STUDIES ON RADIOPAQUE POLYMERS FOR BIOMEDICAL APPLICATIONS

A THESIS PRESENTED BY

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TO

SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY
Thiruvananthapuram

IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF
DOCTOR OF PHILOSOPHY

2011

To my parents

CERTIFICATE

*I, Dawlee S., hereby certify that I have personally carried out the work depicted in the thesis entitled, "**STUDIES ON RADIOPAQUE POLYMERS FOR BIOMEDICAL APPLICATIONS**", except where external help sought are acknowledged. No part of the thesis has been submitted for the award of any other degree or diploma prior to this date.*

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STUDIES ON RADIOPAQUE POLYMERS FOR BIOMEDICAL APPLICATIONS

Submitted by

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for the degree of

Doctor of Philosophy

of

SREE CHITRA TIRUNAL INSTITUTE

FOR

MEDICAL SCIENCES AND TECHNOLOGY

Thiruvananthapuram

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Acknowledgements

I wish to place on record my sincere thanks to my Ph.D supervisor, Dr. M. Jayabalan, Scientist F & Head, Polymer Science Division, SCTIMST for his guidance, help and support throughout the course of investigation. I express my depth of gratitude to my former guide, Dr. A. Jayakrishnan, Vice Chancellor, University of Kerala for his help, inspiration and encouragement during the initial phase of my Ph.D course. I would like to acknowledge my thanks to Dr. K. Sreenivasan, Dr. P. Ramesh and Dr. A. K. Gupta, the members of my Doctoral Advisory Committee for their valuable suggestions and sincere advice.

Thanks are due to the Director, SCTIMST & Head, BMT Wing, SCTIMST for providing the necessary facilities to carry out the work. Financial assistance from Council for Scientific & Industrial Research, New Delhi & Kerala State Council for Science, Technology and Environment, Trivandrum in the form of a senior research fellowship and young scientist award are gratefully acknowledged.

I acknowledge my sincere thanks to Mr. S. Suresh Babu and Mr. P. Rajesh, Bioceramics Laboratory, SCTIMST for their help in IR & EDX analyses. I am grateful to my friends, Dr. P. S. Hema & Ms. V. L. Reena, NIIST, Trivandrum and Ms. Rekha Narayan, NCL, Pune for their help in doing NMR analyses of the samples. I wish to thank Dr. G. Saraswathy, CLRI, Chennai and Ms. P. G. Sreelekha, IIT Bombay for their help with GPC analyses. I am indebted to my friend, Dr. S. Sajeesh, Biosurface Technology, SCTIMST and Dr. Christine Vauthier, Université de Paris, France for their help in getting the iodine estimation done at Service Central d' Analyse, Centre National de la Recherche Scientifique, Verniason, France.

I also express my sincere thanks to Dr. C. Radhakumary, Laboratory for Polymer Analysis, SCTIMST for thermal analyses and my fellow research scholars, Mr. Arjun Namboothiri & Ms. Priya A. Nair, Polymer Processing Division, SCTIMST for DMA. I owe my sincere thanks to Dr. Roy Joseph, Polymer Processing Division, SCTIMST for his help in determining the mechanical properties of the samples. I wish to thank Mr. S. Vijayan, Bioceramics Laboratory, SCTIMST for the XRD analyses of the polyurethanes.

Thanks are also due to Ms. S. Usha Vasudev & Ms. Deepa K. Raj, Tissue Culture Laboratory, SCTIMST for their help in carrying out the cytotoxicity evaluation of the samples. I would like to express my gratitude to Dr. Lissy K. Krishnan & Ms. A. Priyanka, Thrombosis Research Unit, SCTIMST for their assistance in blood compatibility evaluation. I am extremely grateful to Dr. P. V. Mohanan & Ms. C. S. Geetha, Toxicology Division, SCTIMST for their expertise and help in toxicological and implantation studies of the materials. I wish to thank Dr. A. Sabareeswaran, Histopathology Laboratory for his help in doing the histopathology analyses. I am also thankful to Dr. Umashankar & Dr. Sachin J. Shenoy, Division of *In-vivo* Models and Testing, SCTIMST for the X-ray analyses.

I express my irredeemable depth of gratitude to Dr. Nirmala Rachel James, Associate Professor, IIST, Trivandrum for her constructive criticism, help, support and encouragement throughout the duration of my work. I acknowledge my sincere thanks to my friends, Mr. Arun Anirudhan, Dr. Biji Balakrishnan, Dr. K. K. Nishi, Dr. V. N. Sivanandam, Dr. M. R. Rekha, Mr. S. Kiran, Mr. Narahari Mahanta, Ms. T. A. Sonia, Ms. Shelma Sreekanth, Ms. Asha Rani, Mr. G. T. Finosh, Mr. H. Hashim, Ms. M. K. Mitha and Ms. Lekshmi Krishna for their friendly cooperation, warm companionship and whole hearted help.

Lastly, I would like to thank my family for all their love and encouragement. I express my heartfelt thanks to my parents, sister, brother-in-law, uncles and aunts for their support and inexhaustible encouragement throughout my academic career. I owe my sincere thanks to my husband for his help, faithful support and encouragement during the final stages of my Ph.D course.

Dawlee S.

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Abbreviations

AIBN	2,2'-azobis(isobutyronitrile)
ATR	Attenuated total reflectance
BMA	Butyl methacrylate
BPA	Bisphenol-A
CSO	Cotton seed oil
DBTDL	Dibutyltin dilaurate
DMA	Dynamic mechanical analysis
DMF	Dimethyl formamide
EDX	Energy dispersive X-ray analysis
EMA	Ethyl methacrylate
FTIR	Fourier transform infrared spectral analyses
GMA	Glycidyl methacrylate
GPC	Gel permeation chromatography
HDI	Hexamethylene diisocyanate
HEPES	(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)
ISO	International Standards Organisation
MDI	4,4'-methylenebis(phenyl isocyanate)
MMA	Methyl methacrylate
MTT	3-(4,5-dimethylthiazol-2yl)-2,5,diphenyl tetrazolium bromide
NMR	Nuclear magnetic resonance
PBS	Phosphate buffered saline
PMMA	Poly(methyl methacrylate)
PPG	Polypropylene glycol
PS	Physiological saline
PTMG	Polytetramethylene glycol
TGA	Thermogravimetric analysis
THF	Tetrahydrofuran
UHMWPE	Ultra high molecular weight polyethylene
X-ray	X-radiography
XRD	X-ray diffraction

SYNOPSIS

Polymeric biomaterials are increasingly being used for medical applications such as cardiovascular implants, prostheses, orthopedic implants, wound dressings and controlled release devices. It would be highly desirable if these implants and prosthesis could be evaluated to monitor their performance and/or exact location in a non-invasive manner. X-radiography is a low cost imaging technique for such an evaluation. Though most metallic implants used in surgery are highly opaque to X-rays, implants based on polymeric materials are often not radiopaque in nature. Conventional polymers cannot be detected by X-rays because they contain elements such as C, H, O and N which exhibit both low electron density and low specific gravity. Therefore, investigation into strategies to increase electron density and the specific gravity of polymers in order to make them radiopaque assumes great importance.

Polymers could be rendered radiopaque by blending them with radiopacifying agents like barium sulfate or metal powders such as that of tantalum, or by the incorporation of heavy metal salts such as barium bromide, bismuth halides, or uranyl nitrate into an appropriate polymer ligand via chelation. However, the physical and mechanical properties of the base polymer are often adversely affected by the incorporation of these additives. When the radiopaque additive is a metal salt not covalently bound to the polymer, it tends to leach into the body fluids over long periods, which makes the radiopacity a temporary phenomenon apart from producing systemic toxicity. The best method to produce radiopaque polymers is to synthesize reactive monomers having covalently bound heavy atoms and use these monomers as building blocks for biomaterials that can exhibit intrinsic radiopacity or graft radiopacifying agents onto preformed high molecular weight polymers.

Polyurethanes have been employed as blood contacting materials for a number of years because of their excellent mechanical properties that can be tuned for specific applications. Polyurethanes find applications in total artificial heart, heart valves, mammary implants, pacemaker connectors, suture materials and matrices for controlled drug release. Polyurethanes exhibiting X-ray contrast properties would have obvious advantages over their non-radiopaque counterparts in medical and related applications. Therefore, it was relevant to couple iodine-containing compounds having radio contrast properties to polyurethanes to make them X-ray

opaque. The few reports on radiopaque polyurethanes employed brominated compounds to induce radiopacity. When using brominated chain extenders, the polymer must have desired bromine content (about 15% of the weight of polyurethane) to impart useful radiopaque properties. Adequate bromine content can be achieved only by increasing the amount of chain extender. This results in a relatively large hard segment ratio leading to stiffness and toxicity. Polyurethanes could be made radiopaque by employing chain extenders containing radiopacifying elements like iodine. Iodine was chosen to impart radiopacity because of its greater mass attenuation coefficient and since iodinated compounds are routinely employed in interventional radiology. It was possible to introduce sufficient concentration of the radiopacifying elements by using polyiodinated chain extenders.

Methacrylate polymers are widely used for medical applications such as contact lenses, bone cements for partial or total joint replacement, embolic materials and in dentistry as orthodontic and denture base materials. A major drawback of these polymers is that they are radiolucent, since polymers hardly absorb X-ray radiation due to the absence of heavy elements within their structure. Various methods have been employed in an attempt to give methacrylate polymers some degree of X-ray opacity so as to use for medical and dental applications, however, aesthetic and/or mechanical properties usually suffer to such an extent that the polymer would be clinically unacceptable. The only satisfactory method to impart radiopacity in methacrylate polymer is to utilize a halogen containing monomer, or carry out a post-polymerization halogenation of reactive groups. In this respect, we were interested in synthesizing novel radiopaque copolymers composed of glycidyl methacrylate (GMA). Therefore, objectives the investigation were the synthesis and evaluation of novel radiopaque polyurethane and acrylate copolymers for biomedical applications.

The thesis consists of six chapters. Chapter 1 deals with the introduction of the thesis which provides a brief outline of biomaterials, polymeric biomaterials and radiopaque polymers for medical applications. The aim and scope of work is also covered in this chapter.

Chapter 2 deals with the literature review on radiopaque polymeric materials. An overview on different radiopaque polymeric materials that are currently used as medical implants or inserts, the advantages and limitations of each radiopaque polymeric material are summarized.

Chapter 3 provides the details of materials and experimental methods used in the preparation, characterization and evaluation of radiopaque polymers. Radiopaque chain extenders (2,3-diiodo-2-butene-1,4-diol and bis- hydroxyethyl ether of tetraiodo bisphenol A) were prepared for the synthesis of radiopaque polyurethanes. Radiopaque polyurethanes were prepared by employing radiopaque chain extenders, polyols (polytetramethylene glycol (PTMG) and polypropylene glycol (PPG)) and diisocyanates (4,4'-methylenebis(phenyl isocyanate) (MDI) and hexamethylene diisocyanate (HDI)). Radiopaque acrylic copolymers based on GMA and other co-monomers, methyl methacrylate (MMA), ethyl methacrylate (EMA) and butyl methacrylate (BMA) were also prepared. Physicochemical characterization of radiopaque polymers using infrared spectroscopy (IR), energy dispersive X-ray analysis (EDX), elemental analysis, X-ray diffraction (XRD), thermogravimetric analysis (TGA), dynamic mechanical analysis (DMA), X-radiography (X-ray) and gel permeation chromatography (GPC) are described. The studies on *in vitro* biostability are also detailed in this chapter. The *in vitro* biostability of the polymers were studied by aging the polymers in different media for 6 months at 37 $^{\circ}$C. Phosphate buffered saline, Ringer's solution, Papain enzyme and its buffer solution, Alcoholic potassium hydroxide and Hydrogen peroxide were used. The aged polymers were tested for change in weight and surface properties. The biocompatibility was evaluated by *in vitro* cytotoxicity, cell viability and hemocompatibility as per ISO standards. Intracutaneous irritation test were also carried out using extract of the materials as per international standards. The *in vivo* biocompatibility was evaluated by subcutaneous implantation of the materials and histopathological analysis of harvested tissues as per ISO standards.

Chapters 4 and 5 comprise the results and discussion (R&D) of the thesis. These chapters are divided into 6 different sub-sections. Section 1(R&D) deals with the synthesis and characterization of the iodinated chain extenders, 2,3-diiodo-2-butene-1,4-diol and bis-hydroxyethyl ether of tetraiodo bisphenol A. The chain extenders were characterized by ^{1}H and ^{13}C NMR spectra. The percentage iodine content was quantitatively estimated by elemental analyses.

Section 2 (R&D) deals with the synthesis of the novel radiopaque polyurethanes using iodinated chain extenders. Four polyurethanes were developed using PTMG, PPG, MDI, HDI and aliphatic chain extender, 2,3-diiodo-2-butene-1,4-diol. Two polyurethanes were synthe-

sized from PTMG, PPG, MDI and aromatic chain extender, bis-hydroxyethyl ether of tetraiodo bisphenol A. All polyurethanes were synthesized by the conventional two step solution polymerization technique. The polymers were segmented block copolymers having polyol soft segment and diisocyanate-chain extender hard segment.

In section 3 (R&D) the physico-chemical, surface and thermal characterization of the newly synthesized radiopaque polyurethanes are described. High molecular weight polyurethanes were obtained and the percentage iodine content in the polymers was more than sufficient for clinical X-ray visibility. The physico-chemical characterizations have shown that the HDI-based polyurethanes were partially crystalline with phase-separated surface morphology. However, MDI-based polyurethanes have phase-mixed surface morphology which undergoes dynamic surface reorganization in aqueous medium to a phase-separated surface morphology. X-ray analysis of the polyurethanes revealed good radiopacity. The polyurethanes also possessed excellent thermal stability.

Section 4 (R&D) discusses the studies on biostability of radiopaque polyurethane polymers. There was neither significant change in weight nor evidence for bond breaking at the degradation-susceptible urethane or ether groups in the IR spectra of aged polyurethanes in simulated physiological conditions. The studies with accelerated chemical degradation revealed degradation of HDI based polyurethanes. However, no degradation (weight loss) or dimensional change was observed with other polyurethanes.

Section 5 (R&D) deals with the preparation and characterization of radiopaque glycidyl methacrylate based copolymers. High molecular weight copolymers possessing excellent radiopacity were obtained. The iodinated copolymers showed higher glass transition temperature and thermal stability in comparison with unmodified polymers. The presence of bulky iodine atoms in the polymer backbone decreased the flexibility of the macromolecules and created modified polymers with novel properties.

Section 6 (R&D) describes the studies on biocompatibility of the newly prepared radiopaque polyurethane and acrylate copolymers. All the radiopaque polymers were non-cytotoxic to L929 mouse fibroblast cells *in vitro*. The studies on blood-material interaction *in vitro* have shown that these radiopaque polymers were blood compatible. *In vivo* toxicological and im-

plantation studies revealed biocompatibility. The histopathological studies revealed that the tissue response of the test materials was similar to that of control material at the end of 4, 8 and 12 weeks. It also revealed evidence of repair with fibrous tissue capsule consisting fibrocytes and collagen around the implant site and neovascularisation (angiogenesis).

Finally, the Chapter 6 deals with summary, conclusion and future prospects of the investigation. The potential application of the candidate radiopaque polyurethanes and acrylate copolymers are also emphasized in this section.

INTRODUCTION

1 INTRODUCTION

1.1 Biomaterials

A commonly used definition of biomaterial is "a nonviable material used in a medical device, intended to interact with biological systems" (Williams 1987). It can also be defined as any substance other than a drug, or combination of substances, synthetic or natural in origin, which can be used for any period of time as whole or as a part of a system which treats, augments or replace any tissue, organ or function of the body (Williams 1992). Biomedical materials include metals, ceramics, pyrolytic carbon materials, composites and polymers. Of these groups, polymers represent the largest class. The single most important factor that distinguishes a biomaterial from any other material is its ability to exist in contact with tissues of the human body without causing an unacceptable degree of harm to that body. Biomaterials can be used for a wide range of applications where we interface synthetic materials and modified natural materials with biology. Table 1 lists some of these applications, both medical and non medical (Ratner & Bryant 2004).

Biomaterials must have special properties that can be tailored to meet the needs of a particular application. The three fundamental properties essential for the development of biomaterials are mechanical properties, a functional characteristic and biocompatibility. Mechanical strength is required to retain an adequate level of performance. The functional characteristic is required so that the material has the specific property to perform the required task. Biocompatibility is generally defined as the ability of a biomaterial, prosthesis, or medical device to perform with an appropriate host response in a specific application (Black & Black 1992). It can be considered in terms of tissue compatibility and blood compatibility. Biocompatibility or the clinical success of a biomaterial is directly dependent upon the response of the host tissue to the perturbation brought about by the foreign material. Hematological criteria call for materials that do not induce thrombosis, do not alter the stability of soluble or cellular materials in the blood, and do not allow allergic, toxic, aging or cell fragility reactions.

The evaluation of biological responses to a medical device is carried out to determine that the medical device performs as intended and presents no significant harm to the patient or user. Thus the goal of biological response evaluation is to predict whether a biomaterial,

medical device, or prosthesis presents potential harm to the patient or user by evaluating conditions that simulate clinical use.

Table 1: Uses for biomaterials

Medical Uses	Non-medical uses
Artery graft	Arrays for DNA and diagnostics
Breast implant	Bioremediation materials
Cochlear implant	Biosensors
Dental implant	Bioseparations, chromatography
Ear drainage tube	Biofouling-resistant materials
Feeding tube	Biomimetics for new materials
Glaucoma drainage tube	Cell culture
Hydrocephalous shunt	Controlled release for agriculture
Intraocular lens	Electrophoresis materials
Joints (hip, knee, shoulder)	Fuel cells (biomass)
Keratoprosthesis	MEMS
Left ventricular assist device (LVAD)	Muscles (artificial) and actuators
Mechanical heart valve	Nanofabrication
Nerve guidance tube	NEMS
Ophthalmic drug delivery device	Neural computing/biocomputer
Pacemaker	Smart clothing for biowarfare
Renal dialyzer	Yeast array chip
Stent	
Tissue adhesive	
Urinary catheter	
Valve, heart	
Wound dressing	
X-ray guide	
Zirconium knee joint	

1.2 Polymeric biomaterials

Polymers make up by far the broadest and most diverse class of biomaterials, making the medical market, the fourth largest area of plastic application. Polymers have been widely used for biomedical applications due to their favourable structure-property relationships (Tanazawa 1993). Both biopolymers and synthetic polymers are used as biomaterials. Since the density and mechanical properties of many of the synthetic polymers resemble those of biological tissues, more closely than metals, they are most extensively used as biomaterials. The wide applicability of synthetic polymers are also due to the availability in a wide variety of chemical compositions and physical properties, ease of fabrication into complex shapes and structures, easily tailored surface properties and favourable cost performance ratio.

Although hundreds of synthetic polymers are available, only ten or twenty polymers are

mainly used in medical device fabrications from disposables to long term implants. The success of a polymeric biomaterial depends on factors such as material properties, design and biocompatibility and hence these aspects should be rigorously satisfied.

The main disadvantage of polymeric biomaterials for long-term application is their susceptibility to degrade in biological environment. The degradation products of these polymers may be toxic to tissues. Degradation can also lead to change in molecular weight and structure of the polymers, resulting in deterioration of mechanical properties and stability. Table 2 shows some of the polymers currently used as biomaterials and their applications (Vert 2007).

Table 2: Some polymers currently used clinically and some of their applications

Polymers	Biomedical applications
Poly(methyl methacrylate)	Rigid contact lenses, intraocular lens
Polymeric compounds based on methyl methacrylate	Acrylic cements for orthopedy and odontology, facial prostheses, joint surgeries, filling of bone cavities and porous bony tissues
Poly(2-hydroxyethyl methacrylate)	Flexible contact lenses, plastic surgery, hemocompatibility of surfaces
Nylon-type polyamides	Sutures
Poly(vinyl chloride)	Blood pushes, catheters
Poly(ethylene terephtalate)	Vascular protheses, cardiac valves
Polytetrafluoroethylene	Orthopedy, vascular clips
Polyurethanes	Catheters, cardiac pumps
Silicones	Plastic surgery, tubes, oxygenators

1.3 Radiopaque polymers for medical applications

Radiopaque polymeric agents find extensive use in medicine such as implants, catheters, medical adhesives, to detect changes within body cavities, diagnose various disease states, monitoring of embolization process and in dentistry for prosthetic applications, such as dentures or restorative resins. It would be highly desirable if these polymeric biomaterials could be assessed in a non-invasive manner so as to evaluate their performance. X-radiography being a quick, reliable, non-destructive, non-invasive and low cost technique is commonly employed in medical imaging.

