the determination of trace amounts of copper.

The procedure for the preparation of copper dithiocarbamate complex is already detailed in the previous section 3.2.4.2.1. The HPLC system consisted of a Waters 510 pump, C18 column and 7725 Rheodyne injector (Waters Inc., U.S.A). The presence of copper complexes of dithiocarbamates was detected using a variable-wavelength UV detector 486. Separation was achieved on a 3.5 μm particle size, 4.6×75 mm (Waters Inc., Ireland) C18 column. The mobile phase used was acetone-water (90:10, v/v) with a flow rate of 1.0 ml/min. The detection wavelength used was 435 nm. The amount of dithiocarbamate in the sample materials was calculated from

the response factor of the detector relative to respective standard. The response factor is given by the following equation,

$$\text{Response factor} = \frac{Peak\ area}{Concentration} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ 3.1$$

3.2.4.3.1. Assay validation

It has been reported that cobalt(II) chloride could be used as a complexing agent for the estimation of dithiocarbamates (Kaniwa, 1987). Before validating the test method, the effectiveness of copper(II) sulphate and cobalt(II) chloride as complexing agents for ZDEC was compared under conditions used in the present study with respect to the percentage conversion using a solution of known concentration of ZDEC (22 μg/ml). The standard solution of ZDEC was divided into two equal parts; one part was reacted with copper(II) sulphate solution (section 3.2.4.2.1) and the other by cobalt(II) chloride solution. The preparation of cobalt-dithiocarbamate complex was carried out using 10 mM cobalt(II) chloride solution in distilled water. It was found that the cobalt-dithiocabamate complex formed green solutions in dichloromethane. They gave an absorbance peak at a wavelength of 320 nm, which was taken as the detection wavelength for the HPLC analysis of cobalt dithiocarbamate complexes. The experiment was repeated with latex sheets containing ZDEC (formulation DE-5 referred in section 3.4.2). The latex sheets were extracted in artificial sweat, and the residue obtained was dissolved in 10 ml dichloromethane. The amount of ZDEC released into artificial sweat was quantified using HPLC as discussed earlier. Mean values were derived from tests performed on three samples taken from separate latex sheets (DE-5).

The test method was validated by determining parameters such as linearity, precision, accuracy, detection limit (DL) and reliable quantitation limit (RQL) by analyzing replicate samples of ZDEC dissolved in dichloromethane at different concentrations. Stock solution of ZDEC (1 mg/ml) was prepared by dissolving 0.1 g of ZDEC in 100 ml of dichloromethane. Working standard solutions of ZDEC were prepared by diluting aliquots of the stock solution in the same solvent to yield known concentrations of 0.6375, 1.275, 2.55, 5.1, 10.2, and 20.4 μg/ml.

The precision was calculated as the coefficient of variation (CoV).

$$\text{Precision} = \frac{SD}{\bar{x}} \times 100 \quad \ldots\ldots 3.2$$

where SD and $\bar{x}$ are the standard deviation and mean of three measurements respectively. The accuracy, also expressed as percent bias, was determined from the back calculated concentration of ZDEC solutions and the known concentration.

$$\text{Accuracy} = \frac{A - B}{A} \times 100 \quad \ldots\ldots 3.3$$

where, A is the known concentration, and B is the back calculated concentration as determined by HPLC. A calibration curve was drawn by plotting concentration versus peak area. The DL and the RQL of ZDEC were also calculated using linear regression analysis (Chan, 1996). Detection limit is defined as the concentration of the analyte that gives a response that is significantly different from the background response.

$$\text{DL} = \frac{3 \times SE}{slope} \quad \ldots\ldots 3.4$$

Reliable quantitation limit is considered as the lower limit for precise quantitative measurements and is calculated from the regression line data provided at least 75% of the analyte is recovered.

$$\text{RQL} = \frac{10 \times SE}{slope} \quad \ldots\ldots 3.5$$

where, SE is the standard error. The experiment was repeated with standard solutions of ZDBC of concentrations ranging from 5 to 50 μg/ml to determine the DL and RQL for ZDBC as well.

3.2.5. Determination of water-extractable proteins

The water-extractable protein content of some of the commercial brands of NRL gloves was determined as per ASTM D 5712 (1999). This method involves the extraction of aqueous-soluble protein from the gloves using a buffered solution, followed by precipitation to remove interfering aqueous-soluble substances. The precipitated proteins are redissolved and quantified colorimetrically by modified Lowry method using a protein standard. The method also utilizes background

correction to negate the effects of the interferences by aqueous-extractable chemicals that are added to the NRL gloves during manufacturing. The fundamental equation representing the reaction is,

Latex protein + copper tartarate solution → Copper-polypeptide bond

Copper-polypeptide bond + Folin reagent → Blue colored reduction product

A series of standard solutions of ovalbumin of concentrations ranging from 2 to 200 μg/ml were prepared. The gloves were cut in a special manner *i.e.,* cut off each finger and cut them up one side; cut the remaining glove body up the side and then into two pieces. The pieces were then transferred into a clean 500 ml polypropylene bottle and extracted in phosphate buffer (pH 7.4 ± 0.2) at 25 ± 5 °C for 120 ± 5 min. The phosphate buffer was prepared by mixing 82 ml of 0.15 M disodium hydrogen phosphate with 18 ml of 0.15 M sodium dihydrogen phosphate. An extraction ratio of 10 was used. A fixed quantity of the protein standards and samples was mixed with sodium deoxycholate and then with phosphotungstic acid / trichloroacetic acid mixture to precipitate the proteins. The precipitated proteins were then redissolved in a fixed amount of sodium hydroxide. This was then followed by the addition of alkaline sodium tartarate and folin reagent for color development. The mix was allowed to stand for 15 min and the samples were analyzed using a spectrophotometer (Model UV-1601, Shimadzu, Japan) at a wavelength of 750 nm. The unknown protein concentrations were calculated from the calibration curve fitted to second degree polynomial equation.

3.3. DITHIOCARBAMATE-RELEASE FROM GLOVES

Exposure to residual accelerators has been recognized as a risk factor in the development of allergic reactions to NRL products. This part of the work intends to study (i) whether the dithiocarbamate residues present in the latex surgical gloves are released into the hand sweat of human subjects when gloves are donned; (ii) identification and quantification of the dithiocarbamates released into the hand sweat; (iii) comparison of the amount of dithiocarbamates released into hand sweat with that into artificial sweat, and (iv) the factors affecting the dithiocarbamate-release into the hand sweat and artificial sweat.

3.3.1. Dithiocarbamate-release into hand sweat-Experimental design

Natural rubber latex gloves used for this part of the study were from the same batch of a single brand (brand 'H'). It was found that the glove contained ZDEC. All the experiments were performed during the day between 10.00 a.m. and 5.00 p.m. The average room temperature was 27 ± 2 °C and the relative humidity varied between 80-90%. Eight healthy volunteers from both sexes were briefed about the procedure and their consent was obtained. The hands of the volunteers were washed thoroughly with soap and water; the hands were washed then in distilled water and wiped dry. They were asked to don the gloves on both hands and were allowed to do their routine work. Duration of 1 h was given to simulate the use conditions as health care workers frequently change their gloves to reduce the risk of contracting blood borne viral diseases. On elapsing 1 h, the gloves were removed inside out. Each glove surface (left and right) that was in contact with the skin was rinsed with 125 ml deionized water. The washings from each glove in the pair were mixed together and extracted with 100 ml dichloromethane. The extraction procedure was repeated four times with fresh volume of dichloromethane. The dichloromethane-fractions were added together and the solvent was distilled off using a flash evaporator at 60 ± 2 °C. A residue was obtained and analyzed for the presence of dithiocarbamates by TLC and HPLC. This procedure was repeated for other pairs of glove collected from every volunteer.

The amount of ZDEC released into the hand sweat of the volunteers could be used for the exposure assessment of gloves, which essentially involves the estimation of the amount of residual chemicals to which the health care worker is exposed daily (mg/day). Similar studies have been reported in the risk assessment of PVC medical devices containing DEHP (Khaliq *et al.,* 1992). Exposure to chemicals is a function of the surface area and duration. The surface area (SA) of the glove was calculated using the following equation (ASTM D 5712, 1999).

$$SA = \frac{4 \times length(mm) \times width\ (mm)}{10000}\ dm^2 \quad \text{.....3.6}$$

The surface area of the left and right glove (brand 'H') was 4.59 dm^2. Assuming that the health care worker (eg. dentist) wears the glove for 8 h per day (Boyle *et al.,* 2002), an estimate of the amount of ZDEC (mg/day) to which the

health care worker is exposed daily could be calculated using the following equation (Khaliq *et al.*, 1992).

$$Extent\ of\ ZDEC\ \text{exposure} = \frac{\begin{matrix} amount\ of\ ZDEC\ released \\ in\ hand\ sweat\ (\mu g / dm^2 / h) \times SA\ (dm^2) \times 8h \end{matrix}}{1000} \quad \ldots 3.7$$

3.3.2. Measurement of sweat rate and sweat pH of human subjects

The hands of the same volunteers were covered with clean pre-weighed polythene bags, and the open ends of the bags were fastened about 5 cm above the wrist using a surgical tape. A similar procedure has been reported for the whole body sweat collection of exercising volunteers (Shirreffs and Maughan, 1997). Sweat was collected for a duration of 1 h. The weight of the polythene bags after sweat collection was noted separately for the right and left hands. The difference between the initial and final weights gives the sweat rate in g/h. The sweat rate for the right and left hands was calculated individually and their average value was reported. The sweat rates of some of the volunteers could not be determined, as they felt uncomfortable during the experiment. However, the amount of sweat collected was adequate to measure the pH. After determining the sweat rate, the sweat collected from both hands was added together and the pH was measured using a pH meter (Cyberscan 510, Eutech Instruments Pte Ltd., Singapore).

3.3.3. Dithiocarbamate-release into artificial sweat

The amount of ZDEC released into artificial sweat under simulated conditions was determined and compared with that released into hand sweat. It has been suggested that the simulated conditions used for the determination of the amount of residual chemicals released into artificial sweat from gloves should be comparable to the conditions prevailing while donning gloves (Knudsen *et al.*, 2000a). Therefore, in the present study, the end-use conditions are simulated by filling the gloves with artificial sweat. The pH of the artificial sweat used was in the range 5-8 as it was found that the pH of the hand sweat of human subjects varied in the range 5.84-7.45.

The extraction using artificial sweat was conducted with fresh pairs of glove. The inside of the left as well as the right glove that is supposed to come in direct contact with the hands was filled with 500 ml artificial sweat. The extraction was

carried out at seven different pH, namely, 5, 6, 6.5, 7, 7.2, 7.4 and 8 at 37 ± 2 °C for a period of 1 h. The glove was then fastened with a twine and placed in a beaker. The cuff end of the gloves was stretched over the lip of the beaker. The beaker was clamped and the whole assembly was shaken occasionally. The sweat solution from both the right and left gloves was mixed together and extracted with 400 ml dichloromethane. This was repeated four times with fresh volume of dichloromethane. The solvent fractions were combined and the solvent was distilled off using a flash evaporator at 60 ± 2 °C to recover the residue. The residue was then re-dissolved in 10 ml dichloromethane and the amount of ZDEC released into artificial sweat was quantified by HPLC as detailed in section 3.2.4.3.

3.4. PREPARATION OF NRL VULCANIZATES

3.4.1. Preparation of dispersions

To ensure uniform mixing of compounding ingredients, and to avoid subsequent settling, water-soluble ingredients are added to latex as aqueous solutions and water-insoluble ingredients as aqueous dispersions or emulsions (Blackley, 1997b). Distilled water was used for the preparation of dispersions and solutions. Potassium hydroxide was prepared as 10% aqueous solution. Zinc diisononyldithiocarbamate and the antioxidant, MMBI were obtained in the form of dispersions from the suppliers listed in Table 3.3. The chemicals such as sulphur, zinc oxide, ZDEC, ZDBC and ZIBC were prepared as 50% m/m dispersions using a ball mill as per the formulations given in Table 3.5.

Table 3.5. Formulations for the preparation of dispersions

Ingredients	Sulphur	Zinc oxide	Accelerators
	Dry	Dry	Dry
Sulphur	100	-	-
Zinc oxide	-	100	-
Accelerator	-	-	100
Dispersol F	2.5	1	2
Distilled water	97.5	99	98

Sulphur was ball-milled for atleast 72 h to obtain the finest quality dispersion. Aqueous dispersions of dithiocarbamate accelerators and zinc oxide were prepared by ball-milling for 24 h and 18 h respectively. Since NRL is anionic, the surface active agent used was anionic Dispersol F. Before adding the dispersions to latex, the quality of the dispersions was checked by water miscibility test.

3.4.2. Formulation recipes

Various health organizations in Europe and USA regulate the maximum allowable quantities of accelerators and antioxidants in latex medical products (www.sinrubtech.com, 1999). For example, BgVV (Recommendations XXI) in Germany limits the quantity of ZBEC in teats and nipples to a maximum level of 0.5 p.p.h.r (www.bgvv.de).

All the formulations used in the present study were variations of a typical formulation for gloves given by Pendle and Gorton (1980). The different formulations using ZDEC are given in Table 3.6 and Table 3.7. Here, the amount of ZDEC was varied from 0.5-1.0 p.p.h.r keeping the amount of sulphur constant at 0.5 p.p.h.r (Table 3.6). In the next step, the amount of ZDEC was kept at 0.5 p.p.h.r and the amount of sulphur was increased (Table 3.7). Apart from other ingredients, 0.8 p.p.h.r of MMBI was used as an antioxidant in one of the formulations (formulation DE-5A).

Table 3.6. NRL formulations using varying ZDEC level

Ingredients	DE-1	DE-2	DE-3	DE-4
	Dry	Dry	Dry	Dry
60% NRL	100	100	100	100
10% KOH	0.3	0.3	0.3	0.3
50% Sulphur	0.5	0.5	0.5	0.5
50% ZDEC	0.5	0.66	0.82	1.0
50% ZnO	0.5	0.5	0.5	0.5
ZDEC-sulphur ratio	1.0	1.32	1.62	2.0

Table 3.7. NRL formulations using ZDEC at varying sulphur level

Ingredients	DE-1	DE -5	DE -6	DE-5A
	Dry	Dry	Dry	Dry
60% NRL	100	100	100	100
10% KOH	0.3	0.3	0.3	0.3
50% Sulphur	0.5	0.75	1.0	0.75
50% ZDEC	0.5	0.5	0.5	0.5
50% ZnO	0.5	0.5	0.5	0.5
50% MMBI	-	-	-	0.8
ZDEC-sulphur ratio	1.0	0.67	0.5	0.67

Table 3.8 and Table 3.9 show the various formulations using ZDBC as the vulcanization accelerator. The amount of ZDBC was varied from 0.2-1.0 p.p.h.r while maintaining a constant level of sulphur (Table 3.8). In the next step, the ZDBC content was kept constant at 0.5 p.p.h.r and the amount of sulphur was varied (Table 3.9). A 0.8 p.p.h.r of MMBI was used as an antioxidant in one of the formulations (formulation BU-5A).

Table 3.8. NRL formulations using varying ZDBC level

Ingredients	BU-1'	BU-1	BU-2	BU-3	BU-4
	Dry	Dry	Dry	Dry	Dry
60% NRL	100	100	100	100	100
10% KOH	0.3	0.3	0.3	0.3	0.3
50% Sulphur	0.5	0.5	0.5	0.5	0.5
50% ZDBC	0.2	0.5	0.66	0.82	1.0
50% ZnO	0.5	0.5	0.5	0.5	0.5
ZDEC-sulphur ratio	0.4	1.0	1.32	1.62	2

Table 3.9. NRL formulations using ZDBC at varying sulphur level

Ingredients	BU-1	BU-5	BU-6	BU-7	BU-5A
	Dry	Dry	Dry	Dry	Dry
60% NRL	100	100	100	100	100
10% KOH	0.3	0.3	0.3	0.3	0.3
50% Sulphur	0.5	0.75	1.0	1.5	0.75
50% ZDBC	0.5	0.5	0.5	0.5	0.5
50% ZnO	0.5	0.5	0.5	0.5	0.5
50% MMBI	-	-	-	-	0.8
ZDEC-sulphur ratio	1.0	0.67	0.5	0.33	0.67

The latex vulcanizates containing ZBEC were prepared as per formulations given in Table 3.10 and Table 3.11. As explained in the above cases, the effect of varying amounts of ZBEC at constant sulphur content was studied (Table 3.10). It has been reported that the use of ZBEC alone does not impart adequate mechanical properties to the vulcanized latex sheets (Gonlag, 2000). The use of thiazole-ZBEC combination is generally recommended (Gorton, 1988). In the present study, ZBEC-ZMBT accelerator system was employed. Table 3.11 gives the formulations that employ a fixed level of ZMBT (0.2 p.p.h.r) and ZBEC (0.5 p.p.h.r) for varying amounts of sulphur.

Table 3.10. NRL formulations using varying ZBEC level

Ingredients	BZ-1	BZ-2	BZ-3
	Dry	Dry	Dry
60% NRL	100	100	100
10% KOH	0.3	0.3	0.3
50% Sulphur	0.5	0.5	0.5
50% ZBEC	0.5	0.66	0.82
50% ZnO	0.5	0.5	0.5
ZBEC-Sulphur ratio	1.0	1.32	1.62

Table 3.11. NRL formulations using ZBEC-ZMBT accelerator combination

Ingredients	BZ-4	BZ-5	BZ-6
	Dry	Dry	Dry
60% NRL	100	100	100
10% KOH	0.3	0.3	0.3
50% Sulphur	0.5	0.75	1.0
50% ZBEC	0.5	0.5	0.5
50% ZMBT	0.2	0.2	0.2
50% ZnO	0.5	0.5	0.5

Table 3.12 shows the formulations using ZIBC as the vulcanization accelerator. Zinc isobutyldithiocarbamate being the isomeric counterpart of ZDBC, three recipes were formulated with ZIBC.

Table 3.12. NRL formulations using ZIBC at varying sulphur level

Ingredients	IB-1	IB-5	IB-5A
	Dry	Dry	Dry
60% NRL	100	100	100
10% KOH	0.3	0.3	0.3
50% Sulphur	0. 5	0.75	0. 75
50% ZIBC	0.5	0.5	0.5
50% ZnO	0.5	0.5	0.5
50% MMBI	-	-	0.8

Latex vulcanizates using ZDNC were prepared as per the formulations given in Table 3.13 and Table 3.14. Formulations DN-1, 2 and 3 employ a constant level of sulphur (1 p.p.h.r) and the concentration of ZDNC was varied (Table 3.13). In the next stage, the ZDNC was kept constant at 0.5 p.p.h.r and the concentration of sulphur was varied (Table 3.14).

Table 3.13. NRL formulations using varying ZDNC content

Ingredients	DN-1	DN -2	DN -3	DN-1A
	Dry	Dry	Dry	Dry
60% NRL	100	100	100	100
10% KOH	0.3	0.3	0.3	0.3
50% Sulphur	1.0	1.0	1.0	1.0
50% ZDNC	0.5	0.7	1.0	0.5
50% ZnO	0.5	0.5	0.5	0.5
50% MMBI	-	-	-	0.8
ZDNC-sulphur ratio	0.5	0.7	1.0	0.5

Table 3.14. NRL formulations using ZDNC at varying sulphur level

Ingredients	DN-4	DN-5	DN-1
	Dry	Dry	Dry
60% NRL	100	100	100
10% KOH	0.3	0.3	0.3
50% Sulphur	0.5	0.75	1.0
50% ZDNC	0.5	0.5	0.5
50% ZnO	0.5	0.5	0.5
ZDNC-sulphur ratio	1.0	0.67	0.5

3.4.2.1. Compounding and vulcanization

The additives were added into the latex on measures of 'parts of additive per 100 parts of rubber (p.p.h.r)' on a dry weight basis. All the dispersions were added to the latex one by one in the same order as given in the formulation recipes. To achieve an even distribution of additives, each dispersion was stirred slowly into the latex for about 10 min. The colloidal stability of the latex was maintained during and after the addition of ingredients and subsequent handling. The total solid content of the mix was adjusted to 40% and allowed to mature for 19 h. Maturation ensures adequate dispersion of the compounding ingredients, and allows time for any air bubbles in the mix to rise to the surface. After maturation, the mix was strained through a 80-100 mesh stainless steel gauze. The sieved latex compound was cast on leveled glass

plates. The cast sheets were allowed to air dry for at least 48 h. The dried sheets were then vulcanized at 120 ± 2 °C for 15 min followed by leaching in water.

3.4.2.2. Leaching of vulcanized latex sheets in water

All the vulcanized latex sheets were subjected to leaching in water for 1 h at 50 ± 2 °C. A leaching ratio of 100 was maintained. The water was changed every 15 min. An additional leaching in water at room temperature (28 ± 2 °C) for 24 h was also given. The leached sheets were dried in an air oven at 70 ± 2 °C for 30 min.

3.4.2.3. Leaching of vulcanized latex sheets in alkali

Some of the latex vulcanizates were given an additional leaching using a 20% sodium carbonate solution to remove the residual dithiocarbamates from the surface of the latex vulcanizates. After the first leaching in water for 15 min, the sheets were leached in alkali at 70 ± 2 °C for 30 min. A leaching ratio of 100 was employed. Following alkali leaching, the latex vulcanizates were thoroughly washed with water for three times at 50 ± 2 °C for 45 min to remove traces of alkali. An additional leaching in water at room temperature for 24 h was also given. The leached sheets were dried in an air oven at 70 ± 2 °C for 30 min.

3.4.3. Characterization of latex vulcanizates

3.4.3.1. Mechanical properties

Mechanical properties such as tensile strength, elongation at break and modulus at 100, 200 and 300% elongation were determined using an Instron Universal Testing Machine, Model 4411, at a strain rate of 10 min^{-1}. Dumbbell shaped specimens were punched from the vulcanized sheets using pneumatic die cutter (ATSFAAR, Italy) with an ISO Type 2 die (ISO 37(E), 1994). Mean values were calculated from tests performed on six dumbbell specimens. Accelerated aging was also carried out on selected formulations to study the extent of deterioration of mechanical properties (ASTM D 573, 1999). The aging was carried out at a temperature of 70 ± 2 °C for a period of 168 h in an air oven. The effect of alkaline leaching on the mechanical properties of latex vulcanizates before and after aging was also evaluated.

3.4.3.2. Crosslink density

The cross-link density (ρ_c) was determined according to a method developed by Ellis and Welding (1964). In this method, a pre-weighed rubber sample was swelled in toluene and the weight of the swollen samples was noted at various time intervals (1 h, 4 h, 9 h, 25 h, 49 h, 81 h and 100 h). The swelling index is given by the following equation.

$$\text{Swelling index} = \frac{S-T}{T} \quad \text{..... 3.8}$$

where, S is the weight of swollen rubber sample and T is the initial weight of the test sample. The volume fraction (V_r) of the rubber in toluene was determined using the following equation,

$$V_r = \frac{\frac{(D-FT)}{\rho_r}}{\frac{(D-FT)}{\rho_r} + \frac{A_0}{\rho_s}} \quad \text{.....3.9}$$

where, V_r is the volume fraction of rubber in the swollen material, T and D are the weights of the specimen and its deswollen weight respectively, F is the weight fraction of the insoluble components, A_0 is the weight of absorbed solvent corrected for the increment in swelling and ρ_r and ρ_s are the densities of the rubber and the solvent respectively. The correction factor A_o was determined from a plot of swelling index versus $(\text{time})^{1/2}$. The cross-link density (ρ_c) was calculated using the Flory-Rehner equation.

$$\rho_c = \frac{-\ln(1-V_r) + V_r + \chi V_r^2}{V_s\left(V_r^{1/3} - \frac{V_r}{2}\right)} \times \frac{1}{2} \quad \text{..... 3.10}$$

where, ρ_c, cross link density (mol/cc); V_s, molar volume of toluene; V_r, volume fraction of rubber in the swollen gel; and χ, the rubber-solvent interaction parameter.

3.4.3.3. Dithiocarbamate-release into artificial sweat

The extraction of the latex vulcanizates in artificial sweat was carried out as per procedure described in section 3.2.2. The amount of different dithiocarbamates released into artificial sweat was quantified by HPLC (section 3.2.4.3).

3.4.3.4. Determination of residual dithiocarbamates

As ZDEC and ZDBC are the widely used dithiocarbamate accelerators in the latex industry, the effect of the ZDEC-sulphur and ZDBC-sulphur ratios on the amount of residual dithiocarbamates was determined. Recently, Asiah *et al.,* (2005) studied the effect of maturation time on the reduction of chemical residues in NRL products. The amount of residual dithiocarbamates was determined as per the procedure explained in section 3.2.3 and 3.2.4.3.

3.4.3.5. Effect of storage time on ZDEC-release

The effect of storage time on the amount of ZDEC released into artificial sweat was investigated. Latex sheets containing a fixed quantity of sulphur (0.5 p.p.h.r) and varying amounts of ZDEC ranging from 0.5-1.0 p.p.h.r were used. The sheets were packed in polythene bags, sealed and stored at ambient conditions for 1, 8, 24 and 48 weeks. The average room temperature was 28 ± 2 °C, and relative humidity ranged between 70 and 80%. At the end of storage period, the latex sheets were extracted in artificial sweat to determine the amount of ZDEC released. The procedure for the extraction and quantification is already described in sections 3.2.2 and 3.2.4.3 respectively. For comparison, latex sheet containing ZDBC (formulation BU-1) was also extracted in artificial sweat after storing for a period of 16 weeks.

3.4.3.6. Degree of swelling

The degree of swelling of latex vulcanizate in artificial sweat and dichloromethane was studied. Known weight of a rubber sample was immersed in artificial sweat (pH = 6.5) and dichloromethane, and the swollen weight was noted after 24 h. The percentage degree of swelling is given by the following equation.

$$\text{Percentage degree of swelling} = \frac{S-T}{T} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad 3.11$$

where, S is the weight of swollen rubber sample and T is the initial weight of the test sample.

3.5. STATISTICAL ANALYSIS

Statistical analysis of the data was carried out using Spearman's rank correlation test (Hollander and Wolf, 1999) when the data was in the form of grading

or ranking as in the case of *in vitro* cell culture test. For all other data sets, statistical significance was analyzed by analysis of variance (ANOVA) (α=0.05).

3.6. BIOLOGICAL EVALUATION

3.6.1. *In vitro* cell culture cytotoxicity test

In vitro cell culture cytotoxicity of commercial gloves and some latex vulcanizates was assessed as per ISO 10993-5 (1999) by both direct contact and test on extract methods using L929 cell lines. L929 (mouse fibroblast subcutaneous connective tissue) cell lines were procured from National Centre for Cell Science (NCCS, Pune, India). All the test samples were sterilized by ethylene oxide gas before subjecting them to cell culture cytotoxicity test. Samples were then degassed as per a validated procedure, which ensure the removal of residual ethylene oxide. The L929 cell lines were maintained in minimum essential medium (Hi Media, Pune, India) supplemented with 10% fetal bovine serum (Sigma, USA), 100 IU/ml penicillin and 100 µg/ml streptomycin (medical grade). The cells were seeded and incubated at 37 ± 2 °C in 95% humid and 5% carbon dioxide atmosphere till 80% confluency was attained. The starting cell density was 1×10^3 cells/ml for direct contact method and 1×10^2 cells/100 µl for test on extract method.

3.6.1.1. Direct contact test

In the direct contact assay, round-shaped samples of 4 mm diameter were placed in contact with the cells in a 24-well dish. Copper and ultra high molecular weight polyethylene (UHMWPE) discs were used as the positive and negative control respectively. Specimens of positive, negative and the samples were carefully placed in individually prepared cultures and incubated at 37 ± 2 °C in 95% humid and 5% carbon dioxide atmosphere. It is ensured that the test sample placed at the centre of the well covers one-tenth of the cell layer surface. The culture medium and the specimens were removed and the degree of cytotoxicity was determined qualitatively by viewing general morphology, vacuolization, detachment of cells and cell lyses around the material under an inverted phase contrast microscope (Leica DMIL, Germany). Based on these parameters, the material was graded according to a cytotoxicity scale ranging from 0-3 as per a validated test procedure based on ISO 10993-5 (1999). The grading was done as follows: If the confluency of healthy

cells is greater than 80%, it is graded as '0' (non-cytotoxic); if it is between 60-80%, the grade given is '1' (mildly cytotoxic); if it is 40-60%, the material is graded as '2' (moderately cytotoxic) and if it is less than 40%, a grading of '3' (severely cytotoxic) is given. Triplicates were used for both the test material and the controls.

3.6.1.2. Test on extract

The test on extract assay assesses the toxicity of the material extract. The material extract was prepared at 37 ± 2 °C by incubating 0.1 g of the sample in 1 ml of the culture medium supplemented with fetal bovine serum for 24 h. Diluted phenol (1.3 mg/ml) and extract of UHMWPE were used as the positive and negative controls respectively. A monolayer of cells grown in a 96-well dish containing the cell culture medium was taken and the medium was drained off completely. The material extract was added carefully to the multi-well plate without disturbing the cell monolayer. This was then incubated at 37 ± 2 °C for 24 h. Six replicates were used for both the test material and the controls. In the case of commercial gloves, the grading was done in a scale ranging from 0-3 as mentioned in the previous section 3.6.1.1 (ISO 10993-5, 1999). On the other hand, the latex vulcanizates were graded as per the United States Pharmacopeia (USP) method where the cell response was graded in the cytotoxicity scale ranging from 0-4 as per a validated test procedure and is described in Table 3.15 (USP 26, 2003).

Table 3.15. Description of cytotoxicity grading as per USP

Cytotoxicity grade	Cell response
0	Discrete cells, no cell lysis
1	Not more than 20 % cell damage
2	Not more than 50% cell damage
3	Not more than 70% cell damage
4	Nearly complete destruction of the cells

3.6.2. Intracutaneous (intradermal) reactivity test

Intracutaneous irritation tests were carried out to evaluate the potential of the extracts of the vulcanised latex sheets to produce irritation following intradermal injection of extracts into rabbits. Healthy, thin skinned New Zealand albino rabbits of either sex, with body weight not less than 2 kg, were used for the study. The animals

for the study were prepared by clipping the fur on the dorsal side, close to the skin. Extracts from the material were prepared both in normal saline and cottonseed oil (CSO) as per ISO 10993-10 (1995). For this, a 60 cm^2 of the sterilized sample was extracted in 10 ml each of saline and CSO at 70 ± 2 °C for a period of 24 h. A 0.2 ml of the saline extract of latex sheets was injected at five anterior sites on the left side of each rabbit (Figure 3.1).