Based on casting shadows, radiographic imaging technique incorporates the principle of radiopacity, which is the physical property of absorbing X-rays. This technique depends on variations in densities between a specimen and its surroundings. X-rays are characterized by an electromagnetic vibration with a wavelength ranging from 12 to 0.06 $\mathring{A}$ and energy varying between 10 to 100 keV. Absorption occurs by the interaction of X-ray photons with electrons in the specimen, promoting these electrons to higher energy levels at a rate proportional to the concentration of electrons in the specimen, and thus gives rise to radiopacity observed in X-ray films. This absorption is described by the equation:

$$\text{Electrons per mL} = \rho N(Z/A) \tag{1}$$

where ρ = density; N = Avogadro number; A = atomic weight; Z = atomic number. Since $Z/A \sim 0.5$ and N is a constant, the electron concentration depends only on the density of the material. The predominant component of X-ray absorption in the employed energy range is contributed by the photoelectric effect. The relationship between the photoelectric absorption of X-rays and the atomic number of an element is approximated by the equation:

$$\mu = k\lambda^3 Z^4 + 0.2 \tag{2}$$

where μ is the absorption coefficient, k = a constant, λ = wavelength, Z = atomic number, 0.2 = average coefficient of scattering. It is apparent from the dependence of X-ray absorption on Z^4 that a high atomic number is associated with effective X-ray absorption. As a consequence, elements having high atomic numbers such as iodine, bismuth, barium, bromine and tantalum are most frequently used to obtain high radiopacity.

The imaging technique used with radiopaque polymers is the usual roentgenographic technique. The various specimens are radiographed by X-rays and the resulting images obtained are compared with the opacity of an aluminium wedge exposed on the same radiographic film. Radiopacity equivalent to 2.0 mm of aluminium is the required standard for the radiopacity of plastics for medical purpose.

Conventional polymers cannot be detected by commonly used imaging techniques like X-ray and ultrasound because they contain elements such as carbon, hydrogen, oxygen and

nitrogen which exhibit both low electron density and low specific gravity. Sharp X-ray imaging is obtained with materials having high electron density. Therefore, research on methods for increasing the average electron density and the specific gravity of polymers by incorporating heavy elements would lead to the development of novel radiopaque polymers.

Early methods for imparting radiopacity to polymeric systems involved the incorporation of heavy-metal salts as physical mixtures. A major drawback of this method was the creation of non-homogenous mixtures, resulting in the deterioration of the end product. To overcome this problem, single phase radiopaque polymer-salt complexes were produced by the incorporation of a radiopaque heavy metal salt into an appropriate polymer ligand via chelation. However, the physico-mechanical properties and biocompatibility of the base polymer were often adversely affected due to leaching of the non- covalently bound radiopacifying agents. Another approach to make the polymer radiopaque was to covalently link a radiocontrast dye to the polymer. This approach was possible only if the polymer possessed a reactive functional group to which the dye could be attached. The most promising approach to develop polymers with inherent radiopacity would be to covalently bind the radiopacifying elements with the monomers prior to their polymerization. Physico-mechanical and radiocontrast properties of the polymer are not compromised by this method and the secondary release of radiopacifying agent is also prevented.

1.4 Objectives and scope of the investigation

Polyurethanes have been employed as blood contacting materials for a number of years because of their excellent mechanical properties that can be tuned for specific applications. Polyurethanes find applications in total artificial heart, heart valves, mammary implants, pace-maker connectors, suture materials and matrices for controlled drug release. Polyurethanes exhibiting X-ray contrast properties would have obvious advantages over their non-radiopaque counterparts in medical and related applications. Although numerous approaches have been made to impart radiopacity to polymers, there are not much reports on radiopaque polyurethanes with iodinated chain extenders. Iodine is preferred to impart radiopacity in polymers for two reasons. Firstly the greater mass attenuation coefficient of the former, and secondly iodinated compounds are the opacifying agents of modern nonionic X-ray contrast media and toxicolog-

ical aspects of such systems are well documented.

We therefore felt it worthwhile to examine the possibility of coupling iodine-containing compounds having radio contrast properties to polyurethanes to make them X-ray opaque. Polyurethanes could be made radiopaque by employing chain extenders containing radiopacifying elements like iodine. It would be possible to introduce sufficient concentration of the radiopacifying elements by using polyiodinated chain extenders. Therefore, one of the objectives of the study was to explore the development of radiopaque polyurethanes. In the case of radiopaque polymers meant for medical applications, the physico-chemica properties are very important and intoducing heavy elements such as iodine into the polymer backbone should not make the properties worse. The suface properties of the polyurethanes after iodination would be examined by IR, EDX and contact angle measurements. The mechanical properties would be determined to assess their suitability for various applications. The polyurethanes will also be subjected to X-rays to assess their visibility during clinical applications.

Polyurethanes are known to trigger mechanisms of blood coagulation and thrombus formation and tend to degrade upon long term implantation. Numerous approaches have been tried to modify polyurethanes to enhance bio- and blood compatibility and biostability. None of the commercial or experimental polyurethanes available at present fulfills all the requirements for the ultimate biomedical material. In this work, attempts have been made to study the biostability of radiopaque polyurethanes in different physiological media and by accelerated chemical degradation. In order to examine the potential of polyurethanes for biomedical applications, *in vitro* and *in vivo* biocompatibility studies will be carried out on the most promising modified polyurethanes.

Acrylic polymers that contain epoxy functional groups as pendant units have been studied extensively due to the ability of epoxide groups to enter into a large number of chemical reactions. Copolymers based on glycidyl methacrylate (GMA) are thus of great value for binding enzymes and other biologically active species. Acrylate copolymers are also used as dental and bone cement and in ophthalmology. A major drawback of these copolymers is that they are radiolucent, since polymers hardly absorb X-ray radiation due to the absence of heavy elements within their structure. Yet, when a polymeric biomaterial is used inside the body (either

temporarily or permanently), it is often required that the material can be visualized through X-ray fluoroscopy. Various methods have been employed in an attempt to give acrylate polymers some degree of X-ray opacity so as to use for medical and dental applications, however, aesthetic and/or mechanical properties usually suffer to such an extent that the polymer would be clinically unacceptable. The only satisfactory method to impart radiopacity in acrylic polymer is to utilize a halogen-containing monomer, or carry out a post-polymerization halogenation of reactive pendant groups.

In this respect, it is of interest to synthesize several radiopaque copolymers composed of GMA and other methacrylate monomers such as methyl methacrylate (MMA), ethyl methacrylate (EMA) and butyl methacrylate (BMA) for their potential use in biomedical applications. The copolymers would be characterized with respect to composition, molecular weight, thermal properties, and radiopacity. The vital aim of the work is to evaluate the materials for biomedical applications. With this intention, tissue and blood compatibility and cytotoxicity of the polymers will examined with the most promising iodinated copolymer.

The detailed objectives of the investigation are:

1. To synthesize and characterize iodinated aliphatic and aromatic diols which can be used as chain extenders for the preparation of radiopaque polyurethanes.

2. To prepare radiopaque polyurethanes based on aliphatic and aromatic diisocyanates by employing different polyols and iodinated chain extenders.

3. To characterize the polyurethanes for physico-chemical properties viz molecular weight, surface properties using FTIR, EDX and contact angle, thermal properties using TGA, mechanical properties viz tensile properties and toughness, viscoelastic properties using dynamic mechanical analysis and X-ray visibility.

4. *In vitro* studies on the biostability of these polyurethanes in simulated physiological media by aging in hydrolytic ionic media, hydrolytic enzyme (papain and its buffer) solution and in harsh chemical conditions.

5. To synthesize radiopaque copolymers based on GMA-MMA, GMA-EMA and GMA-BMA.

6. To characterize the radiopaque copolymers with respect to composition, molecular weight, thermal properties, and radiopacity.

7. *In vitro* biological evaluation of radiopaque polyurethanes and radiopaque copolymers for cytotoxicity, cell viability and blood material interaction (hematology).

8. *In vivo* toxicological studies of most promising radiopaque polyurethane and radiopaque copolymer for intracutaneous irritation and subcutaneous implantation for biocompatibility and hitocompatibility.

9. To identify potential biomedical applications of newly synthesised radiopaque polymers.

LITERATURE REVIEW

2 LITERATURE REVIEW

2.1 *Radiopaque polymeric systems*

Polymers possessing X-ray contrast properties are classified in to three main prototypes such as radiopaque polymer blends, radiopaque-polymer salt complexes and polymerization products of radiopaque monomers.

2.1.1 Radiopaque polymer blends

Radiopaque polymer blends were prepared by incorporating the radio-opacifying agents such as heavy metal powders, inorganic salts of a heavy element, or organic compounds containing a heavy atom substituent as physical mixtures with the polymer (Silberman-Hazony 1988). These physical mixtures formed heterogenous dispersions with an uneven distribution of the radiopacifying agent. The most commonly used heavy elements were iodine, barium, bismuth, zircon and tantalum (Combe 1972, Chandler et al. 1971*a*, Tanaka et al. 1993, Watts 1987, Rawls et al. 1992, 1990, Taira et al. 1993). The incompatibility of low polarity resins with highly polar ionic substances lead to the deterioration of the physical and mechanical properties of the polymer because of phase separation. Permanent radiopacity could not be ensured by these blends because radiopacifying agents were not chemically incorporated into the polymer and with time the radiopaque additives progressively leached out.

Isobutyl 2-Cyanoacrylate along with a radiopaque additive was introduced as a polymerizing agent for the endovascular treatment of arteriovenous malformations (Zanetti & Sherman 1972). Based on the observation of a low potential to have a mutagenic effect in rats, Isobutyl 2-Cyanoacrylate is no longer produced (Vinters et al. 1985, Brothers et al. 1989). As a substitute for Isobutyl 2-Cyanoacrylate, a chemically similar monomer such as N-butyl 2-cyanoacrylate with a nonionic contrast agent (Lipiodol) was proposed (Brothers et al. 1989). Use of additives for rendering cyanoacrylates radiographically visible interfered with the polymerization time and inability to position the implant precisely into a target limited the use of this system. The monomer methyl cyanoacrylate was inserted in the presence of an X-ray opaque agent into the oviduct and polymerized in situ as part of a sterilization technique to block the fallopian tubes in females (Hoffmann 1982). The radiopaque material indicated the location

and degree of blockage. Esters of cyanoacrylic acid were polymerized in the presence of urogranoic acid having iodide substituents and used for medical adhesion of joints and insertion of catheters. The radiopacifying compound leached out and the product was radiopaque only for 3-5 months.

Poly(methyl methacrylate) has been used widely in dental applications as resins, dentures, restoratives, impression materials and in dental surgery as bone cement for partial or total joint replacement and as a percutaneous biomaterial for vertebroplasty (Chang 1981, Chandler et al. 1971*a*, Rawls et al. 1990). Radiopaque dental materials have been developed to locate and remove accidentally ingested dental resins (Willems et al. 1991, Omer et al. 1986, Espelid et al. 1991). Metal inserts such as gold gauze and lead foil have been employed to develop dental methacrylic resins (Davy & Causton 1982). However, these resins were of little use as the additives degraded the dentures. Small quantities of finely divided inorganic salts have been added to Poly(methyl methacrylate) to make it radiopaque. Most of these salts were incompatible with the polymer and the addition of inorganic fillers to Poly(methyl methacrylate) did not modify hardness, solubility, and water absorption of the material to a great extent, but it increased the compressive strength and reduced transverse strength (Chang 1981). It also decreased the optical translucency and resulted in the concentration of stresses leading to fracture (Chandler et al. 1971*b*). Metal salts of vinyl monomer such as barium and zinc acrylates have been copolymerized with methyl methacrylate to impart radiopacity to the dental implants, but the ionic nature of these resins lead to significant absorption of water and subsequent hydrolysis resulting in the loss of the opacifying atom (Williams et al. 1993).

A formulation based on the addition of silane-treated radiopaque glass to Poly(methyl methacrylate) was developed, but the product was fragile and it caused tooth breakage (Chandler et al. 1971*b*,*a*,*c*). Organo iodine compounds were added to Poly(methyl methacrylate) to encounter the problems associated with inorganic salt additives (Combe 1969). Even though the radiopacity was satisfactory, the photostability of the material was reduced. Barium salts are frequently used as an additive commercially for dental resins and bone cements, though it reduced the mechanical strength and fracture toughness of bone cement (Molino & Timmie Topoleski 1996). Toxicological screening of the derivative indicated low toxicity, but in

situ polymerization caused problems associated with residual monomer and the exothermic reaction. Although the radiopaque agents did not give serious toxic effects initially, it showed subsequent toxicity due to its secondary release in soft tissues and systemic absorption by the host. Bismuth and barium-containing glasses have been used as X-ray opacifying media in Poly(methyl methacrylate), but the amount of glass required significantly increased the weight of the resin and made it brittle (Combe 1971).

Poly(methyl methacrylate) has also found application in vertebroplasty procedures. Vertebroplasty involved the percutaneous injection of Poly(methyl methacrylate) cement into a lesion of a vertebral body under radiological control (Jensen et al. 1997, Cotten et al. 1998). Powder Poly(methyl methacrylate) and liquid methyl methacrylate were combined prior to injection and radiopacity of the system was ensured with tantalum or tungsten salts. The side effects observed were inflammatory reaction, heat related damage and embolic potential associated with leakage of monomer into perivertebral spaces and draining veins.

Non-biodegradable Poly(methyl methacrylate) microspheres impregnated with barium sulphate was synthesized for their potential use in embolization (Thanoo & Jayakrishnan 1989). These radiopaque microspheres were found to be very stable in aqueous medium without significant leaching of the radiopacifying agent. Radiopaque microspheres encapsulated with 40-50% barium sulphate have been developed from Poly(2-hydroxyethyl methacrylate) too for their use as particulate emboli in endo vascular embolization (Jayakrishnan et al. 1990). However, long term stability of the microspheres were not addressed in the study.

Poly(vinyl alcohol) and poly(vinyl acetate) particles were also used as embolic material (Berenstein & Kricheff 1979). The radiopacity was imparted by the addition of inorganic salts or iodinated contrast medium. Poly(vinyl alcohol) microspheres impregnated with barium sulphate and methyl iothalamate have been evaluated as radiopaque particulate emboli (Thanoo et al. 1991). Silicone resins have been cured in an aqueous medium using Poly(vinyl alcohol) as suspension stabilizer to generate microspheres for embolization technique (Thanoo & Jayakrishnan 1991). Incorporation of radiopacifying agents such as barium sulphate or methyl iothalmate was found to destabilize the suspension, where as radiopaque tantalum powder could be encapsulated without affecting the stability, although it led to the formation of larger

microspheres. Cellulose acetate incorporated with barium oxide as radiopacifying agent has also been used for the embolization of aneurysms (Kinugasa et al. 1992, 1994). The material showed a low viscosity and precipitated as hard mass in aqueous media.

Radiopaque polyurethanes are commercially available as heterogenous dispersions with additives like barium sulphate, bismuth subcarbonate or certain metals such as tungsten. The main disadvantages of additive incorporated polyurethanes are degradation and leaching resulting in the systemic absorption by the host, deterioration of mechanical and radio contrast properties. Radiopaque polyurethane-silicone network resin compositions prepared as blends of polyurethane, diiodo or tetraiodo benzoates have been reported (Flynn 1986, 1981).

Patent literature also describes the use of polyurethane coatings containing radiopaque metal powders such as tin, lead and bismuth, for use in catheters (Jeckel & Glen Falls 1967). Addition of metals results in the acceleration of the urethane reaction, thereby limiting the pot-life of the biomaterial. Small amount of diglycolic acid is used along with the polyurethane coatings to control or halt the catalytic action of the heavy metal powder on the urethane reaction. Optically transparent and radiopaque catheters were also produced by blending a small amount of a diol of tetrabromopthalic anhydride and a plasticizer with the polyurethane employed in forming the catheter (Slingluff 1973, 1975). Goossens & Molari Jr (1980) suggested that optically clear radiopaque catheters could be formed from terpolymers of polycarbonatepolydiorganosiloxane having carbonate, halocarbonate and polydiorganosiloxane constituents.

Tantalum loaded polyurethane microspheres possessing radiopacity were developed for embolization and they proved that the size distribution of microspheres was not adversely affected by the addition of the radiopacifying agent (Thanoo & Jayakrishnan 1991). Toxicity studies were limited to demonstrating the non hemolytic behaviour of these radiopaque microspheres in *in vitro* tests.

2.1.2 Radiopaque polymer salt complexes

The polymer salt complexes were basically proposed to control dental filling radiologically using acrylic resins. These systems were produced by the incorporation of a radiopaque, heavy metal salt into an appropriate polymer ligand by chelation (Rawls et al. 1990). These

complexes were homogenous and possessed both polymeric and ionic character. The types of polymers used as ligands typically constitute a polar section that can strongly bind cations and a non-polar part that allows solubilization in non-polar media. This property enables the ligands to dissolve inorganic salts in low polarity organic media.

The polymeric ligands can chelate a wide range of inorganic salts, such as barium, cesium, bismuth, lead or mercury derivatives, in order to produce single phase systems. Limiting release and constant radiopacity are ensured by entrapment of the polymer-salt complex in a crosslinked interpenetrating network. These complexes are not covalently bound and the halogenated functional groups might tend to hydrolyze or degrade and consequently, form leachable compounds. Release of radiopaque salts is favored in the presence of acidic environment of living tissues. In addition to the potential toxic effects, the leaching of bromide and other halogens leads to discolouration of the resin, converting the polymer to a radiolucent material. The presence of halogenated organic additives may also contribute to the degradation of the polymer.

Copolymers of methyl methacrylate and poly(methoxyethylene glycol) with an average of 22 ethylene oxide units were synthesized and chelated with barium bromide (Xia et al. 1985). Permanent radiopacity was not achieved with these derivatives, and this limited their potential for clinical application. Polymer salt complexes possessing X-ray contrast property were prepared by cation-chelating monomers to achieve complete solubilization of heavy metal salts (Smid et al. 1987). Heavy metal compounds were chelated or solubilized to obtain a single phase with the macromolecular structure of the resin having optical transparency and stability. Homogenous materials of high radiopacity have been obtained with Poly(methyl methacrylate) and other acrylic based resins. Blends of Poly(methyl methacrylate) and heavy metal salts were developed by dissolving bismuth tribromide and bismuth chloride in methyl methacrylate (Smid et al. 1987, Rawls et al. 1990). The high solubility of the salt resulted from the interaction between the carbonyl group and bismuth. Poly(methyl methacrylate) containing an organobismuth radiopacifying additive such as triphenyl bismuth have also been reported (Rawls et al. 1996, Delaviz et al. 1990, Smid et al. 1993, 1990). A minimum of 23% weight of halogenated derivative was necessary to obtain the same radiopacity as the aluminium standard. The bis-

muth compound acted as a plasticizer reducing the glass transition temperature and a slight elevation in cytotoxicity was described due to a reduction in monomer conversion.

Radiopaque miscible polymer coordination complex of poly(methyl methacrylate) and uranyl nitrate has been reported (Smid et al. 1989) (Cabasso & Sahni 1990). The polymer does not degrade through the main chain and the biocompatibility has not been evaluated, nor has the long-term stability of the complex *in vivo* been established. Radiopaque coordination complex of bismuth bromide and uranyl hexahydrate with polymers prepared from acrylated phosphoryl esters has also been developed for biomedical applications (Cabasso & Sahni 1990). The phosphoryl group was selected to provide stronger coordination sites for the bismuth and uranium salts and to impart adhesive properties toward hard tissues. Preliminary biocompatibility data indicated satisfactory performance, but the polymer does not degrade through the main chain and the long term stability of the complex *in vivo* have not been reported.

2.1.3 Radiopaque polymerised monomers

Theses systems are produced by introduction of the radiopacifying elements covalently as a substituent into the polymer backbone. Here the radiopaque derivative is molecularly bound as a structural unit and such polymers could form clear, homogenous, non leachable materials with mechanical properties, which are substantially equivalent to that of parent polymer.

Dental resins of acceptable radiopacity were prepared from acrylic materials such as barium acrylate by adding acrylic acid to a suspension of barium hydroxide in water (Combe 1971). The water soluble barium acrylate formed was polymerized by heating in the presence of ammonium persulfate as initiator. The poly(barium acrylate) synthesized showed extensive crosslinking due to the presence of divalent barium ions. Barium acrylate was also copolymerized with methyl methacrylate to form dental cements. However, the addition of barium weakened and reduced the impact and transverse strength of resins, even though it had very little effect on the hardness. Crosslinked structure of poly (barium acrylate) resulted in harder and brittle polymers and affected their solubility. Barium induces higher brittleness in the dental resins and increases the quantity of monomer required to form satisfactory dental dough. Moreover, the ionic nature of the barium acrylate lead to the absorption of significant amounts of water and the slow hydrolysis of the resin resulted in the loss of the opacifying atom (Davy

& Causton 1982).

Monomers containing phenyl bismuth moiety was also used as radiopacifiers in the preparation of dental resins (Smid et al. 1990, Ignatious, Sein, Delaviz, Cabasso & Smid 1992, Ignatious, Delaviz, Cabasso & Smid 1992). Transparent, hard materials were obtained by copolymerizing methyl methacrylate and styryldiphenyl bismuth with benzoyl peroxide as initiator. Permanent, chemical incorporation into the polymer structure prevented the leaching out of the heavy metal X-ray contrast agent in any kind of solvent. Identical copolymerizations were obtained with other monomers such as styrene and other vinyl monomers (Delaviz et al. 1990). Poly(methyl methacrylate) was made radiopaque by the incorporation of bromine into the Poly(methyl methacrylate) resin (Davy & Causton 1982). The radiopacifying agent 2,3-dibromo propyl methacrylate was synthesized by refluxing methacrylic acid and 2,3-dibromopropanol in toluene. 2,3-dibromo propyl methacrylate was homopolymerized to obtain polymer with high radiopacity, but unfortunately it was very brittle. Inorder to improve the mechanical properties of the brominated polymer, poly (2,3-dibromo propyl methacrylate) was copolymerized with methyl methacrylate using azo-isobutryl nitrile as initiator. The synthesized copolymers had a crosslinked structure and were hydrophobic in nature. Upon increasing the poly (2,3-dibromo propyl methacrylate) content, the effect on the impact strength and tensile strength was minimal until 60% bromine content. The flexural strength decreased continuously while the elasticity modulus increased proportionally to the content of the brominated polymer.

Grafting of iodine-containing molecules onto preformed high molecular weight polymers have been investigated so as to prepare radiopaque hydrogel spherical particles by acylation of poly(2-hydroxyethyl methacrylate) beads using triiodobenzoic acid derivatives or non-toxic contrast media used clinically, such as iothalmic acid and iopanoic acid (Horak et al. 1998, Jayakrishnan et al. 1990). Homopolymerization and copolymerization of iothalmic ester of poly(2-hydroxyethyl methacrylate) with hydroxyethyl methacrylate and methyl methacrylate failed to give high molecular weight polymers, probably because of the presence of bulky iodine atoms in the monomers which stearically hinder the polymerization propogation step. In contrast, copolymerization of the triiodobenzoic acid derivative with hydroxyethyl methacrylate was possible. Sufficient radiopacity was obtained by binding 25 to 30% weight of iodine, even

though it was possible to bind more than 40% weight of iodine to the resin backbone. However, such microspheres with high iodine content were found to be hard, brittle, less hydrophilic, and non-swelling. The incorporation of iodine into the resin backbone did not decrease the water absorbing capacity of the microspheres. Non radiopaque microspheres possessed equilibrium water content of about 62%, while microspheres containing 29% weight of iodine had an equilibrium water content superior to that of microspheres with 40% weight of iodine, even though many of the reactive hydroxyl groups had been used in the acetylation reaction. This high water absorbing capacity was attributed to the highly porous structure of the microspheres. Preliminary subcutaneous implantation studies of the iodinated microspheres in rats or rabbits did not demonstrate adverse tissue reactions. Histological examination indicated that radiopaque emboli did not cause any damage or inflammation to the surrounding tissue. The blood compatibility screening has shown that the iodinated microspheres were non hemolytic but they promoted blood coagulation and would be suitable only for patients with pronounced blood hypocoagulation.

Radiopaque microspheres were also formed by the dispersion polymerization of the monomer 2-methacryloyloxyethyl(2,3,5- triiodobenzoate) in 2-methoxyethanol as the continuous phase and polyvinyl-pyrrolidone as the stabilizer (Galperin & Margel 2006). The radiopacity of these particles dispersed in water and in the dry state was demonstrated with an imaging technique based on X-ray absorption usually used in hospitals. These microspheres possessed a relatively narrow size distribution and may be used for different X-ray imaging purposes, such as blood pooling, embolization, dental composition, implants, prostheses, and nanocomposites.

Iodinated radiopaque polymeric nanoparticles of sizes ranging between 30 and 350 nm were synthsised by emulsion polymerization of the monomer 2-methacryloyloxyethyl(2,3,5-triiodobenzoate) in the presence of sodium dodecyl sulfate as surfactant and potassium persulfate as initiator for X-ray imaging applications (Galperin et al. 2007). The polymeric nanoparticles were composed of approximately 58% by weight iodine, and were therefore expected to possess significant radiopaque nature. *In vitro* radiopacity of the iodinated nanoparticles of 30.6$\pm$5.0 nm diameter, dispersed in water and in the dry state, was demonstrated with a CT scanner. *In vivo* CT-imaging performed in a dog model by intravenous administration

of the uniform 30.6±5.0 nm diameter radiopaque nanoparticles dispersed in saline demonstrated significant enhanced visibility of lymph nodes, liver, kidney and spleen. The results indicated that the nanoparticles may be useful as new efficient contrast agents for X-ray imaging applications. The nanoparticle aqueous dispersion, however, was not stable and tended to agglomerate, particularly at weight concentration of dispersed nanoparticles above ~0.3%. To overcome this limitation, iodinated radiopaque copolymeric nanoparticles were synthesized by the emulsion copolymerization of 2-methacryloyloxyethyl (2,3,5-triiodobenzoate) and glycidyl methacrylate (Aviv et al. 2009). *In vivo* CT preliminary trials with the nanoparticles dispersed in a 5% dextrose aqueous continuous phase demonstrated the possible clinical use of these particles as contrast agents for medical X-ray imaging applications. Significant enhanced visibility of the blood pool, lymph nodes, and the liver and spleen of model animals has been demonstrated. The efficiency of these copolymeric nanoparticles for the diagnosis of cancerous liver has also been illustrated.

Radiopaque micron sized non-crosslinked polystyrene-poly(2-methacryloyloxyethyl(2,3,5-triiodobenzoate) particles of narrow size distribution were prepared by a single-step swelling of uniform polystyrene template microspheres with emulsion droplets of methylene chloride containing the initiator benzoyl peroxide and the iodinated monomer 2-methacryloyloxyethyl(2,3,5-triiodobenzoate), followed by the polymerization of the monomer within the swollen template particles (Galperin & Margel 2006). Radiopaque micron-sized uniform crosslinked polystyrene/poly(2-methacryloyloxyethyl(2,3,5-triiodobenzoate)-divinylbenzene) composite particles were prepared similarly with emulsion droplets of methylene chloride containing divinylbenzene, in addition to the initiator and the iodinated monomer. The radiopacity of these iodinated particles was demonstrated by an imaging technique based on X-ray absorption usually used in hospitals.

X-ray visible injectable polymeric microspheres were prepared from methyl methacrylate and 2-[2',3',5'-triiodobenzoyl]oxoethyl methacrylate for their potential utility as traceable bulking agents (Saralidze et al. 2003). Biocompatibility of injected radiopaque microspheres was investigated *in vivo* by using the mouse as a model (Emans et al. 2005). Microspheres were injected subcutaneously or intramuscularly and examined after 3 months. X-ray fluoroscopy gave clear images of the microspheres as an ensemble, and it was found that no migration

occurred during the 3 months. Histopathology confirmed that all microspheres stayed close to the site of the injection and it was well tolerated by the animal. Injectable radiopaque and surface functionalized polymeric microparticles based on a methacrylate monomer with covalently bound iodine atoms have been reported for their applications as a biomaterial in different clinical fields, such as cosmetic surgery, reconstructive surgery, and urology (Saralidze et al. 2003). These particles featured excellent cytocompatibility *in vitro* and *in vivo*. A method to engineer the surface of the particles through immobilization of collagen to control anchoring of the particles in soft tissue has also been explored.

Intrinsically radiopaque hydrophilic microspheres were developed by suspension polymerization from radiopaque monomer 2-[4-iodobenzoyl]-oxo-ethyl methacrylate and hydroxyethyl methacrylate or 1-vinyl-2-pyrrolidinone as hydrophilic component (van Hooy-Corstjens et al. 2007). It has been shown that for clinically relevant X-ray visibility the spheres should contain at least 20% weight of iodine. The advantage of these microspheres over commercially available embolization particles was that the fate of the spheres could be monitored using X-ray fluoroscopy. It would enable the interventional radiologist to determine precisely the site of injection and the amount of spheres that has to be injected to obtain sufficient occlusion without the risk of migration. A further advantage would be to ascertain postoperatively the position of the spheres radiographically.

Acrylic acid derivatives of iodine containing compounds such as triiodophenol and iothalmic acid were prepared with a view of producing radiopaque polymers for medical applications were found to be highly resistant to homopolymerization and copolymerization with monomers such as hydroxyethyl methacrylate and methyl methacrylate under normal conditions by free radical polymerization (Jayakrishnan & Thanoo 1992). This was attributed to the bulky nature of iodinated aromatic nucleus sterically hindering the propagation step during polymerization. However, the monomers in concentrations up to 25% weight could be polymerized with hydroxylethyl methacrylate in the presence of small concentrations (1-5% weight) of a cross linking agent such as ethyleneglycol dimethacrylate. While triiodo phenyl methacrylate based polymers were found to be hemolytic in nature, polymer based on iothalmic acid was found to be non hemolytic in *in vitro* tests. Introduction of a spacer

arm between the bulky halogenated aromatic nucleus and the vinyl group reduced the steric hindrance in the next generation of radiopaque polymers (Horak et al. 1997, Benzina et al. 1996). This allowed the facile homopolymerization and copolymerization of monomers such as 2-methacryloxyethyl(2,3,5-triiodobenzoate), 2-methacryloxypropyl(2,3,5-triiodobenzoate) and 3-(methacryloylamidoacetamido)-2,4,6 triiodobenzoic acid with hydroxyethyl methacrylate or methyl methacrylate by dispersion, suspension, and bulk polymerization (Horak et al. 1997, Benzina et al. 1996, Emans et al. 2005, Davy & Causton 1982).

Radiopaque hydroxyethyl methacrylate have been used as a terpolymer composed of 2-[p-iodobenzoyl]-ethyl methacrylate, methyl methacrylate and hydroxylethyl methacrylate (Benzina et al. 1994). Radiopaque iodomethacrylate was obtained in standard esterification reaction between hydroxylethyl methacrylate and 4-iodo benzoic acid. The radiopaque terpolymer exhibited low *in vitro* thrombogenicity and microscopic evaluation after subcutaneous implantation in rats showed that this derivative was well tolerated in subcutaneous tissues. The combined results revealed that the terpolymer was very suitable for prosthetic applications in the cardio vascular system.

Radiopaque polymeric materials were synthesised from methyl methacrylate, hydroxyethyl methacrylate and a radiopacifying agent having covalently bound iodine atoms for their possible use as radiopaque implant biomaterials (Kruft et al. 1994). The radiopacifying agent was prepared from 4-iodo phenol and methacryloyl chloride. Thrombogenicity of the polymers was determined by an *in vitro* thrombin generation test procedure and the materials exhibited low thrombogenicity. The materials were visible even as fibers with a diameter of approximately 0.3 mm using routine imaging X-ray techniques. The radiopaque polymers exhibited promising properties with respect to applications as construction materials for endovascular stents.

Iodinated aromatic methacrylate monomers were prepared by the esterification of hydroxyl containing methacrylate esters with 2,3,5 triiodobenzoic acid or by the ring opening nucleophilic addition of various halogenated phenols or 2,3,5 triiodobenzoic acid to 2,3 epoxypropyl methacrylate (Davy et al. 1997). The monomers were soluble in a variety of mono and dimethacrylate esters and could be thermally polymerized to give clear X-ray opaque polymers to be used in denture base acrylics and orthopedic bone cement applications. In these sys-

tems, the iodinated aromatic molecules were introduced into the polymer as a side group via ester linkages. Upon *in vitro* degradation, the radiopaque iodinated aromatic compound was cleaved and excreted, leaving the non-radiopaque polymethacrylate chain.

Iodinated and brominated derivatives of aromatic dihydroxy monomers were prepared and polymerized to form radiopaque polycarbonates and polyarylates to be used as medical implants and drug delivery devices (Kohn et al. 2002, Kohn & Zeltinger 2005). The monomers were also employed as radiopacifying, biocompatible, non-toxic additives for other polymeric biomaterials. These systems covalently bind the iodinated portion into the main chain of the polymer, yielding polymers with mechanical properties comparable to the parent polymers. As these systems were based on polycarbonates, they were slow degrading relative to other polymer systems such as polyanhydrides. Poly(anhydride esters) based on iodinated versions of salicylic acid were synthesized to generate radiopaque biomaterials (Carbone et al. 2008). Cytotoxicity studies using mouse fibroblasts indicated that iodinated salicylate based poly(anhydride esters) were biocompatible with cells at low polymer concentrations.

Iodine containing methacrylate monomer, 3,4,5-triiodobenzoyloxyethyl methacrylate was synthesized by coupling 2-hydroxy ethyl methacrylate with 3,4,5 triiodo benzoic acid (Lakshmi et al. 2003). Homopolymerization and copolymerization of the monomer with methyl methacrylate was carried out using 2,2'-azobis isobutyronitrile as the initiator. A terpolymer of 3,4,5-triiodobenzoyloxyethyl methacrylate, methyl methacrylate and hydroxylethyl methacrylate were also synthesized. The polymers were found to be freely soluble in common solvents for acrylic polymers. These copolymers could be used in medical and dental areas where radiopacity would be a desirable feature of the implants.

Radiopaque iodine containing bone cement has been prepared from a copolymer of methyl methacrylate and 2-[4-iodobenzoyl]-oxo-ethylmethacrylate (van Hooy-Corstjens, Govaert, Spoelstra, Bulstra, Wetzels & Koole 2004). The composition of the iodinated cement was adjusted such that similar handling properties and radiopacity as for the commercial cement were obtained. The intrinsic mechanical behaviour of the radiopaque was far superior to the commercially available bone cements.

Acrylic radiopaque cements have been synthesized from an iodine containing methacry-

late, 2,5-diiodo-8-quinolyl methacrylate (Vázquez et al. 1999). The cement could be formulated by the incorporation of the radiopaque additive to the liquid phase in concentrations of 5 or 7.5% weight with respect to the liquid phase. This concentration was sufficient to render the cement radiopaque and it provided good values of curing parameters and an improvement in mechanical properties with respect to cements containing 10% weight of barium sulphate. Biocompatibility was studied by implantation of rods of the cements into rats and histological analysis of the surrounding tissue indicated that only a very thin fibrous capsule was formed around the rod implanted without any chronic inflammatory response.

Polymers with inherent radiopacity based on the copolymers of methyl methacrylate and 2-[2',3',5'-triiodobenzoyl]oxoethyl methacrylate were evaluated for their potential applications as dental or bone cements (Zaharia et al. 2008). The copolymers were analyzed on an X-ray microcomputed tomography and they proved to be radiopaque even at low concentrations of 2-[2',3',5'-triiodobenzoyl]oxoethyl methacrylate. The biocompatibility was tested both *in vitro* and *in vivo* in the rat. These materials were found to be non toxic and were well tolerated in long term experiment by the animal.

Radiopaque acrylic bone cements based on 2-[2',3',5'-triiodobenzoyl]ethyl methacrylate and 3,5-diiodine salicylic methacrylate have also been reported and the mechanical properties of these cements were better than those of the commercially available barium sulphate-containing cement (Artola et al. 2003). The radiopacity obtained was comparable to that of the aforementioned cement and polymers exhibited showed good biocompatibility.

Vertebroplasty and balloon kyphoplasty are widely used for the augmentation of osteoporosis-induced vertebral compression fractures. Almost invariably, an injectable poly(methyl methacrylate) bone cement that contains a large amount of barium sulphate particles is used in these procedures. To avoid the deleterious effect of this radiopacifier, a highly-radiopaque iodine-containing acrylic bone cement was developed for use in augmentation of vertebral compression fractures (Boelen et al. 2008). Radiopacity was introduced by incorporating an iodine-containing methacrylic copolymer into the cement powder. This copolymer was prepared via suspension polymerization of methyl methacrylate and a methacrylic monomer containing covalently-bound iodine (2-[4'-iodobenzoyl]-oxo-ethyl methacrylate).

Radiopaque polymeric spinal cages based on an iodinated copolymer prepared from methylmethacrylate and 2-[4-iodobenzoyl]-oxo-ethylmethacrylate have been reported (van Hooy-Corstjens, Aldenhoff, Knetsch, Govaert, Arin, Erli & Koole 2004). Cytocompatibility experiments revealed that the material contained no toxic leachables and the cells could adhere to and proliferate on the iodinated copolymer. Compression experiments at physiologically relevant strains disclosed mechanical characteristics comparable to that of commercially available cages. The presence of iodine atoms ensured the X-ray visibility of the cages.

Cellulose was made radiopaque by coupling triiodobenzoic acid with the hydroxyl groups of cellulose for possible applications as embolic agents (Mottu et al. 2002). A sheep model was used to assess *in vivo* the radiopacity and precipitation properties of a highly concentrated solution of cellulose acetate 2,3,4-triiodobenzoate mixed ester. Cellulose acetate iodobenzoate mixed esters dissolved in diglyme or dimethyl isosorbide could be useful embolic liquids for the treatment of cerebral aneurysms or arteriovenous malformations. Iodinated starch suspension has also been prepared as a model particulate hepatic contrast agent (Cohen et al. 1981). The triiodobenzoyl derivative of starch, containing about 61% of covalently bound iodine, forms a stable suspension. The biodistribution study demonstrated that satisfactory contrast was obtained in the liver and spleen as the material was taken up by the reticuloendothelial cells. Limited acute toxicity studies were performed in mice and it was found to be non toxic.

Radiopaque polymer hydrogels were prepared from iodine containing poly(methyl methacrylates) for their potential use in biomedical applications (Kruft et al. 1997). The monomers were ortho and para-iodo and 2,3,5-triiodobenzoic acid esters of 2-hydroxymethyl methacrylate, and the para-iodophenol ester of methyl methacrylic acid. The monomers were copolymerized with one or more non-iodinated analogs and a small amount of crosslinkers to produce polymer hydrogels with varying iodine contents. It was reported that the hydrogels were well tolerated by subcutaneous tissue and the presence of iodine did not severely alter the swellability of the hydrogel. No tissue necrosis, abscess formation or acute inflammation was observed, although all implants were surrounded by a fibrous capsule. However, these materials also did not degrade through the main polymer chain, and upon side chain ester cleavage, became radiolucent due to loss of iodine atoms in the ester side chain.

In order to pursue a possibility of application of radiopaque polymer hydrogels to vascular embolization, studies were done on iodine-containing copolyanion and their hydrogels with polycation via formation of polyion complexes (Okamura et al. 2002). Acrylamide derivatives having triiodophenyl and carboxyl groups were synthesized and copolymerized with sodium styrene sulfonate. Hydrogels were prepared by mixing aqueous solutions of the obtained radiopaque copolyanions and polyallylamine. Embolization was examined by injection of these hydrogels into vein of a removed porcine kidney as a preliminary test for transcatheter arterial embolization for hepatocellular carcinoma. It was found that the hydrogels prepared from the copolycation gave high X-ray contrasts of the vein and remained there, though copolycations with low molecular weights had a tendency to drain through the capillaries to the peripheral tissues.

Radiopaque degradable Poly(vinyl alcohol) hydrogels were synthesized by replacing 0.5% of the pendent alcohol groups on the Poly(vinyl alcohol) with 4-iodobenzoylchloride (Mawad et al. 2007). This level of substitution rendered the polymer adequately radiopaque and could be assessed by X-ray fluroscopy. The subsequent modification of 0.8% of the pendent hydroxyl groups with an ester acrylate functional group allowed for crosslinking of the macromers. The radiopaque hydrogels degraded over a time span of 140 days. Rheology data suggested that the macromer solutions were appropriate for injection.