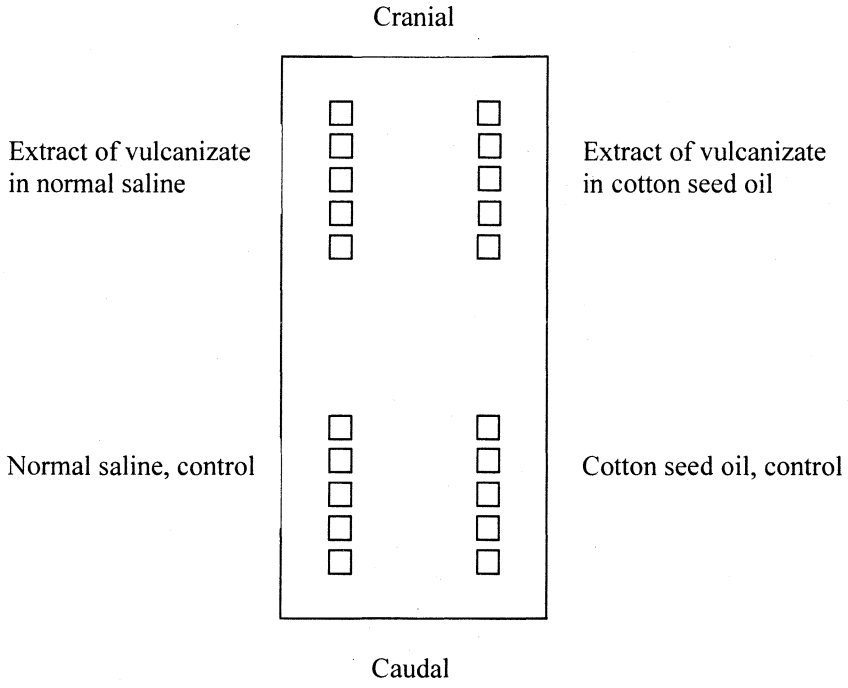


Figure 3.1. Arrangement of injection sites for intracutaneous irritation studies

Similarly, 0.2 ml of saline control was injected at five posterior sites on the same side of each rabbit. The above procedure was repeated for CSO extracts of the latex sheets and control solutions on the right side of each rabbit. The sites were then evaluated for skin reactions at 24, 48, and 72 h following the injection. Toxicity associated symptoms such as erythema, and oedema at 24, 48, and 72 h was recorded and the average irritation score was calculated. The primary irritation index is determined by adding the primary irritation score of each site of the animal and dividing the total score by the number of animals and number of periods. The primary irritation index is categorized into four as given in Table 3.16.

Table 3.16. Primary irritation responses categories in rabbit

Mean score	Responses categories
0 to 0.4	Negligible
0.5 to 1.9	Slight
2 to 4.9	Moderate
5 to 8	Severe

3.6.3. Sensitization

Skin sensitization by closed patch sensitization method as per ISO 10993-10 (1995) was carried out in guinea pigs to evaluate the response of the animal's immune system to the extracts of the latex vulcanizates. The material extract was prepared by extracting a 60 cm^2 of the sterilized latex sheets in 10 ml saline at 70 ± 2 °C for a period of 24 h. Four-ply gauze was used as the control material. A total of 15 Hartley strain guinea pigs, weighing between 300-500 g of either sex were selected for the experiment. Prior to each application period, the fur on the dorsal area on either side of the vertebral column of each animal was clipped. The test was carried out in two phases: an induction phase followed by a challenge phase. In the induction phase, the skin was lightly swabbed with 70% alcohol and air dried. A saturated patch of four ply gauze in physiological saline extract of the material was applied topically on the clipped upper back region of 10 animals (test animals). Similarly a saturated patch of four ply gauze in physiological saline was topically applied as control to other 5 animals. This was then covered with occlusive dressings. The occlusive dressings from the test and the control sites were removed after 6 h. The reaction at the site of application was observed at 24 h and 48 h for evidence of any erythema and oedema. This procedure was repeated at weekly intervals for three more weeks.

Fourteen days after the last application (induction period) all the test and control animals were challenged with the physiological saline extract of the test material. For this, the hair on the animal's flank area (untested area) was removed and the area was lightly swabbed with 70% alcohol and a saturated patch of test material was topically applied. This was then covered with occlusive dressings. The occlusive dressings from the test and the control were removed after 6 h. The reaction at the site of application was observed at 24 and 48 h for the evidence of any erythema and oedema. The numerical grading was recorded as per ISO 10993-10 (1995).

CHAPTER 4

EVALUATION OF COMMERCIALLY AVAILABLE NRL GLOVES AND STANDARDISATION OF ANALYTICAL TEST PROCEDURES

4.1. INTRODUCTION

In response to the growing concern over the allergic reactions to NRL products, regulatory agencies such as the FDA, EEC, and ASTM put forward essential requirements with regard to protein, chemical and powder levels in medical gloves. Although the threshold level of proteins, chemicals and powder-borne aeroallergens for sensitization has not been established till now, the upper limit of water-extractable proteins, glove powder and total accelerators in the finished medical gloves has been set by various agencies. The manufacturer is expected to meet these minimum standards while claiming conformity of the product with the quality certification by regulatory agencies. The chapter presents the results of the evaluation of the medical gloves marketed in India with regard to the cytotoxicity potential, dithiocarbamates released into artificial sweat, residual dithiocarbamates, and water-extractable proteins.

4.2. RESULTS AND DISCUSSION

4.2.1. *In vitro* cell culture test

Of the eleven brands of commercially available gloves tested, eight were of surgical type and three were of examination type. Table 4.1 gives the cytotoxicity grades obtained for the different brands of NRL medical gloves in the direct contact test and test on extract.

Table 4.1. Cytotoxicity grade obtained for the different brands of latex gloves

Sl. No.	Sample codes	Glove type	Cytotoxicity grade	
			Direct contact	Test on extract
1	A	Surgical, pre-powdered	1	0
2	B	Surgical, pre-powdered	3	0
3	C	Surgical, pre-powdered	1	1
4	D	Surgical, pre-powdered	2	a
5	E	Surgical, pre-powdered	0	0
6	F	Surgical, pre-powdered	0	0
7	G	Surgical, pre-powdered	0	3
8	H	Surgical, pre-powdered	1	0
9	I	Examination, powder free	0	0
10	J	Examination, powdered	3	3
11	K	Examination, powder free	0	0

0-non-cytotoxic; 1-mildly cytotoxic; 2- moderately cytotoxic; 3-severely cytotoxic; a-tests not conducted

The cell culture test showed that nearly 63% of the total gloves selected were cytotoxic to L929 cells. Seventy five percent of the surgical and 33% examination gloves were found to be cytotoxic. The cell responses varied from non-cytotoxic (grade '0') to severely cytotoxic (grade '3') with the majority of the gloves exhibiting cytotoxicity in the direct contact test. The wide variation in the cell response is attributed to the inherent variability associated with the production of these commercial gloves.

Figure 4.1 shows the typical optical micrograph of the L929 cells in direct contact with the NRL gloves. The cytotoxicity potential of latex gloves was graded taking into account the morphological changes and cytopathic or cell death exhibited

by the cell line. The grading was done in comparison with the cell response of the negative and the positive controls (Figure 4.2).

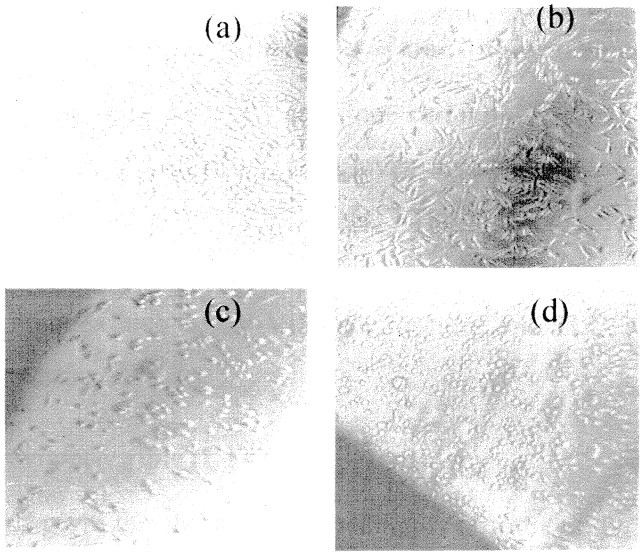


Figure 4.1. Optical micrograph of L929 cells incubated with latex gloves: (a) non cytotoxic; (b) mildly cytotoxic; (c) moderately cytotoxic; and (d) severely cytotoxic

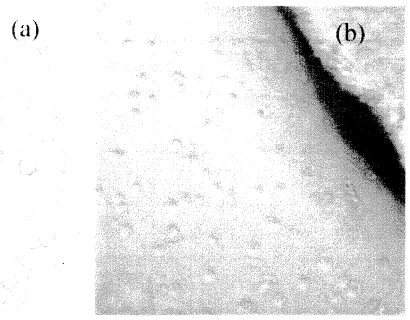


Figure 4.2. Optical micrograph of L929 cells incubated with control materials: (a) negative control showing non cytotoxicity; and (b) positive control showing severe cytotoxicity

In the case of non cytotoxic gloves, L929 cells retained their spindle shaped morphology (Figure 4.1(a)). A similar response was observed for the negative control and is shown in Figure 4.2(a). On the other hand, exposure to the toxic gloves caused changes in the cell morphology and the damaged cells appeared nearly spherical in shape (Figure 4.1(b-d)). Some of the gloves showed severe cytotoxicity as is evident from Figure 4.1(d), which showed a cell response similar to that of the positive control (Figure 4.2(b)). The *in vitro* cell culture cytotoxicity of gloves indicated that

the residual chemicals were released into the culture medium inducing toxicity to the surrounding cells.

4.2.2. Identification and quantification of zinc dithiocarbamates

As zinc dithiocarbamates are the commonly used accelerators in the manufacture of latex dipped goods, the gloves were analysed to identify and quantify the dithiocarbamates present in them. The identification of the type of dithiocarbamates released into artificial sweat or dichloromethane was carried out by analytical techniques such as UV-VIS spectroscopy, and TLC, and the quantification was performed using HPLC.

4.2.2.1. Ultraviolet-visible (UV-VIS) spectroscopy

Table 4.2 gives the absorption wavelengths of some of the commonly used zinc dithiocarbamates and antioxidants.

Table 4.2. Absorption wavelengths of zinc dithiocarbamates and some antioxidants

Name of chemical	Absorption wavelengths (nm)
ZDEC	270
ZDBC	265
ZBEC	269
ZIBC	268
ZDNC	270
Vanox ZS	266 & 228
Vanox MBPC	286 & 232
Wingstay L	285 & 229

Figure 4.3 shows a typical UV-VIS spectrum of the sweat extract of brand 'E' and the control ZDEC sample.

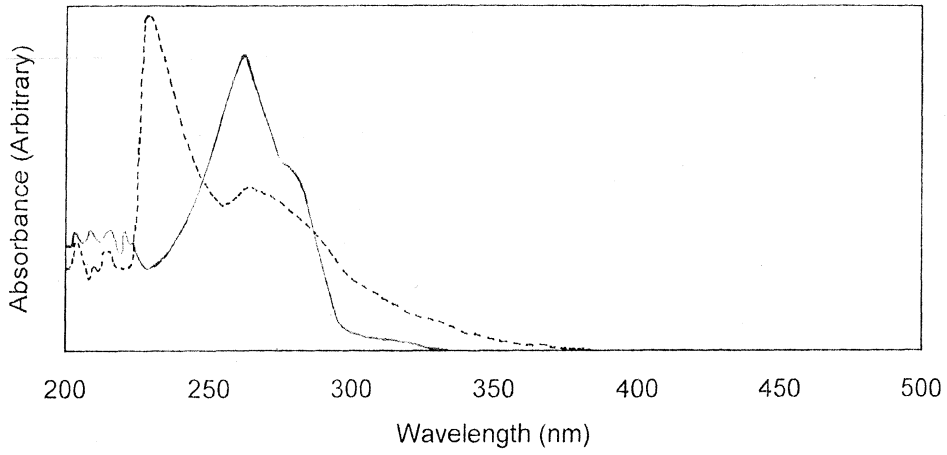


Figure 4.3. UV-VIS spectrum of ZDEC (—) and the sweat extract of brand 'E' (---)

It was found that both the control and the brand 'E' showed an absorption band at 274 nm indicating the release of residual chemicals (accelerators and/or antioxidants) into artificial sweat from gloves. However, as the different dithiocarbamates and the phenolic antioxidants gave an absorption maximum in the range 265-270 nm, it was difficult to distinguish the type of residual chemicals released into artificial sweat by UV-VIS spectroscopy. An additional peak at 228 nm was present in the spectrum of brand 'E', probably due to the presence of lactic acid or the antioxidant residues in the sweat extract. Lactic acid is found to absorb around 228 nm. Owing to its solubility in dichloromethane, lactic acid migrated from artificial sweat into dichloromethane fraction during the recovery of ZDEC from artificial sweat solution.

4.2.2.2. Thin layer chromatography (TLC)

It has been reported that the residual dithiocarbamates in gloves contributed to the cell toxicities more than antioxidants (Nakamura *et al.*, 1990). Therefore, only the dithiocarbamates released into the artificial sweat were identified and quantified. Zinc dithiocarbamates gave diffused spots on the TLC plate, which made the distinction between the different types of dithiocarbamates impossible. On the other hand, their copper complexes gave tighter spots on the chromatographic plate. A similar observation has been noticed by Parker and Berriman (1954). The R_f values of the spots formed by standard dithiocarbamates were calculated, and are given in Table 4.3.

Table 4.3. R_f values of the copper complex of different zinc dithiocarbamates

Sample Codes*	R_f value
CDEC	0.41 ± 0.01
CDBC	0.87 ± 0.01
CIBC	0.86 ± 0.02
CBEC	0.66 ± 0.03
CDNC	0.96 ± 0.01

* CDEC-Copper diethyldithiocarbamate; CDBC-Copper dibutyldithiocarbamate; CIBC-Copper diisobutyldithiocarbamate; CBEC-Copper dibenzyldithiocarbamate; CDNC-Copper diisononyl dithiocarbamate

It is apparent that the copper complexes of different zinc dithiocarbamates showed characteristic R_f values. However, the R_f value of the copper complex of ZDBC (CDBC) and its isomeric counterpart, ZIBC (CIBC) were so close and that the differentiation between them was impossible. Table 4.4 gives the R_f values of the copper complexes of dithiocarbamate released into artificial sweat from commercial gloves.

Table 4.4. R_f values of copper complex of dithiocarbamates released into artificial sweat from gloves

Sample Codes	R_f value
A	0.40
B	0.41
C	0.41
D	0.41
E	0.41
F	0.41
G	0.41
H	0.41
I	a
J	0.42
K	a

a- not found

It was observed that except for brands 'I' and 'K', the chromatograms of the sweat extracts of glove brands contained a common spot with an R_f value around 0.41 corresponding to ZDEC. The TLC analysis indicated that the ZDEC residues were released into the artificial sweat from the gloves.

4.2.2.3. High performance liquid chromatography (HPLC)

Figure 4.4 shows the chromatogram of the standard solution of ZDEC when subjected to HPLC.

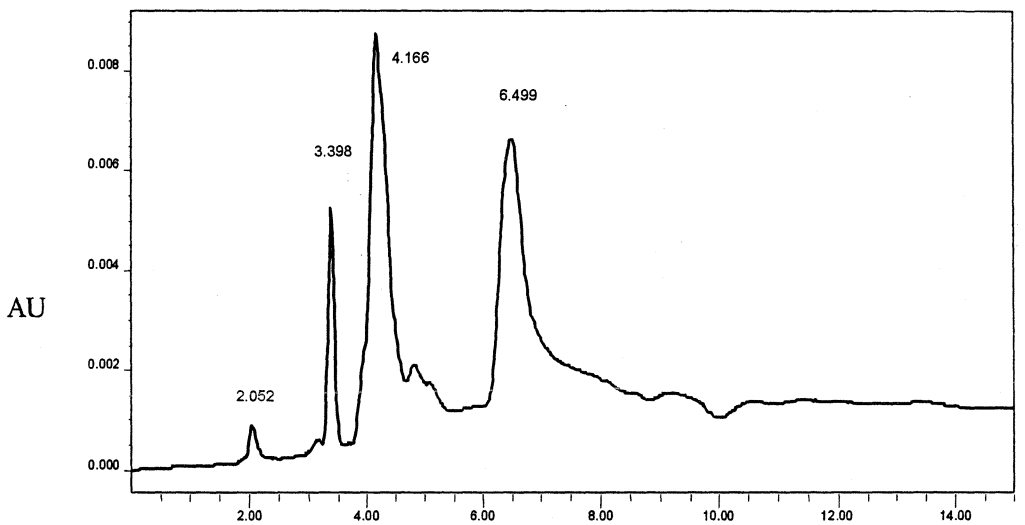


Figure 4.4. Chromatogram of ZDEC

It is apparent that ZDEC gave a complex chromatogram containing many peaks, which made the quantification difficult. The formation of multiple peaks is ascribed to the substitution reaction of ZDEC with the metal parts of the HPLC column. It has been reported that the zinc dithiocarbamates underwent exchange reactions with the metal parts of the chromatographic column resulting in the formation of multiple peaks (Hutchins *et al.*, 1982; Mathieu *et al.*, 2000). Problems associated with the lability of the dithiocarbamate ligands were overcome by converting them into more stable copper complexes. Addition of copper(II) sulphate to standard solutions of zinc dithiocarbamates facilitated the formation of brown colored copper-dithiocarbamate complexes. The intensity faded to light brown, and finally to light yellow as the concentration of the standard solutions of zinc dithiocarbamates was gradually decreased. Prior to the quantification of copper-

dithiocarbamates, the UV-VIS spectrum of the copper complexes of standard zinc dithiocarbamates was recorded. Figure 4.5 shows a typical UV-VIS spectrum of copper complex formed from ZDEC (CDEC).

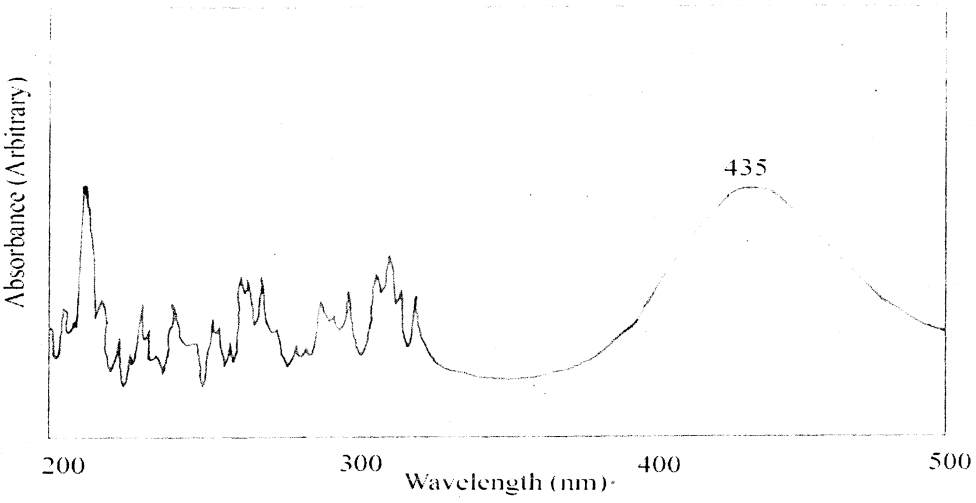


Figure 4.5. UV-VIS spectrum of CDEC

The UV-VIS spectrum indicated that CDEC absorbed at 435 nm. A similar observation was made in the case of copper complexes of ZDBC, ZBEC, ZIBC and ZDNC. Trifunovic *et al.* (2002) reported that the copper complex of a polydentate dialkyldithiocarbamic acid ligand gave an absorption maximum at 438 nm. The spectral band at 435 nm is ascribed to the $dxy \rightarrow dxz$ transitions of the Cu(II) ion (Fabretti *et al.,* 1984; Trifunovic *et al.,* 2002). The geometry of copper-dithiocarbamate complex is reported to be square planar where the bidentate dithiocarbamate ligands coordinate to Cu(II) ion through both the sulphur atoms (Kaludjerovic *et al.,* 2002; Trifunovic *et al.,* 2002).

As the copper-dithiocarbamate complexes absorbed at 435 nm, the detection wavelength for copper dithiocarbamates during HPLC analysis was set at 435 nm. The HPLC chromatograms of the copper complexes of different dithiocarbamates are shown in Figure 4.6.

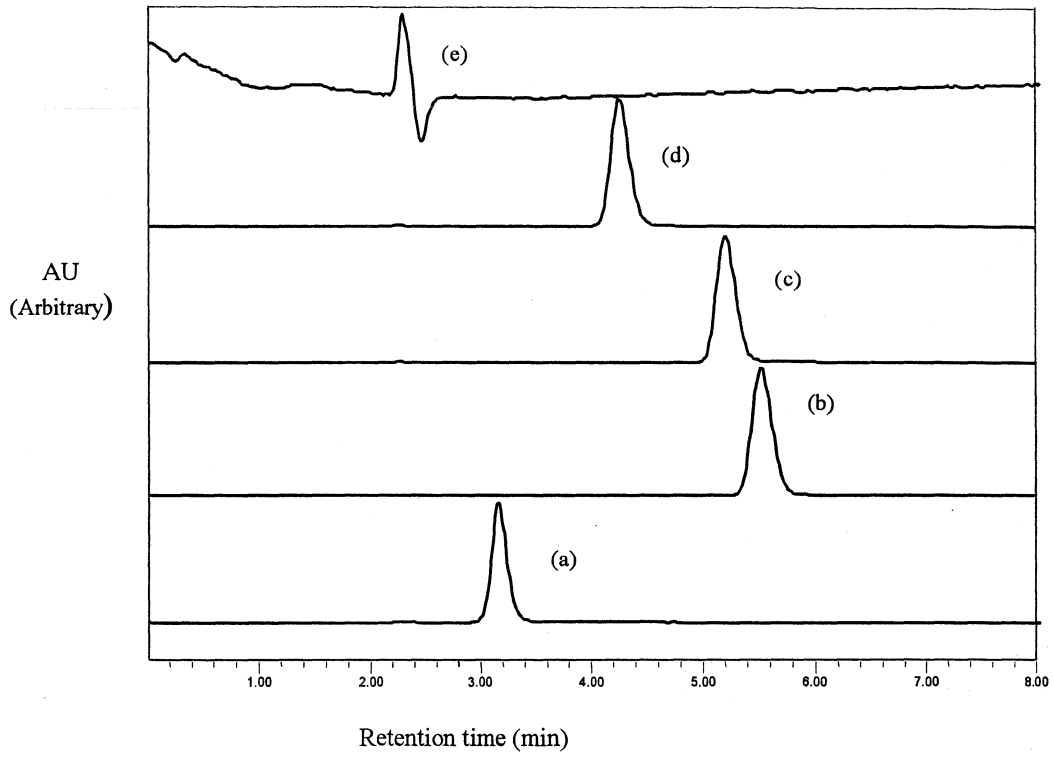


Figure 4.6. Chromatograms of copper complexes of different zinc dithiocarbamates: (a) CDEC; (b) CDBC; (c) CIBC; (d) CBEC; and (e) CDNC

Under the chromatographic conditions specified, the retention times of the copper-dithiocarbamate complexes were different. Table 4.5 gives the retention times of the copper complexes of different zinc dithiocarbamates.

Table 4.5. Retention times of copper complex of different zinc dithiocarbamates

Sample Codes	Retention time (min)
CDEC	3.20 ± 0.05
CDBC	5.72 ± 0.30
CIBC	5.51 ± 0.40
CBEC	4.48 ± 0.30
CDNC	2.25 ± 0.04

As the retention times differ from one another, the type of dithiocarbamate compounds could be identified by looking at the retention times. However, the retention times of the copper complexes of ZDBC and its isomeric counterpart ZIBC (CDBC and CIBC respectively) were closer making the differentiation between them difficult.

4.2.2.3.1. Assay Validation

Table 4.6 compares the percentage conversion of ZDEC into its copper and cobalt complexes using copper(II) sulphate and cobalt(II) chloride respectively.

Table 4.6. Percentage conversion of ZDEC using different complexing agents

Complexing agent	Concentration of the standard ZDEC (μg/ml)	Amount of ZDEC quantified (μg/ml)	% Conversion
copper(II) sulphate	22	24	109
cobalt(II) chloride	22	8	36

$$\%\ Conversion = \frac{Amount\ of\ ZDEC\ quantified}{Concentration\ of\ the\ standard} \times 100$$

It was found that the amount of ZDEC quantified from a known concentration of ZDEC solution using copper(II) sulphate was greater compared to cobalt(II) chloride. A similar observation was noticed in the case of latex vulcanizates as well. Table 4.7 compares the amount of ZDEC quantified from artificial sweat extract of the latex vulcanizate (DE-5) using copper(II) sulphate and cobalt(II) chloride.

Table 4.7. Comparison of the amount of ZDEC quantified from latex vulcanizate using different complexing agents

Sl. No.	Amount of ZDEC released into artificial sweat ± SD (μg/g)	
	Using copper(II) sulphate	Using cobalt(II) chloride
1	11.49	5.0
2	12.14	4.03
3	11.65	3.59
Average	11.76 ± 0.34	4.2 ± 0.72

The results indicated that copper(II) sulphate is a better complexing agent which ensures complete conversion of ZDEC into the respective copper complex. Hence, the validation was carried for the method using copper(II) sulphate only. The linearity, accuracy, precision, DL, and RQL were determined. The standard ZDEC solutions with concentration ranging from 0.6375-20.4 μg/ml gave excellent linear plots when direct absorbance measurements at 435 nm were made and plotted against concentration. The concentration was back calculated by linear regression analysis,

and a correlation coefficient of 0.99 was obtained. The linear regression curve for the determination of ZDEC is given in Figure 4.7.

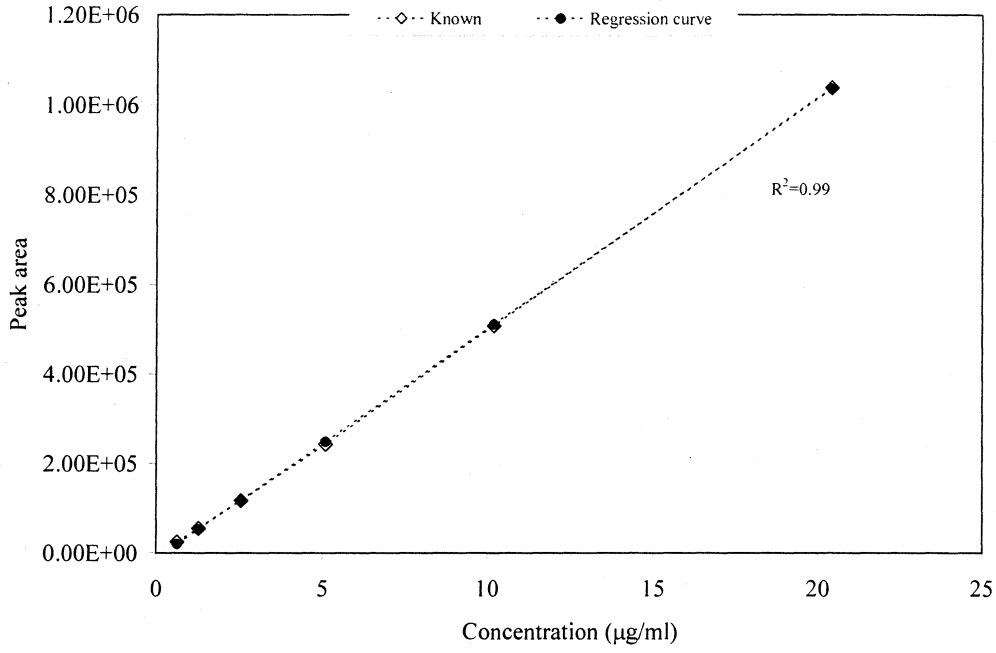


Figure 4.7. Linear regression curve for the quantification of ZDEC

Table 4.8 gives precision and accuracy of the HPLC method for the quantification of ZDEC.

Table 4.8. Accuracy and precision data for the quantification of ZDEC

Known concentration (µg/ml)	Back calculated concentration ± SD (µg/ml)	Precision (% coefficient of variation)	Accuracy (% bias)
0.6375	0.743 ± 0.0034	0.46	16.5
1.275	1.328 ± 0.004	0.31	4.15
2.55	2.518 ± 0.015	0.59	1.25
5.1	4.981 ± 0.03	0.62	2.33
10.2	10.13 ± 0.032	0.32	0.68
20.4	20.46 ± 0.084	0.41	0.29

The results given in Table 4.8 showed that high precision (< 1%) was achieved in the range of the concentration studied. Accuracy of the method was good except for the lowest value of concentration studied (0.6375 µg/ml). Back calculated

concentration of ZDEC was within 5% of the original values for the range of concentration from 1.275-20.4 µg/ml. The DL and the RQL were also calculated from the regression curve. The DL of ZDEC was 0.25 µg/ml, and the RQL was found to be 0.84 µg/ml. Thus the method for the determination of ZDEC was found to be accurate, precise and linear.

In the case of ZDBC, a plot of peak area versus concentration gave linear plots with a correlation coefficient of 0.99. The linear regression curve is shown in Figure 4.8.