Biodegradable Poly(ϵ-caprolactone-co-α-iodo-ϵ-caprolactone) having X-ray contrast property was synthesized by binding iodine to Poly(ϵ-caprolactone) chain bearing carbanionic site on α-position of carbonyl groups using lithium diisopropylamide (Nottelet et al. 2006). Covalent binding of an iodine atom on a Poly(ϵ-caprolactone) backbone was achieved by a rapid and easy two steps one pot substitution reaction of iodine on the macropolycarbanion formed by addition of lithium diisopropyl amide on Poly(ϵ-caprolactone). Substitution degrees up to 25% were obtained. Iodinated Poly(ϵ-caprolactone) being a radiopaque degradable aliphatic copolyester containing no additional radiopaque filler, is expected to have potential applications in temporary reconstructing material or drug delivery system. The new Poly(ϵ-caprolactone) based copolymer also exhibited good mechanical strength and radiopacity.

Poly (urethane urea) was rendered radiopaque by attaching 3,4,5 triiodobenzoic acid onto

polymer backbone (James & Jayakrishnan 2007). By optimizing the reaction conditions, the modifications were carried out without adversely affecting the properties of the starting polymer significantly. Though the radiopaque polymer exhibited altered thermal characteristics, the degradation behavior remained unchanged. The observed changes in thermal characteristics were explained on the basis of possible inter-phase mixing and the changes in the close packing of the polymer chains by the introduction of bulky iodine atoms.

Grafting of iodine containing molecule such as N-(2,6-diiodocarboxy phenyl)-3,4,5 triiodo benzamide onto commercially available medical grade polyurethane, Tecoflex 80A have also been investigated for their potential use as vascular stents and catheters (James et al. 2006). The end product obtained showed about 8% weight of iodine in the polymer and radiopacity was almost equivalent to that of a 2mm thick aluminium wedge. However, the modified polymer showed reduced thermal stability compared to the starting polymer. This anomaly was explained on the basis of the reduced extent of intermolecular hydrogen bonding among the hard segments of the end product.

Implantable medical devices fabricated from polyurethanes with grafted radiopaque groups have also been investigated (Wang 2008). Stents having radiocontrast properties were prepared from degradable polymer segments having diurethane linkages with radiopaque functional groups such as triiodo benzoyl chloride or triiodo benzoic acid. However, the reaction sites available in the urethane groups (secondary amino functional groups) of polyurethane for grafting radiopacifiers were limited to achieve the required degree of radiopacity.

Polyurethanes prepared using halogenated polyols and halogenated polyisocyanates as reactants are disclosed in patents (Cambron et al. 1988, Onwumere 2003). Although these polyurethanes provide sufficient radiopacity, they have a high flex modulus and are not as soft as desired in simulated physiological medium. Optically transparent radiopaque polyurethanes that are dimensionally stable under standard sterilization conditions would be desirable as externally communicating devices such as catheters. Stability of the polyurethanes could be enhanced by using halogenated chain extenders.

Patent literature describe the synthesis of radiopaque polyurethanes using halogenated chain extenders like tetrabromodipentaerythritol, dibromoneopentyl glycol and tetrabromo-

bisphenol A bis(2-hydroxyethyl ether) (Markush & Sarpeshkar 1993, 1994). When using brominated chain extenders, the polymer must have minimum amount of bromine (approximately 15%) to impart useful radiopaque properties. Although a bromine content of at least about 10 or 15% by weight was sufficient to confer radiopacity, it has been found that a bromine content of less than about 20% by weight on the particular brominated chain extender resulted in optically opaque or translucent polyurethanes. Adequate bromine content can be achieved only by increasing the amount of chain extender which results in a relatively large hard segment ratio of the polyurethanes leading to stiffness and toxicity.

It would be particularly advantageous to make medical or dental devices from a polymeric material in which the radiopaque component is incorporated as a structural unit of the polymer. Because the radiopacity is obtained from halogenated moieties contained within the structure of the polymer, these moieties do not leach out and create non-uniformities in the polymer.

MATERIALS AND METHODS

3 MATERIALS AND METHODS

3.1 Materials

2-Butyne-1,4 diol, dibutyltin dilaurate (DBTDL), 4,4'-methylenebis(phenyl isocyanate) (MDI) and hexamethylene diisocyanate (HDI) were obtained from Sigma-Aldrich, MO, USA and used as such. Polytetramethylene glycol (PTMG) (Terathane® molecular weight 1000) (Sigma-Aldrich) and Polypropylene glycol (PPG) (molecular weight 1000) (Sigma-Aldrich) were degassed at 50 °C under reduced pressure for 24 h. Bisphenol-A (4,4'-isopropylidenediphenol) (BPA) (Sigma-Aldrich) was recrystallized from dry toluene before use. Diethyl ether, Hexane, Ethyl acetate, Ethylene chlorohydrin, Potassium carbonate, Potassium iodide, sodium bicarbonate, and cuprous iodide were procured from S.D Fine Chemicals, Mumbai, India and used as such. Dimethyl formamide (DMF) (S.D Fine Chemicals, Mumbai, India) was vacuum distilled and kept over 4Å molecular sieves prior to use.

GMA (Sigma-Aldrich), MMA (Sigma-Aldrich), EMA (Sigma-Aldrich) and BMA (Sigma-Aldrich) were washed free of the inhibitor using sodium hydroxide solution followed by water, dried over anhydrous sodium sulfate, and distilled under reduced pressure prior to use. Iodine (Merck, Darmstadt, Germany) and catalyst o-phenylenediamine (Merck) were used as such. Initiator 2,2'-azobis(isobutyronitrile) (AIBN) (Merck) was purified through recrystallization from methanol before use. Tetrahydrofuran (THF) (Merck) was refluxed over sodium and distilled prior to use. Methanol (Merck) and Dichloromethane (Merck) were used as supplied without any further purification.

3.2 Methods

3.2.1 Synthesis and characterization of iodinated chain extenders

3.2.1.1 Synthesis and characterization of aliphatic chain extender 2,3 diiodo-2-butene-1,4 diol

Polyurethanes can be made radiopaque by employing iodinated chain extenders with X-ray contrast properties. Patent literature disclose the versatility of 2,3-diiodo-2- butene-1,4-diol (IBOL) as a parent compound of an antimicrobial derivative, 2,3-diiodo-1,4-dithiocyano-2-butene (Merianos 1978). The chain extender IBOL used for the preparation of radiopaque

polyurethanes was synthesised from 2-butyne-1,4-diol as per the general procedure by Merianos and John (Priebe 2002). In a round bottom flask, 10.75 g (0.12 mol) of 2-butyne-1,4 diol was stirred with about 125 mL of water and 2.5 g cuprous iodide, surrounded by an ice bath. Cold solution of 32.5 g (0. 25 mol) iodine in 250 mL of 20% aqueous potassium iodide was added to the reaction mixture. Stirring was continued for overnight at room temperature and then the mixture was filtered under suction, washed with 250 mL of cold 5% sodium bicarbonate solution and distilled water. The yield of product obtained after reaction was around 42 g, which is almost 100% conversion and yield.

The crude IBOL was recrystallized out of a mixture of organic solvents such as ethyl acetate and hexane in various proportions. Melting point was determined using a Barnstead mel-temp apparatus (Barnstead- 1002D, USA). NMR (^{1}H and ^{13}C) analysis of IBOL was done using a Bruker 300 MHz instrument (Bruker AC-300, USA) with TMS as the internal standard and DMSO d^{6} as the solvent.

3.2.1.2 Synthesis and characterization of aromatic chain extender bis hydroxyethyl ether of tetraiodo bisphenol A

Iodination of BPA to synthesize tetraiodo bisphenol A was carried out according to the procedure reported by Kevin and Edgar for iodination of phenols (Edgar & Falling 1990). BPA, 2.00g (0.0087 mol) was dissolved in 50 mL of methanol. Six equivalents of sodium iodide (7.8 g, 0.052 mol) and two equivalents of sodium hydroxide (0.69 g, 0.0176 mol) were added and the solution was cooled to 0 °C. Aqueous sodium hypochlorite (98.3 mL, six equivalents, 0.052 mols of sodium hypochlorite) was then added dropwise over 75 min at 0 - 3 °C. As each drop hit the solution, a red color appeared and faded almost instantly, resulting in a yellow colored solution. It was stirred for 1 h at 0-2°C and was then treated with 20 mL of 10% aqueous sodium thiosulphate and was precipitated by adding 10% HCl. A white solid was formed which was further washed with methanol to remove unreacted BPA, as BPA dissolves in methanol while tetraiodo bisphenol A does not.

Iodinated bisphenol A (2g, 0.0027 mol), ethylene chlorohydrin (0.47 g, 0.0059 mol) and anhydrous potassium carbonate (2.23 g, 0.0162 mol) were placed in a minimum amount of DMF and heated at 90 °C for 21-24 h. This was then brought to room temperature, poured

into an excess of water, and extracted with diethyl ether. The organic layers were combined and washed first with a dilute sodium hydroxide solution and then with water. The diethyl ether layer was dried over anhydrous sodium sulfate and concentrated to get a pale yellow solid.

The crude IBPA was purified by column chromatography with 30% ethyl acetate in hexane as the eluant to get a white, crystalline solid. Melting point was determined using a Barnstead mel-temp apparatus (Barnstead- 1002D, USA). NMR (^{1}H and ^{13}C) analysis of IBPA was done using a Bruker 300 MHz instrument (Bruker AC-300, USA) with TMS as the internal standard and $CDCl_3$ as the solvent.

3.2.2 Synthesis and purification of radiopaque polyurethanes

All the polyurethanes were synthesized by the conventional two step pre-polymer method using solution polymerization technique. The synthesized polymers were extracted repeatedly with hot ethanol in Soxhlet extractor to remove catalyst and the leachable low molecular weight oligomers, if any.

3.2.3 Synthesis of polyurethanes using aliphatic chain extender 2,3 diiodo-2-butene-1,4 diol.

Aromatic and aliphatic polyurethanes were prepared by the conventional two-step solution polymerization using 2,3- diiodo-2- butene-1,4-diol (IBOL).

3.2.3.1 Aromatic polyurethanes (PTMGMDIBOL & PPGMDIBOL)

4,4'-Methylenebis(phenyl isocyanate) (MDI), polyols, PTMG & PPG, and chain extender, IBOL were used for the synthesis of aromatic polyurethanes (PTMGMDIBOL & PPGMDIBOL). In a typical synthesis, MDI, 1.61 g (0.0063 mols) and PTMG or PPG, 2.90 g (0.0029 mols) were dissolved in 25 mL DMF in a round bottom flask and the reaction was carried out at 80 $^{\circ}C$ for 4 h with constant stirring under nitrogen atmosphere. DBTDL (0.5 wt% of reactants) was then added. 1.15 g (0.0034 mols) of IBOL dissolved in 25 mL of DMF was added through a pressure-equalized addition flask. The reaction was continued at the same temperature for 1 h and then cooled to room temperature. The reaction mixture was kept under stirring at ambient conditions for another 18 h. Polyurethanes (PTMGMDIBOL or PPGMDIBOL) were precipitated

from cold water, washed with methanol and dried.

3.2.3.2 Aliphatic polyurethane (PTMGHDIBOL & PPGHDIBOL)

Hexamethylene diisocyanate (HDI), polyols, PTMG & PPG and chain extender, IBOL were used for the synthesis of aliphatic polyurethane (PTMGHDIBOL & PPGHDIBOL). In a typical synthesis, PTMG or PPG, 2.90 g (0.0029 mols) dissolved in 25 mL DMF, 1.27 g (0.0075 mols) of HDI, 1.15 g (0.0034 mols) of chain extender IBOL dissolved in 25 mL DMF and DBTDL (0.5 wt % of reactants) were used. The reaction conditions narrated for aromatic polyurethanes were followed.

3.2.4 Synthesis of polyurethanes using aromatic chain extender bis hydroxyethyl ether of tetraiodo bisphenol A.

Polyurethanes were prepared by the conventional two-step solution polymerization using bis hydroxyethyl ether of tetraiodo bisphenol A (IBPA)

3.2.4.1 Aromatic polyurethanes (PTMGMDIBPA & PPGMDIBPA)

4,4'-Methylenebis(phenyl isocyanate) (MDI), polyols, PTMG & PPG, and chain extender, IBPA were used for the synthesis of aromatic polyurethanes (PTMGMDIBPA & PPGMDIBPA). PTMG or PPG (1.2g, 0.0012 mol) dissolved in 5 mL DMF, MDI (0.66 g, 0.0026) dissolved in 20 mL DMF, IBPA (1.14g, 0.0014) and DBTDL (0.5 wt % of reactants) were used. The reaction conditions narrated for aromatic polyurethanes were followed.

3.2.5 Characterization of radiopaque polyurethanes

3.2.5.1 Fourier transform infrared spectra (FTIR)

The IR spectra of the polyurethanes were recorded using a FTIR spectrophotometer (Nicolet, Impact 410, USA) having an ATR-KRS5 accessory.

3.2.5.2 Gel permeation chromatography (GPC)

GPC measurements were performed on a Jasco HPLC system having PU2080 pump, rheodyne injector, phenogel columns, Borwin software and Merck LaChrom RI detector L-

7490. DMF was used as mobile phase at a flow rate of 1 mL/min.

3.2.5.3 X-ray diffraction (XRD)

XRD analyses were carried out with Siemens D-5005 diffractometer, using Cu Kα radiation at an operating voltage of 40 KV, 30mA current and a 2θ range of 10 to 90°.

3.2.5.4 Energydispersive X-ray analysis (EDX)

EDX analyses of polyurethanes were conducted using an EDX attached to an Environmental Scanning Electron Microscope (FEI, Quanta 200, The Netherlands).

3.2.5.5 Elemental analysis

Elemental analyses of iodine were performed at Service Central d' Analyse, Centre National de la Recherche Scientifique, Solaise, France.

3.2.5.6 Thermal analyses

The TGA of polyurethanes were carried out using SDT-2960, TA Instruments Inc, USA, thermogravimetric analyzer. Measurement was performed in nitrogen atmosphere at a heating rate of 10°C/min.

3.2.5.7 Dynamic mechanical analysis (DMA)

Dynamic mechanical analysis was measured using Tritec 2000B DMA (Triton Technology Limited, UK). Polyurethane films were tested in tensile mode at a scan rate of 1°C/min, frequency of 1 Hz and temperature range from -150 to 150°C.

3.2.5.8 Determination of mechanical properties

The mechanical properties were determined by tensile property experiments using an Instron model 3345 testing machine. Tests were performed as per standards using a crosshead speed of 100 mm/min, an extensometer and a load cell of 100N [ISO 527-5A, Appendix].

3.2.5.9 Studies on X-ray opacity

The radiopacity of polyurethanes was assessed using a standard clinical General Electric X-ray instrument equipped with 2.5 mm aluminium filtration set at 40 KvP with 10 mA current for 0.2 s. The relative X-ray opacity of the polyurethanes was determined visually by comparison with an aluminium step wedge (0.5-3 mm thick in 0.5 mm steps) and non-iodinated polyurethanes (2 mm thickness) exposed on the same radiograph. The polymeric films having 2mm thickness were radiographed and the resulting images were compared with the opacity of 2 mm thick aluminium wedge, a widely used radiographic standard (Mottu et al. 1999).

The greyness of the films in the resulting image was measured using Photoshop CS® (Adobe Inc. software). The X-ray absorbance was determined as A = 255-grey level and ranged from 0 (no X-ray absorption) to 255 (100% absorption). The radiopacity of the films was then quantitatively evaluated as the ratio of their greyness to that of aluminium (thickness 2 mm) as reported elsewhere (Boelen et al. 2008).

3.2.5.10 Studies on surface properties under aqueous conditions

Studies on surface characteristics of the polyurethanes were carried out by investigating the contact angle in aqueous media in dynamic conditions. Dynamic contact angle analysis was performed by the Wilhelmy plate technique, in deionised water at 25°C. A tensiometer KSV SIGMA model 701 was used. Neat and clean polyurethane strips (5x1x0.05cm) free from surface heterogeneity were used. The rate of immersion-emersion was 5 mm/min in water and immersion depth was 5 mm in standard conditions. The measurements were the average of 5 contact angle measurements of the polyurethane film. The dynamic surface changes was investigated by incubating the polyurethane films in deionised water for 24h at 25°C.

3.2.6 Studies on *in vitro* biostability of polyurethanes

The *in vitro* biodurability of the polyurethanes was evaluated by aging the materials in simulated biological environment. The polymers were aged in simulated physiological fluid, Ringers solution, Phosphate Buffered Saline (PBS) and enzyme media. Biodurability of the polyurethanes was also evaluated using an oxidizing agent. Experiments were done as per

ISO standards [ISO 10993-13, Appendix]

3.2.6.1 Evaluation of *in vitro* hydrolytic stability

The *in vitro* hydrolytic stability of the polymers was evaluated by aging the polymers in Ringers solution and PBS (Appendix). PBS was prepared as per the standard procedures (Dawlee et al. 2005). Solutions of 0.2 M disodium hydrogen phosphate and 0.2 M sodium dihydrogen phosphate in 0.9% saline were prepared separately and mixed in the ratio 77:23 (v/v). The pH was adjusted to 7.4 by adding dilute solution in distilled water (1:2) of disodium hydrogen phosphate (if acidic) or sodium dihydrogen phosphate (if alkaline). Ringer's solution (Appendix) was prepared as per the procedure by (Ritchie et al. 1990).

Accurately weighed polymer films (10 mm diameter & 2 mm thickness; n=3) were immersed in the hydrolytic media in screw capped bottles and aged for 6 months at 37°C. To ensure the activity of the media, the media were changed with fresh solution for every 7 days interval. The aged samples were washed in distilled water, vacuum dried and hydrolytic degradation was assessed by FTIR spectral analyses. The aged polymers were also tested for any weight loss. The percentage weight loss was calculated by the formula,

$$\text{Weight loss} = \left(\frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \right) \times 100$$

3.2.6.2 Evaluation of *in vitro* oxidative stability

The polymers were aged in oxidative medium, 3% H_2O_2 solution as per the ISO protocol. Accurately weighed polymer samples were immersed in the medium and aged at 37°C for 6 months. The aged polymers were tested as described in the previous section.

3.2.6.3 Evaluation of degradation in hydrolytic enzyme

In order to investigate the biostability in hydrolytic enzyme environment, the polymers were aged in *in vitro* papain medium and its buffer (control). 10 mg of papain enzyme (2-8 units/mg solid; 1 unit can hydrolyse 1.0 mol -NH-CO- group) was used in 10 mL HEPES buffer of pH 6.8 (Appendix). The accurately weighed polymer films (n=3) having diameter 10 mm & 1

mm thickness were immersed in enzyme and also in buffer (control) media and aged 37°C for 6 months. The activity of enzyme was maintained by adding fresh enzyme (10 mg) for every 3 days. The aged samples were rinsed with distilled water, vacuum-dried at 60°C and characterized by FTIR analysis. The aged polymers were also evaluated for change in weight.

3.2.6.4 Studies on accelerated chemical degradation of polyurethanes

The stability of polymers under accelerated chemical degradation was evaluated using harsh chemical treatment. Aging of polymers in boiling water for 100 h and also in boiling alcoholic potassium hydroxide (0.5 N) for 4h were carried out (Thomas & Muthu 2008). Weight loss and visible changes of samples were noted.

3.2.7 Synthesis and purification of radiopaque acrylic copolymers

Glycidyl methacrylate-based acrylate copolymers were synthesized using GMA and acrylic monomers such as MMA, EMA and BMA. In an RB flask, a mixture of 0.02 mol of GMA and 0.0004 mol of AIBN were dissolved in 15 mL of THF. Then 0.02 mol of acrylic comonomer (MMA or EMA or BMA) was added to the RB flask and the mixture was flushed with nitrogen for 30 min. Each flask was then kept in an oil bath maintained at 70°C for 24 h with constant stirring. The copolymers were precipitated from methanol, washed with methanol several times and dried under vacuum at room temperature for 24 h. The glycidyl methacrylate-based acrylate copolymers based on MMA, EMA and BMA are coded as P(GMA-co-MMA), P(GMA-co-EMA) and P(GMA-co-BMA) respectively.