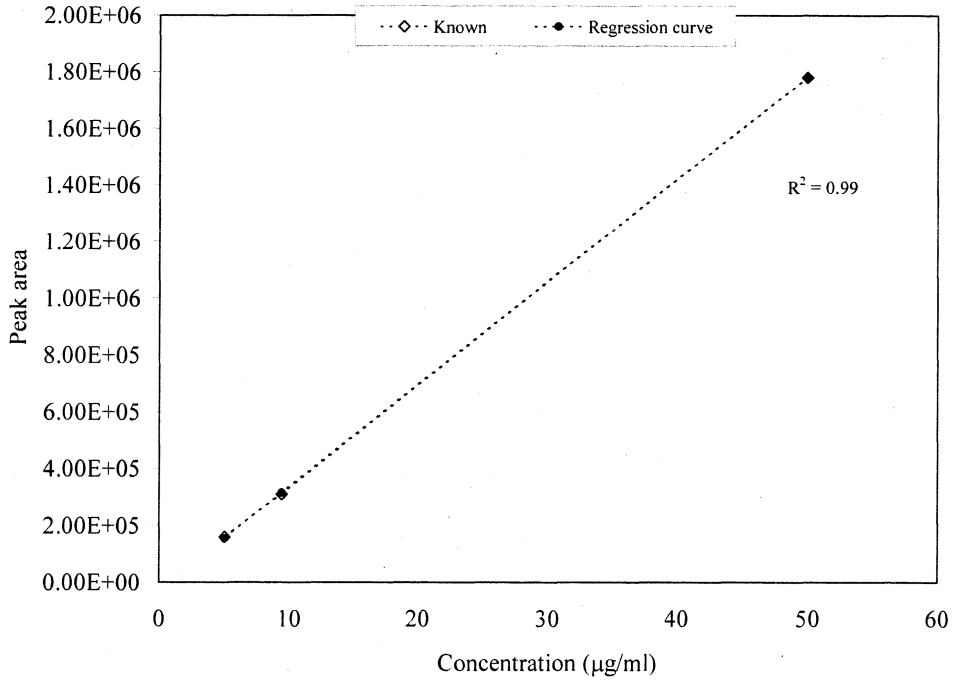


Figure 4.8. Linear regression curve for the quantification of ZDBC

Table 4.9 gives the accuracy of the test method for the quantification of ZDBC.

Table 4.9. Accuracy data for the quantification of ZDBC

Known concentration (µg/ml)	Back calculated concentration (µg/ml)	Accuracy
5.04	4.45	14
9.45	8.7	7.9
49.98	49.31	1.3

The DL and RQL of ZDBC were found to be 0.61 µg/ml and 2.0 µg/ml respectively. It was interesting to note that the DL and RQL of ZDBC lie in the higher range compared to ZDEC. A similar observation was made by Depree *et al.* (2004) who employed cobalt(II) chloride, and the DL was reported to be 5 and 10 µg/ml for ZDEC and ZDBC respectively. They also reported that the lowest concentration at which ZDEC could be detected visually was 62.5 µg/ml. On the other hand, the present method facilitated the detection of ZDEC with concentration as low as 0.6375 µg/ml.

4.2.2.3.2. Determination of ZDEC in gloves

The amount of dithiocarbamates released into artificial sweat and dichloromethane from the gloves was quantified using HPLC. Since the brands 'I' and 'K' did not contain any detectable ZDEC, they were excluded from quantification steps. As all the remaining nine brands of gloves contained ZDEC, only six brands of gloves that differed in their cytotoxic response were selected for sweat extraction studies. Prior to the quantification of ZDEC released into artificial sweat (sweat-extractable ZDEC), the UV spectrum of the copper complexes of both the standard ZDEC (CDEC) and the dithiocarbamate present in the sweat extract of a commercial glove (brand 'E') were recorded, and are given in Figure 4.9.

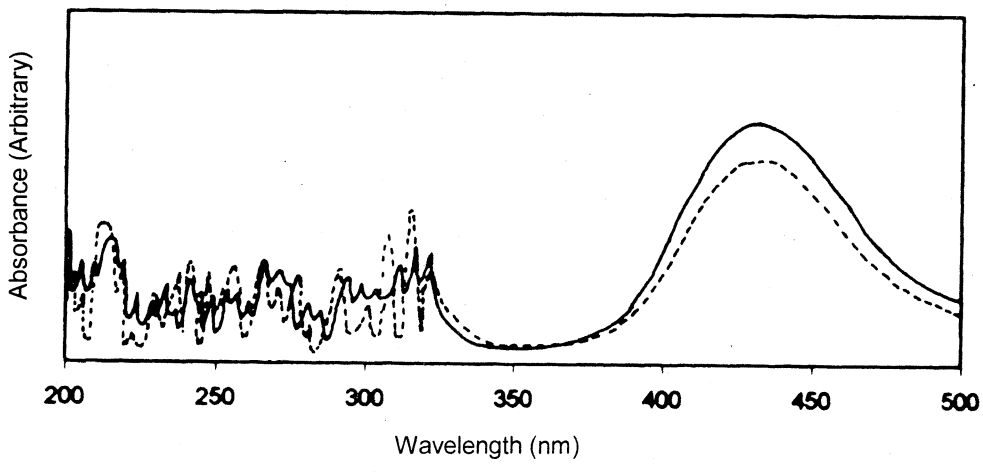


Figure 4.9. UV-VIS spectrum of copper complexes of standard ZDEC (—) and the dithiocarbamate present in the sweat extract of brand 'E' (---)

The spectrum contained a characteristic absorption maximum at 435 nm for both the sample and the standard confirming the presence of ZDEC in the sweat extract of the glove Figure 4.10 shows a typical chromatogram of the copper

complexes of both the standard ZDEC (CDEC) and the dithiocarbamate present in the sweat extract of a commercial glove (brand 'E')

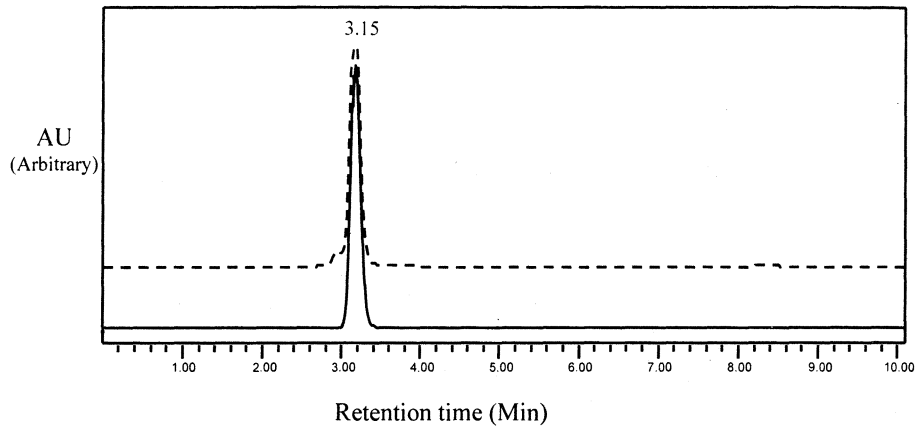


Figure 4.10. Chromatogram of copper complexes of standard ZDEC (—) and the dithiocarbamate present in the sweat extract of brand 'E' (---)

The presence of ZDEC in the sweat extract of glove brand 'E' was evident from the chromatogram. Figure 4.10 showed that the retention time of copper complex of both ZDEC and sweat extract of brand 'E' was 3.15 min, a value quite different from that of ZDBC (5.7 min) and ZBEC (4.5 min), indicating that the residual ZDEC was released into the artificial sweat solution from the gloves. Table 4.10 gives the amount of sweat-extractable ZDEC and residual ZDEC in the different brands of gloves.

Table 4.10. Amount of sweat-extractable and residual ZDEC in commercial gloves

Sample codes	Sweat-extractable ZDEC (μg/g)	Residual ZDEC (mg/g)
A	94	1.25
F	39	0.20
C	78	2.36
E	61	3.05
H	101	3.84
J	173	3.16

The results showed that the amounts of both the sweat-extractable and the residual ZDEC varied widely from brand to brand. The amount of sweat-extractable ZDEC ranged from 39 μg/g (brand 'F') to as high as 173 μg/g (brand 'J'), while the amount of residual ZDEC varied from 0.20 mg/g (brand 'F') to 3.84 mg/g

(brand 'H'). Such a variation is expected in the case of commercial gloves, as different manufacturers adopt different processing techniques for the production of latex products. As the amount of sweat-extractable ZDEC varied between the different brands of gloves, it is expected that the allergenicity and/or cytotoxicity of the different brands of glove may also vary. Knudsen *et al.* (1993) found that gloves releasing higher amounts of thiurams and/or dithiocarbamates in artificial sweat elicited more number of positive reactions during patch testing of gloves. It has been reported that ZDEC in concentrations of about 0.25% weight of the penrose drain (2.5 mg/g) induced local toxicity and delayed wound healing in mice (Nicolaysen *et al.,* 2004). It is to be noted that four out of the six gloves contained residual ZDEC in quantities ≥ 2.5 mg/g. The high residual ZDEC content in medical gloves cautions the Indian latex industry to take adequate measures to reduce the amounts of residual ZDEC as low as reasonably practicable.

As far as the extraction in dichloromethane is concerned, the amount of ZDEC released was much greater compared to that in artificial sweat, and is attributed to the ability of the dichloromethane to swell the NRL gloves. It was found that the NRL gloves swell in dichloromethane to an extent of 450% compared to a mere 12% in artificial sweat. Apparently, dichloromethane extracted ZDEC residues from the interior of the glove pieces, which accounts for the higher residual ZDEC content. Figure 4.11 shows the relationship between the residual ZDEC content and sweat-extractable ZDEC in the different brands of gloves.

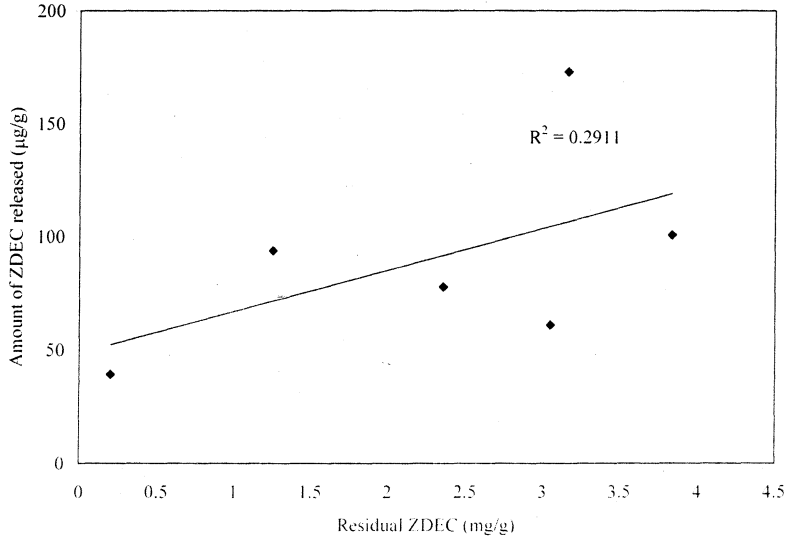


Figure 4.11. Relationship between the residual and sweat-extractable ZDEC

Figure 4.11 showed a low correlation between the amounts of residual and sweat-extractable ZDEC in the commercial brands of gloves indicating that the release of ZDEC into artificial sweat is not related to the amount of residual ZDEC. A similar observation has been made by Tinkler (1996) who reported that the amount of dithiocarbamates released into physiological solutions was not directly proportional to the residue levels in the gloves. The low correlation is again attributed to the inherent variability in the processing of NRL associated with the production of latex products (Tinkler, 1996).

4.2.3. Relationship between cytotoxicity grade and sweat-extractable ZDEC

The TLC and HPLC analysis of the commercial medical gloves showed the presence of ZDEC, which is reported to be the most cytotoxic compound among the different dithiocarbamates and antioxidants used in the latex industry (Nakamura *et al.,* 1990; Ikarashi *et al.,* 1992). It is, therefore, expected that the major contributing factor to the cytotoxicity of gloves is the presence and availability of ZDEC. It may be noticed that the brands 'I' and 'K' with no detectable ZDEC showed a non cytotoxic response to L929 cells.

An attempt was made to correlate the cytotoxicity response to the amount of sweat-extractable ZDEC of the commercial gloves. Ideally, the amount of ZDEC released into the cell culture medium should be determined, and correlated with the cytotoxicity response. However, this was not possible due to the poor separation between the aqueous culture medium and the solvent medium (dichloromethane) used to extract the ZDEC residues from the culture medium. Figure 4.12 shows the relationship between the cytotoxicity grade of the latex gloves in the direct contact assay and the sweat-extractable ZDEC. Figure 4.13 shows the relationship between the cytotoxicity grade of the latex gloves in the test on extract assay and the sweat-extractable ZDEC.

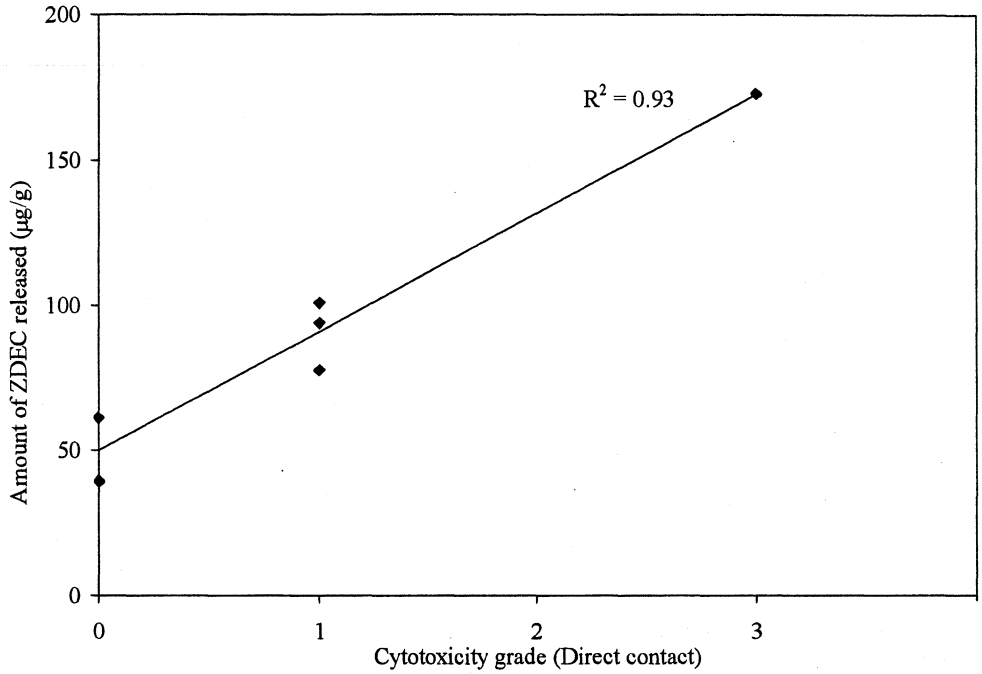


Figure 4.12. Plot of cytotoxicity grade (direct contact) Vs. sweat-extractable ZDEC

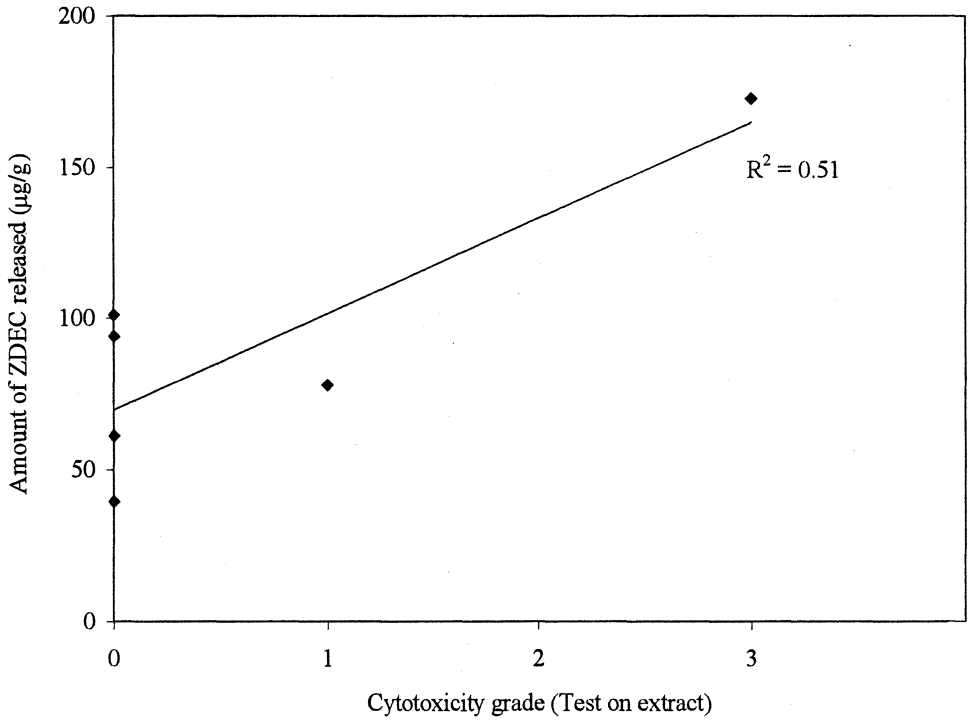


Figure 4.13. Plot of cytotoxicity grade (test on extract) Vs. sweat-extractable ZDEC

It is apparent that a linear relationship existed between the cytotoxicity grade in the two types of *in vitro* cell culture methods and the sweat-extractable ZDEC indicating that the cytotoxic potential of the gloves increased with an increase in the

amount of sweat-extractable ZDEC. The effect of the amount of sweat-extractable ZDEC on the cytotoxic potential of the latex gloves was statistically significant in both direct contact ($p<0.001$) and test on extract assay ($p=0.038$). Similar observation has been made by Talja *et al.*, (1993) who reported that there existed a correlation between the IC_{50} values and the zinc concentration (of the accelerators) in urinary catheters. Tsuchiya *et al.* (1993) reported that the degree of cytotoxicity of polyurethane films containing dithiocarbamates increased with an increase in the amount of dithiocarbamates. Similar observations have been reported in the case of urinary catheters, which caused strictures in patients. Wilksch *et al.* (1983) reported an excellent correlation between the cytotoxic effects of soluble extracts from urinary catheters and the degree of acute and chronic inflammation induced during the subcutaneous implantation in rats.

The Spearman's rank correlation coefficients between the sweat-extractable ZDEC and cytotoxicity grade were 0.93 and 0.51 for the direct contact assay and the test on extract assay respectively. The good correlation obtained between direct contact assay and amount of ZDEC released compared to the extract assay is attributed to the fact that the direct contact assay was more sensitive to detect the weak cytotoxicity exhibited by glove materials. This is clearly evident from the cytotoxicity response of brands 'A' and 'H'. In the direct contact test, they showed a mild cytotoxicity response while in the test on extract assay a non toxic response was observed. Tsuchiya *et al.* (1993) reported direct contact method as a more sensitive method compared to extraction method in the colony assay of materials containing dithiocarbamates.

Figure 4.12 showed that gloves having extractable ZDEC equal to or less than 61 μg/g of glove exhibited a non-cytotoxic response. A mildly cytotoxic behavior was observed when the amount of sweat-extractable ZDEC was in the range 78-100 μg/g of glove. The material was severely cytotoxic when the sweat-extractable ZDEC rose to about 173 μg/g of glove. It may be also noted that the sweat-extractable ZDEC of brand 'C' (78 μg/g) is lower than that of brand 'A' (94 μg/g) but its cytotoxicity grade in the test on extract is '1'. The exact cause of this discrepancy in the cytotoxic response of brands 'C' and 'A' is not known owing to the wide variation associated with the production of medical gloves.

4.2.4. Determination of water-extractable proteins

Table 4.11 and Table 4.12 give the amount of water-extractable proteins in some of the commercially available brands of gloves in the local market and international market respectively.

Table 4.11. Amount of water-extractable proteins in some brands of commercially available gloves marketed in India

Brand code	Extractable protein, Mean ± SD (CoV)	
	μg/dm^2 of glove	μg/g of glove
A	260 ± 40 (15.3)	243 ± 23 (9.4)
B	158.6 ± 1.6 (0.98)	151 ± 1.2 (0.8)
D	143.2 ± 44 (30.7)	101 ± 30.6 (30)
E	136.1 ± 1.8 (1.3)	158.95 ± 2 (1.3)
I	22.7 ± 6.89 (30.3)	25.5 ± 6.9 (27)

SD- standard deviation; CoV- Coefficient of variation

Table 4.12. Amount of water-extractable proteins in some brands of gloves in the international market

Brand code	Extractable protein, Mean ± SD (CoV)	
	μg/dm^2 of glove	μg/g of glove
PO	56.9 ± 16.4 (28.8)	36.3 ± 10.5 (29.0)
OP	24.5 ± 4.9 (20.1)	26.9 ± 5.7 (21.1)
NE	24.3 ± 9.8 (40.5)	19.8 ± 8.8 (44.5)
UL	6.03 ± 1.9 (32.1)	4.8 ± 1.4 (29.3)
DC	7.3 ± 2.2 (30.5)	8.1 ± 2.5 (31.4)
DG	93.3 ± 10.6 (11.4)	91.7 ± 8.5 (9.2)

The gloves obtained from the international market were some popular brands manufactured by M/s Ansell and M/s Johnson & Johnson. It was found that the amount of water-extractable proteins was generally high in the gloves available in the Indian market compared to those in the international market, probably due to insufficient leaching of gloves during manufacturing. The recommended level of water-extractable proteins specified by standards such as ASTM D 3577 (2002) and

ASTM D 3578 (2002) for both surgical and examination gloves is below 200 μg/dm^2. Though users and manufacturers believe that there is a safe limit of protein level in gloves, many agencies, for example MDA (Medical Device Agency, UK) or EEC do not provide any definitive guidance to purchasers on what protein levels can be regarded as safe (Phillips, 2004; EEC/93/42, 2004). However, the reduction on the level of proteins will greatly reduce the chances of sensitizing more people and reduces frequency of allergic reactions to a previously sensitized patient or health care worker.

4.3. CONCLUSIONS

Some of the commercially available medical gloves made of NRL were subjected to *in vitro* cell culture test and chemical analysis. Analytical techniques were developed in order to identify and quantify extractable zinc dithiocarbamates present in these gloves. It was found that the UV-VIS spectroscopy may be used for a preliminary investigation to detect the presence of residual chemicals, eventhough no conclusive evidence regarding the type of dithiocarbamates could be made. Thin layer chromatography could be used as a rapid and specific technique to distinguish the different zinc dithiocarbamates based on their R_f values. On the other hand, HPLC could be successfully used for both identification and quantification of zinc dithiocarbamates. The identification could be made by looking at the retention time characteristic of each zinc dithiocarbamate. It was found that the amount of ZDEC recovered using copper(II) sulphate was greater compared to cobalt(II) chloride, another complexing agent used for the quantification of zinc dithiocarbamates. The HPLC method was validated with standard solutions of ZDEC and ZDBC. The test method for the determination of ZDEC was found to be linear, accurate and precise. Good linearity and accuracy of the test method were observed for the determination of ZDBC as well. The DL of ZDEC and ZDBC was 0.25 and 0.61 μg/ml and the RQL was 0.84 and 2.0 μg/ml respectively. The present methodology based on a precolumn derivatization using copper(II) sulphate facilitated the identification and quantification of the zinc dithiocarbamates present in the NRL products/vulcanizates. The HPLC method could be used as a simple test to monitor the level of ZDEC in latex medical products.

The *in vitro* cell culture test indicated that the residual chemicals present in the finished latex gloves were released into the culture medium and caused cell lysis. Nearly 63% of the total gloves selected were found to be cytotoxic in nature. All the gloves except two contained ZDEC. The amount of both the sweat-extractable and the residual ZDEC varied widely from brand to brand. The amount of sweat-extractable ZDEC ranged from 39 μg/g to as high as 173 μg/g, and the amount of residual ZDEC varied from 0.20 mg/g to 3.84 mg/g. The cytotoxicity grade showed a linear relationship with the amount of sweat-extractable ZDEC indicating that the cytotoxic potential of gloves increased with an increase in the amount of sweat-extractable ZDEC. The effect of the amount of sweat-extractable ZDEC on the cytotoxic potential of the latex gloves was statistically significant in both direct contact and test on extract assay. A potential benefit that accrues from the sweat-extraction study of the latex gloves is that it may be developed as a test method to screen new latex products containing dithiocarbamates before subjecting them to expensive *in vivo* biological tests. However, further studies with significantly higher number of latex gloves/latex formulations are needed to establish a threshold limit for sweat-extractable ZDEC that exhibits toxicity in *in vitro* cell culture evaluation.

The amount of water-extractable proteins was generally high in the gloves marketed in India compared to those in the international market. The results in general pointed to the fact that the quality of the gloves marketed in India is a matter of concern with regard to the residual ZDEC and water-extractable protein levels.

CHAPTER 5

DITHIOCARBAMATE-RELEASE FROM SURGICAL GLOVES

5.1. INTRODUCTION

Significant exposure of health care workers as well as patients to zinc dithiocarbamates is likely to arise from the use of NRL medical devices. Exposure to dithiocarbamates is a serious concern in the industrial sector as well. The route of exposure and the resultant uptake by the body depend on the nature of the NRL product. In the case of gloves, dermal contact is the major route of exposure to dithiocarbamates. Hands perspire when gloves are donned, and the sweat solubilises the chemicals present on the surface of the gloves. When these chemicals became bioavailable, allergic reactions may occur. It has been reported that the amount of bioavailable chemicals depends on the amount of chemicals released into hand sweat (Knudsen *et al.,* 1993). As the release and the subsequent exposure to residual chemicals contributed to the development of allergic reactions in NRL gloves, the extent of dithiocarbamate-release from gloves under real-use (using human subjects) and simulated-use conditions (using artificial sweat) was determined. The amount of dithiocarbamate released into hand sweat and artificial sweat was compared to check whether the artificial sweat extraction of gloves could be used as a simulated test to estimate the exposure of users to dithiocarbamates.

5.2. RESULTS AND DISCUSSION

5.2.1. Dithiocarbamate-release from gloves into hand sweat

Table 5.1 gives the R_f values of the copper complex of the zinc dithiocarbamate released into the hand sweat of the human subjects from the glove.

Table 5.1. R_f values of copper complex of dithiocarbamates released into hand sweat

Human subject code	R_f value
A	0.41
B	0.41
C	0.41
D	0.40
E	0.39
F	0.41
G	0.40
H	0.41

It is apparent that the sweat extract of the human subjects gave only a single spot with an R_f value of around 0.41 indicating that ZDEC was released into the hand sweat from the latex gloves. This observation was further confirmed in HPLC. The retention time of the copper complex formed from both the standard ZDEC and the dithiocarbamate released into the hand sweat of the human subjects was 3.16 min indicating that ZDEC was released into the hand sweat from the gloves. Typical HPLC chromatograms of the copper complex of standard solutions of ZDEC (CDEC) and that of the sweat extract of the human subject 'F' are shown in Figure 5.1.

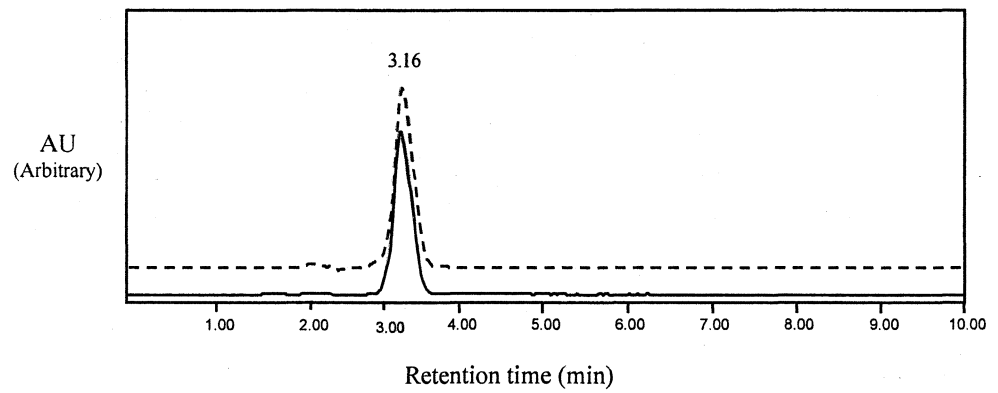


Figure 5.1. Chromatograms of CDEC (—) and the copper complex of the dithiocarbamate released into the hand sweat of human subject 'F' (---)

Table 5.2 gives the sweat rate, pH of the hand sweat, and the amount of ZDEC released into the hand sweat of human subjects. An estimate of the extent of exposure to ZDEC in health care workers was also carried out using equation 3.7 (section 3.3.1) and is also given in Table 5.2.

Table 5.2. Sweat rate, sweat pH, amount of ZDEC released into the hand sweat of the human subjects and the approximate extent of exposure to ZDEC

Subject code	Sweat rate (g/h)	pH of hand sweat	Amount of ZDEC released		Extent of exposure to ZDEC (mg/day)
			($\mu g/dm^2/h$)	($\mu g/g/h$)*	
A	1.262	5.84	48	54	1.76
B	-	7.04	55	62	2.02
C	-	7.14	54	61	1.98
D	0.779	7.15	13	15	0.48
E	0.667	7.24	5	6	0.184
F	0.717	7.36	74	83	2.72
G	0.706	7.40	116	130	4.26
H	-	7.45	85	95	3.12

* to convert dm^2 into g, a conversion factor of 0.8904 ± 0.001 was used for this particular brand of glove

The extent of ZDEC-release into hand sweat varied among the human subjects, and the values ranged from 5 $\mu g/dm^2$ to 116 $\mu g/dm^2$ despite using the same brand of gloves. This may be due to the inherent variations in the sweat rate, sweat pH, hand exercise, glove-fit etc among the human subjects. It was found that the sweat rate and sweat pH varied among the human subjects participated in the present study. Variations in sweat rate with ethnicity and rate of exercise among individuals has previously been reported by Dill *et al.* (1983). It was interesting to note that the amount of ZDEC released into the hand sweat of volunteers D, E, F, and G varied widely despite having comparable sweat rates indicating that the sweat rate has no substantial effect on the amount of ZDEC released into hand sweat.