Glycidyl methacrylate-based acrylate copolymers were converted to inherently radiopaque acrylate copolymers by regioselective ring opening of epoxide groups of GMA units and subsequent covalent attachment of elemental iodine. In a 100 mL RB flask, 0.01mol of glycidyl methacrylate-based acrylate copolymer [P(GMA-co-MMA) or P(GMA-co-EMA) or P(GMA-co-BMA)] was dissolved in 15 mL of dichloromethane. To this, 0.001 mol of o-phenylenediamine was added as the catalyst and 0.01 mol of iodine dissolved in 25 mL of dichloromethane was then added through a pressure equalized addition funnel in drops. The reaction was then kept at room temperature overnight with constant stirring. After the reaction, iodinated copolymer was precipitated out from methanol. It was washed again with methanol, dried under vacuum

at room temperature for 24 h and yield noted . The iodinated copolymer was then dissolved in dichloromethane and cast to form films of desired dimensions for further characterization. The radiopque copolymers based on MMA, EMA and BMA are represented as IP(GMA-co-MMA), IP(GMA-co-EMA) and IP(GMA-co-BMA) respectively.

3.2.8 Characterisation of radiopaque acrylic copolymers

3.2.8.1 Fourier transform infrared spectra (FTIR)

The IR spectra of the copolymers were recorded using a FTIR spectrophotometer (Nicolet, Impact 410, USA) as KBr pellets.

3.2.8.2 Nuclear magnetic resonance spectra (NMR)

^{1}H and ^{13}C NMR spectra were recorded using a Bruker 300 MHz instrument (Bruker AC-300, USA) with TMS as the internal standard and CDCl3 as the solvent.

3.2.8.3 Gel permeation chromatography (GPC)

The molecular weights of the copolymers were determined by GPC measurements using Waters HPLC system with Styragel HR columns, and R401 Differential refractometer. THF was used as the mobile phase at a flow rate of 1 mL/min.

3.2.8.4 Energydispersive X-ray analysis (EDX)

EDX analyses of polymers were conducted using an EDX attached to an Environmental Scanning Electron Microscope (FEI, Quanta 200, The Netherlands).

3.2.8.5 Elemental analysis

Elemental analyses of iodine were performed at Service Central d' Analyse, Centre National de la Recherche Scientifique, Solaise, France.

3.2.8.6 Thermal analyses

The thermal stability was determined by thermogravimetric analysis using SDT-2960 Instruments Inc, USA, thermogravimetric analyzer. Measurements were performed in nitrogen atmosphere at a heating rate of 10°C/min. DSC measurements were conducted with DSC-2960, TA Instruments Inc. The calorimeter was calibrated with indium metal as a standard. Measurements were performed in nitrogen atmosphere at a heating rate of 10°C/min.

3.2.8.7 Studies on X-ray opacity

X-radiographs were obtained using a standard clinical General Electric X-ray instrument equipped with 2.5mm aluminium filtration set at 40 KvP with 10 mA current for 0.2 s. X-ray opacity of the iodinated copolymers was evaluated by the same method as described for polyurethanes. The greyness of the films in the resulting image was measured using Photoshop CS® (Adobe Inc. software) and radiopacity was determined quantitatively.

3.2.9 Studies on biocompatibility of radiopaque polymers

In vitro biocompatibility of the newly synthesized polymers was assessed by cell culture tests for cytotoxicity and hemocompatibility studies. Clean and sterile polymers were used for the studies.

3.2.9.1 Cleaning and Sterilization of Polymers

Since ultrasonic cleaning is known to improve the surface quality, the polymeric samples were cleaned ultrasonically. Polymeric samples (purified by Soxhlet extraction with methanol & ethanol) with required dimension and quantity (as per standard) were sonicated for 20 min in 0.5% liquid soap solution (Cleansol®, International Biological Laboratories, India) and 5 min each twice in distilled water. Samples were rinsed three times after each sonication using distilled water. Cleaned wet samples were dried at 50 °C for 48 h in clean room facility. The samples were packed, sealed (separately for each analysis) and sterilized using ethylene oxide gas.

3.2.9.2 Evaluation of *in vitro* cytotoxicity

In vitro cytotoxicity testing was done using the direct contact method with the polymers based on ISO standards [ISO 10993-5, Appendix]. *In vitro* cytotoxicity was evaluated using circular disc samples, 6 mm diameter and 1mm thickness. Test samples, negative controls (high density polyethylene) and positive controls (copper wire) in triplicate were placed on subconfluent monolayer of L929 mouse fibroblast cells. L929 cells were subcultured from stock culture (National Centre for Cell Sciences, Pune, India) by trypsinization and seeded onto multi-well tissue culture plates (Nunc, Denmark). Cells were fed with Dulbecco's minimum essential medium supplemented with bovine serum and incubated at 37 °C in 5% carbon dioxide atmosphere. When the cells attained a monolayer, the material was kept in contact with the cells in triplicate.

After incubation of cells with test samples at 37 °C for 24 h, cell culture was examined microscopically (Leica, WILD MPS32, Germany) for cellular response around test sample. Cellular responses were scored as 0, 1, 2, and 3 according to non-cytotoxic, mildly cytotoxic, moderately cytotoxic and severely cytotoxic.

Cytotoxicity of the polymers was quantitatively assessed further by MTT assay (Balakrishnan & Jayakrishnan 2005) which measures the metabolic reduction of yellow colored 3-(4,5-dimethylthiazol-2yl)-2,5,diphenyl tetrazolium bromide to a purple coloured formazan by viable cells. Toxicity was evaluated on the extract of the material (0.1 cm^2/mL for iodinated polyurethanes & 0.1 g/mL for iodinated copolymers) in medium containing serum. MTT dissolved at a concentration of 0.05g/mL in sterile PBS, filtered through a 0.22 μm filter to remove any formazan crystals was used as working solution. Cells were cultured as before in multi-well tissue culture plates and when monolayer was attained, culture medium was removed, rinsed with phosphate buffered saline (PBS) and 100 μL each of extracts of copolymer and negative control (high density polyethylene) and 100 μL of diluted phenol (positive control) were added to different pre-labeled wells containing cells. Cells with medium alone served as control. Culture medium (100 μL) was used as reagent blank. Plates were incubated for 24 h at 37°C in 5% carbon dioxide atmosphere. After 24 h, the extracts/medium was removed and 200 μL of MTT working solution was introduced using a multi channel pipette in to each

well. Plates were wrapped with aluminum foil and incubated at 95% humidified atmosphere at 37°C for 8 h. After removing the reagent solution and rinsing with PBS, 200 μL of isopropanol was added to each well and incubated for 20 min at 37°C in a shaker incubator (Labline Instruments, Melrose Park, USA). The absorbance of the resulting solution in each well was recorded immediately at 570 nm using automated micro plate reader (Bio-Tek Instruments, Vermont, USA).

3.2.9.3 Evaluation of *in vitro* blood compatibility

Blood compatibility evaluations of the polyurethanes were carried out using blood from human volunteers. Blood was collected into sodium citrate which acts as the anticoagulant in the ratio 9:1. The consumption of red blood cells (RBC), white blood cells (WBC) and platelets on contact with the sample was analyzed. The polymeric films (PTMGMDIBOL & PTMGMDIBPA) were placed in wells of tissue-culture grade polystyrene Petri dishes and wetted using PBS. Each material was exposed with 1 mL of blood for 30 min under agitation at 75 $\pm$ 5 rpm using an environmental bath shaker (Labline Instruments Inc., Illinois, USA) thermostated at 37 °C. Samples were withdrawn for analysis immediately after mixing and after 30 minutes of exposure. A well without any material was used as the reference. The consumption of RBC and WBC was evaluated using a haematology analyzer (Cobas Minos, Roche Diagnostics, France) calibrated using traceable standards.

For evaluating the effect of material on blood coagulation, partial thromboplastin time (PTT) and fibrinogen concentration were determined. Fibrinogen content was detected using Coagulation Analyzer (Diagnostica Stago, France) and was based on the principle that, when excess concentration of thrombin was added to diluted plasma, clotting time varied directly with fibrinogen concentration. The automated coagulation analyzer detects the clotting time and a calibration curve was generated using WHO traceable control plasma. From the calibration curve, the concentration of fibrinogen was determined. PTT was analyzed on samples exposed to fresh citrated human blood immediately and after 30 min of exposure. Polymeric films were equilibrated for 1 h in PBS, exposed to blood under agitation at 100 rpm using the environmental bath shaker at 37 °C. After 1 and 30 min exposure, blood was centrifuged to

obtain platelet poor plasma. Plasma was then mixed with cephalin reagent and incubated for 3 min after adding $CaCl_2$ solution to initiate clotting. Clotting time was noted using an automated coagulation analyzer (Diagnostica Stago, France). Blood compatibility evaluations were done according to ISO standards [ISO 10993-4, Appendix].

3.2.9.4 Evaluation of hemolytic potential

The haemolytic potential of the material is the measure of the extent of haemolysis that may be caused by the material when it comes in contact with blood. The total hemoglobin in the initial samples was measured using automatic hematology analyser (Sysmex-K 4500, Diamond Diagnostics, USA). The free hemoglobin liberated into the plasma after exposure was measured in each sample using HP 8453 Diode Array Spectrophotometer and the percentage hemolysis was calculated using the formula

$$\text{Percentage haemolysis} = \left(\frac{\text{Free Hb}}{\text{Total Hb}}\right) \times 100. \tag{3}$$

3.2.9.5 Evaluation of *in vivo* biocompatibility

Experiments with laboratory animals for biocompatibility were done using iodinated polyurethane, PTMGMDIBPA and iodinated copolymer, IP(GMA-co-MMA) with approval of the Institutional Animal Ethics Committee constituted under the provisions of the Prevention of Cruelty to Animals Act, 1960 (GOI). The newly synthesised polymers were extracted with methanol to remove any residual monomer and cleaned untrasonically, dried and sterilized with ethylene oxide before the evaluation of biocompatibility.

3.2.9.5.1 *In vivo* intracutaneous irritation test In order to evaluate the local tissue response to the extracts of iodinated polymers in rabbit, intracutaneous irritation test was conducted as per the ISO protocol [ISO 10993-10, Appendix]. Albino Rabbit animal models were used for the study. The physiological saline (PS) and cotton seed oil (CSO) extracts of the polymers, PTMGMDIBPA or IP (GMA-co-MMA) was aseptically injected into 5 sites with the dosage of 0.2 mL/site on the upper left hand side and right hand side of 2 rabbits. The control extracts (PS and CSO alone) were injected into 5 sites on the lower left hand side and right

hand side of the same rabbits. The grading of erythema and oedema of test and control sites of all animals at 24, 48 and 72 h were recorded.

3.2.9.5.2 *In vivo* Biocompatibility by Subcutaneous implantation Tissue compatibility of iodinated polymers PTMGMDIBPA and IP(GMA-co-MMA) were evaluated by subcutaneous implantation in Wistar rat animal models for 3 months according to ISO standards [ISO 10993-6, Appendix]. For implantation in subcutaneous tissue, six Wistar rats aged 6 weeks (weight 100-120g) were used for each polymer. Surgery was performed under anesthesia using xylaxin (5 mg/kg body wt) and ketamine (100 mg/kg body wt). Under aseptic precautions, incisions were made on the skin and subcutaneous pockets were made by blunt dissection for introduction of the polymer. In each animal, three test specimens and three control specimens (biocompatible ultra high molecular weight polyethylene, UHMWPE) were implanted. The test specimens made of the polymer films were of 10 mm in diameter and 1 mm in thickness. The test and control were inserted in the dorsal subcutaneous tissue of adult rats proximally and distally. The subcutaneous pockets were closed and the skin was secured by a suture. The rats were then caged and had free access to standard rat food and water. The rats were also subjected to X-rays to evaluate the *in vivo* radiopacity of the copolymer after 12 weeks of implantation.

The implants were extracted by sacrificing two rats every 4 weeks until 12 weeks. Samples were immediately removed, fixed by immersion in 10% buffered formalin for 24ĥ, dehydrated in ethanol, clarified in xylene and embedded in paraffin. Histological sections were obtained and stained by hematoxylin and eosin. The biological response was evaluated by assessing the macroscopic and histopathological responses as function of time. The responses to the test sample were compared to the responses obtained at the control sites.

RESULTS

4 RESULTS

4.1 Synthesis & characterization of iodinated chain extenders

Thermoplastic linear polyurethane elastomers are generally produced by the reaction of a low molecular weight hydroxyl-terminated aliphatic polyether with diisocyanates and low molecular weight diols used as chain extenders. In the present studies, iodinated chain extenders were used for the synthesis of radiopaque polyurethanes.

4.1.1 Synthesis and characterization of aliphatic chain extender 2,3 diiodo-2-butene-1,4 diol

The chain extender IBOL was synthesized by the general procedure for iodination of alkynes. Melting point of the synthesized IBOL was found to be 178 °C. The iodine content in the sample was estimated quantitatively as 74.89%. The ^{1}H and ^{13}C NMR spectra of IBOL is given in Figures 1 and 2. On the basis of the spectral data and melting point, the structure was confirmed to be that of IBOL.

^{1}H NMR (300 MHz,DMSO d^6, δ): 4.2 [-CH_2, 4H, d], 5.5 [-OH, 2H, s]

^{13}C NMR (300 MHz, DMSO d^6, δ): 73.88 [-CH_2,], 105.15 [C=C]

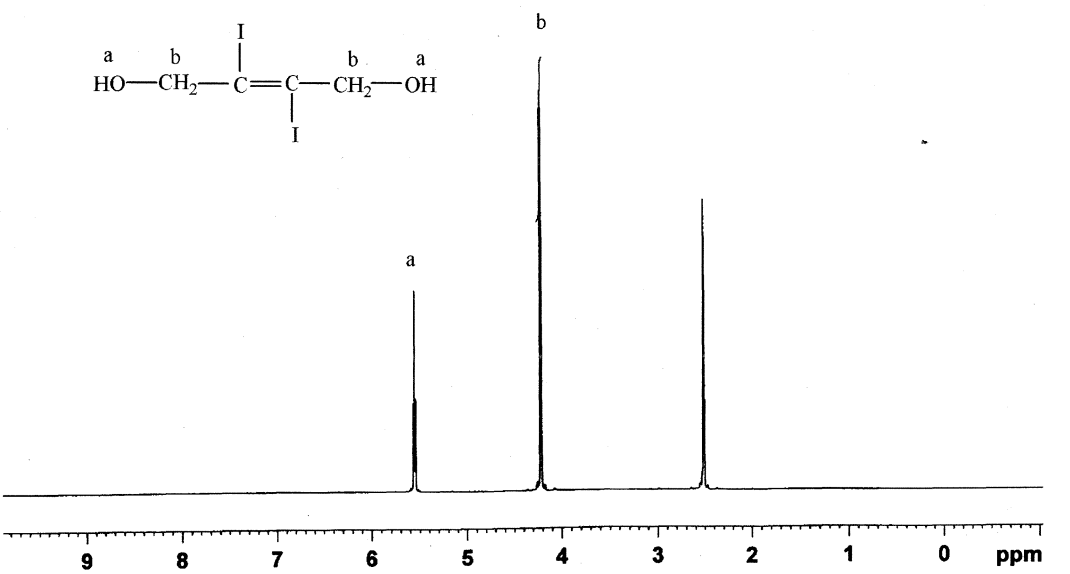

Figure 1: ^{1}H NMR Spectra of 2,3 diiodo-2-butene-1,4-diol

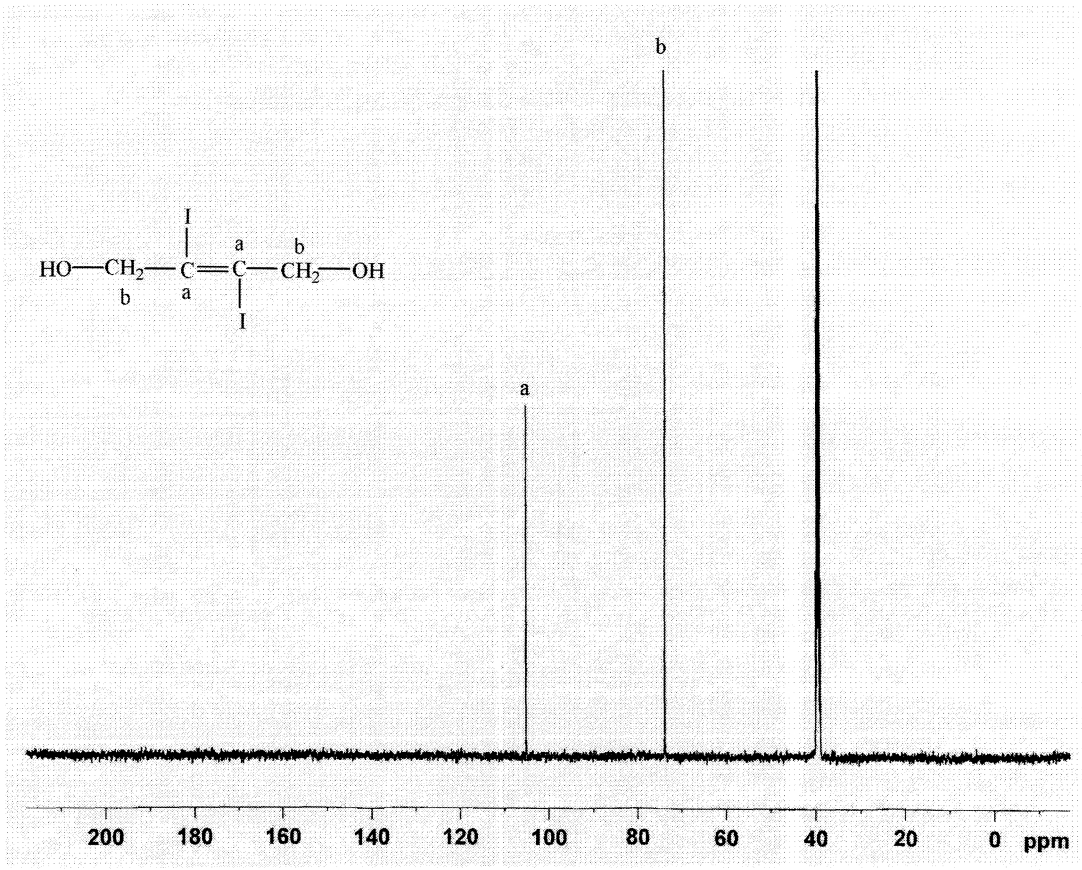

Figure 2: ^{13}C NMR Spectra of 2,3 diiodo-2-butene-1,4-diol

4.1.2 Synthesis and characterization of aromatic chain extender bis hydroxyethyl ether of tetraiodo bisphenol A

The iodinated aromatic chain extender, IBPA was synthesized using bisphenol A as the starting material. The iodine content in the sample was estimated as 59.9%. The purified IBPA with a melting point of 151-153 °C was identified further by its ^{1}H and ^{13}C NMR spectra (Figures 3 and 4).