Figure 5.2 gives the plot of the amount of ZDEC released into the hand sweat of human subjects versus sweat pH when the glove was donned for 1 h.

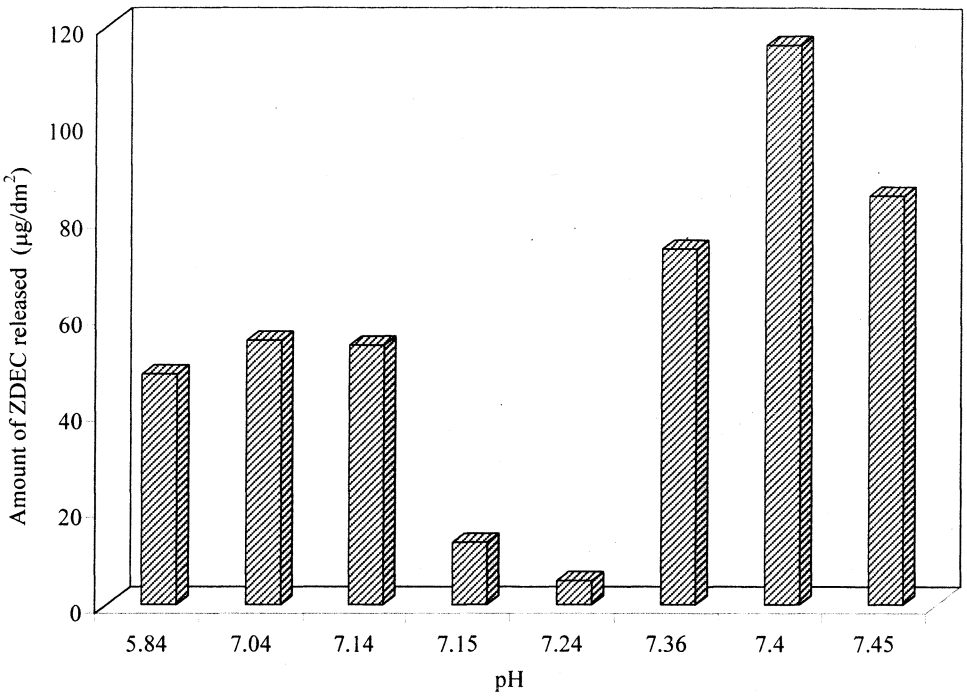


Figure 5.2. Amount of ZDEC released into the hand sweat Vs. hand sweat pH

It may be noted that the pH of the hand sweat of all but one human subject fell in the alkaline range. The release of ZDEC was generally high under alkaline conditions. A wide variation in the amount of ZDEC-release was observed with respect to small changes in the pH of the hand sweat (7.14-7.15 and 7.36-7.45). In general, the results indicated lack of a clear relationship between sweat-extractable ZDEC and pH of the hand sweat. The results indicated that the extent of ZDEC-release into hand sweat may be affected by one or more of the factors such as pH, sweat rate, hand exercise, glove-fit etc, which may vary from person to person.

The exposure assessment was based on direct measurement of exposure to ZDEC among users and comparing it with a threshold value for an adverse effect. It was observed that the health care worker is exposed (assuming a donning time of 8 h per day) to high amounts of ZDEC daily and the extent of exposure varied between 0.18 and 4.25 mg/day (Table 5.2). At present, no threshold value of ZDEC for sensitization among population is available (SCMPMD, 2000). Researchers reported wide range of ZDEC levels at which patients showed positive responses during patch testing of gloves (Kaniwa *et al.*, 1988; Knudsen *et al.*, 1993; Kaniwa *et al.*, 1994).

Knudsen *et al.* (1993) reported that gloves releasing higher quantities of dithiocarbamates would elicit a higher number of positive reactions in patch test than those releasing smaller quantities. Kaniwa *et al.,* (1988) reported that surgical rubber glove containing as low as 77 μg/g of ZDEC induced positive reactions in 6.4% of the 78 patients subjected to patch testing. Moreover, it has been reported that repeated exposure to chemicals over a period of time is more effective in inducing sensitization than a single dose (Boeniger, 2002). It is to be noted that the glove released high amounts of residual ZDEC (> 77 μg/g) into the hand sweat of some of the human subjects. Although none of the human subjects showed any sign of irritation while donning gloves for 1 h, it may be possible that repeated exposure to high amounts of residual ZDEC will cause irritation and/or sensitization among users over a period of time.

Similar exposure assessment studies of dithiocarbamate compounds have been reported. Recently, the daily intake of ethylenebisdithiocarbamate pesticides levels in food samples has been determined and compared with the acceptable daily intake as a part of the exposure assessment of these compounds (Caldas *et al.,* 2004). The Swedish Chemical Inspectorate estimated that the health care worker wearing gloves for 2 h daily will receive a DEHP (plasticizer) dose of 0.007 mg/kg/day (KEMI, 2000).

Extensive studies on the permeation data of chemicals through human skin have shown that molecular weight and solubility were the two major factors affecting the permeability of chemicals through the skin (Boeniger, 2002). Chemicals with molecular weight less than 400 exhibited a higher permeation flux through the skin (Boeniger, 2002). This implies that ZDEC, having a molecular weight of 362, permeates through the skin at a faster rate than its higher homologues such as ZDBC or ZBEC. In addition, there are many other factors that affect the permeation of chemicals through the skin. Occlusion of the skin for long times led to chronic impairment of the barrier functions of the skin, and may enhance the permeation of chemicals through the skin (Ramsing and Agner, 1996). Also, there exists a fairly linear dose-response relationship to sensitizing compounds among humans (Boeniger, 2002). This follows that occlusion of the skin by latex glove containing high quantities of residual ZDEC may enhance the permeation of residual chemicals

through the skin and induce allergic reactions. As surgeons and dentists are required to change the gloves quite often at work place, they are expected to be more susceptible to sensitization if gloves containing high amounts of residual dithiocarbamates are used. In such cases where the use of gloves could not be avoided, gloves containing lower quantities of residual dithiocarbamates should be used to reduce the exposure to sensitizing chemicals.

5.2.2. Dithiocarbamate-release from gloves into artificial sweat

Quantity of ZDEC released into the artificial sweat as a function of sweat pH is given in Figure 5.3.

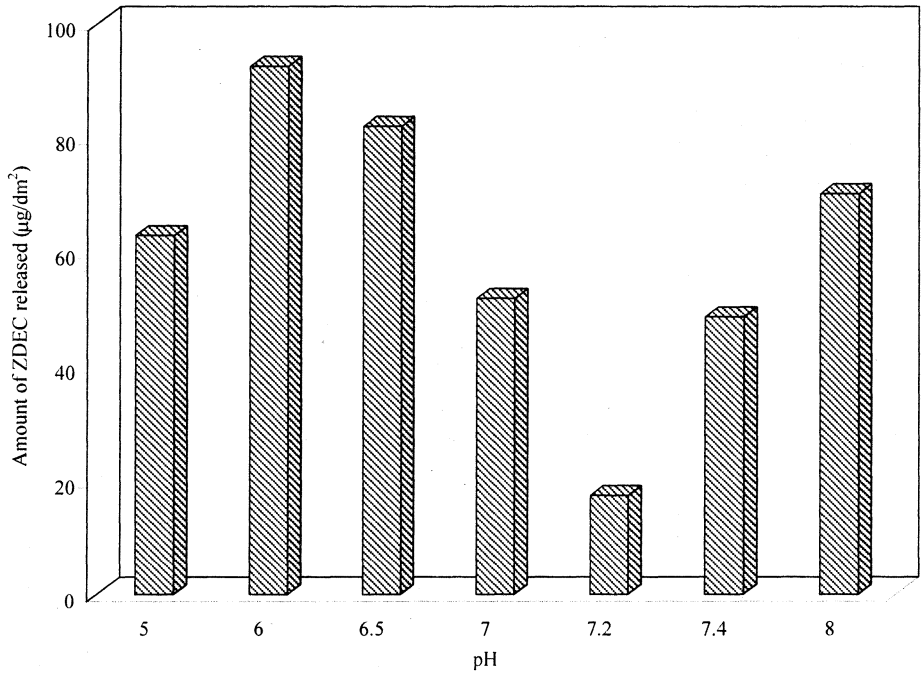


Figure 5.3. Amount of ZDEC released into artificial sweat Vs. pH of artificial sweat

The results showed that the pH of artificial sweat seems to affect the quantity of ZDEC released ($p<0.0001$). It has been reported that the release of benzothiazole accelerators into artificial sweat varied with change in pH (Emmet *et al.*, 1994). Figure 5.3 showed that the amount of ZDEC released was generally high under acidic conditions used. However, a lower value for sweat-extractable ZDEC was obtained at pH 5 probably due to the decomposition of ZDEC. It has been reported that zinc dithiocarbamates rapidly get decomposed under acidic conditions (Aspila *et al.*, 1969; Van Leeuwen 1986; Heasook, 1995). The half life of ZDEC at pH 2.2 and 3.4 was

reported to be 7.3 and 14 sec respectively indicating their instability in highly acidic conditions (Aspila *et al.*, 1969).

A comparison of the amount ZDEC released into the hand sweat of the human subjects and that released into the artificial sweat from the glove in 1 h is shown in Figure 5.4.

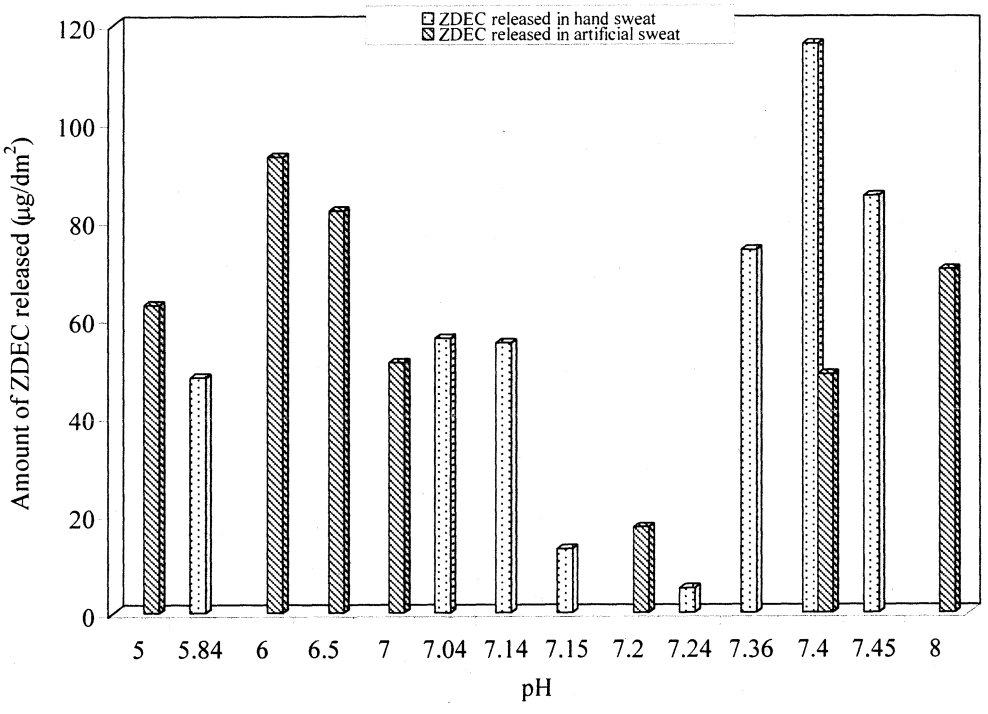


Figure 5.4. Comparison of the amounts of ZDEC released into hand sweat and artificial sweat (pH of artificial sweat were 5, 6, 6.5, 7, 7.2, 7.4 & 8 and the remaining pH values plotted on theX-axis corresponds to the pH of hand sweat)

The statistical analysis by ANOVA indicated that there is no significant relationship between the amount of ZDEC released into the hand sweat and artificial sweat ($p>0.05$). The wide variations in the factors such as pH, sweat rate, hand exercise, glove-fit etc among the human subjects might have contributed to the low correlation between the amount of ZDEC released into hand sweat and that released into the artificial sweat.

The results of the present study indicated that the glove wearers are potentially exposed to the residual ZDEC present in the medical gloves. The European Commission's guidelines on medical devices containing NRL recommends that the anticipated exposure to patients and users to residual chemicals from NRL products

may be estimated (EEC/93/42, 2004). Direct measurements of exposure are the only way to establish unequivocally whether and to what extent individuals are exposed to ZDEC. But it is neither affordable nor technically feasible to measure exposures for everyone in all populations of interest. It therefore, necessitates the development of a simulated chemical test that may give an idea about the possible exposure of humans to dithiocarbamates. It is important that the experimental model should not underestimate the dithiocarbamate-release levels significantly, but, at the same time, not overestimate the release levels to such an extent that experimental model would offer no advantage. Based on the results of the present study, a possible experimental model could be extraction of gloves in artificial sweat. It is important that the extraction conditions should closely match the real use conditions of gloves. It is therefore, appropriate to fill the gloves with artificial sweat to estimate the dithiocarbamate-release as done in the present case.

As considerable degree of variability was noticed in the extent of ZDEC-release into the hand sweat of human subjects, it is prudent to estimate the ZDEC-release and subsequent exposure to dithiocarbamates on a "worst case" basis. It was found that the maximum release of ZDEC (93µg/dm^2 equivalent to 104µg/g) into artificial sweat occurred when the pH was 6. At pH 6.5, the amount of ZDEC released into artificial sweat was slightly lower (82 µg/dm^2 equivalent to 92 µg/g). A pH below 6 seems unsuitable for extraction of gloves in artificial sweat as ZDEC is known to decompose under acidic conditions (Aspila *et al.,* 1969). In general, a pH of 6-6.5 may be suitable for the extraction of gloves in artificial sweat to estimate the ZDEC-release from gloves on a "worst case" basis. Interestingly, the amount of ZDEC released into the sweat of human subject 'G' (116 µg/dm^2) exceeded the "worst case" level of ZDEC-release in artificial sweat (93-82 µg/dm^2). Although a slight underestimation was observed in the case of one out of the eight human subjects, the study, in general, indicated that the extraction of gloves in artificial sweat at pH 6-6.5 under simulated-use conditions closely approximated the real-use conditions of gloves and may, therefore, be used for the estimation of dithiocarbamate-release and subsequent exposure of users to dithiocarbamates from gloves.

5.3. CONCLUSIONS

Exposure to residual ZDEC occurs when gloves are donned. The extent of ZDEC-release into hand sweat varied among human subjects despite wearing the same brand of glove. The results indicated a lack of clear relationship between the sweat-extractable ZDEC and sweat rate or sweat pH. On the other hand, the pH of artificial sweat seems to affect the quantity of ZDEC released. A comparison between the extent of ZDEC-release under real-use (using human subjects) and simulated-use conditions (artificial sweat at pH 6-6.5) indicated that the ZDEC-release under simulated-use conditions closely approximated the real-use conditions of gloves. This follows that filling the glove with artificial sweat at pH 6-6.5 and subsequent extraction for 1 h under simulated-use conditions may be used for the estimation of ZDEC-release and the anticipated exposure of users to ZDEC from gloves.

CHAPTER 6

FACTORS AFFECTING RELEASE OF DITHIOCARBAMATES INTO ARTIFICIAL SWEAT

6.1. INTRODUCTION

In the absence of adequate information on the factors contributing to dithiocarbamate-release, the exposure assessment of dithiocarbamates would be incomplete. As commercially available medical gloves widely vary in their composition, NRL vulcanizates using different dithiocarbamates and dithiocarbamate-sulphur ratios were prepared to study the dithiocarbamate-release. The present study investigates the effect of (i) dithiocarbamate type, (ii) amount of ZDEC initially added, (iii) storage time, (iv) water leaching, and (v) alkaline leaching on the extent of dithiocarbamate-release into artificial sweat from latex vulcanizates.

6.2. RESULTS AND DISCUSSION

6.2.1. Effect of dithiocarbamate type

Latex vulcanizates with same initial dithiocarbamate levels were prepared, and extracted in artificial sweat to study the effect of dithiocarbamate type on the amount of dithiocarbamates released into artificial sweat. Figure 6.1 shows the amount of different zinc dithiocarbamates released into the artificial sweat from the latex vulcanizates having a dithiocarbamate-sulphur ratio of 1.0. All the latex compounds

DE-1, BU-1, IB-1, BZ-1, and DN-4 contained 0.5 p.p.h.r each of sulphur and the respective dithiocarbamate (Figure 6.1). On molarity basis, the formulations DE-1 contained 1.38×10^{-3} moles of ZDEC; BU-1 contained 1.05×10^{-3} moles of ZDBC; IB-1 also contained 1.05×10^{-3} moles of ZIBC; BZ-1 contained 8.2×10^{-4} moles of ZBEC and DN-4 contained 6.6×10^{-4} moles of ZDNC.

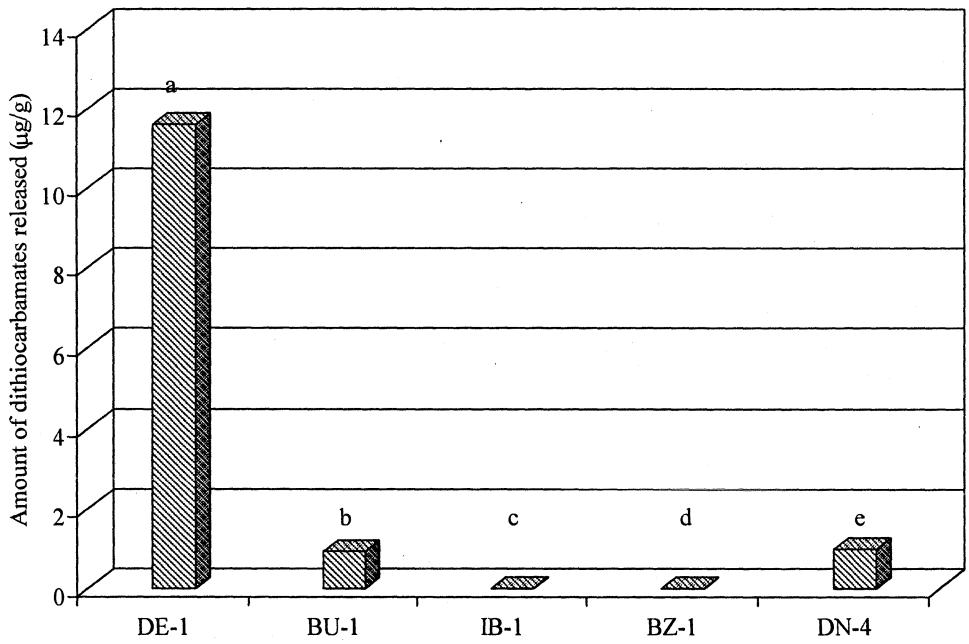


Figure 6.1. Amount of (a) ZDEC; (b) ZDBC; (c) ZIBC; (d) ZBEC; and (e) ZDNC released into artificial sweat from latex vulcanizates (all latex compounds contained 0.5 p.p.h.r of the respective dithiocarbamate)

It is evident that the extent of release of ZDEC into artificial sweat from latex sheets was substantial (~12 µg/g) compared to ZDBC or higher homologues despite using the same amount of dithiocarbamate accelerators for vulcanization. It is to be noted that the number of moles of ZDEC, ZDBC and ZIBC did not differ significantly. Negligible quantities (<1 µg/g) of ZDBC, ZIBC, ZBEC and ZDNC were released into artificial sweat from latex vulcanizates. A similar trend was observed in the case of latex vulcanizates with a different dithiocarbamate-sulphur ratio (0.67) and is shown in Figure 6.2.

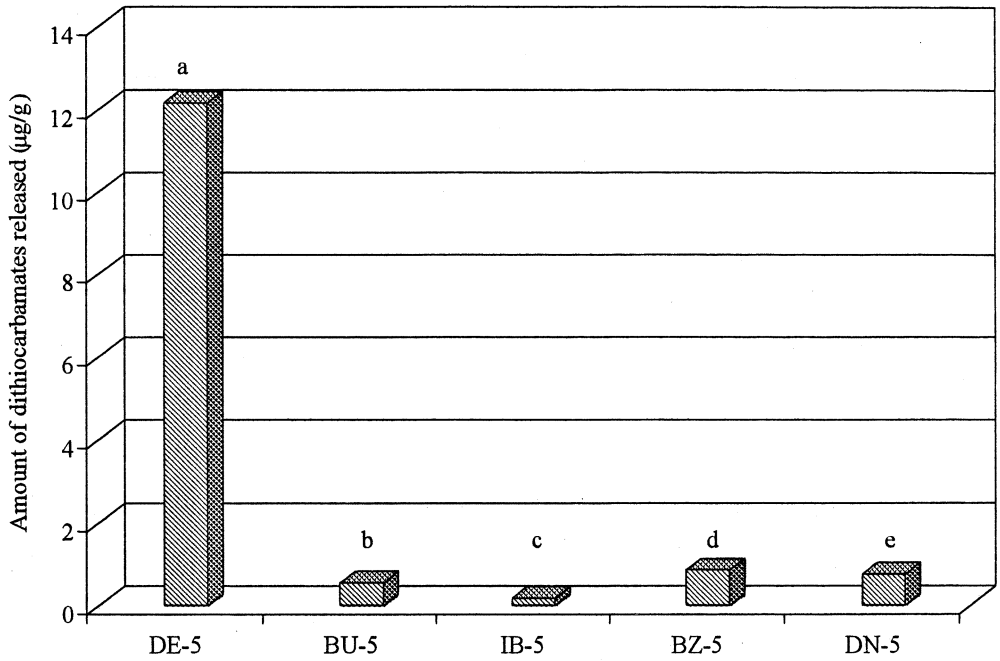
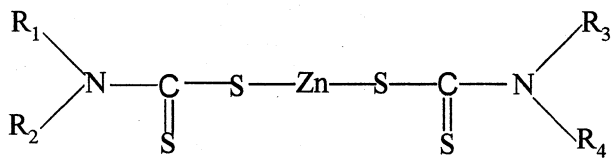


Figure 6.2. Amount of (a) ZDEC; (b) ZDBC; (c) ZIBC; (d) ZBEC; and (e) ZDNC released into artificial sweat from latex vulcanizates (all latex compounds contained 0.75 p.p.h.r of sulphur and 0.5 p.p.h.r of the respective dithiocarbamate)

The variation in the extent of release of different dithiocarbamates into artificial sweat is attributed to the difference in their solubility in rubber. The general structure of dithiocarbamates is given in Scheme 6.1.



ZDEC, R_1, R_2, R_3, R_4 = Ethyl (i.e., $-CH_2-CH_3$)

ZDBC, R_1, R_2, R_3, R_4 = Butyl (i.e., $-CH_2-CH_2-CH_2-CH_3$)

ZBEC, R_1, R_2, R_3, R_4 = Benzyl (i.e., $-CH_2-C_6H_5$)

ZIBC, R_1, R_2, R_3, R_4 = Isobutyl (i.e., $-CH_2-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_3$)

ZDNC, R_1, R_2, R_3, R_4 = Isononyl (i.e., $-CH_2-\underset{\displaystyle CH_3}{\underset{|}{CH}}-(CH_2)_5-CH_3$)

Scheme 6.1. General structure of zinc dithiocarbamates

The structure showed that dithiocarbamates differed only in the nature of the *N*-substituted alkyl/aryl group. Zinc diethyldithiocarbamate contained a non-bulky ethyl group as the *N*-substituted alkyl group. In contrast, all other dithiocarbamate compounds contained bulky alkyl or aryl groups with higher hydrocarbon content which makes them more rubber-soluble compared to ZDEC. Solubility experiments with dithiocarbamates in natural rubber showed that the solubility of ZDBC is much higher compared to ZDEC (Morris and Thomas, 1995; Weng *et al.,* 2000). The limit of solubility of ZDEC and ZDBC in natural rubber is found to be 0.8 and 2.0 p.p.h.r respectively (Weng *et al.,* 2000). The high rubber-solubility of ZDBC explains its substantially lower extent of release into artificial sweat compared to ZDEC. A similar argument holds for ZIBC, the isomeric counterpart of ZDBC. Zinc diisononyldithiocarbamate is also reported to have high solubility in rubber leading to a low release in artificial sweat (Morris, 1995; Pendle, 1997).

Another factor which is expected to govern the extent of dithiocarbamate-release is the residual dithiocarbamate content. The amounts of residual dithiocarbamates of vulcanizates containing ZDEC and ZDBC were determined, and are given in Table 6.1.

Table 6.1. Amount of residual ZDEC and ZDBC

Formulation code	Residual ZDEC (mg/g)	Residual ZDBC (mg/g)
DE-1	1.92	-
BU-1	-	4.75
DE-5	1.0	-
BU-5	-	4.03

It was found that the vulcanizate 'BU-1' contained a higher amount of residual ZDBC of about 4.75 mg/g compared to vulcanizate 'DE-1' which contained 1.92 mg/g of residual ZDEC. Despite containing high levels of residual ZDBC, the vulcanizate 'BU-1' released negligible amounts of ZDBC into the sweat solution. A similar observation was noticed in the case of vulcanizates DE-5 and BU-5. Therefore, it may be inferred that the rubber solubility of dithiocarbamates in rubber is a major factor controlling their release into artificial sweat.

At this point, it is worth mentioning that the dithiocarbamates are capable of inducing allergy to the general population if available at the surface of the skin. Accelerators which are more rubber soluble remain trapped in the rubber matrix thereby minimizing the availability to the human skin. Minimal contact with human skin is expected to reduce the chances of sensitization and the resultant allergy.

6.2.2. Effect of varying amounts of ZDEC

Figure 6.3 shows the variation in the amount of sweat-extractable ZDEC with an increase in the amount of ZDEC initially added to the latex formulations (DE-1, DE-2, DE-3 and DE-4). The variation in the amount of sweat-extractable ZDEC with respect to residual ZDEC content of these vulcanizates is also shown in Figure 6.3.

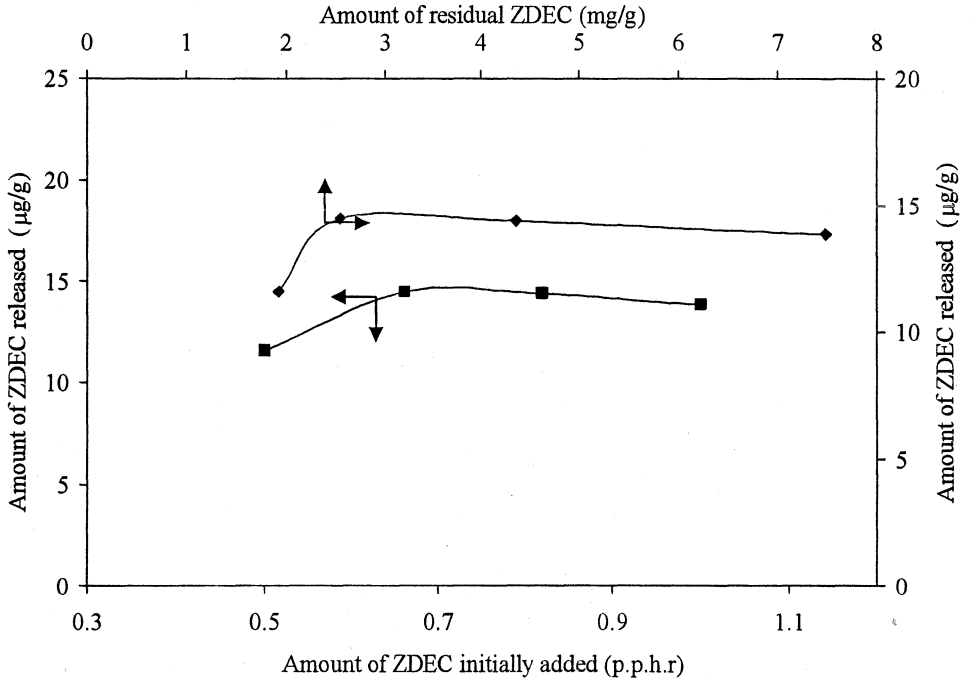


Figure 6.3. Variation in the amount of sweat-extractable ZDEC with respect to (■) varying amounts of ZDEC initially added and (◆) residual ZDEC content

Figure 6.3 shows that the amount of ZDEC released into artificial sweat showed an initial increase and then remained almost constant irrespective of an increase either in the amount of ZDEC initially added to the latex formulations or in the amount of residual ZDEC. It is to be noted that the amount of residual ZDEC content increased with an increase in the amount of ZDEC initially added. The

variation of the amount of residual ZDEC content with respect to amount of ZDEC initially added is given in Figure 6.4.

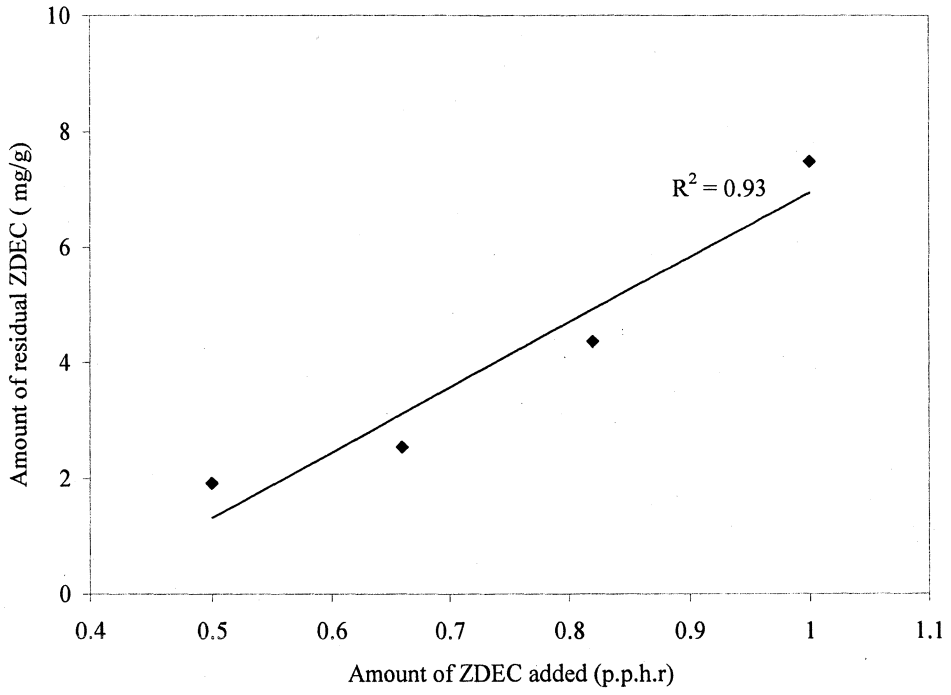


Figure 6.4. Variation of residual ZDEC content with respect to the amount of ZDEC added initially to the latex formulations

The constant release of ZDEC into artificial sweat from latex vulcanizates containing different initial levels of ZDEC/residual ZDEC is attributed to the inability of the aqueous sweat solution to swell the latex sheets. The percentage degree of swelling of all latex vulcanizates in artificial sweat amounts to only around 12% after 24 h. It could therefore be inferred that the sweat solution wets only the surface of the latex vulcanizates. As a result, the release of ZDEC is confined to the surface of the latex vulcanizates. This condition may be relevant in cases when the latex vulcanizates were stored for short periods. A different situation may arise when the latex vulcanizates containing varying amounts of residual ZDEC were stored for longer durations.

6.2.3. Effect of storage time

Migration of chemical additives before, during and after vulcanization could be of benefit in some cases; but at the same time may prove detrimental in some other instances. In certain cases, for example, waxes rely on the migration to the surface of

the article to provide optimum protection against ozone degradation. In other cases like tyre building, migration of additives raises concern. In the health care sector, migration of residual accelerators and antioxidants in products such as gloves, condoms, and catheters, which are intended to come in contact with the skin or mucosal membrane is of particular concern. Understanding the accelerator migration through the latex vulcanizate would indeed help the manufacturers to choose the right accelerator for dipped products.

The effect of storage time (shelf time) on the extent of ZDEC-release into artificial sweat was studied using latex vulcanizates containing varying amounts of ZDEC (DE-1, DE-3 and DE-4). All the sheets were water leached. Figure 6.5 shows the variation in the amount of ZDEC released into artificial sweat from latex vulcanizates with storage time.

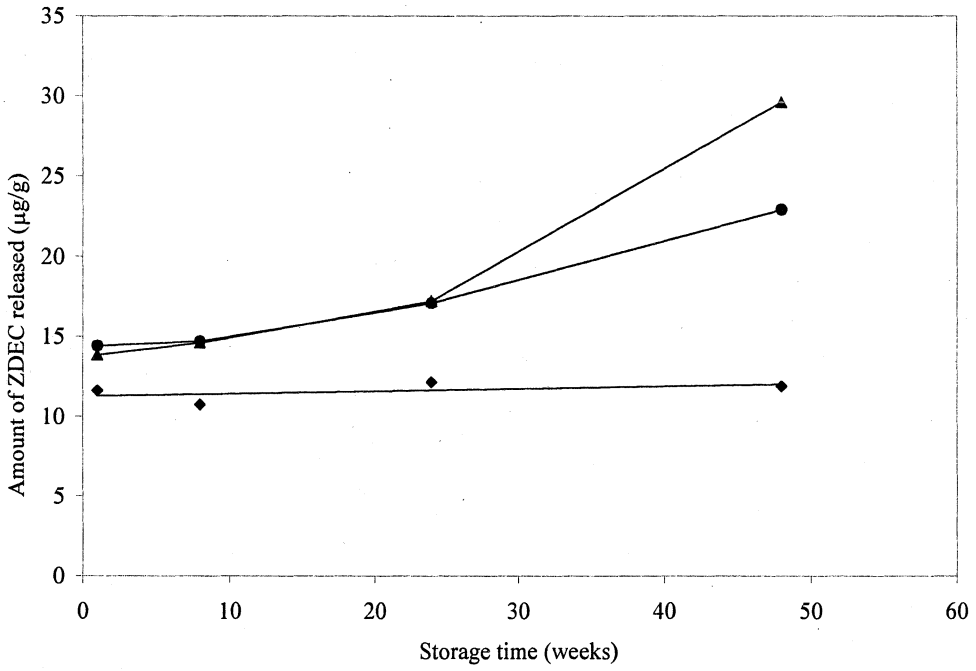


Figure 6.5. Amount of ZDEC released into artificial sweat from latex vulcanizates (◆) DE-1; (●) DE-3; and (▲) DE-4 stored for different periods of time

Within the time frame of the experiment, the results indicated that the amount of sweat-extractable ZDEC significantly increased with an increase in the storage time ($p < 0.05$). The increase in the amount of sweat-extractable ZDEC upon storage is attributed to the migration of ZDEC residues through the rubber phase. Lederer *et al.,* (1982) reported that the magnitude of change in curative concentration across

the rubber interface increased with increasing storage time indicating the migration of curatives. The extent of migration is found to depend on the nature of the curative and the rubber matrix (Lewis *et al.,* 1969; Lederer *et al.,* 1982). It has been reported that the accelerators with high rubber solubility showed a lower tendency to migrate across the rubber phase (Pendle, 1997). Zinc diethyldithiocarbamate, being less rubber-soluble, migrated across the natural rubber vulcanizate over a period of time resulting in an increase in its concentration at the surface which in turn accounts for the increase in the amount of sweat-extractable ZDEC.

It was also found that with an increase in the amount of ZDEC added to the latex formulations, the amount of sweat-extractable ZDEC from the vulcanizates tended to increase upon storage (Figure 6.5). The rate of migration of ZDEC across the rubber phase was calculated from the slope of the curves in Figure 6.5 (Hoover *et al.,* 2003). The slopes of the curves for DE-1, DE-3 and DE-4 were 0.02, 0.19 and 0.34 respectively. It is apparent that there was a steady increase in the slope with an increase in the amount of initial level of ZDEC. This indicated that the rate of migration increased with an increase in the amount of initial ZDEC content in the latex vulcanizates. This, in fact, is due to the increase in the amount of residual ZDEC in these latex vulcanizates (Figure 6.4). The residual ZDEC content was found to be directly proportional to the initial ZDEC content in the formulations. As the amount of residual ZDEC was increased, they migrated at a faster rate to the surface of the vulcanizates resulting in an increase in its concentration at the surface upon storage. This accounts for the increase in the amount of sweat-extractable ZDEC with the increase in the initial ZDEC content. The results indicated that the amount of residual ZDEC is a major factor controlling the migration of ZDEC through the rubber phase, which in turn affected the extent of ZDEC-release into artificial sweat. This is clearly depicted in Figure 6.6.

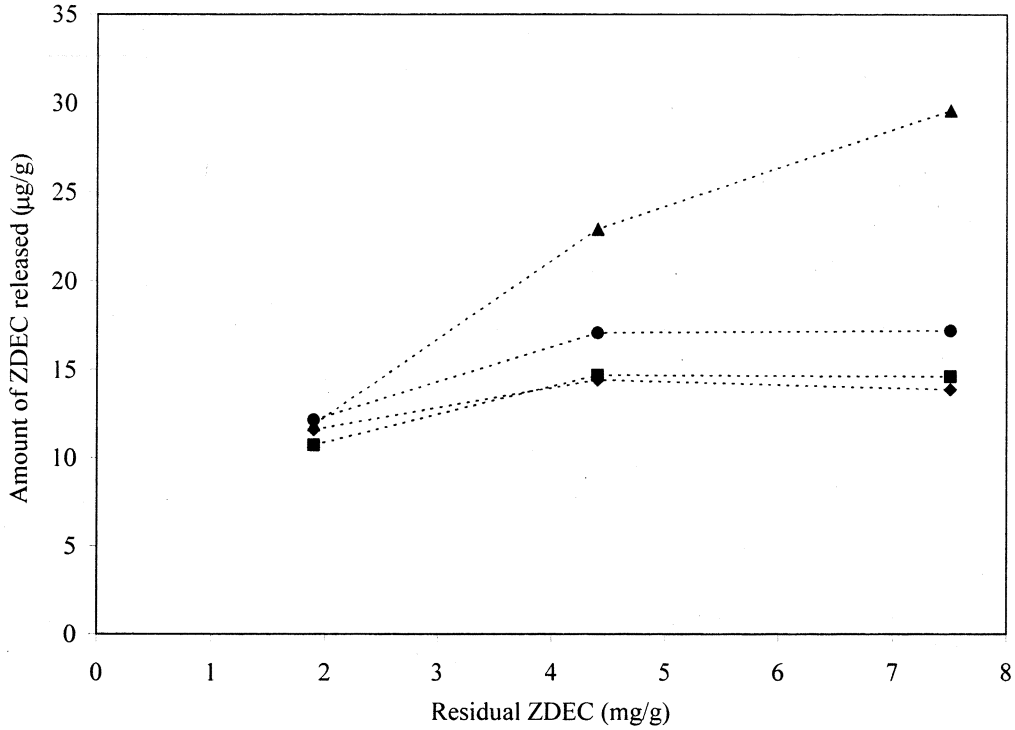


Figure 6.6. Effect of the amount of residual ZDEC on the sweat-extractable ZDEC in latex vulcanizates stored for (◆) 1 week; (■) 8 weeks; (●) 24 weeks; and (▲) 48 weeks

It is evident that as the residual ZDEC content increased, the sweat-extractable ZDEC showed substantial increase upon storage. It was also interesting to observe a drastic and linear increase in the amount of sweat-extractable ZDEC with an increase in the amount of residual ZDEC content at 48 weeks of storage of latex vulcanizates. Thus, the results showed that the migration of ZDEC residues to the surface of the latex vulcanizates was substantial at higher residual ZDEC contents and longer storage time. This is of particular concern as it would lead to the accumulation of ZDEC at the surface of latex products over a period of time placing the user the risk of sensitization to latex products. It was found that the medical gloves marketed in India contained ZDEC. It was also noticed from the labeling claims that the gloves differed in the expiry dates, which spanned from 3 to 5 years. Such a long period may result in the migration of residual ZDEC leading to the accumulation of ZDEC residues at the surface of the gloves. In such situations, the allergencity of the glove may increase upon storage leading to an increase in the incidence of glove-related allergic reactions. A linear dose-response relationship has been reported in many of the patch testing of gloves in patients (Knudsen *et al.*, 1993; Boeniger, 2002). Therefore, the amount of residual ZDEC should be kept as low as reasonably

practicable in the latex products intended for skin and mucosal contact applications. At present, the expiry date of gloves, as proposed by FDA, is supported by the stability (accelerated aging tests) and sterility studies. As storage resulted in the migration of ZDEC to the surface of the vulcanizates, the migration of ZDEC may also be considered while fixing the expiry date of latex products containing ZDEC.

The diffusion coefficients of ZDEC could be calculated using the migration data obtained in the present study. Detailed studies on the migration of additives through rubber have shown that the migration is controlled by diffusion obeying the Fick's Second Law (Morris and Thomas, 1995; Hoover *et al.,* 2003). Migration of an additive from a polymer above the glass transition temperature is controlled by molecular diffusion of the migrant in the polymer and is described by Fick's second law as given in equation 6.1.

$$\frac{\partial Cp}{\partial t} = D_p \frac{\partial^2 Cp}{\partial x^2} \quad \text{.....6.1}$$

where Cp is the concentration of the migrant in the film at a time t and position x. A solution of equation 6.1 for moderate and large migration times is given in equation 6.2 (Crank and Park, 1968).

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2 \pi^2} \exp\left[\frac{-D_P (2n+1)^2 \pi^2 t}{4l^2}\right] \quad \text{.....6.2}$$

where M_t is the amount of additive migrated at time t, M_∞ is the amount of migrant released at equilibrium, D_p is the diffusion coefficient of the migrant, t is the storage time and l is the thickness of the polymer film. The equation 6.2 can be simplified to give another equation 6.3 assuming that the medium into which release occurs does not contain any migrant initially.

$$\frac{M_t}{M_0} = \frac{2}{l}\left(\frac{D_p t}{\pi}\right)^{0.5} \quad \text{.....6.3}$$

where M_0 is the amount of migrant present in the polymer initially. The equation 6.3 is known to provide accurate estimates of diffusion coefficients for complete migration assuming that resistance to mass transfer is negligible

(Chung *et al.*, 2002). The migration model given in equation 6.1 based on Fick's Second Law has been extensively used for the assessment of migration of additives through rubber (Morris and Thomas, 1995).

The diffusion coefficients of ZDEC at each concentration was calculated from the plot of $\frac{Mt}{M_0}$ versus $\sqrt{t}$ using initial migration data and linear regression analysis. Theoretically it is possible to calculate D_p using a single datum, however, the use of multiple data points provides a reliable estimate of D_p. Figure 6.7 shows the plots of $\frac{Mt}{M_0}$ versus $\sqrt{t}$ indicating that the migration of ZDEC followed the characteristic Fickian behavior, i.e. the amount of ZDEC migrated is linearly related to the square root of time.

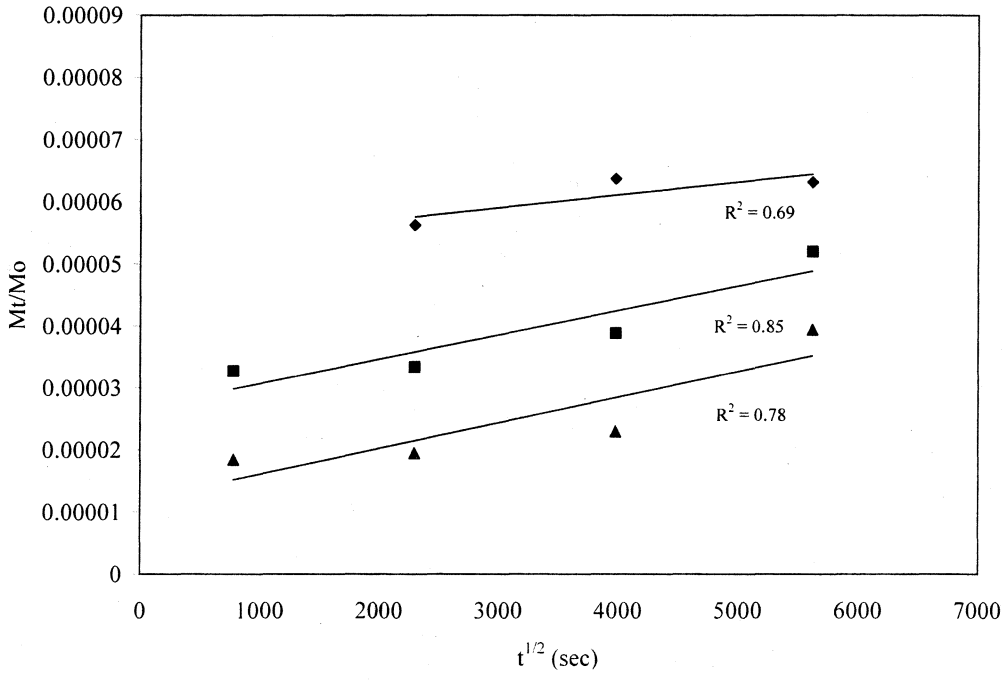


Figure 6.7. Typical plot of $\frac{Mt}{M_0}$ versus $\sqrt{t}$ using the migration data of latex vulcanizates DE-1, DE-3 and DE-4 containing (◆) 0.5 p.p.h.r; (■) 0. 82 p.p.h.r; (▲) 1.0 p.p.h.r of ZDEC respectively

The diffusion coefficient was calculated using the migration data available at each concentration and is given in Table 6.2.

Table 6.2. Diffusion coefficient (D_p) of ZDEC

Initial level of ZDEC (p.p.h.r)	Residual ZDEC (mg/g)	D_p (cm^2/s), at 28 °C
0.5	1.92	2.07×10^{-9}
0.82	4.36	3.94×10^{-9}
1.0	7.48	4.14×10^{-9}

The diffusion coefficient of ZDEC was found to be concentration dependent. A similar observation was made by Lederer *et al.,* (1982) who reported a small rationalizable effect of concentration on the diffusion coefficient of curatives in rubber matrix. The concentration dependence of diffusion coefficient may be due to the high concentration of residual ZDEC in the rubber matrix (Begley, 1997).

It was interesting to note that the latex vulcanizate 'BU-1' released negligible amounts of ZDBC (below 1 μg/g) into artificial sweat upon storage despite containing large amounts of residual ZDBC (4.75 mg/g). The low extent of the release of ZDBC into artificial sweat is attributed to the lower rate of migration of ZDBC through the rubber phase owing to its highly rubber-soluble nature (Morris and Thomas, 1995; Weng *et al.,* 2000). This is further confirmed by comparing the extent of release of dithiocarbamates from vulcanizates BU-1 to that from DE-3 having a similar residual dithiocarbamate content (residual ZDEC = 4.36 mg/g). It was found that the the vulcanizate DE-3 showed an increase in the amount of sweat-extractable ZDEC upon storage indicating that rubber-solubility of dithiocarbamates controls their migration through the rubber phase. Apart from rubber-solubility, the molecular weight of the diffusing material was found to affect the rate of migration in an inverse proportion (Lederer *et al.,* 1982). The high molecular weight and high rubber-solubility of ZDBC are therefore responsible for the lower extent of release of ZDBC upon storage despite the presence of high residual ZDBC in the latex vulcanizates. This makes ZDBC an ideal candidate for the manufacture of latex health care products that are marketed with long expiration dating.

6.2.4. Effect of leaching

The effect of water and alkali leaching on the amount of ZDEC released into artificial sweat from latex vulcanizates (DE-5) was investigated. The vulcanizates

were leached as detailed in section 3.4.2.3. One set of latex vulcanizates of DE-5 was given only water leaching and the other set an additional alkali leaching. Unleached sheets of DE-5 (both unvulcanized and vulcanized) were used as the controls. The effect of leaching of latex sheets in water and alkali on the sweat-extractable ZDEC is shown in Figure 6.8.

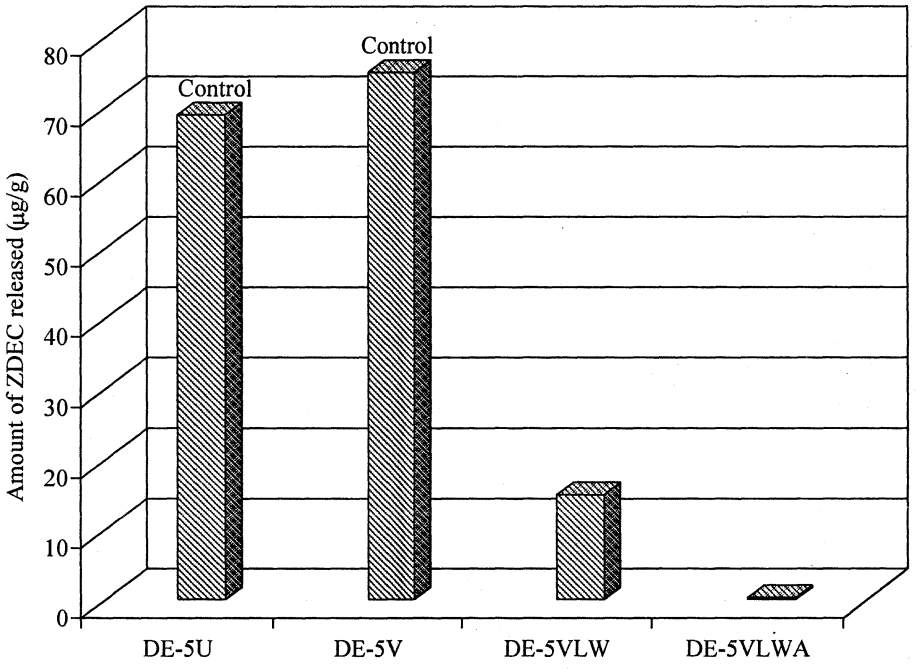


Figure 6.8. Effect of leaching on the amount of ZDEC released into artificial sweat (DE-5U-unvulcanized and unleached; DE-5V-vulcanized & unleached; DE-5VLW-vulcanized & leached in water; DE-5VLWA- vulcanized & leached in water and alkali)

It is apparent that the unleached sheets (DE-5U & DE-5V) released high amounts of ZDEC residues into artificial sweat compared to the leached sheets (DE-5VLW & DE-5VLWA). A drastic reduction of about 80% in the amount of sweat-extractable ZDEC was observed in the case of latex vulcanizates (DE-5VLW) subjected to water-leaching. Further decrease in the amount of sweat-extractable ZDEC was observed when the latex vulcanizates was subjected to an additional leaching in alkali (DE-5VLWA), and an overall decrease of 98% was observed. This reduction in the amount of sweat-extractable ZDEC from the leached sheets is attributed to the removal of ZDEC residues from the surface of the sheets. It was interesting to note that the vulcanized but unleached sheets (DE-5V) released slightly higher amount of residual ZDEC into the sweat solution compared to the

unvulcanized sheets (DE-5), probably due to the migration of residual ZDEC to the surface of latex sheets during vulcanization. In fact, such migration to the surface of vulcanizates during vulcanization has been reported in the case of proteins (Shamsul *et al.*, 1993; Yeang *et al.*, 1995).

Hot and cold water leaching has been extensively used in the dipping industry as an effective method to reduce the residual proteins and chemicals as well as to improve the mechanical properties of latex sheets. Many manufacturers have made their own modifications in the leaching process to reduce the allergen content as low as possible. Generally, leaching includes both wet gel and dry leaching of dipped goods. The results showed that the leaching in water removed ZDEC residues from the surface of latex vulcanizates. A near complete removal of ZDEC from the surface of latex vulcanizates could be achieved by subjecting them to an additional washing with alkali. As the allergic reactions are mainly due to the bioavailability of residual accelerators present in the finished latex goods, it is necessary to keep their amounts as low as possible. Thus the removal of residual ZDEC is expected to reduce the incidence of allergic responses among users and is facilitated by subjecting them to a combination of water and alkali leaching.

It is expected that the treatment of latex vulcanizates with alkali solution may cause deterioration in the mechanical properties. The sheets that have been subjected to alkali leaching were, therefore, mechanically tested in a universal testing machine to estimate the extent of deterioration in the mechanical properties. The mechanical properties of latex sheets before and after alkali leaching are shown in Table 6.3.

Table 6.3. Effect of alkali leaching on the mechanical properties of ZDEC-vulcanized latex sheets

Sample code	Tensile strength (MPa)	Elongation at break (%)
DE-5VLW	25.3 ± 1.8	738 ± 21
DE-5VLWA	22.6 ± 1.9	698 ± 50

There was only a marginal decrease in the tensile strength and elongation at break following alkali leaching and the percentage decrease was about 11% and 5% respectively.

6.3. CONCLUSIONS

Despite the addition of the same amount of dithiocarbamate accelerators, the latex vulcanizates released negligible quantities of ZDBC and higher homologue dithiocarbamates into artificial sweat compared to ZDEC. It was found that ZDEC-release into artificial sweat showed a near constant release despite a steady increase either in the amount of ZDEC initially added to the latex formulations or residual ZDEC content in the latex vulcanizates. The extent of ZDEC-release was found to be dependent on the storage time. Within the time frame of the study (48 weeks), it was found that the amount of sweat-extractable ZDEC from latex vulcanizates increased with an increase in the storage time. The rate of migration of ZDEC increased with an increase in the amount of residual ZDEC content. The effect was more pronounced at higher residual ZDEC contents (> 1.92 mg/g) and longer storage times (≥ 24 weeks). It was found that the diffusion coefficient of ZDEC was dependent on concentration. Interestingly, ZDBC showed a lower tendency for migration across the rubber phase compared to ZDEC upon storage.

A combination of water and alkali leaching effectively reduced the amount of residual ZDEC present on the surface of latex sheets to negligible levels. The mechanical properties remain largely unaffected by alkali leaching.

CHAPTER 7

EFFECT OF DITHIOCARBAMATE-SULPHUR RATIO ON RESIDUAL DITHIOCARBAMATE CONTENT

7.1. INTRODUCTION

As the amount of residual ZDEC contributed considerably to its migration and subsequent accumulation on the surface of the vulcanizates, it is required to keep their amount as low as reasonably practicable in the finished latex goods, at the same time maintaining adequate levels of mechanical properties. The objective of the present study is to optimize the dithiocarbamate-sulphur ratio so that most of the accelerators is consumed during vulcanization and only minimal residues remain.

7.2. RESULTS AND DISCUSSION

The effect of varying dithiocarbamate-sulphur ratio on the amount of residual ZDEC and ZDBC was studied. In the first step, the amount of ZDEC and ZDBC was varied keeping the amount of sulphur constant (increasing dithiocarbamate-sulphur ratio, Table 3.6 and Table 3.8). In the next step, the sulphur content was increased keeping the ZDEC and ZDBC content constant (decreasing dithiocarbamate-sulphur ratio, Table 3.7 and Table 3.9).

Figure 7.1 shows the effect of varying amounts of ZDEC and ZDBC on the amount of residual dithiocarbamates in the latex vulcanizates.

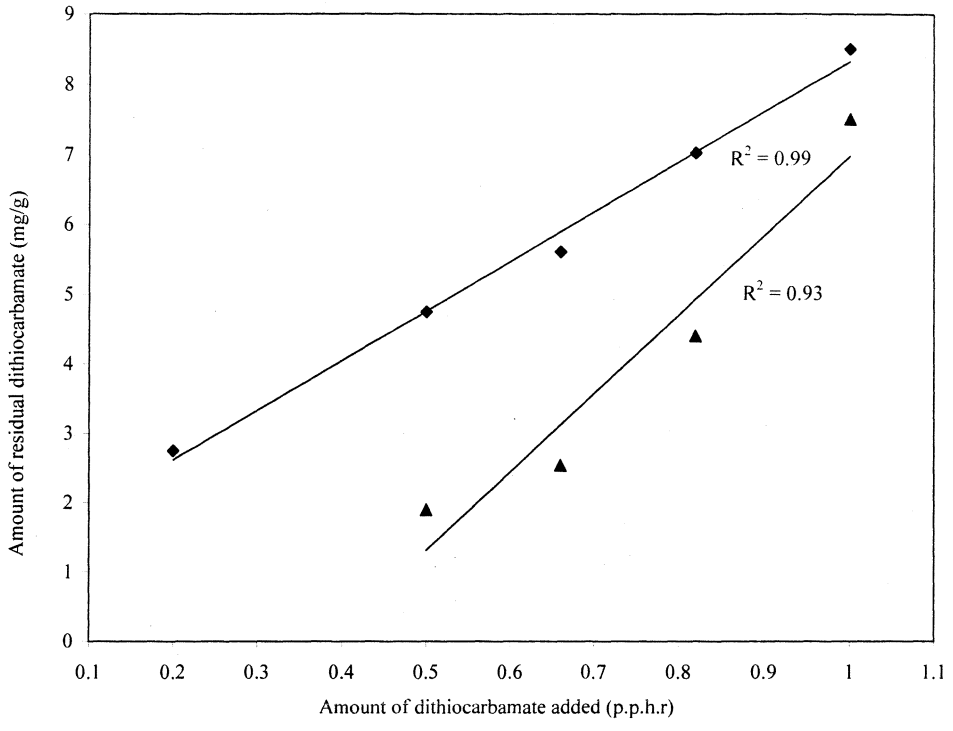


Figure 7.1. Effect of varying amounts of dithiocarbamate on the amount of (▲) residual ZDEC; and (◆) residual ZDBC

The amount of residual dithiocarbamates was found to be directly proportional to the amount of dithiocarbamate initially added to the latex formulations. This could be explained as follows. It has been reported that the presence of sulphur is required for the transfer of dithiocarbamates into the rubber phase and that the transfer of sulphur occurs rapidly compared to the dithiocarbamate accelerators (Blackley, 1997a). It is to be noted that all the above latex formulations contained a fixed quantity (0.5 p.p.h.r) of sulphur, and the level of dithiocarbamates was increased gradually up to 1 p.p.h.r. At higher levels of initial dithiocarbamates, the amount of sulphur might not be enough to facilitate the transfer of dithiocarbamates into the rubber phase. Hence, excess dithiocarbamates present in the latex compounds remain unavailable for vulcanization, and this explains the steady increase in the amount of residual dithiocarbamates. This is clearly depicted in Figure 7.2, which shows the variation in the crosslink density and residual dithiocarbamate contents with an increase in the amount of dithiocarbamates initially added to the latex formulations.

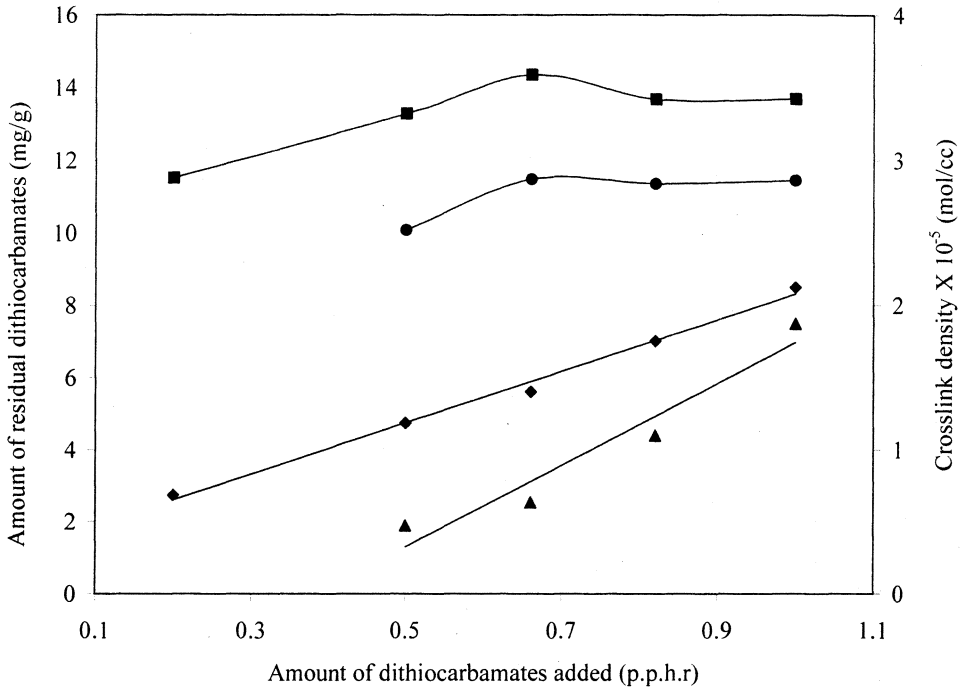


Figure 7.2. Effect of varying amounts of dithiocarbamate on the (▲) amount of residual ZDEC; (●) crosslink density of ZDEC-cured vulcanizates; (◆) amount of residual ZDBC; and (■) crosslink density of ZDBC-cured vulcanizates

The crosslink density showed a slight initial increase and then remained almost constant irrespective of the steady increase in the amount of dithiocarbamates initially added to the latex formulations.