^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.57 [$C(CH_3)_2$, 6H, s], 2.4 [-OH, 2H, s], 4.0 [-O-CH_2-CH_2-OH, 4H, t], 4.1[-O-CH_2-CH_2-OH, 4H, t], 7.55 [Ar-H, 4H, s]

^{13}C NMR (300 MHz, $CDCl_3$, δ): 30.62 [$C(CH_3)_2$], 41.45 [$C(CH_3)_2$], 62.13 [-O-CH_2-CH_2-OH], 74.27 [-O-CH_2-CH_2-OH], 90.84 [Ar-C (ortho)], 138.15 [Ar-C (meta)], 149.12 [Ar-C (quarternary)], 155.31[Ar-C (quarternary)]

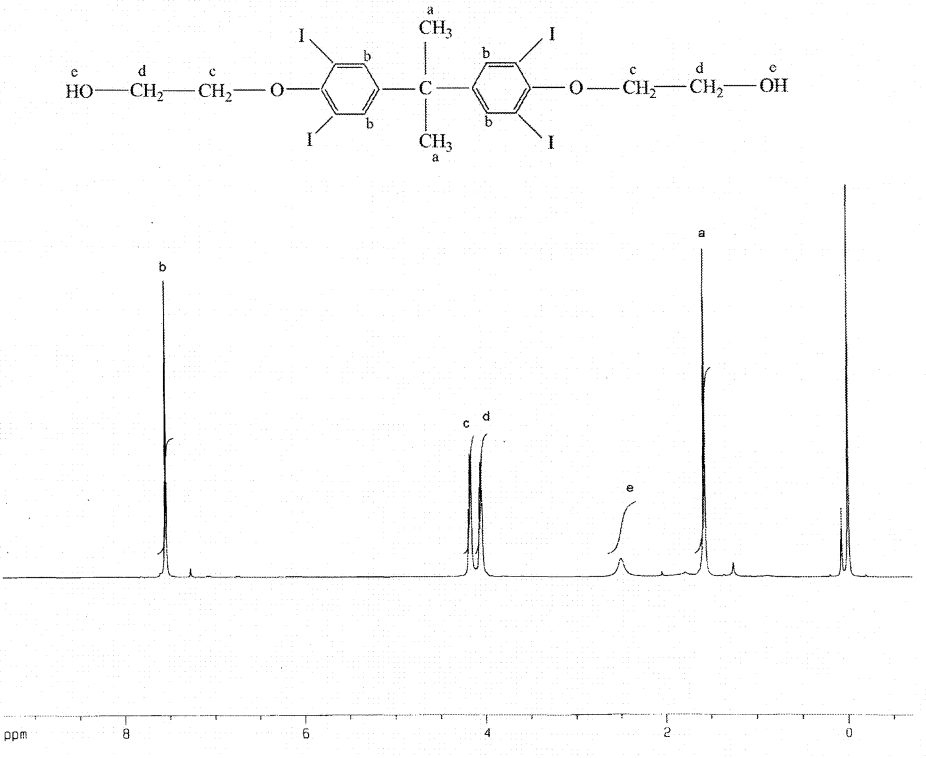

Figure 3: ^{1}H NMR Spectra of bis hydroxyethyl ether of tetraiodo bisphenol A

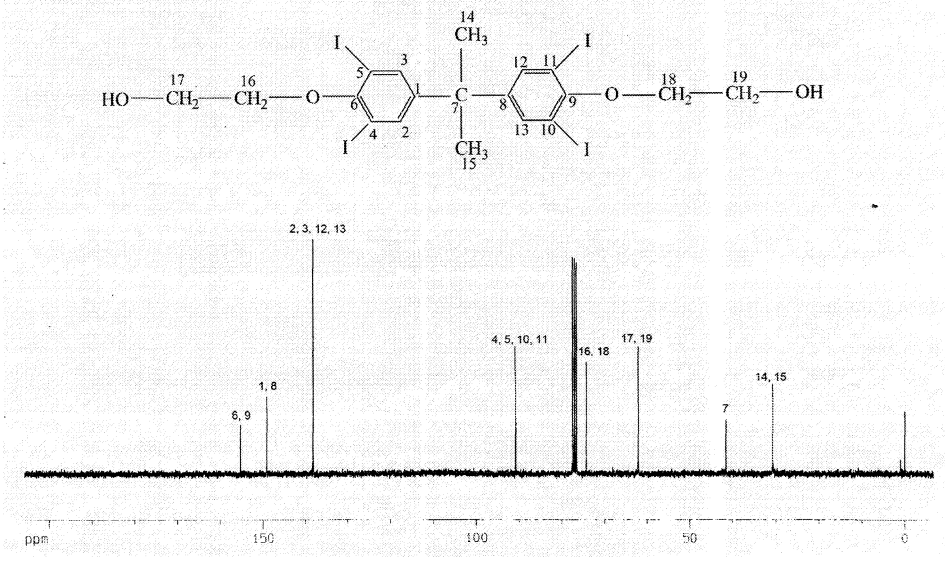

Figure 4: ^{13}C NMR Spectra of bis hydroxyethyl ether of tetraiodo bisphenol A

4.2 Synthesis of radiopaque polyurethanes

4.2.1 Synthesis of polyurethanes using iodinated chain extender, 2,3 diiodo-2-butene-1,4 diol

Aromatic and aliphatic radiopaque polyurethanes based on PTMG, PPG and IBOL have been synthesized from MDI and HDI by the conventional two-step solution polymerization reactions (Figure 5). The formulation variables for the synthesis of various polyurethanes are given in Table 3. The percentage composition of soft segment and hard segment in polyurethanes are given in Table 4.

Table 3: Formulation variables of polyurethanes based on IBOL

	Reactant Concentration (mol)				
	Diisocyanate		Polyol		Chain extender
Polyurethane	MDI	HDI	PTMG	PPG	IBOL
PTMGMDIBOL	0.0063	-	0.0029	-	0.0034
PTMGHDIBOL	-	0.0075	0.0029	-	0.0034
PPGMDIBOL	0.0063	-	-	0.0029	0.0034
PPGHDIBOL	-	0.0075	-	0.0029	0.0034

Table 4: Chemical composition of polyrethanes based on IBOL

Polyurethane	Hard segment (mol %)	Soft segment (mol %)	Isocyanate index
PTMGMDIBOL	76.98	23.01	1.0
PTMGHDIBOL	78.98	21.01	1.2
PPGMDIBOL	76.98	23.01	1.0
PPGHDIBOL	78.98	21.01	1.2

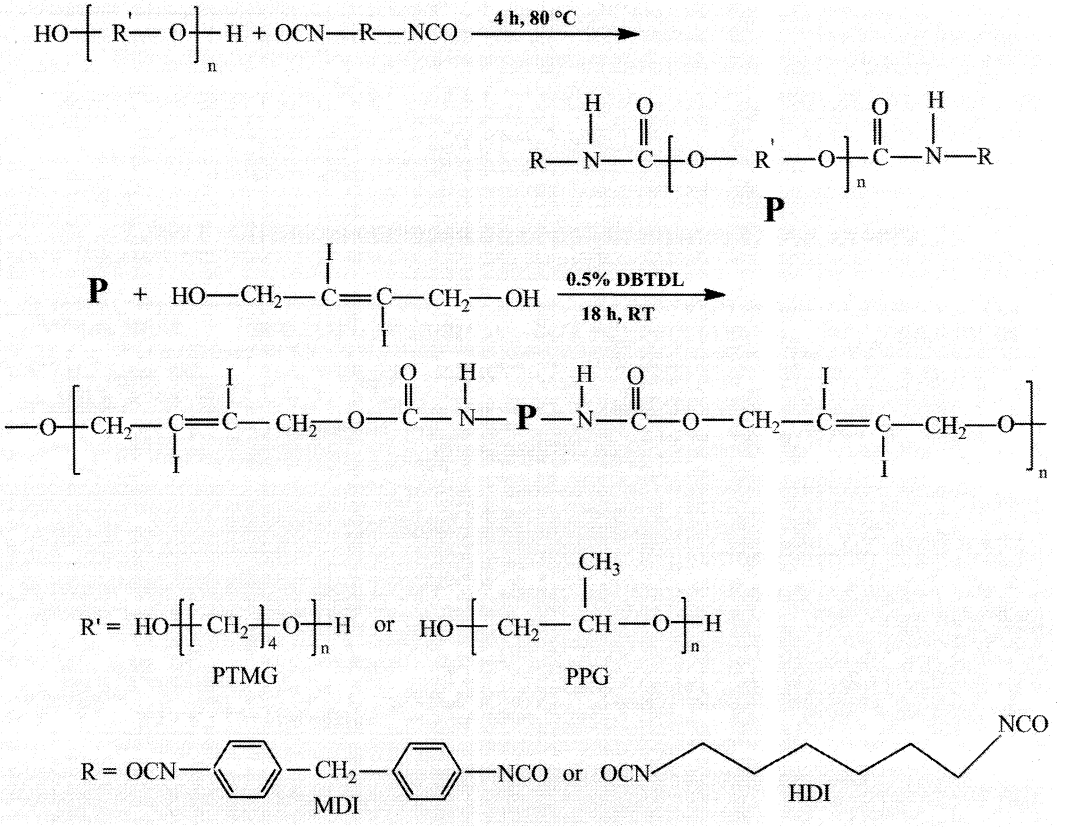

Figure 5: General synthesis of radiopaque polyurethanes using chain extender 2,3 diiodo-2-butene-1,4 diol

4.2.2 Synthesis of polyurethanes using iodinated aromatic chain extender, bis hydroxyethyl ether of tetraiodo bisphenol A

Polyurethanes were synthesized using MDI, PTMG, PPG and IBPA as chain-extender, as per scheme given in Figure 6. The formulation variables for the synthesis of various polymers are given in Table 5. The percentage composition of soft segment and hard segment are given in Table 6.

Table 5: Formulation variables of polyurethanes based on IBPA

	Reactant Concentration (mol)			
	Diisocyanate	Polyol		Chain extender
Polyurethane	MDI	PTMG	PPG	IBPA
PTMGMDIBPA	0.0026	0.0012	-	0.0014
PPGMDIBPA	0.0026	-	0.0012	0.0014

Table 6: Chemical composition of polyurethanes based on IBPA

Polyurethane	Hard segment (mol %)	Soft segment (mol %)	Isocyanate index
PTMGMDIBPA	76.92	23.07	1.0
PPGMDIBPA	78.92	21.07	1.0

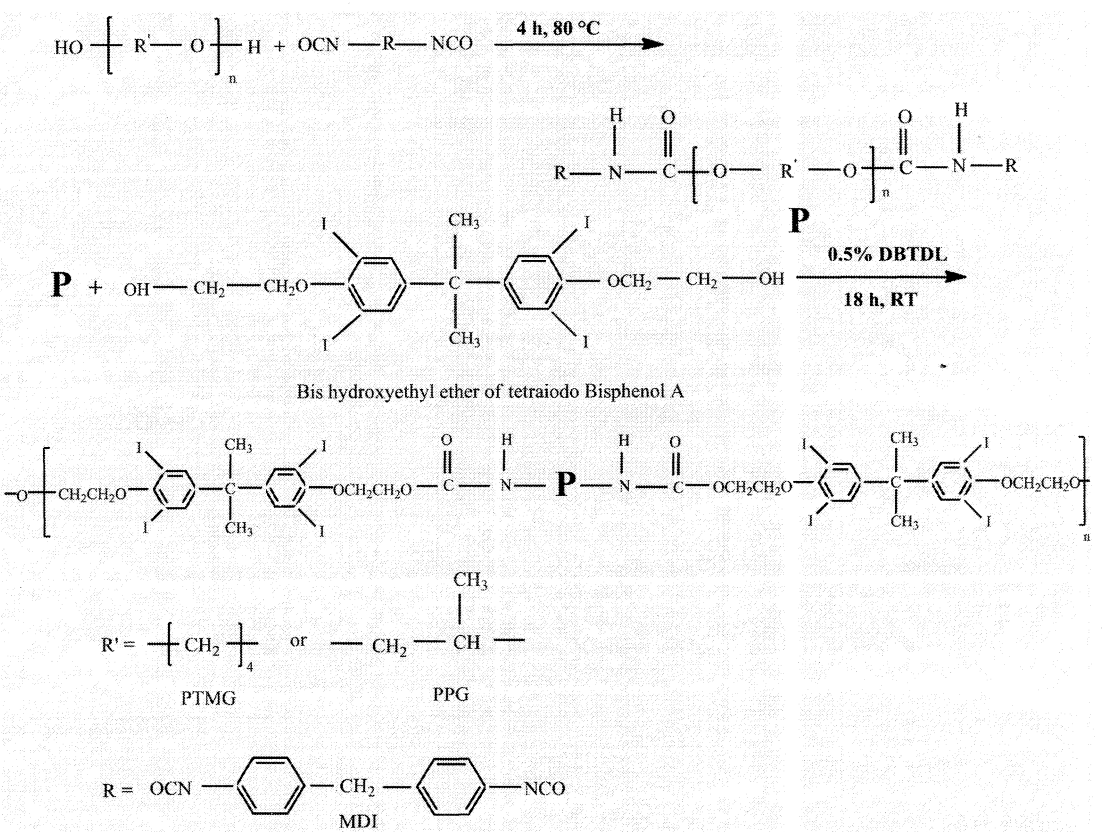

Figure 6: General synthesis of radiopaque polyurethanes using chain extender bis hydroxyethyl ether of tetraiodo bisphenol A

4.3 *Characterization of radiopaque polyurethanes*

4.3.1 Fourier transform infrared spectral analyses

The Fourier transform infrared-attenuated total reflectance (FTIR - ATR) spectral analyses were used to characterize polyurethanes (Figure 8 and 9). The major ATR-IR peak responses of polyurethanes are given in Table 7. Absorption bands in the carbonyl and N-H stretching in the ATR-IR Spectra indicated the presence of hydrogen bonds. The presence of hydrogen bonded carbonyl absorption peak, (1688 cm^{-1} and 1700 cm^{-1}) in HDI based polyurethanes revealed the inter urethane hydrogen bonding of the hard segment domains which may lead to a higher degree of micro-phase separation in the surface as shown in Figure 7. The absence of peak at 1702-1708 cm^{-1} for C=O stretching (bonded) and presence of peak between 3302 cm^{-1} and 3319 cm^{-1} for N-H stretching (bonded) for MDI based polyurethanes reveal hydrogen bonding interactions between ether groups of the soft segments and the urethane amide in the hard segments as represented in Figure 7.

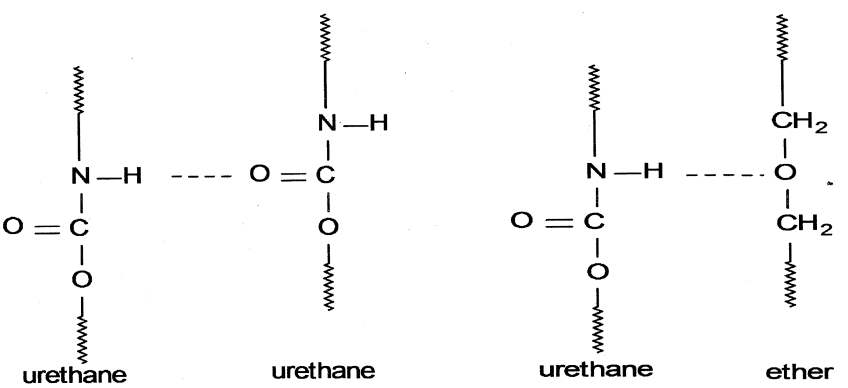

Figure 7: Hydrogen bonding interactions in polyurethane

Table 7: ATR-IR absorption band assignments

Observed wave number (cm^{-1})						
PTMGMDIBOL	PTMGHDIBOL	PPGMDIBOL	PPGHDIBOL	PTMGMDIBPA	PPGMDIBPA	Spectral assignment
3297	3319	3302	3320	3303	3302	N-H bonded stretching
2936	2933	2969	2932	2936	2923	C-H asymmetric stretching
2852	2853	2867	2853	2852	2854	C-H symmetric stretching
1727	-	1722	-	1728	1742	C=O non bonded stretching
-	1688	-	1700	-	-	C=O bonded stretching
1596	-	1595	-	1595	1595	C=C stretching in benzene ring
1534	1533	1534	1529	1533	1534	N-H bending + C-N stretching
1411	-	1411	-	1412	1411	C-C stretching in benzene ring
1219	1249	1224	1243	1218	1220	Combined effect of ν(C-N)+ δ(N-H)
1101	1102	1074	1100	1101	1056	C-O-C stretching of polyol
815	-	815	-	815	813	C-H bending in benzene ring

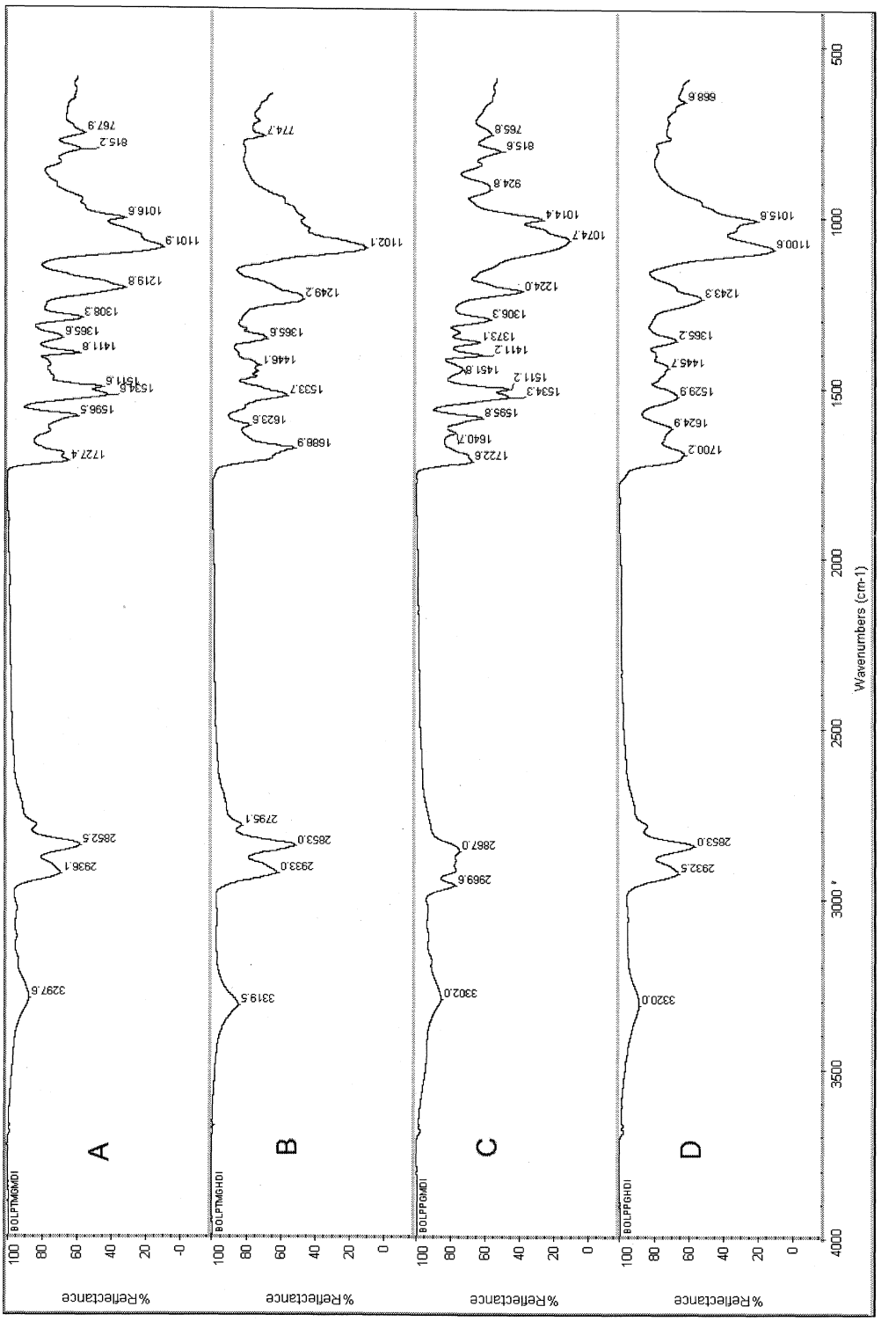

Figure 8: IR spectra of the polyurethanes PTMGMDIBOL (A), PTMGHDIBOL (B), PPGMDIBOL (C), and PPGHDIBOL (D)

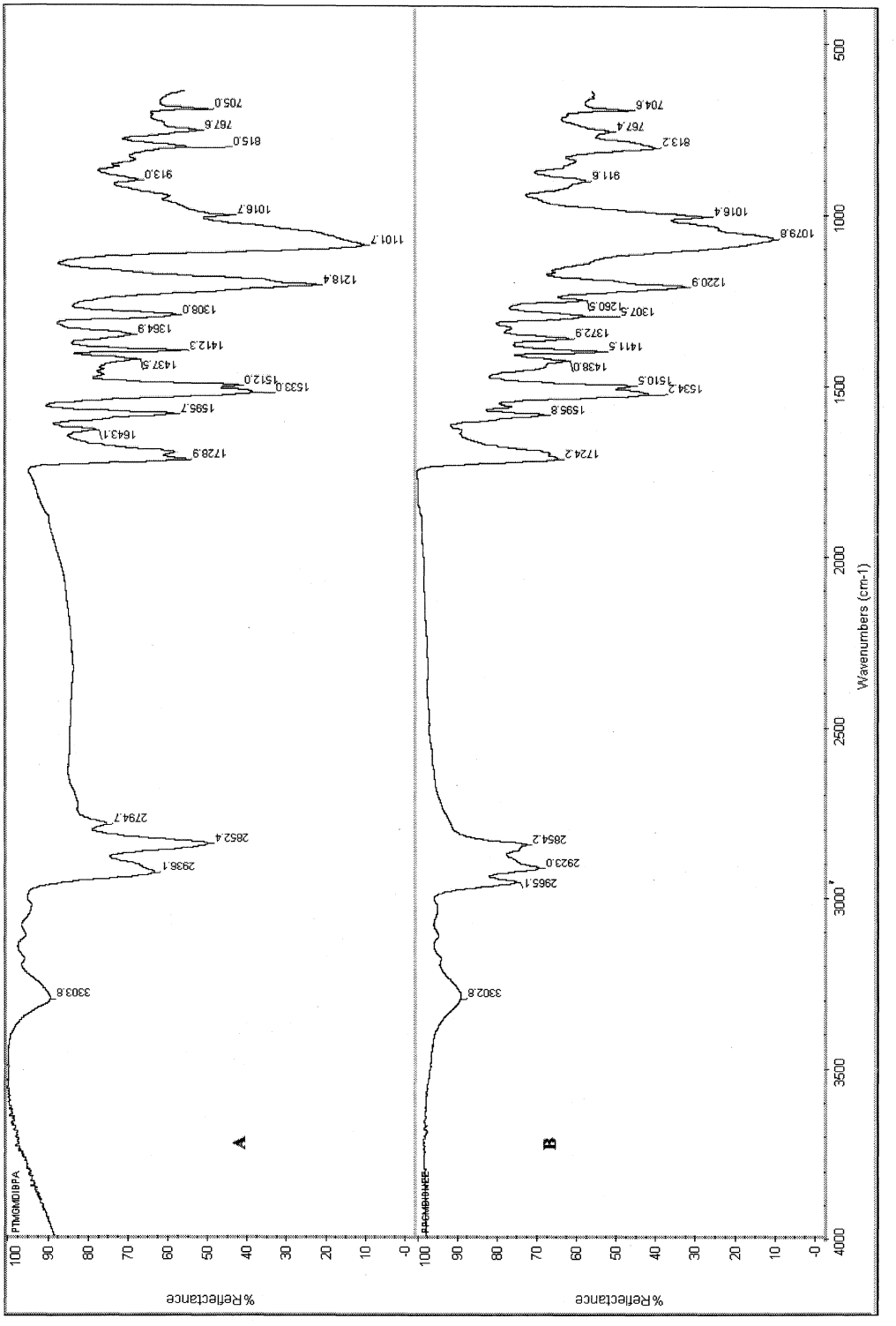

Figure 9: IR spectra of the polyurethanes PTMGMDIBPA (A) and PPGMDIBPA (B)

4.3.2 Gel permeation chromatography

Molecular weights of polyurethanes were determined by GPC (Table 8). High molecular weight polyurethanes were obtained with MDI based polyurethanes when compared to analogous HDI based polyurethanes. The GPC data further indicated that with MDI, the reaction proceeded to completion to form high molecular weight polyurethanes.