A situation similar to the present study has been observed in the vulcanization of dry natural rubber, where less amounts of ZDEC are getting used up with an increase in the amount of ZDEC initially added (Scheele and Birghan, 1958). Loh (1982) reported an entirely different situation in the case of prevulcanization of NRL using ZDBC where the rate of disappearance of ZDBC increased with an increase in the initial amount of ZDBC, and is attributed to the presence of the aqueous phase. It has been reported that the aqueous phase facilitated the transfer of the accelerator or some derivative of it to the rubber phase in a prevulcanization reaction, and factors such as the rubber-soluble nature of ZDBC and high temperature accelerated the transfer process (Blackley, 1997a). The continuous transfer of accelerators into the rubber particles during the whole process of prevulcanization ensures the gradual removal of ZDBC molecules from the latex compound, which accounted for the observation made by Loh (1982).

Figure 7.3 shows the effect of varying amounts of sulphur on the amount of residual dithiocarbamates in the latex vulcanizates.

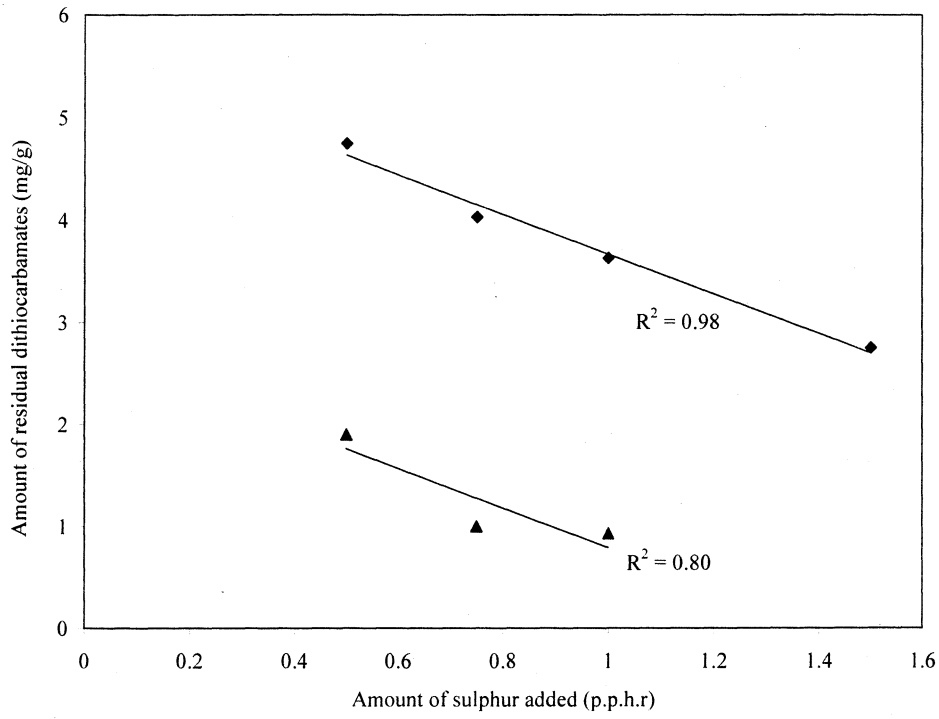


Figure 7.3. Effect of varying amounts of sulphur on the (▲) amount of residual ZDEC; and (◆) amount of residual ZDBC

It was found that the amount of residual dithiocarbamates showed a linear decrease with an increase in the amount of sulphur initially added to the latex formulations. The high level of sulphur in the latex formulations facilitated the transfer of dithiocarbamates or some derivative of it to the rubber phase. As a result, the dithiocarbamates get used up during vulcanization, which accounts for the steady decrease in the amount of residual dithiocarbamates with an increase in the amount of sulphur added initially. The crosslink density data of the latex vulcanizates support the above observation. Figure 7.4 shows the effect of varying amounts of sulphur on the crosslink density and the amount of residual dithiocarbamates.

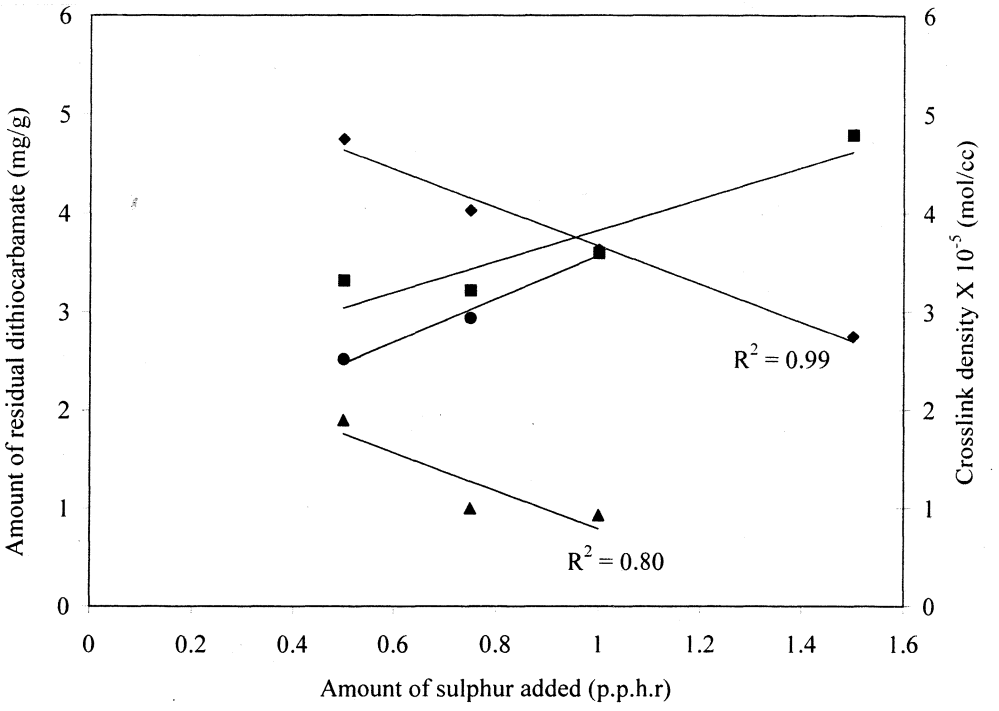


Figure 7.4. Effect of varying amounts of sulphur on the (▲) amount of residual ZDEC; (●) crosslink density of ZDEC-cured vulcanizates; (◆) amount of residual ZDBC; and (■) crosslink density of ZDBC-cured vulcanizates

It is apparent that with an increase in the amount of sulphur added, the crosslink density steadily increased and the amount of residual dithiocarbamates decreased indicating that the utilization of dithiocarbamates increased as the sulphur content was increased.

It was also found that the crosslink density of latex vulcanizates cured with ZDBC showed higher values than those containing ZDEC at equivalent dithiocarbamate levels (Figure 7.2 and Figure 7.4). This is attributed to the greater ability of ZDBC to accelerate the vulcanization process. It has been showed that ZDBC exhibited the greatest ability to accelerate the combination of sulphur with rubber during the prevulcanization process even at room temperature (Loh, 1982; Teik and Poh, 1988). The most obvious reason for this accelerative ability of ZDBC arises from the balance of the two opposing factors, the solubility of the accelerator in the aqueous phase of the latex and the ability of the accelerator to adsorb on the rubber particle (Blackley, 1997a). The solubility of ZDBC in the aqueous phase is less

compared to ZDEC, and therefore, it gets adsorbed on the rubber and participates in the process of vulcanization.

Figure 7.5 summarizes the results of the effect of varying the dithiocarbamate-sulphur ratio on the amount of residual dithiocarbamates.

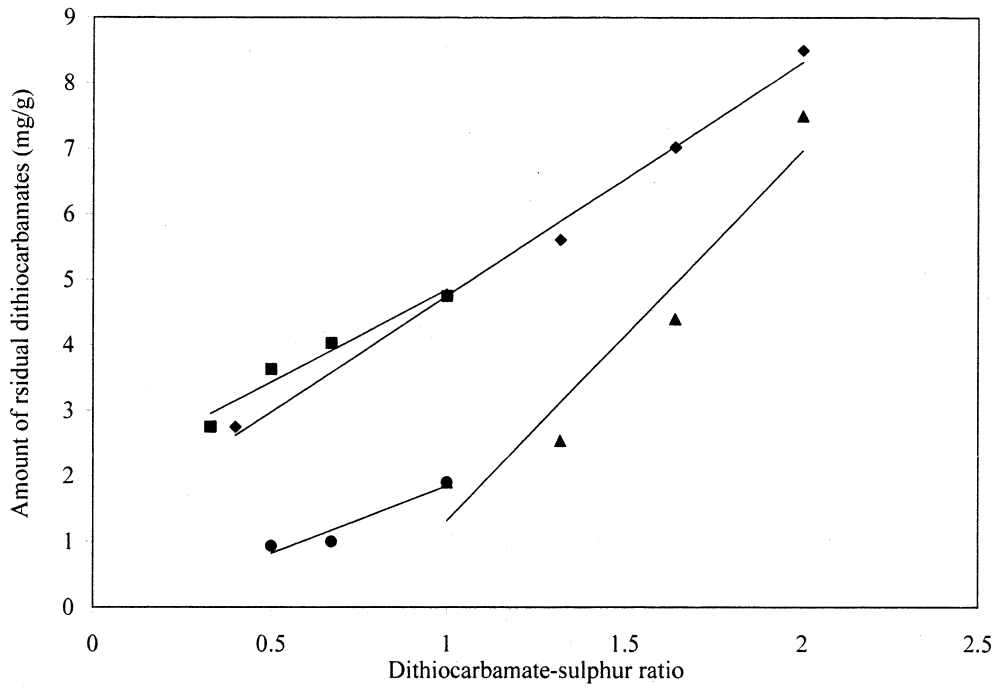


Figure 7.5. Variation in the amount of (▲) residual ZDEC at varying ZDEC level; (●) residual ZDEC at varying sulphur level; (◆) residual ZDBC at varying ZDBC level; and (■) residual ZDBC at varying sulphur level

It is apparent that the residual ZDEC and ZDBC linearly increased with an increase in the dithiocarbamate-sulphur ratio. A relatively steep rise in the amount of residual ZDEC and ZDBC was observed when the dithiocarbamate level was increased compared to when the sulphur level was increased.

The results showed that the amount of residual dithiocarbamates in the finished product could be controlled by adjusting the dithiocarbamate-sulphur ratio. As a linear dose-response relationship was observed in the patch testing of gloves (Knudsen *et al.*, 1993), it is hoped that the chances of allergic reactions would be reduced by keeping the amount of residual accelerators as low as reasonably practicable. It was also detailed in the previous chapter that the rate of migration of ZDEC through the rubber phase is substantial at higher residual ZDEC levels. This again necessitates the formulator to keep the level of residual ZDEC in the latex products as low as possible,

which could be achieved by reducing the dithiocarbamate-sulphur ratio. It is to be noted that the dithiocarbamate-sulphur ratio affects not only the residual dithiocarbamate content but the network structure of the vulcanizates as well. The network structure in turn controls the mechanical properties of the latex vulcanizates. Care must, therefore, be exercised to select an optimum dithiocarbamate-sulphur ratio to maintain the balance between mechanical properties and residual dithiocarbamate content in the finished latex goods.

7.3. CONCLUSIONS

The amount of residual ZDEC and ZDBC increased linearly with an increase in the amount of ZDEC or ZDBC initially added to the latex formulations while maintaining the level of sulphur a constant. On the other hand, the amount of residual ZDEC and ZDBC decreased linearly with an increase in the amount of sulphur added initially to the latex formulations at constant level of dithiocarbamates. In other words, the amount of residual dithiocarbamates increased linearly with an increase in the dithiocarbamate-sulphur ratio. At equivalent initial dithiocarbamate levels, the latex formulations containing ZDBC gave higher (i) residual ZDBC content and (ii) crosslink density compared to that containing ZDEC.

CHAPTER 8

MECHANICAL PROPERTIES

8.1. INTRODUCTION

An acceptable latex formulation is the one which possesses an 'adequate level' of mechanical properties as well as low residual dithiocarbamate content. The effect of dithiocarbamate-sulphur ratio on the amount of residual dithiocarbamates was already discussed in the previous chapter. This chapter details the fine tuning or the optimization of the mechanical properties of latex vulcanizates by varying the dithiocarbamate-sulphur ratio. The vulcanizate properties which are of interest to a manufacturer of latex articles are tensile strength, elongation at break and stiffness (modulus).

8.2. RESULTS AND DISCUSSION

Latex vulcanizates with varying dithiocarbamate-sulphur ratio were prepared using the five different accelerators. In the first step, the latex vulcanizates were prepared by increasing the amounts of dithiocarbamates at a fixed level of sulphur (increasing dithiocarbamate-sulphur ratio). In the second step, the sulphur content was increased while maintaining a constant level of dithiocarbamate (decreasing dithiocarbamate-sulphur ratio). One set of sample was conditioned at ambient temperature and humidity to determine the mechanical properties. As latex articles are generally susceptible to degradation on storage, some selected formulations were subjected to heat aging to simulate the effects of long term storage on the mechanical

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properties. The effect of aging on the mechanical properties was determined. The effect of alkali leaching on the mechanical properties was also studied.

8.2.1. Mechanical properties

The term 'adequate level' means the minimum physical properties required for products as proposed by regulatory agencies. The ASTM and BIS require that the medical gloves should meet the mechanical properties as specified in Table 8.1.

Table 8.1. Physical properties of NRL gloves as required by ASTM and BIS

Type	Before aging		After aging (ASTM D 573, 1999)	
	TS*, (MPa)	EB*, (%)	TS*, (MPa)	EB*, (%)
Surgical gloves (ASTM D 3577, 2002; IS 4148, 1989)	24 (ASTM)	750 (ASTM)	18 (ASTM)	560 (ASTM)
	18 (IS)	700 (IS)	15.5 (IS)	630 (IS)
Examination gloves (ASTM D 3578, 2002)	14	700	14	500

* TS-Tensile strength; EB-Elongation at break

For surgical gloves, ASTM specifies a minimum value of 24 MPa and 18 MPa for tensile strength before and after aging respectively. With regards to the elongation at break, the glove requires a minimum value of 750% before aging and 560% after aging. As far as the examination gloves are concerned, the minimum required values for tensile strength and elongation at break are lower compared to surgical gloves. The requirements for medical gloves by BIS are less stringent compared to ASTM.

Figure 8.1 shows the effect of varying amounts of ZDEC (increasing ZDEC-sulphur ratio) on the tensile strength and elongation at break of the latex sheets prepared from various formulations as given in Table 3.6. The effect of varying amount of sulphur (decreasing ZDEC-sulphur ratio) on the mechanical properties of latex vulcanizates (Table 3.7) is given in Figure 8.2.

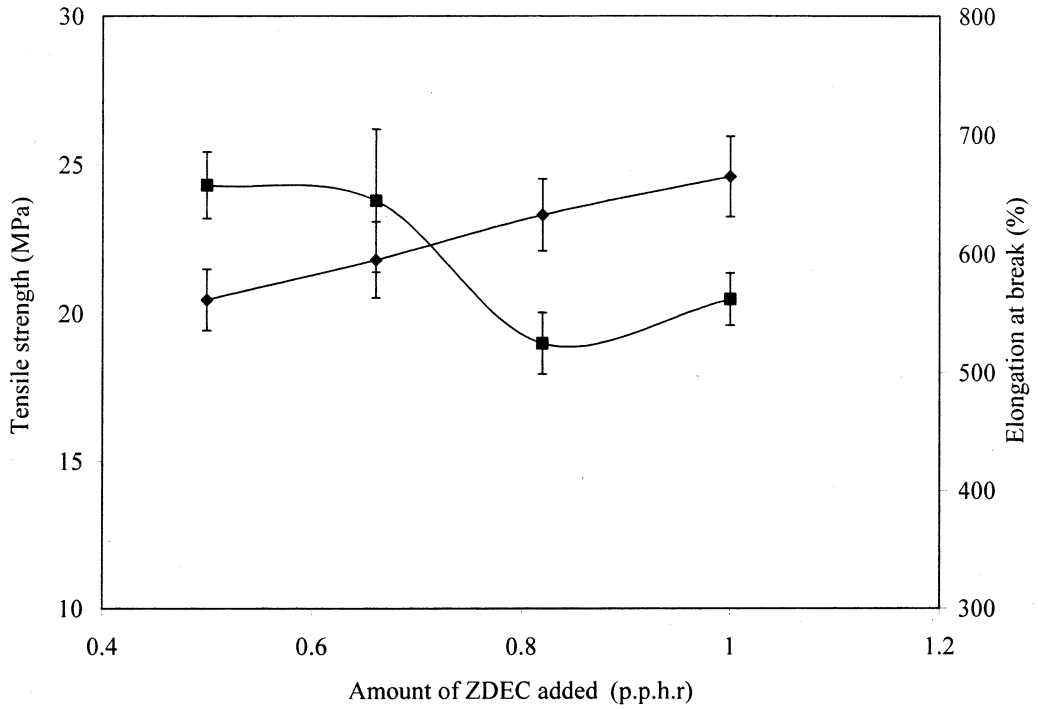

Figure 8.1. Effect of varying amounts of ZDEC on the (◆) tensile strength; and (■) elongation at break at constant level of sulphur

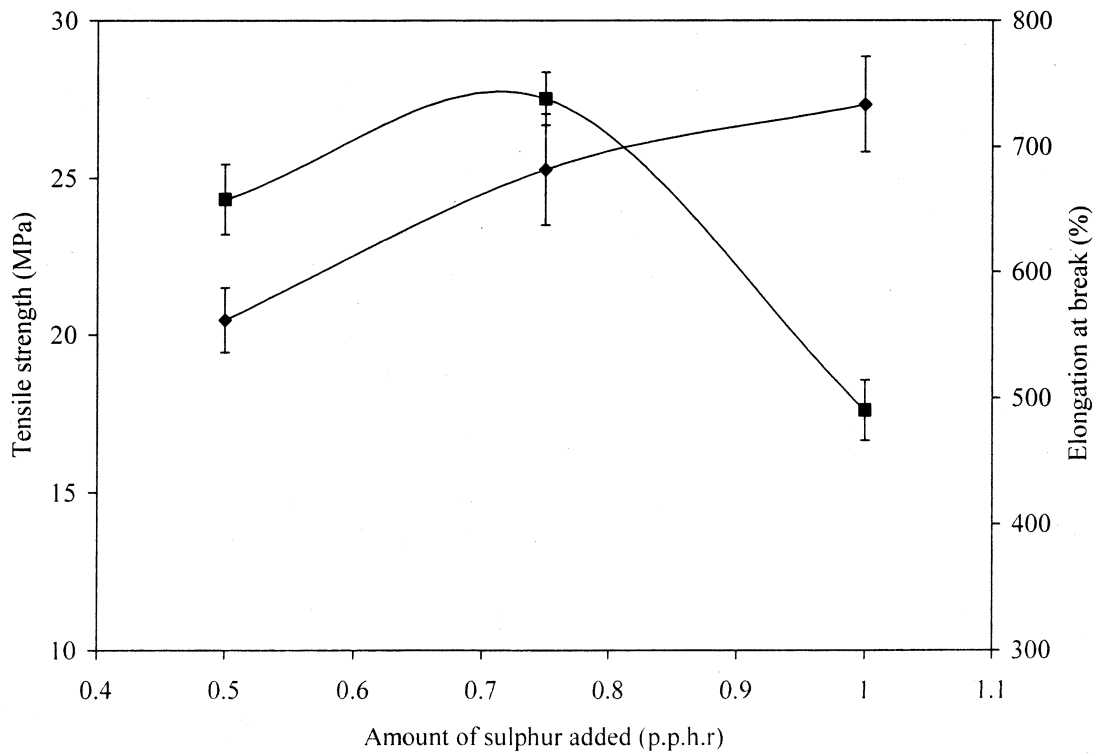

Figure 8.2. Effect of varying amount of sulphur on the (◆) tensile strength; and (■) elongation at break at constant level of ZDEC

It is apparent from Figure 8.1 and Figure 8.2 that the tensile strength increased with an increase in concentration of either ZDEC or sulphur. However, the magnitude of the increase was high when the sulphur content was increased while maintaining the level of ZDEC a constant (Figure 8.2). The elongation at break decreased at high curative levels. This variation in the tensile strength and elongation at break is attributed to the difference in the crosslink densities in the vulcanizates. The effect of crosslink density on the mechanical properties of ZDEC-vulcanized latex sheets is shown in Figure 8.3.

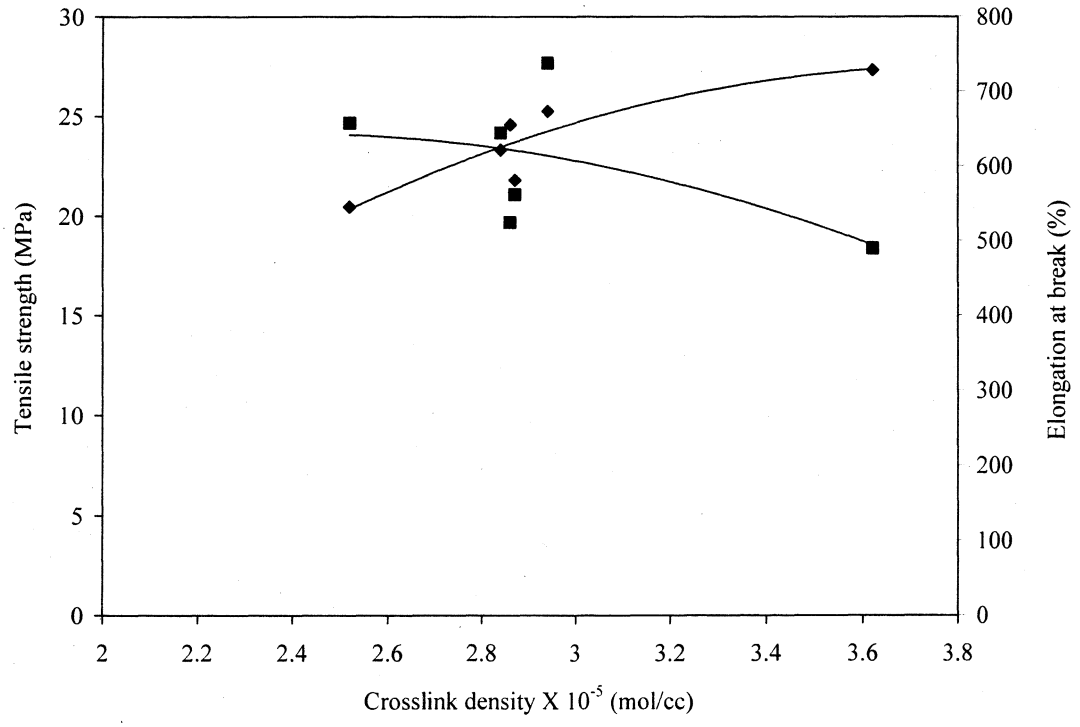

Figure 8.3. Effect of crosslink density on the (◆) tensile strength; and (■) elongation at break of ZDEC-vulcanized latex sheets

It is apparent from Figure 8.3 that the tensile strength increased with an increase in the concentration of crosslinks formed in the vulcanizate, and the elongation at break decreased. It has been well documented that the tensile strength increased with an increase in the crosslink density and passed through a maximum and that the elongation at break decreased (Porter, 1988; Akiba and Hashim, 1997; Coran, 2003). The modulus at 100 and 300% extension of all the latex vulcanizates was less than 5.5 MPa, the maximum limit put forward by ASTM D 3577 (2002). The mechanical properties of some of the selected latex vulcanizates containing ZDEC following aging are given in Table 8.2.

Table 8.2. Effect of aging on the mechanical properties of ZDEC-vulcanized latex sheets

Sample code	Tensile strength (MPa)		Elongation at break (%)	
	Before aging	After aging	Before aging	After aging
DE-1	20. 5 ± 1.0	9.44 ± 1.9	658 ± 28	736 ± 42
DE-4	24.6 ± 1.4	25.4 ± 3.4	562 ± 22	668 ± 37
DE-5	25.3 ± 1.8	19.9 ± 1.5	738 ± 21	652 ± 15
DE-5A	22.2 ± 0.9	17.9 ± 1.5	654 ± 12	683 ± 35
DE-6	27.3 ± 1.5	15.7 ± 1.0	490 ± 24	597 ± 47

The latex vulcanizates deteriorated upon thermal aging as evidenced from the decrease in the mechanical properties (Table 8.2). Aging led to crosslink degradation of the vulcanizates which in turn contributed to the deterioration in the mechanical properties (Blackman and McCall, 1970). It was interesting to note that the latex vulcanizates using the formulation DE-4 showed excellent retention in the tensile strength upon aging compared to DE-1. This is attributed to the antioxidant activity of the residual ZDEC. Table 8.3 gives the amount of residual ZDEC in DE-1 and DE-4 before subjecting them to aging experiment.

Table 8.3. Amount of residual ZDEC in latex vulcanizates

Sample code	Residual ZDEC (mg/g)
DE-1	1.92
DE-4	7.5

It is evident that the amount of residual ZDEC in DE-4 is much greater compared to DE-1, which explains the excellent aging resistance of DE-4. It is well known that the dithiocarbamates show antioxidant activity owing to their capability to degrade the hydroperoxides, the compound causing degradation of the crosslinks (Holdsworth *et al.*, 1964; Al-Malaika *et al.*, 1983). It, therefore, follows that the higher the amount of residual ZDEC, the higher would be the retention of tensile strength upon aging. The mechanical properties of latex formulation containing antioxidant (DE-5A) showed deterioration but it was within the acceptable limits.

Acceptable latex formulations were selected based on (i) the requirements with regard to the mechanical properties specified by ASTM D 3577 (2002) and

ASTM D 3578 (2002) (Table 8.1), and (ii) the amount of residual dithiocarbamate content. It is desirable to keep the amount of residual dithiocarbamates as low as possible in order to reduce the incidence of allergic reactions (Knudsen *et al.,* 1993). There were a few latex formulations using ZDEC such as DE-1, DE-4, DE-5, DE-5A and DE-6, which had adequate mechanical properties before aging. The list reduced to formulations DE-4, DE-5, and DE-5A as DE-1 and DE-6 did not show aging resistance as required by ASTM D 3577 (2002). The residual ZDEC content was in the order DE-4 >> DE-5A > DE-5. As the formulation DE-4 had a higher residual ZDEC content, DE-4 was eliminated in the final step. Thus, the two acceptable formulations selected were DE-5, and DE-5A. However, the elongation at break of DE-5A was found to be slightly lower with regard to the specifications put forward by ASTM or BIS. It is expected that these formulations give better mechanical properties when manufactured by dipping process, which is the commonly used method for the manufacture of gloves.

Figure 8.4 shows the effect of varying amounts of ZDBC on the mechanical properties of the latex sheets prepared using latex formulations as given in Table 3.8.

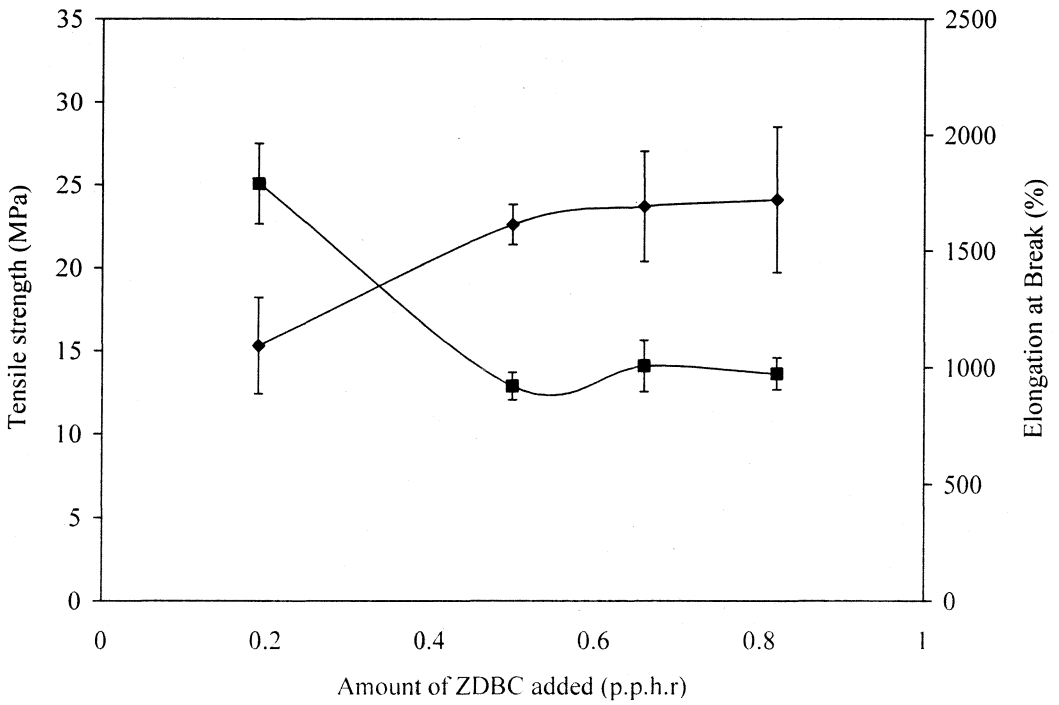

Figure 8.4. Effect of varying amount of ZDBC on the (◆) tensile strength; and (■) elongation at break at constant level of sulphur

The tensile strength showed a sharp increase as the amount of ZDBC was increased to 0.5 p.p.h.r and remained constant afterwards, and the elongation at break showed an opposite behaviour. The effect of varying amounts of sulphur on the mechanical properties of latex vulcanizates prepared as per formulations given in Table 3.9 is shown in Figure 8.5.