Table 8: Molecular weights of polyurethanes estimated by GPC

Polyurethane	M_w	M_n	Polydispersity
PTMGMDIBOL	94354	77239	1.2
PTMGHDIBOL	74540	40420	1.8
PPGMDIBOL	138770	58240	2.3
PPGHDIBOL	25353	14065	1.8
PTMGMDIBPA	206517	111763	1.8
PPGMDIBPA	156210	56269	2.7

4.3.3 X-Ray diffraction studies

X-ray diffraction curves of polyurethanes are given in Figures 10 and 11. The presence of crystalline region in polyurethanes was confirmed by X-ray diffraction analysis (Table 9).

Table 9: XRD peak positions for polyurethanes

Polyurethane	Diffraction Angles, $2\theta(^\circ)$	d-Spacing (A°)
PTMGHDIBOL	19.0	4.6
	20.8	4.2
	28.5	3.1
PPGMDIBOL	17.8	4.9
	28.5	3.1
PPGHDIBOL	18.9	4.6
	20.8	4.2
	28.6	3.1
PPGMDIBPA	11.4	7.7
	17.3	5.1
	23.3	3.8
	25.1	3.5

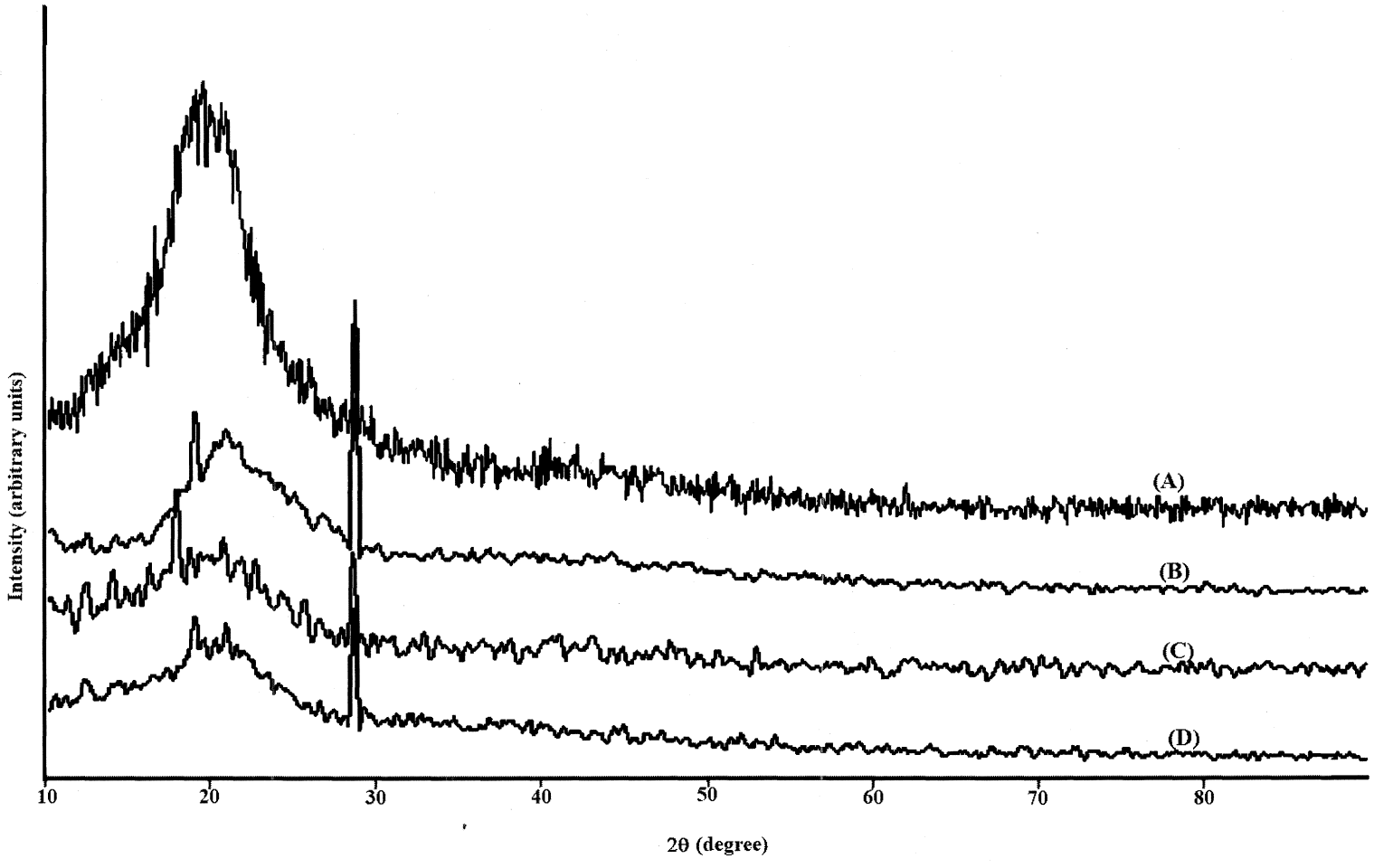

Figure 10: X-ray diffraction patterns of the polyurethanes PTMGMDIBOL (A), PTMGHDIBOL (B), PPGMDIBOL (C) and PPGHDIBOL (D)

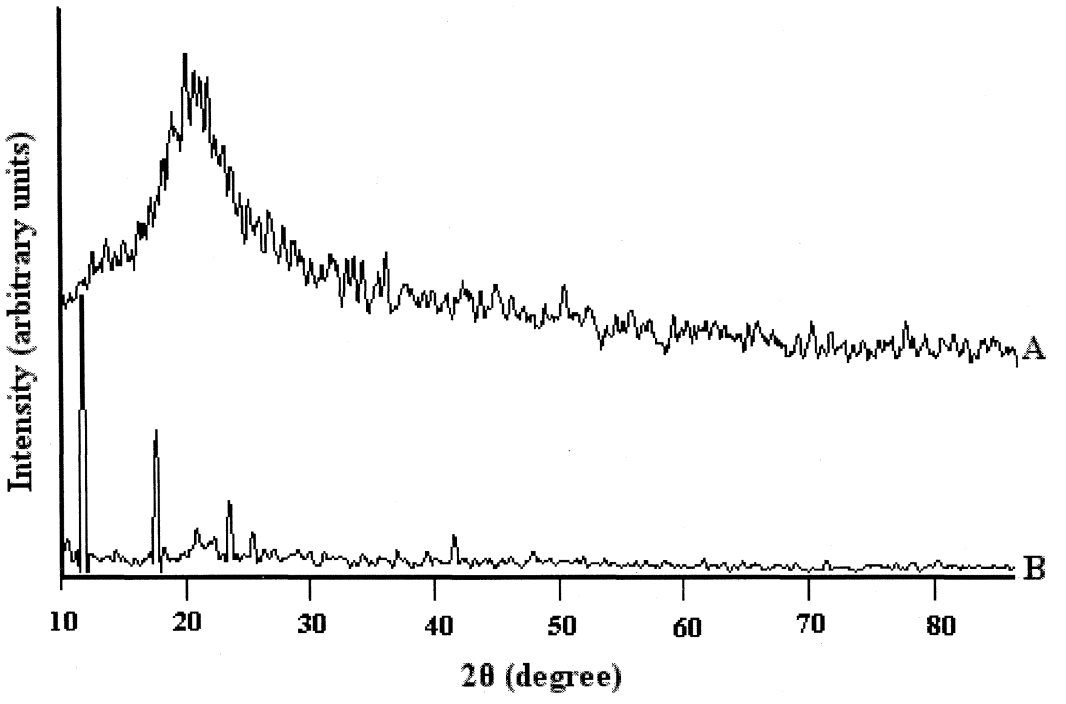

Figure 11: X-ray diffraction patterns of the polyurethanes PTMGMDIBPA (A), PPG-MDIBPA (B)

4.3.4 Energy dispersive X-ray analyses

The presence of iodine in the polyurethanes could be elucidated by EDX (Figures 12 and 13). In the EDX spectrum of polyurethanes which was prepared using iodinated chain extenders, peaks corresponding to iodine atoms in the area 3.5-5 KeV were obtained. Thus, the presence of iodine resulting from incorporation of chain extenders in the polyurethane was confirmed.

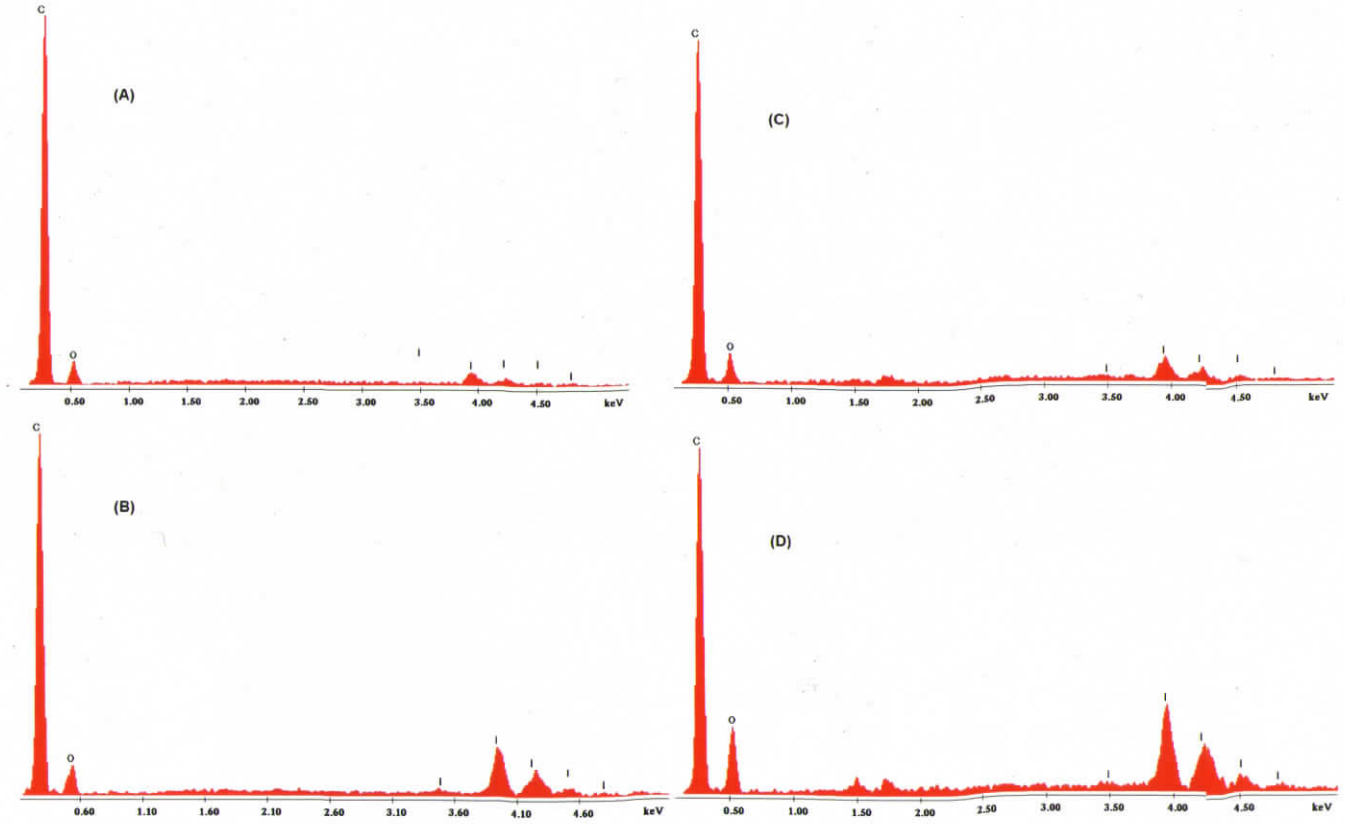

Figure 12: EDX images of polyurethanes PTMGMDIBOL (A), PTMGHDIBOL (B), PPGMDIBOL (C) and PPGHDIBOL (D)

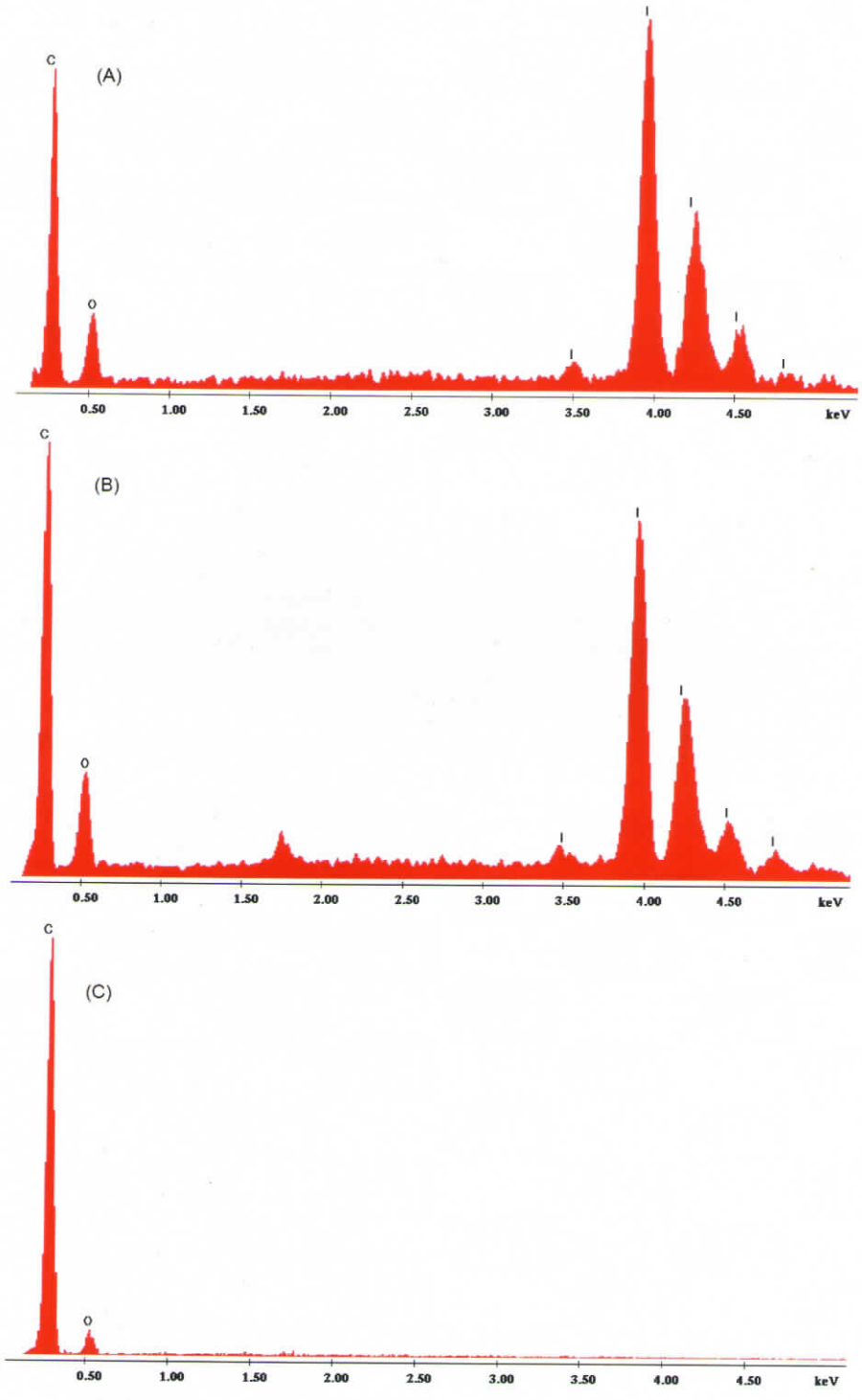

Figure 13: EDX images of polyurethanes PTMGMDIBPA (A), PPGMDIBPA (B)

4.3.5 Elemental analyses

The iodine content of polyurethanes was quantitatively estimated by standard elemental analysis procedure (Table 10). The percentage iodine content in the polymers were found to be sufficient to be adequately radioapque for medical applications.

Table 10: Quantitative estimation of iodine by elemental analyses

Polyurethane	Iodine content (%)
PTMGMDIBOL	3.11
PTMGHDIBOL	10.27
PPGMDIBOL	4.71
PPGHDIBOL	9.42
PTMGMDIBPA	15.57
PPGMDIBPA	13.63

4.3.6 Thermal analyses

The thermal characteristics of the polyurethanes were assessed using thermogravimetric analyses (TGA). TGA curves of polyurethanes with chain extender IBOL are as shown in Figure 14. TGA traces of polyurethanes synthesized using chain extender IBPA are given Figure 15. The thermogravimetric analyses data are given in Table 11. The temperature at which first stage of decomposition occurred is given as Td_1 and temperature at 50% decomposition is given as $Td_{1/2}$.