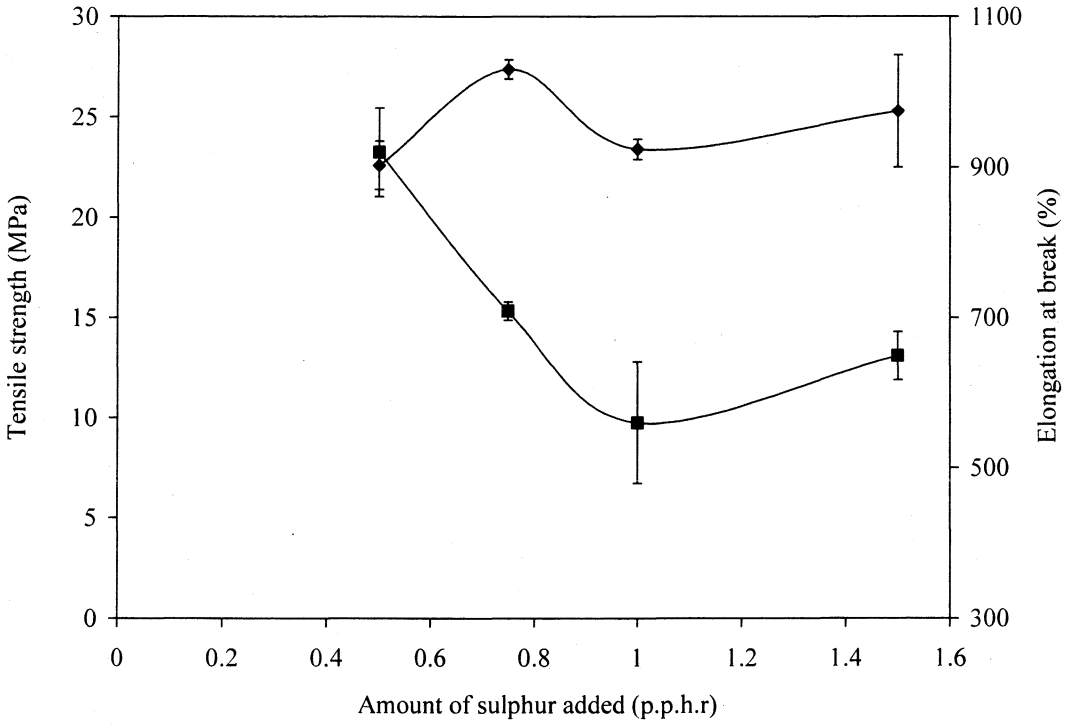

Figure 8.5. Effect of varying amount of sulphur on the (◆) tensile strength; and (■) elongation at break at constant level of ZDBC

It is seen that the tensile strength passed through a maximum, and the elongation at break decreased with an increase in the amount of sulphur added. The variation in the mechanical properties is attributed to the difference in the crosslink density of the vulcanizates. Figure 8.6 shows the variation in the mechanical properties with crosslink density of the latex vulcanizates cured with ZDBC.

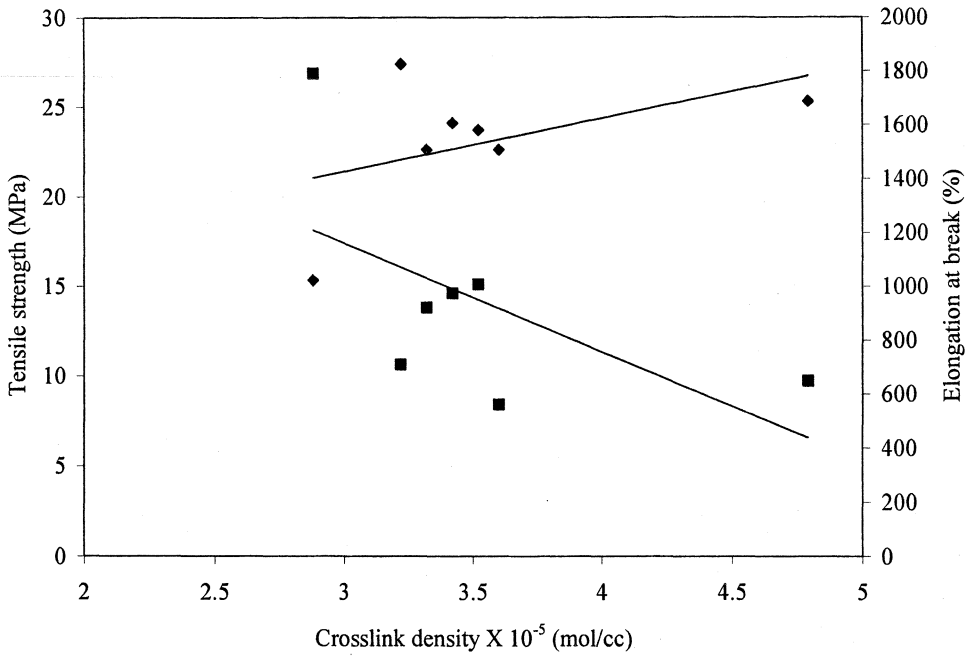

Figure 8.6. Variation of the (◆) tensile strength; and (■) elongation at break of ZDBC-vulcanized latex sheets with crosslink density

As explained earlier, the tensile strength increased and elongation at break decreased with an increase in the crosslink density. It was also found that the elongation at break of ZDBC-vulcanized latex sheets was higher compared to ZDEC-vulcanized latex sheets. This is attributed to the difference in the network structure of the ZDBC-vulcanized latex vulcanizates. It has been reported that the ZDBC affects prevulcanization at ambient temperature leading to the formation of polysulfidic linkages, while ZDEC has little effect (Porter, 1988; Blackley, 1997a). This explains the high elongations of the ZDBC-vulcanized latex sheets. Table 8.4 gives the effect of aging on the mechanical properties of latex sheets vulcanized with ZDBC.

Table 8.4. Effect of aging on the mechanical properties of ZDBC-vulcanized latex sheets

Sample code	Tensile strength (MPa)		Elongation at break (%)	
	Before aging	After aging	Before aging	After aging
BU-1	22.6 ± 1.2	31.3 ± 0.9	920 ± 59	628 ± 23
BU-5	27.4 ± 0.5	30.7 ± 0.5	709 ± 12	697 ± 42
BU-5A	24.7 ± 0.6	25.2 ± 2.8	637 ± 68	586 ± 39

It was found that the latex vulcanizates containing ZDBC showed good aging resistance even in the absence of antioxidant compounds (formulations BU-1 and BU-5). An interesting comparison of the formulations BU-1 and BU-5 with the ZDEC-vulcanized latex sheets (DE-1 and DE-5) showed that the ZDBC-vulcanized latex sheets exhibited better retention of mechanical properties compared to ZDEC-vulcanized latex sheets. In the case of ZDBC-vulcanized latex sheets, the tensile strength increased, and the elongation at break decreased after aging. This means that the ZDBC-cured vulcanizates underwent crosslink formation rather than crosslink breaking during aging and is attributed to the greater accelerative ability of the residual ZDBC to crosslink the rubber. It is to be noted that the vulcanizates BU-1 and BU-5 contained much greater amounts of residual ZDBC (4.75 and 4.03 mg/g respectively) compared to DE-1 or DE-5 (Table 8.3), and the high amounts of residual ZDBC aided the crosslink formation during aging. Also, they acted as antioxidants, which also contributed to the aging resistance of ZDBC-vulcanized latex sheets.

The results indicated that the formulations BU-1, BU-2, and BU-3 gave an acceptable level of mechanical properties as specified by ASTM or BIS. In the previous chapter, it was found that the amount of residual ZDBC content of ZDBC-vulcanized latex sheets was in the order BU-1 < BU-2 < BU-3, and hence, the formulation BU-1 seems acceptable. On the other hand, among the formulations given in Table 3.9, formulations BU-5 and BU-5A seem to meet the mechanical properties specified by ASTM and BIS.

Table 8.5 gives the tensile strength and elongation at break before and after aging of latex sheets vulcanized with ZIBC accelerator, the isomeric compound of ZDBC.

Table 8.5. Mechanical properties of latex sheets using ZIBC

Sample code	Tensile strength (MPa)		Elongation at break (%)	
	Before aging	After aging	Before aging	After aging
IB-1	26.8 ± 2.4	29.6 ± 1.0	666 ± 33	688 ± 65
IB-5	24.8 ± 2.2	26.7 ± 2.8	706 ± 33	657 ± 50
IB-5A	23.8 ± 0.9	23.8 ±1.4	722 ± 10	638 ± 27

Similar to ZDBC-vulcanized sheets, the latex sheets vulcanized with ZIBC exhibited a good retention in the mechanical properties after aging in the absence of antioxidant compounds. The formulation IB-5A seems to meet the requirements put forward by ASTM or BIS with regard to the mechanical properties.

Figure 8.7 shows the effect of varying amounts of ZBEC on the mechanical properties when sulphur was kept constant at 0.5 p.p.h.r. The influence of varying amounts of sulphur on the tensile properties while maintaining a constant level of ZMBT (0.2 p.ph.r) and ZBEC (0.5 p.ph.r) is shown in Figure 8.8.

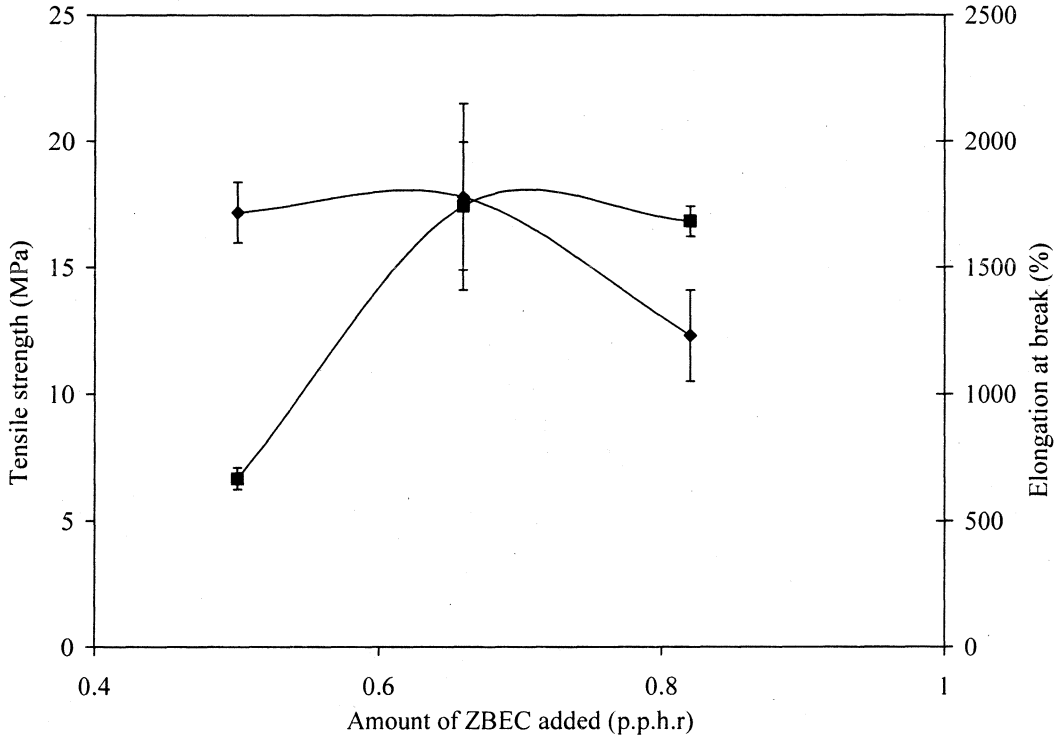

Figure 8.7. Effect of varying amount of ZBEC on the (◆) tensile strength; and (■) elongation at break at constant level of sulphur

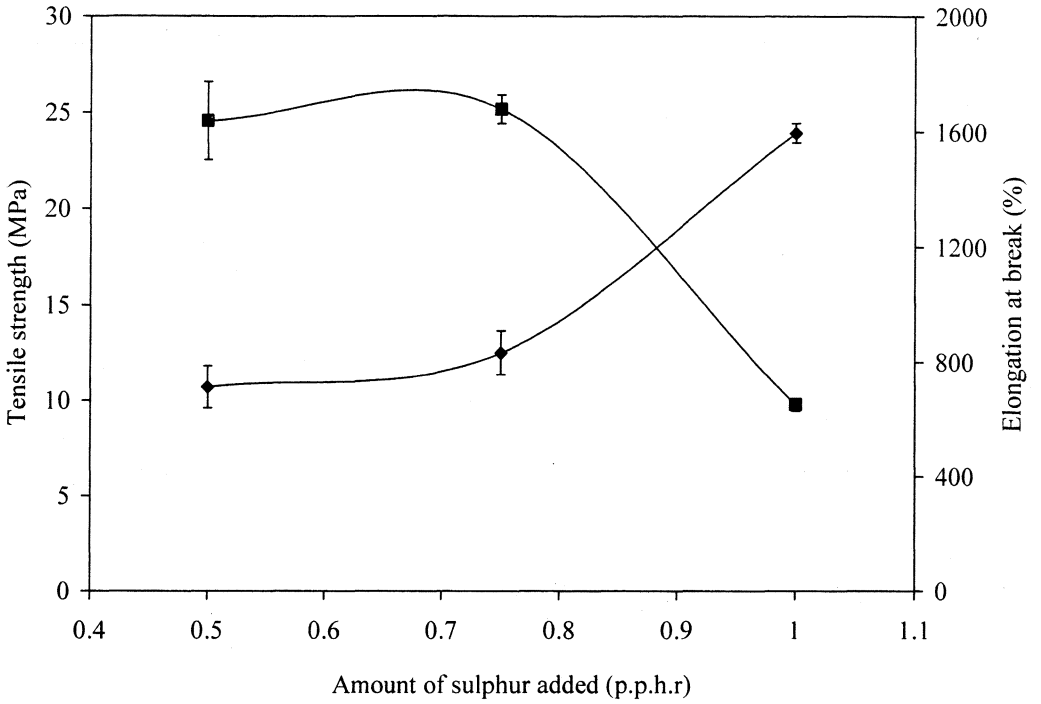

Figure 8.8. Effect of varying amount of sulphur on the (◆) tensile strength; and (■) elongation at break at constant level of ZBEC and in the presence of ZMBT

Figure 8.7 showed that ZBEC did not impart sufficient tensile strength to the latex sheets. The presence of a secondary accelerator, namely, ZMBT imparted adequate tensile strength. This was achieved at a higher loading of sulphur, namely, 1 p.p.h.r (Figure 8.8). With regard to the elongation at break, the formulation did not meet the requirements put forward by ASTM or BIS.

The variation in the mechanical properties of the latex vulcanizates (Table 3.13) with respect to varying amounts of ZDNC is shown in Figure 8.9. The highest tensile strength of 30 MPa was achieved when the ZDNC content was 0.5 p.p.h.r and the sulphur content was 1.0 p.p.h.r. On further increasing the ZDNC content, the tensile strength reduced and levelled off. The elongation at break, on the other hand, remained unchanged irrespective of the amount of ZDNC added. Figure 8.10 shows the variation in the mechanical properties with an increase in sulphur content when the amount of ZDNC was fixed at 0.5 p.p.h.r (Table 3.14).

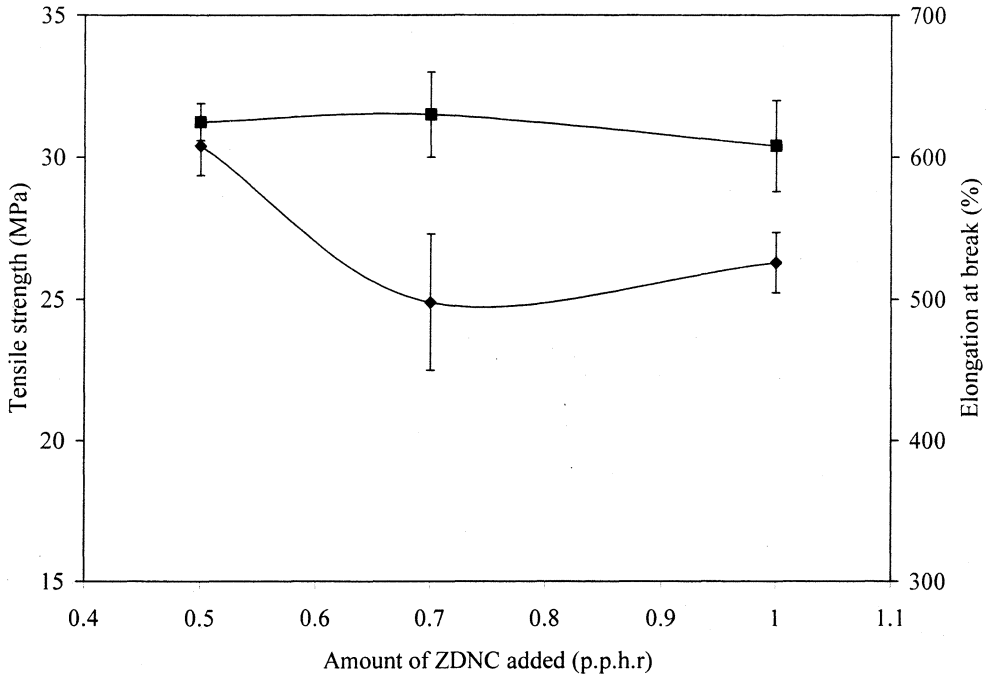

Figure 8.9. Effect of varying amount of ZDNC on the (◆) tensile strength; and (■) elongation at break at constant level of sulphur

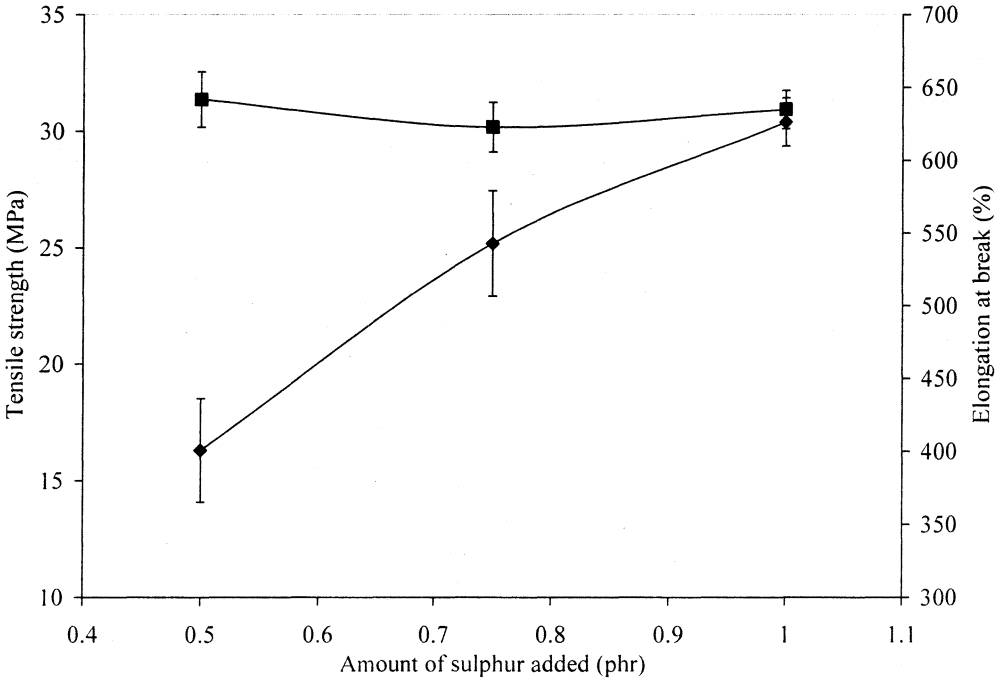

Figure 8.10. Effect of varying amount of sulphur on the (◆) tensile strength; and (■) elongation at break at constant level of ZDNC

It is evident from Figure 8.10 that the tensile strength progressively increased as the amount of sulphur was increased. On the other hand, the elongation at break remained largely unaffected with varying sulphur content. The results indicated that higher levels of sulphur (≥ 0.75 p.p.h.r) are needed to impart adequate tensile strength

to the latex vulcanizates when the concentration of ZDNC was maintained at a minimum of 0.5 p.p.h.r. Figure 8.11 shows the variation in the mechanical properties with crosslink density of the latex vulcanizates cured with ZDNC. It is apparent that the tensile strength increased with an increase in the crosslink density, and the elongation at break remains largely unaffected.

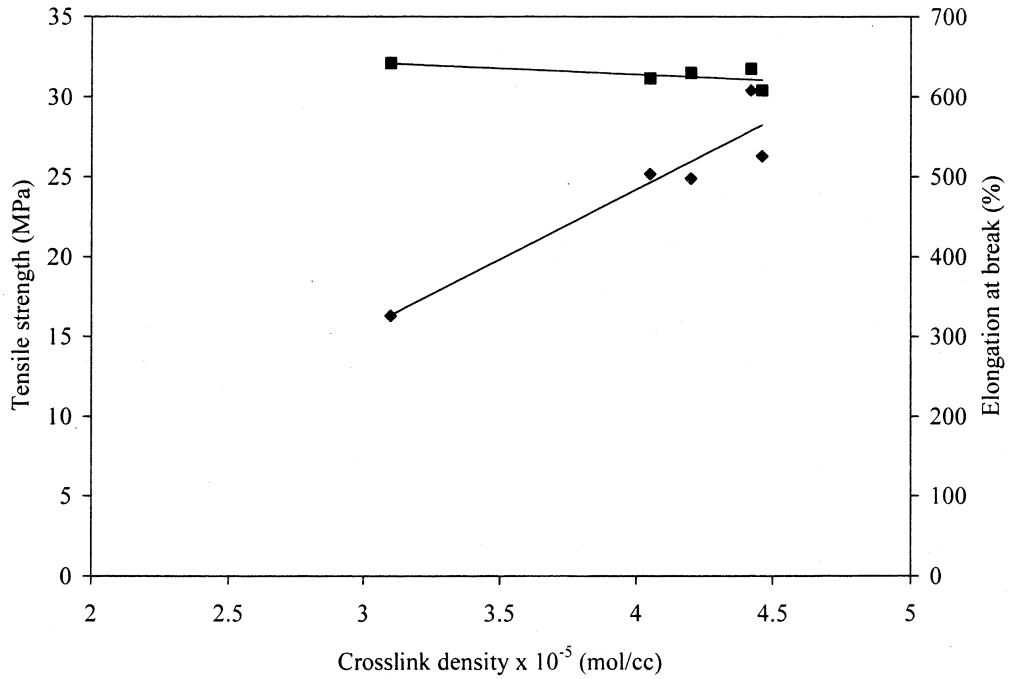

Figure 8.11. Variation of the (◆) tensile strength; and (■) elongation at break with crosslink density of ZDNC-vulcanized latex sheets

Table 8.6 gives the effect of aging on the mechanical properties of the latex vulcanizates vulcanized with ZDNC.

Table 8.6. Effect of aging on the mechanical properties of ZDNC-vulcanized latex sheets

Sample code	Tensile strength (MPa)		Elongation at break (%)	
	Before aging	After aging	Before aging	After aging
DN-1	30.4 ± 1.0	27.6 ± 3.8	635 ± 13	549 ± 27
DN-5	25.2 ± 2.3	25.3 ± 2.6	623 ± 17	559 ± 35
DN-1A	30.5 ± 1.4	28.2 ± 2.7	528 ± 51	659 ± 53

It was found that the ZDNC-vulcanized sheets showed good retention in the mechanical properties even in the absence of antioxidant compounds (DN-1 and DN-5). All the formulations given in Table 8.6 met the requirements by ASTM and

BIS with regard to the tensile strength while, the elongation at break was found to be slightly lower with regard to the specifications given in Table 8.1.

The results of the tensile testing of various latex vulcanizates revealed that the tensile properties varied considerably depending on the type of accelerator used. It has been reported that the crosslink density and hence, the mechanical properties were found to be dependent on the type of accelerator used for the vulcanization (Murgic and Jelencic, 2000). The present study generated a number of formulations using different types of dithiocarbamate accelerators. Some of these formulations that met the specifications put forward by either ASTM or BIS include DE-5, DE-5A, BU-1, BU-5, BU-5A, IB-5A, DN-1, DN-5 and DN-1A. The elongation at break was found to be slightly below the specified limit in some of these formulations; however, it is expected to get improved when dipping process is employed for the manufacture of latex products.

8.2.2. Effect of alkali leaching on mechanical properties

It was found that leaching of the latex sheets in alkali solution removed the dithiocarbamate residues from the surface of latex sheets. The alkali leaching is of benefit only if it does not cause deterioration of the mechanical properties. The effect of alkali leaching on the mechanical properties of latex vulcanizates was studied using selected formulations and is shown in Figure 8.12 and Figure 8.13 respectively.

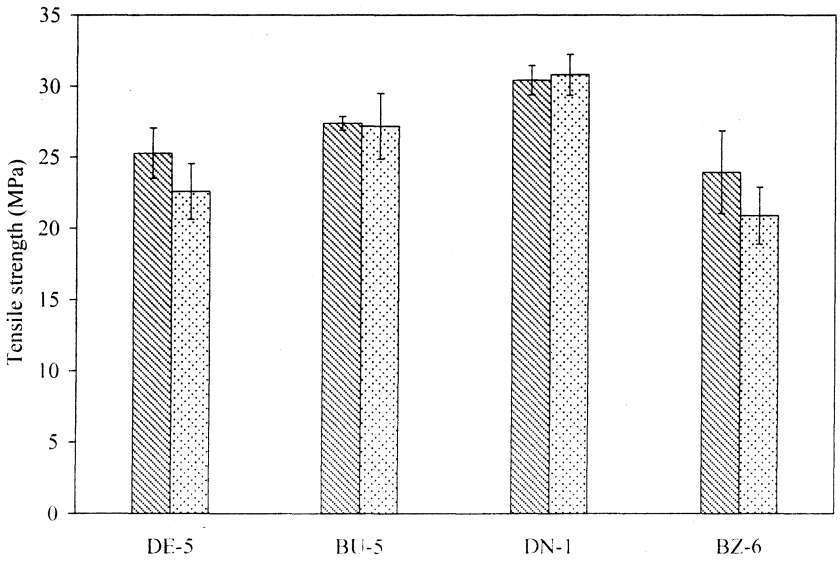

Figure 8.12. Effect of alkaline washing on the tensile strength (▨) before alkaline wash; and (▩) after alkaline wash

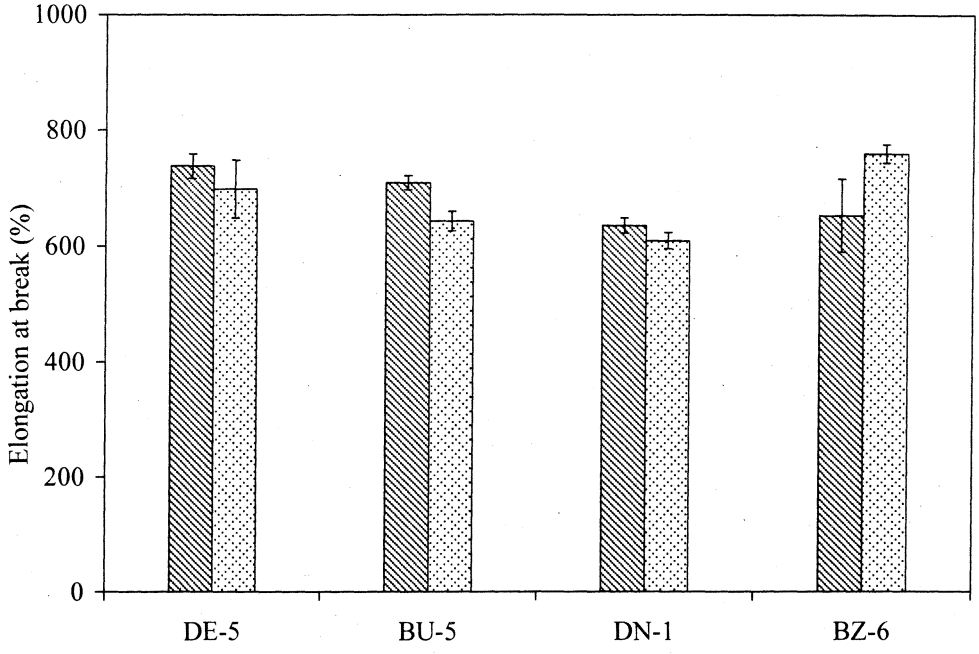

Figure 8.13. Effect of alkaline washing on the elongation at break (▨) before alkaline wash; and (▩) after alkaline wash

The formulations DE-5 and BZ-6 showed a percentage decrease of 11 and 13 respectively with regard to the tensile strength. For all the other formulations studied, the change in tensile strength was negligible. As far as the elongation at break was concerned, there was only a marginal change.

The long-term effects of alkaline washing on the mechanical properties were also studied by carrying out the aging studies. The effect of alkali leaching on the mechanical properties of some of the formulations following aging is shown in Table 8.7.

Table 8.7. Effect of aging on the mechanical properties of alkali-leached latex sheets

Sample code	Before aging		After aging	
	Tensile strength (MPa)	Elongation at break (%)	Tensile strength (MPa)	Elongation at break (%)
DE-5A	22.5 ± 0.5	603 ± 55	17.2 ± 1.7	739 ± 5.9
BU-5A	24.7 ± 0.6	637 ± 68	25.2 ± 2.2	611 ± 20

It was observed that the deterioration in the tensile strength and elongation at break was well within the acceptable limits specified by ASTM (ASTM D 3577,

2002; ASTM D 3578, 2002). Also, ZDBC-vulcanized latex sheets showed good retention in mechanical properties compared to ZDEC-vulcanized latex sheets.

8.3. COST ESTIMATION OF NRL FORMULATIONS

The cost of the formulations (on a dry weight basis) was calculated and is given in Table 8.8.

Table 8.8. Cost of the different latex formulations

Sample code	(Rs./kg)	Sample code	(Rs./kg)
DE-1	69.6	BU-1'	69.4
DE-2	69.7	BU-1	70.1
DE-3	69.9	BU-2	70.4
DE-4	70.2	BU-3	70.8
DE-5	69.7	BU-5	70.2
DE-6	69.7	BU-6	70.2
BZ-1	71.2	BU-7	70.2
BZ-2	71.9	BZ-5	71.7
BZ-3	72.7	BZ-6	71.7

It is apparent that the cost of the formulations using different dithiocarbamate accelerators did not vary much. It follows that substitution of ZDEC with more rubber-soluble accelerators like ZDBC would not increase the cost of the latex product substantially. This could be illustrated by taking formulations DE-5 and BU-5, which used 0.5 p.p.h.r each of the respective accelerator. Assuming that each glove weighs 10 g, the increase in the cost of the glove using formulation BU-1 is only 5 paise.

8.4. CONCLUSIONS

The effect of varying amounts of dithiocarbamate and sulphur on the mechanical properties was studied. It was found that the mechanical properties varied considerably depending upon the type of the dithiocarbamate accelerator used. In general, the mechanical properties improved when the sulphur content was in the range 0.75-1.0 p.p.h.r while maintaining the accelerator level to a minimum possible level of 0.5 p.p.h.r. On the other hand, the mechanical properties showed only a

marginal increase with an increase in the amount of dithiocarbamates initially added to the latex vulcanizates while keeping the sulphur level constant. Under identical processing conditions, it was noticed that ZDBC, ZIBC, and ZDNC gave improved mechanical properties compared to ZDEC. The lowest activating accelerator was found to be ZBEC. However, ZBEC imparted adequate tensile strength in the presence of a secondary accelerator, namely, ZMBT. With regard to aging, good retention in mechanical properties was achieved in the case of latex vulcanizates employing ZDEC, ZDBC, ZIBC and ZDNC. The percentage retention in the mechanical properties was greater for latex vulcanizates containing ZDBC and higher homologues. It was noticed that as the amount of residual dithiocarbamates (ZDEC and ZDBC) increased, the resistance to aging also increased. As expected, the formulations containing MMBI antioxidant compound showed a good retention in the mechanical properties.

The alkaline washing caused slight (11-13%) deterioration in the mechanical properties in the case of latex vulcanizates vulcanized with ZDEC and ZBEC. In contrast, latex vulcanizates containing ZDBC and ZDNC showed negligible change in the mechanical properties. It was found that the alkali-leached sheets (formulations DE-5A and BU-5A) showed good aging resistance and the percentage deterioration of mechanical properties was within the acceptable limits put forward by ASTM and BIS.

The present study generated a number of latex formulations such as DE-5, DE-5A, BU-1, BU-5, BU-5A, IB-5A, DN-1, DN-5 and DN-1A that meet the requirements for mechanical properties put forward by either ASTM or IS. However, the elongation at break was slightly lower in the case of some of the formulations, which is expected to improve when dipping process is used for the manufacture of latex vulcanizates of latex products.

CHAPTER 9

BIOLOGICAL EVALUATION

9.1. INTRODUCTION

The evaluation of biocompatibility of medical devices and materials has been a complex task owing to the diverse range of materials and their various intended uses, with body contact ranging from transient skin contact to blood contact to permanent implantation. Biocompatibility is the term used to describe the state of affairs when a biomaterial exists within a physiological environment, without either the material adversely and significantly affecting the body, or the environment of the body adversely and significantly affecting the material. The typical description of an ideal biocompatible material had been considered as a list of negatives such as non degradable, non irritant, non toxic, non carcinogenic and non allergenic (Williams, 1990). This concept of biocompatibility has been questioned as different materials are associated with different responses in different situations, and the appropriateness of the response will vary from one situation to another. It is therefore reasonable that biocompatibility should be centred around the material-host complex. Biocompatibility is, therefore, defined as 'the ability of a material to perform with an appropriate host response in a specific application' (Williams, 1990; 2004).

Establishing biocompatibility of medical devices and their materials is of vital importance in ensuring product safety. Biocompatibility is generally demonstrated by testing device materials, and their leachable chemicals, using toxicological principles. The evaluation of any new biomaterial or device intended for human use requires data

from systematic testing to ensure that the benefits provided by the final product outweigh the potential risks produced by them. When selecting the appropriate tests for the biological evaluation of a medical device, one must consider the chemical characteristics of the material and the nature, degree, frequency and duration of its exposure to the body. In general, the tests include: acute, sub chronic and chronic toxicity; irritation to skin, eyes and mucosal surfaces; sensitization; hemocompatibility; genotoxicity; carcinogenicity; and effects on reproduction including developmental effects. In cases when these general tests may not be sufficient to demonstrate the safety of some specialized devices, additional tests for specific target organ toxicity, such as neurotoxicity and immunotoxicity may be necessary. Some devices are made of materials that have been well characterized chemically and physically and have a long history of safe use. In such situations, it may not be necessary to conduct all the tests; however, the manufacturer must document the use of this particular material in a legally marketed device with comparable patient exposure.

There are several national and international consensus standards that address the toxicological evaluation of medical devices. In 1986, FDA, Health and Welfare Canada, and Health and Social Services UK issued the Tripartite Biocompatibility Guidance for the evaluation of medical devices. This guidance has been used for selecting appropriate tests to evaluate the adverse biological responses to medical devices. Since that time, the ISO, in an effort to harmonize biocompatibility testing, has developed some standards (18 parts) for biological evaluation of medical devices (ISO 10993). The medical devices are categorized into three as per ISO: surface devices, externally communicating devices and implant devices (ISO 10993-1, 1999). Each category is further classified into subcategories depending on the type of contact to which the patient is exposed. The choice of test program for a device in a given category depends on the duration of the contact: limited (<24 h); prolonged contact (24 h-30days); and permanent contact (>30 days).

This chapter discusses the biological evaluation of some latex vulcanizates that are having acceptable level of mechanical properties and low in residual dithiocarbamate content. The biological performance of the latex vulcanizates was

assessed by the *in vitro* cell culture test, intracutaneous irritation test and dermal sensitization tests, which were carried out according to ISO 10993 standards.

9.2. RESULTS AND DISCUSSION

9.2.1. *In vitro* cell culture cytotoxicity test

The results of the *in vitro* cell culture cytotoxicity test of some of the latex vulcanizates by direct contact are summarized in Table 9.1.

Table 9.1. Results of the *in vitro* cell culture cytotoxicity test of latex sheets prepared using different formulations

Sample code	Cell response (direct contact test)
DE-5	Non cytotoxic
DE-5A	Non cytotoxic
BU-1	Non cytotoxic
BU-6	Non cytotoxic
IB-1	Non cytotoxic
DN-1	Non cytotoxic
DN-1A	Non cytotoxic
DN-5	Non cytotoxic
BZ-6	Non cytotoxic

It was found that the vulcanizates containing different dithiocarbamate accelerators exhibited a non cytotoxic response in the direct contact test. As the Indian latex industry largely employs ZDEC as the accelerator, the latex vulcanizates DE-5 and DE-5A were selected for further biological evaluation. Fresh batches of these latex vulcanizates were prepared and again subjected to direct contact test. The extracts of these latex vulcanizates were prepared and tested by the test on extract. Typical cell response of the latex formulation DE-5 to L 929 cells in the direct contact test and test on extract is shown in Figure 9.1 and Figure 9.2 respectively.

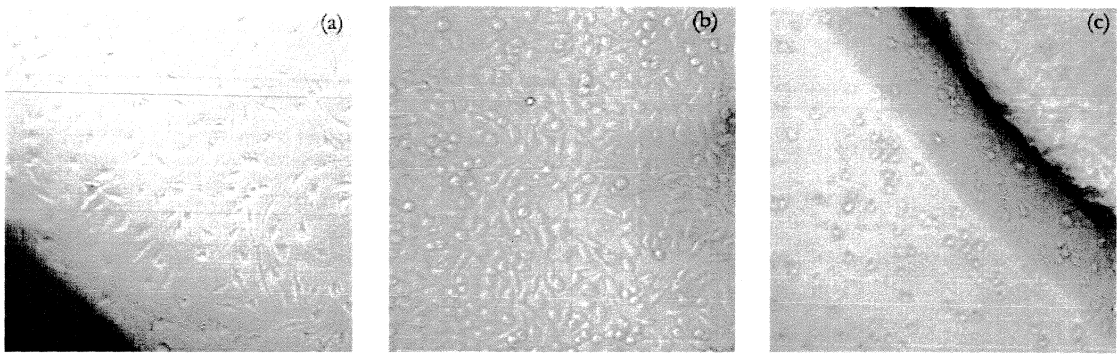

Figure 9.1. L 929 cells incubated with (a) DE-5; (b) negative control; and (c) positive control in the direct contact test

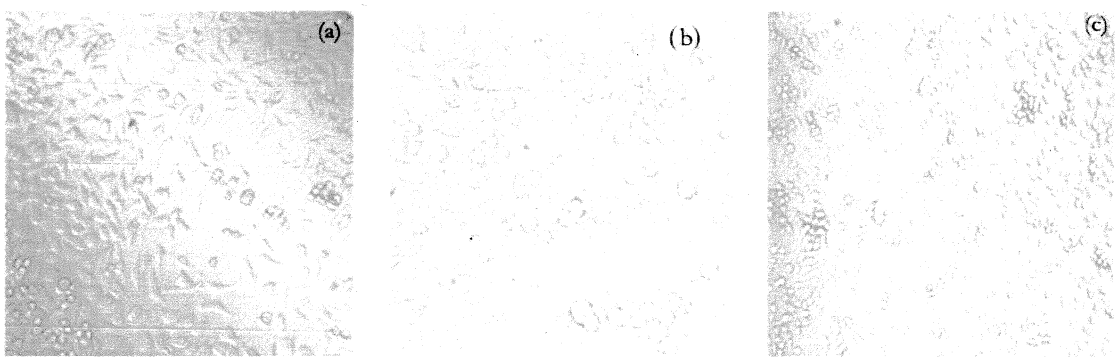

Figure 9.2. L 929 cells incubated with extracts of (a) DE-5; (b) negative control; and (c) positive control in the test on extract

Figure 9.1 and Figure 9.2 showed that the cells retained their spindle shaped morphology in both direct contact and test on extract indicating that DE-5 vulcanizates were non toxic to the L929 cells. The formulation DE-5A also showed a non toxic response in both the direct contact test and test on extract (Figure 9.3)

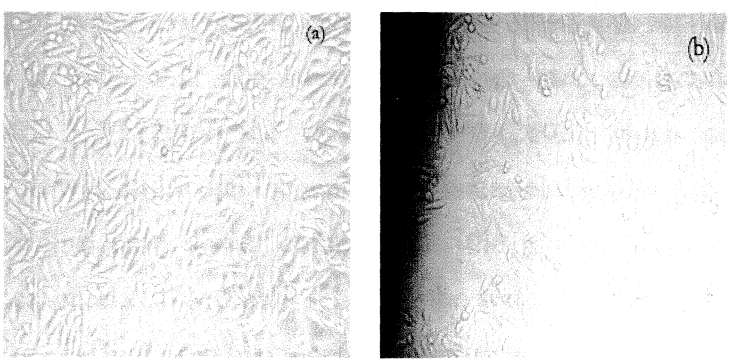

Figure 9.3. L 929 cells incubated with extracts of DE-5A in (a) direct contact test; and (b) test on extract

The cell response of the latex formulations BU-1, BU-6, IB-1, DN-1, DN-1A, and BZ-6 to L 929 cells in the direct contact test and test on extract is shown in Figure 9.4 and Figure 9.5.

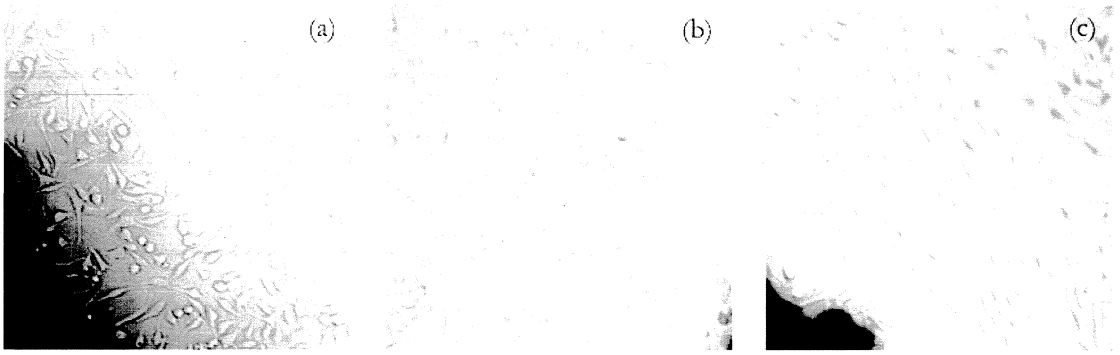

Figure 9.4. L 929 cells incubated with (a) BU-1; (b) BU-6; and (c) IB-1 in the direct contact test

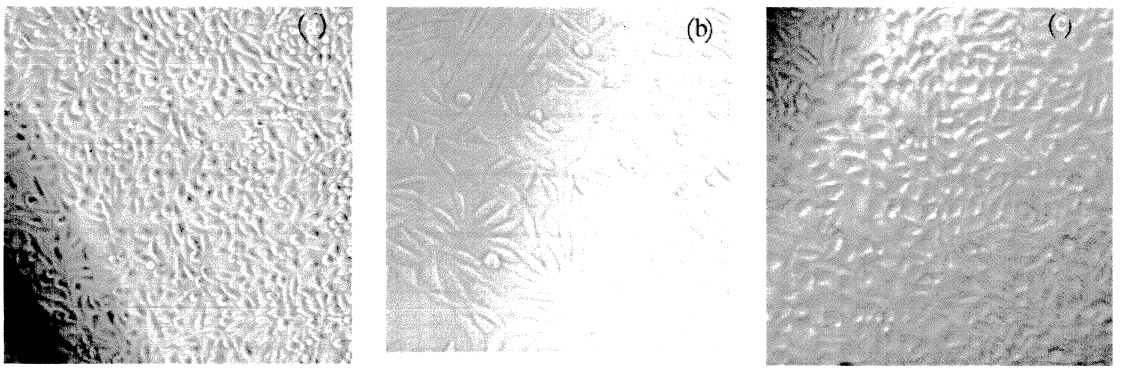

Figure 9.5. L 929 cells incubated with (a) DN-1; (b) DN-1A; and (c) BZ-6 in the direct contact test

It is apparent that the cells in contact with the latex sheets retained their spindle shaped morphology indicating the non cytotoxicity of the latex vulcanizates.

9.2.2. Water-extractable proteins

The amount of water extractable proteins in the latex vulcanizates DE-5 and DE-5A are given in Table 9.2. The latex vulcanizates meet the requirements put forward by ASTM D 3577 (2002) and ASTM D 3578 (2002), which specify a value below 200 μg/dm^2. It was found that the amount of water-extractable proteins in DE-5 and DE-5A was significantly lower compared to the gloves which are currently available in the local market (see Table 4.11). In the wake of more and more people getting sensitized to residual proteins, many regulatory agencies recommend to keep the amount of water-extractable proteins as low as reasonably practicable. For example, in Germany, KAN proposed an upper limit of proteins at 30 μg/g.

Table 9.2. Amount of water-extractable proteins in DE-5 and DE-5A

Sample code	Water-extractable proteins (μg/g)
DE-5	50
DE-5A	29

9.2.3. Intracutaneous irritation studies

The intracutaneous irritation score for DE-5 and DE-5A are given in Table 9.3 and Table 9.4.

Table 9.3. Intracutaneous irritation score for DE-5

Animal	Primary irritation score			
	Erythema		Oedema	
	Saline	CSO	Saline	CSO
Animal 1	0	0.2	0	0
Animal 2	0	0	0	0
Animal 3	0	0	0	0

Table 9.4. Intracutaneous irritation score for DE-5A

Animal	Primary irritation score					
	Erythema			Oedema		
	Saline	CSO	Artificial sweat*	Saline	CSO	Artificial sweat*
Animal 1	0	0.7	0	0	0	0
Animal 2	0	1.1	0	0	0	0
Animal 3	0	0.7	-	0	0	-

* test was performed according to ISO 10993-10 (2002)

The primary irritation index of the latex vulcanizates DE-5 and DE-5A is given in Table 9.5.

Table 9.5. Primary irritation index of DE-5 and DE-5A

Material code	Primary irritation index		
	Saline	CSO	Artificial sweat
DE-5	0	0.02	NT
DE-5A	0	0.28	0

NT-not tested

The primary irritation index for all test specimens in saline and artificial sweat was zero. On the other hand, the primary irritation index of DE-5 and DE-5A in cotton seed oil was 0.02 and 0.28 respectively. This is attributed to the ability of CSO to swell rubber thereby extracting the residual chemicals from the interior of the rubber matrix. It was visually observed that the CSO swelled the latex sheets. DE-5A gave a higher value for primary irritation index in CSO compared to DE-5 and is due to the presence of residual antioxidant chemical, MMBI that get released into CSO.

9.2.4. Sensitization test

Table 9.6 and Table 9.7 give the response to closed patch sensitization studies for erythema and oedema of the control and test materials.

Table 9.6. Responses to closed patch sensitization test recorded for DE-5 for test and control animals (challenge application)

Animal	Skin reaction			
	Erythema		Oedema	
	24 h	48 h	24 h	48 h
Test	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
Control	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0

Table 9.7. Responses to closed patch sensitization test recorded for DE-5A for test and control animals (challenge application)

Animal	Skin reaction			
	Erythema		Oedema	
	24 h	48 h	24 h	48 h
Test	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
Control	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0

For all observation period the numerical grading for erythema and oedema for test specimens was zero.

9.3. CONCLUSIONS

The present study generated some non-cytotoxic formulations, which include DE-5, DE-5A, BU-1, BU-6, IB-1, DN-1, DN-1A, DN-5, and BZ-6. The latex formulations using ZDEC (formulations DE-5 and DE-5A) were further subjected to intracutaneous irritation and skin sensitization tests. The intracutaneous irritation test of the extracts of the latex vulcanizates showed that the material does not cause potential local irritation. Sensitization studies carried out on latex vulcanizates revealed that the material had no potential to elicit the immune response in guinea pigs indicating that these latex formulations could be used for medical applications.

CHAPTER 10

SUMMARY, CONCLUSIONS AND FUTURE PROSPECTS

10.1. SUMMARY AND CONCLUSIONS

Although synthetic latex products were introduced to address the problems posed by NRL medical products, NRL remains the material of choice for the production of barrier products such as gloves and condoms owing to the unique characteristics such as superior barrier protection, elasticity, tear resistance, durability, excellent tactile sensitivity and comfort. In response to the growing concern over the allergic reactions to NRL products, many regulatory agencies recommended that the risks from proteins, residual chemicals and glove powder should be assessed and controlled. Although considerable progress has been made to address the problems posed by proteins and glove powder, little has been done with regard to residual chemicals. The present study was undertaken to study the release of the allergologically relevant, bioavailable residual zinc dithiocarbamate accelerators and the subsequent exposure of users to residual dithiocarbamates. Due to the wide variations associated with the production of commercial gloves, NRL vulcanizates of known compositions using different zinc dithiocarbamate accelerators and dithiocarbamate-sulphur ratios were prepared to evaluate the various factors controlling the dithiocarbamate-release. It was also the intention to develop some latex formulations employing ZDEC, the widely used accelerator in the Indian latex industry, suitable for medical glove manufacture.

The study started with the analysis of eleven brands of commercially available medical gloves marketed in India. The *in vitro* cell culture test of the gloves and their extracts indicated that the residual chemicals present in the finished latex gloves were released into the culture medium, and caused cell death. Nearly 63% of the total gloves selected were found to be cytotoxic in nature. The cell responses varied from non-cytotoxic (grade '0') to severely cytotoxic (grade '3') with the majority of the gloves exhibiting cytotoxicity in the direct contact test. It was also found that the amount of water-extractable proteins was high in some of these gloves compared to those in the international market. The past experience of serious allergic reactions world wide prompted many manufacturers to reduce the level of water-extractable proteins as low as possible to reduce the incidence of sensitization and subsequent allergic elicitations. The present study indicated that adequate measures should be taken to improve the quality of latex medical products marketed in India with regard to the cytotoxicity and water-extractable proteins.

Analytical techniques were employed to identify and quantify the various zinc dithiocarbamates. It was found that the UV-VIS spectroscopy may be used for a preliminary investigation to detect the presence of residual chemicals, eventhough no conclusive evidence regarding the type of dithiocarbamates could be made. On the other hand, TLC could be used as a rapid and specific technique to identify the different zinc dithiocarbamates (ZDEC, ZDBC, ZIBC, ZBEC and ZDNC) based on their R_f values. High performance liquid chromatography could be successfully used for both identification and quantification of dithiocarbamates. The identification could be made by looking at the retention time characteristic of each dithiocarbamate. Before subjecting to TLC and HPLC, the zinc dithiocarbamates were converted into their respective copper complexes by reacting with copper(II) sulphate in order to avoid metal-exchange reactions with the chromatographic (HPLC) column and to obtain a good separation on the silica gel (TLC) plate. The HPLC method was validated with solutions of known concentrations of ZDEC and ZDBC. The test method for the determination of ZDEC was found to be linear, accurate and precise. Good linearity and accuracy of the test method were observed for the determination of ZDBC as well. The detection limit of ZDEC and ZDBC was 0.25 and 0.61 μg/ml, and the quantitation limit was 0.84 and 2.0 μg/ml respectively. Under the conditions used in the present study, copper(II) sulphate was found to be a better complexing agent for

dithiocarbamates compared to cobalt(II) chloride, another complexing agent reportedly used for the quantification of zinc dithiocarbamates.

All the gloves except two contained ZDEC, the most cytotoxic compound among the commonly used dithiocarbamate accelerators in the latex industry. It was found that ZDEC residues were released into artificial sweat, a physiologically simulated medium, from the gloves. The amounts of ZDEC released into artificial sweat (sweat-extractable ZDEC) and dichloromethane (residual ZDEC) varied widely from brand to brand. The amount of sweat-extractable ZDEC ranged from 39 μg/g (brand 'F') to as high as 173 μg/g (brand 'J'), while the amount of residual ZDEC varied from 0.20 mg/g (brand 'F') to 3.84 mg/g (brand 'H'). The amount of sweat-extractable ZDEC showed a statistically significant effect on the cytotoxicity potential of the latex gloves in both the direct contact and test on extract assays indicating that the artificial sweat-extraction of the latex gloves may be used as a test method to screen new latex products containing dithiocarbamates before subjecting them to expensive *in vitro* and *in vivo* biological tests. As many of these gloves contained residual ZDEC in quantities sufficient to cause sensitization to an atopic population, appropriate measures should be taken to optimize the vulcanization system for the production of NRL gloves to keep the amount of residual dithiocarbamates a minimum as well as to maintain the required mechanical properties.

The residual ZDEC present in the glove (brand 'H') was released into the hand sweat of the human subjects when the gloves were donned. The extent of ZDEC-release, and hence the resultant exposure of users to ZDEC varied widely among human subjects despite using the same brand of glove. There was no clear relationship between the amount of ZDEC released into hand sweat and sweat rate or sweat pH of the volunteers. On the other hand, the pH of the artificial sweat seems to affect the quantity of ZDEC released. A comparison between the extent of ZDEC- release under real-use (using human subjects) and simulated-use conditions (using artificial sweat at pH 6-6.5) indicated that the ZDEC-release under simulated-use conditions closely approximated the real-use conditions of the gloves. It, therefore, follows that filling the glove with artificial sweat at pH 6-6.5 and subsequent extraction for 1 h under simulated-use conditions may be used for the estimation of ZDEC-release, and the 'anticipated exposure' of users to ZDEC from gloves.

Despite the addition of the same amount of dithiocarbamate accelerators initially, the latex vulcanizates released negligible quantities of residual ZDBC, ZIBC, ZBEC and ZDNC into artificial sweat compared to ZDEC. It was observed that the major factor controlling the extent of release of different dithiocarbamates was the rubber solubility. At this point, it is worth mentioning that the dithiocarbamates are capable of inducing allergy to users if they are released from the latex products and become available to the surface of the skin. It is, therefore, desirable to use more rubber-soluble (low-releasing) accelerators such as ZDBC or higher homologues for latex products that are intended to come into contact with the human skin or mucosal membrane.

The extraction of latex vulcanizates cured with varying ZDEC-sulphur ratio in artifical sweat showed that the ZDEC-release showed a near constant release irrespective of the amount of ZDEC initially added (up to 1 p.p.h.r) to the latex formulations. Upon storage, the extent of ZDEC-release from these latex vulcanizates increased which is attributed to the migration of ZDEC through the rubber phase to the surface of the vulcanizates. The effect was more pronounced at higher residual ZDEC contents (> 1.92 mg/g) and longer storage times (≥ 24 weeks). Interestingly, ZDBC showed a lower tendency for migration across the rubber phase compared to ZDEC upon storage owing to its rubber-soluble nature. The dependence of ZDEC-release on the shelf time and the amount of residual ZDEC content have serious implications with regard to the quality of latex products stored for long durations. The use of migrating accelerators such as ZDEC in gloves will cause the accumulation of ZDEC at the surface of latex sheets over a period of time. The residual ZDEC present at the surface of the gloves will then be released into the hand sweat while donning gloves and may sensitize the user. As the latex products are usually marketed with long expiration dating periods, it is desirable to use more rubber-soluble accelerators like ZDBC. The substitution of ZDEC in latex formulations by ZDBC was found to be cost-effective as well.

Leaching is a well adopted technique to remove the residual proteins and chemicals from dipped goods. It was found that the leaching in water removed the ZDEC residues from the surface of the latex vulcanizates. Interestingly, an additional leaching in alkali reduced the amount of residual ZDEC present on the surface of

latex sheets to negligible levels. With regard to the mechanical properties of these sheets before aging, the alkaline washing caused slight (11-13%) deterioration in the case of latex vulcanizates vulcanized with ZDEC and ZBEC. In the case of latex vulcanizates containing ZDBC and ZDNC, negligible change in the mechanical properties was observed following alkaline washing. The alkali-leached sheets containing ZDBC showed better retention in mechanical properties compared to ZDEC-vulcanized latex sheets after aging. As alkali leaching (for 30 min at 70 °C) did not cause substantial degradation of the mechanical properties of latex vulcanizates, it could be used as an effective method to remove the residual dithiocarbamates from the surface of the latex products.

As the amount of residual ZDEC contributed considerably to its migration and subsequent accumulation on the surface of the vulcanizates, it is required to keep their amount as low as reasonably practicable in the finished latex goods, at the same time maintaining adequate level of mechanical properties. The amount of residual dithiocarbamates (both ZDEC and ZDBC) was found to increase linearly with an increase in the amount of dithiocarbamate initially added to the latex formulations while maintaining the sulphur level a constant. On the other hand, the amount of residual ZDEC and ZDBC decreased linearly with an increase in the amount of sulphur added initially at constant level of dithiocarbamates. In other words, the amount of residual dithiocarbamates increased linearly with a corresponding increase in the dithiocarbamate-sulphur ratio. The optimum dithiocarbamate-sulphur ratio is the one which maintains a balance between residual dithiocarbamate content and mechanical properties.

It was found that an increase in the amount of dithiocarbamate accelerators had not much influence on the mechanical properties of latex vulcanizates; at the same time, it increased the residual dithiocarbamate contents. Improved mechanical properties were achieved when the sulphur content was in the range 0.75-1.0 p.p.h.r while maintaining the accelerator level at the minimum possible level of 0.5 p.p.h..r. At these sulphur levels, the utilization of ZDEC, and ZDBC was greater leading to lower residual dithiocarbamate contents. The optimized level of sulphur in terms of residual dithiocarbamate content and mechanical properties was in the range 0.75-1.0 p.p.h.r when the amount of dithiocarbamate initially added was 0.5 p.p.h.r.

Under identical processing conditions, it was noticed that ZDBC, ZIBC and ZDNC imparted improved mechanical properties compared to ZDEC and ZBEC. The lowest activating accelerator was found to be ZBEC. However, ZBEC imparted adequate tensile strength in the presence of a secondary accelerator (ZMBT) but the elongation at break was low. With regard to aging, good retention in mechanical properties was achieved in the case of latex vulcanizates which employed ZDBC, ZIBC and ZDNC. It was also noticed that as the amount of residual dithiocarbamates (ZDEC and ZDBC) increased, the resistance to aging also increased. With regard to the tensile strength, the present study generated a number of latex formulations that meet the requirements put forward by either ASTM or IS. However, the elongation at break is slightly on the lower side in the case of some formulations. It is expected that the mechanical properties will be improved during dipping, which is most widely used technique for the manufacture of gloves and other latex health care products.

The preliminary cytotoxicity studies of some latex vulcanizates by direct contact test gave a number of latex formulations that were non cytotoxic to L929 cells. As the Indian latex industry largely employed ZDEC in the manufacture of medical gloves, further biological studies were performed using vulcanizates containing ZDEC only. Two latex formulations (DE-5 and DE-5A) meeting adequate mechanical properties were further subjected to skin sensitization and intracutaneous irritation tests, and were found to be neither sensitizing nor causing irritation. These formulations, therefore, could be used for the manufacture of medical gloves.

10.2. FUTURE PROSPECTS

The results of the present study widen the knowledge-base of NRL industry with regard to the various issues related to zinc dithiocarbamate accelerators. As dithiocarbamates are also used in the manufacture of synthetic latex products, the results of the present study would be useful to synthetic latex industry as well. The future work includes the following:

(i) Development of a standard test method for the determination of dithiocarbamates in latex medical gloves by HPLC using copper(II) sulphate.

(ii) Study on the dithiocarbamate-release under real-use and simulated use conditions with statistically significant number of human subjects in order to develop the artificial sweat extraction as a standard test method for the estimation of the 'anticipated exposure' of users to residual dithiocarbamates in latex medical gloves.

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