Table 11: Thermal characteristics of polyurethanes

Polyurethane	Td_1 (°C)	Weight remained at Td_1(%)	$Td_{1/2}$ (°C)
PTMGMDIBOL	260	96	406
PTMGHDIBOL	260	79	324
PPGMDIBOL	236	93	349
PPGHDIBOL	245	90	330
PTMGMDIBPA	267	94	296
PPGMDIBPA	271	93	306

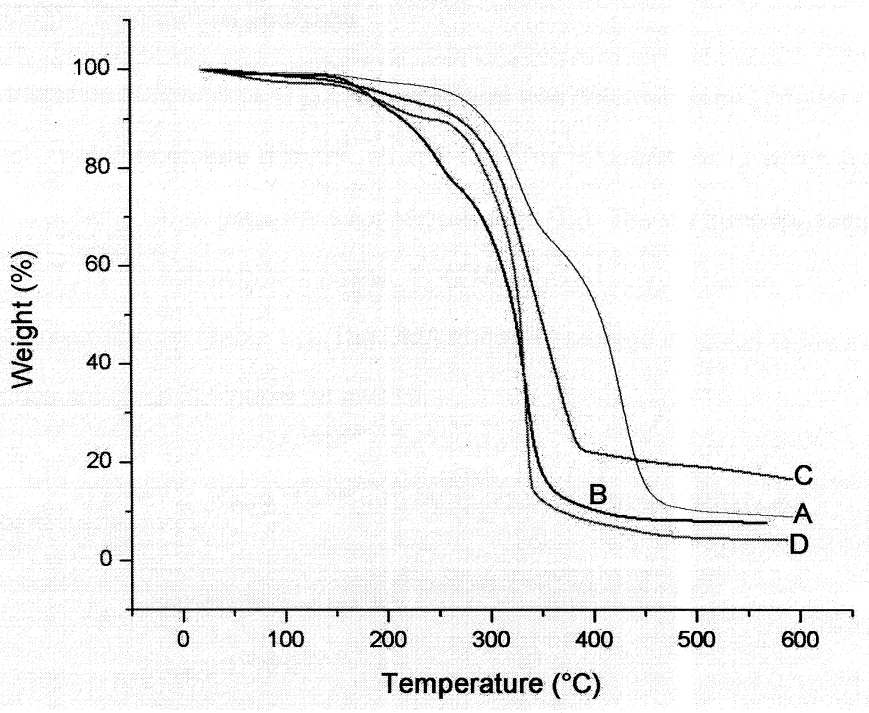

Figure 14: TGA thermograms of polyurethanes PTMGMDIBOL (A), PTMGHDIBOL (B), PPGMDIBOL (C) and PPGHDIBOL (D)

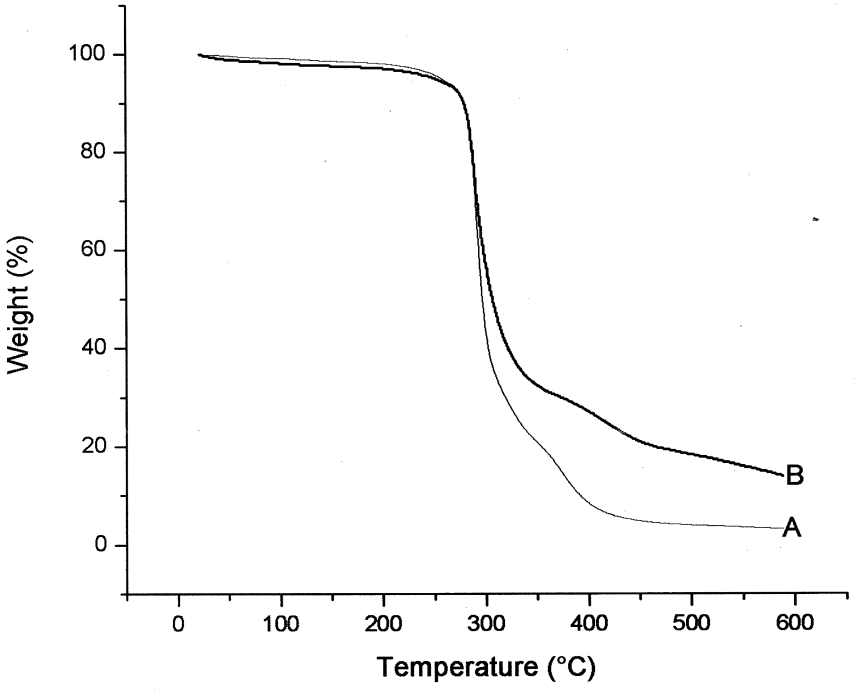

Figure 15: TGA thermograms of polyurethanes PTMGMDIBPA (A), PPGMDIBPA (B)

4.3.7 Dynamic mechanical analyses

Glass transition temperature (T_g) of the polymers was determined by DMA by plotting tan δ as a function of temperature (Figures 16 and 17). The temperature, at which tan δ attains maximum, is determined as glass transition temperature (T_g). The low transition temperature is related to the T_g of the soft-segment phase. T_g of MDI-based polyurethanes is the higher than HDI based polyurethanes (Table 12). The DMA traces for storage modulus of the investigated polyurethanes are given in Figures 18 and 19.

Table 12: Glass transition temperature of polyurethanes

Polyurethane	Glass transition Temperature (°C)
PTMGMDIBOL	-37 °C
PTMGHDIBOL	-45 °C
PPGMDIBOL	1 °C
PPGHDIBOL	-24 °C
PTMGMDIBPA	-26 °C
PPGMDIBPA	-12 °C

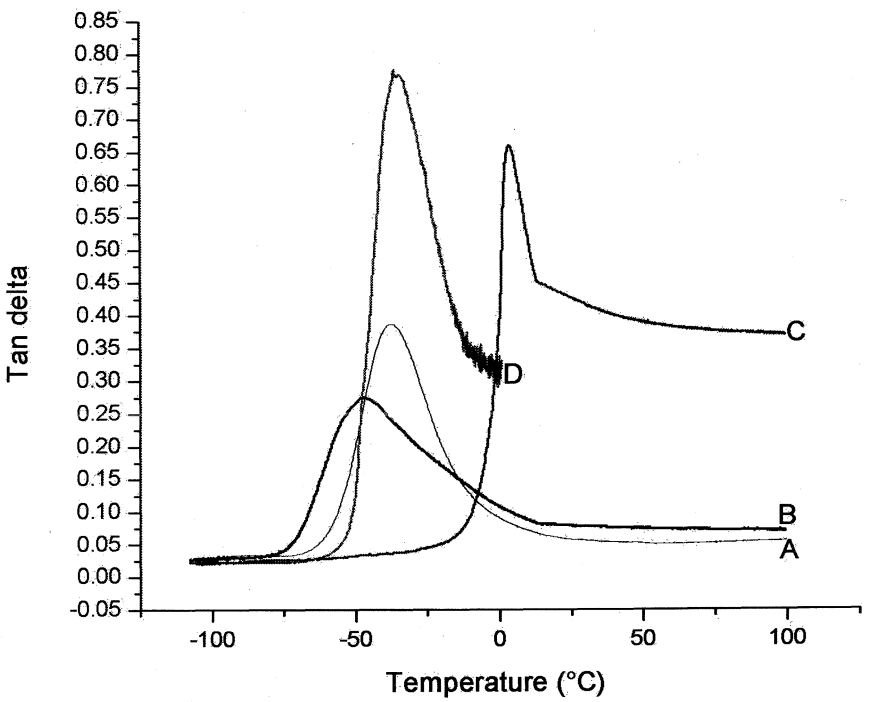

Figure 16: The dynamic mechanical analysis of polyurethanes PTMGMDIBOL (A), PTMGHDIBOL (B), PPGMDIBOL (C) and PPGHDIBOL (D)

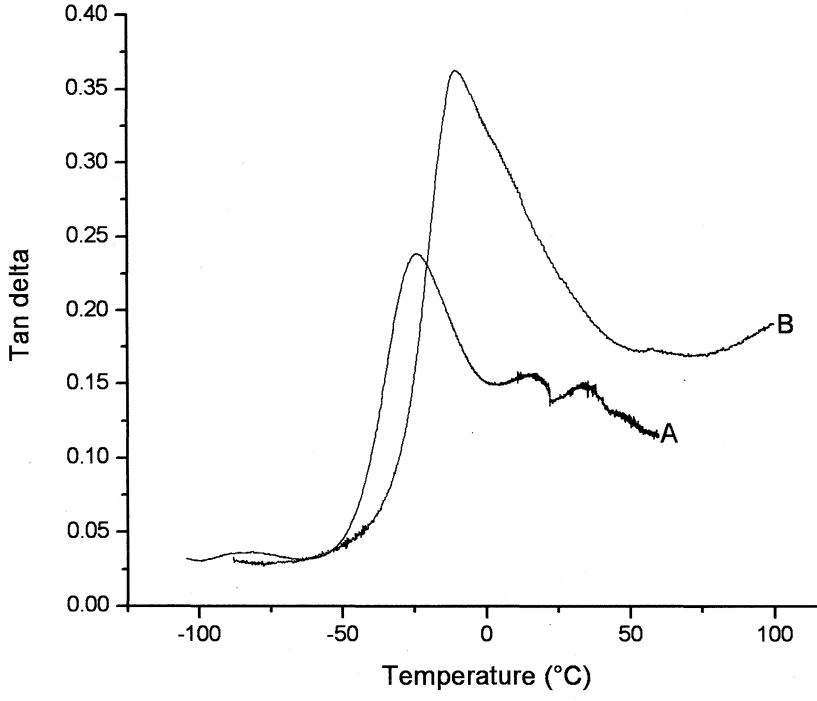

Figure 17: The dynamic mechanical analysis of polyurethanes PTMGMDIBPA (A), PPG-MDIBPA (B)

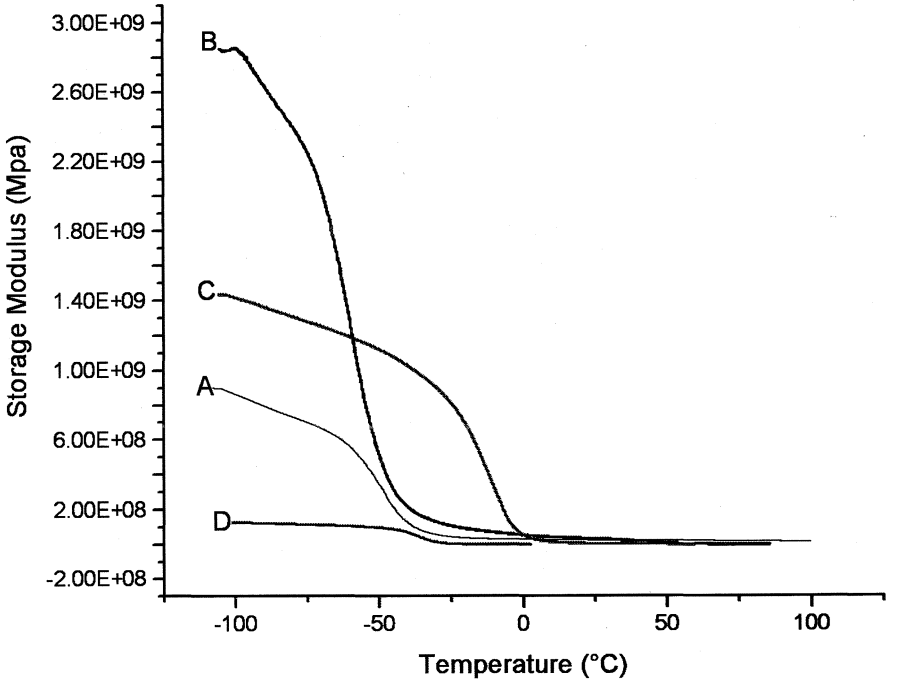

Figure 18: Storage modulus v temperature curves for polyurethanes PTMGMDIBOL (A), PTMGHDIBOL (B), PPGMDIBOL (C) and PPGHDIBOL (D)

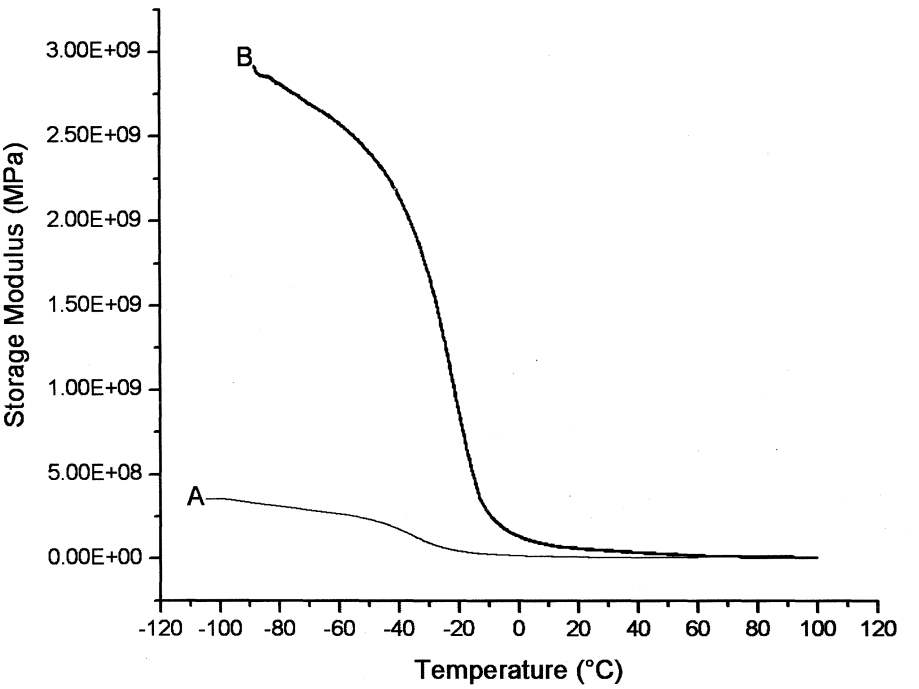

Figure 19: Storage modulus v temperature curves for polyurethanes PTMGMDIBPA (A), PPGMDIBPA (B)

4.3.8 Determination of mechanical properties

The data on tensile property of the polyurethanes are presented in Table 13. PTMGMDI-BOL had higher tensile strength and ultimate elongation than all other polyurethanes.

Table 13: Mechanical properties of polyurethanes

Polyurethane	Tensile strength (Mpa)	Ultimate elongation (%)	Modulus (Mpa)	Toughness (Mpa)
PTMGMDIBOL	35.76 $\pm$ 2.08	752.23 $\pm$ 68.21	12.10 $\pm$ 8.17	106.87 $\pm$ 12.81
PTMGHDIBOL	4.37 $\pm$ 0.83	482.78 $\pm$ 46.73	10.08 $\pm$ 0.07	11.57 $\pm$ 2.30
PPGMDIBOL	2.46 $\pm$ 0.59	675.15 $\pm$ 71.83	41.62 $\pm$ 7.67	7.63 $\pm$ 1.97
PPGHDIBOL	0.74 $\pm$ 0.06	64.26 $\pm$ 9.55	33.99 $\pm$ 6.39	0.28 $\pm$ 0.05
PTMGMDIBPA	5.70 $\pm$ 0.48	692.93 $\pm$ 50.08	131.37 $\pm$ 8.77	21.67 $\pm$ 2.85
PPGMDIBPA	7.28 $\pm$ 0.36	500.61 $\pm$ 89.12	61.26 $\pm$ 13.99	20.53 $\pm$ 0.68

4.3.9 Evaluation of radiopacity

All the polyurethanes were subjected to X-radiographic examination to compare their visibility in X-radiogram(Figure 20). It can be seen from the radiograph that images of polyurethanes films having 2 mm thickness are comparable with that of the aluminium wedge having same dimensions. Quantitaive evaluation of radiopacity confirmed that the radiopacity of polyurethanes were more or less equivalent to the radiopacity of 2 mm thick aluminum wedge (Figure 21).

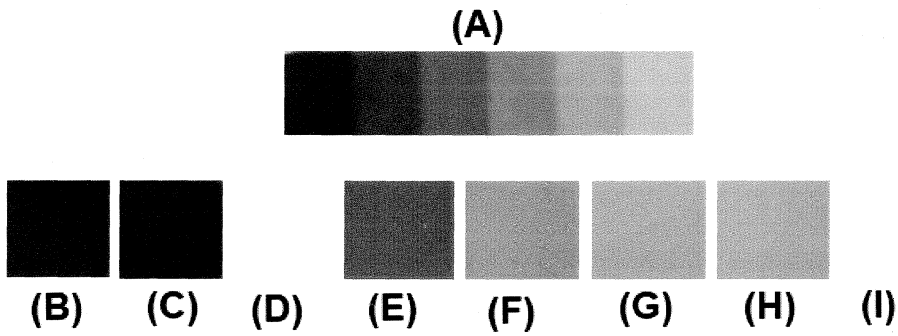

Figure 20: Positive print of a radiograph showing: an aluminium step wedge 0.5 -3 mm thick in 0.5 mm steps (right to left) (A), PPGMDIBPA (B), PTMGMDIBPA (C), non-iodinated polyurethane with chain extender bis hydroxyethyl ether of bisphenol A (not visible in print) (D), PTMGHDIBOL (E), PPGHDIBOL (F), PPGMDIBOL (G), PTMGMDIBOL (H), non-iodinated polyurethane with chain extender 1,4 butane diol (not visible in print)

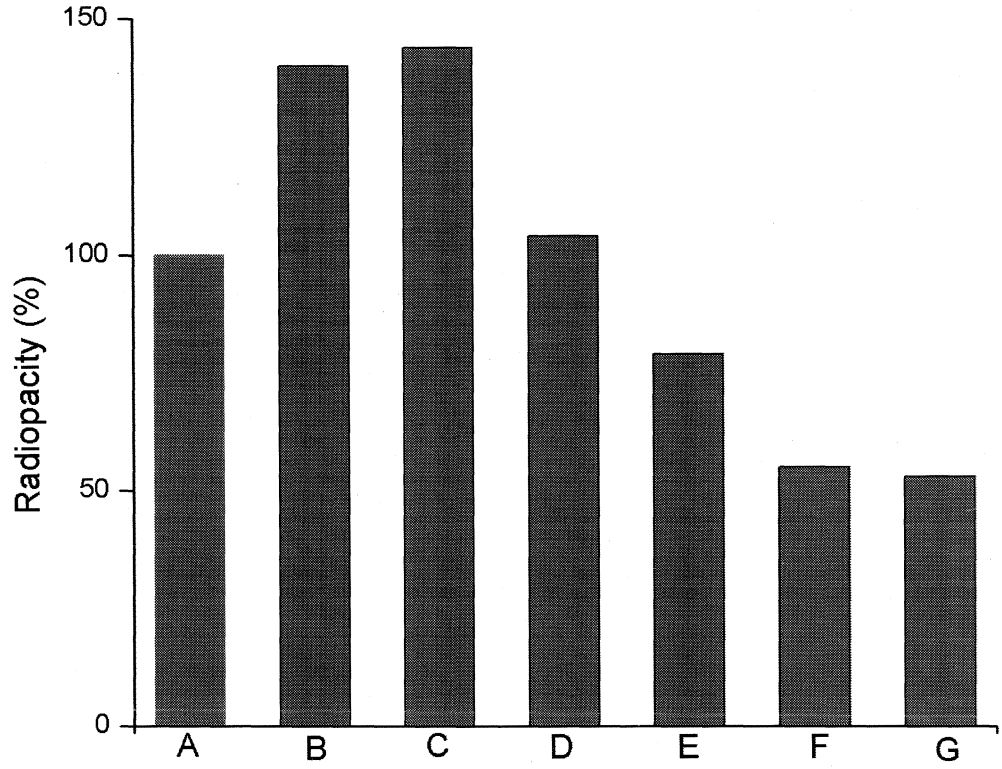

Figure 21: Graph illustrating the radiopacity of polyurethanes evaluated as ratio of absorption relative to standard 2mm thick Al wedge (A), PPGMDIBPA (B), PTMGMDIBPA (C), PTMGHDIBOL (D), PPGHDIBOL (E), PPGMDIBOL (F), PTMGMDIBOL (G)

4.3.10 Contact angle measurement

The contact angle measured for the present polyurethanes are given in Table 14. It is observed that all the present polyurethanes are hydrophilic in nature.

Table 14: Dynamic contact angles values of the polyurethane films in contact with water

Polyurethane	Advancing Angle	Receding Angle
PTMGMDIBOL	$42 \pm 1°$	$35 \pm 3°$
PTMGHDIBOL	$45 \pm 2°$	$38 \pm 2°$
PPGMDIBOL	$52 \pm 4°$	$48 \pm 4°$
PPGHDIBOL	$51 \pm 3°$	$43 \pm 3°$
PTMGMDIBPA	$52 \pm 1°$	$44 \pm 2°$
PPGMDIBPA	$53 \pm 2°$	$48 \pm 4°$

4.4 Biostability of radiopaque polyurethanes

In vitro biodegradation studies were performed to assess the biostability of the radiopaque poly(ether urethanes) synthesized.

4.4.1 Evaluation of hydrolytic stability in ionic medium

The percentage weight loss of aged polyurethanes is given in Table 15. Characteristic ATR-FTIR spectra of all aged polyurethane films incubated for 6 months in PBS and Ringer's solution are shown in Figures 22 - 25. ATR-FTIR spectra of aged polyurethane films are compared with the untreated control (Figures 26 and 27)

Table 15: Gravimetric analysis of polyurethane films aged in hydrolytic medium

	Weight loss (%)		
Polyurethane	PBS	Ringer's	Control
PTMGMDIBOL	0.5 ± 0.2	0.5 ± 0.4	0.5 ± 0.2
PTMGHDIBOL	1.2 ± 0.8	0.9 ± 0.2	0.6 ± 0.1
PPGMDIBOL	0.4 ± 0.5	0.6 ± 0.2	0.8 ± 0.1
PPGHDIBOL	1.1 ± 0.2	1.7 ± 0.3	0.9 ± 0.3
PTMGMDIBPA	0.2 ± 0.1	0.9 ± 0.3	0.4 ± 0.2
PTMGHDIBPA	0.9 ± 0.6	0.8 ± 0.2	0.1 ± 0.1

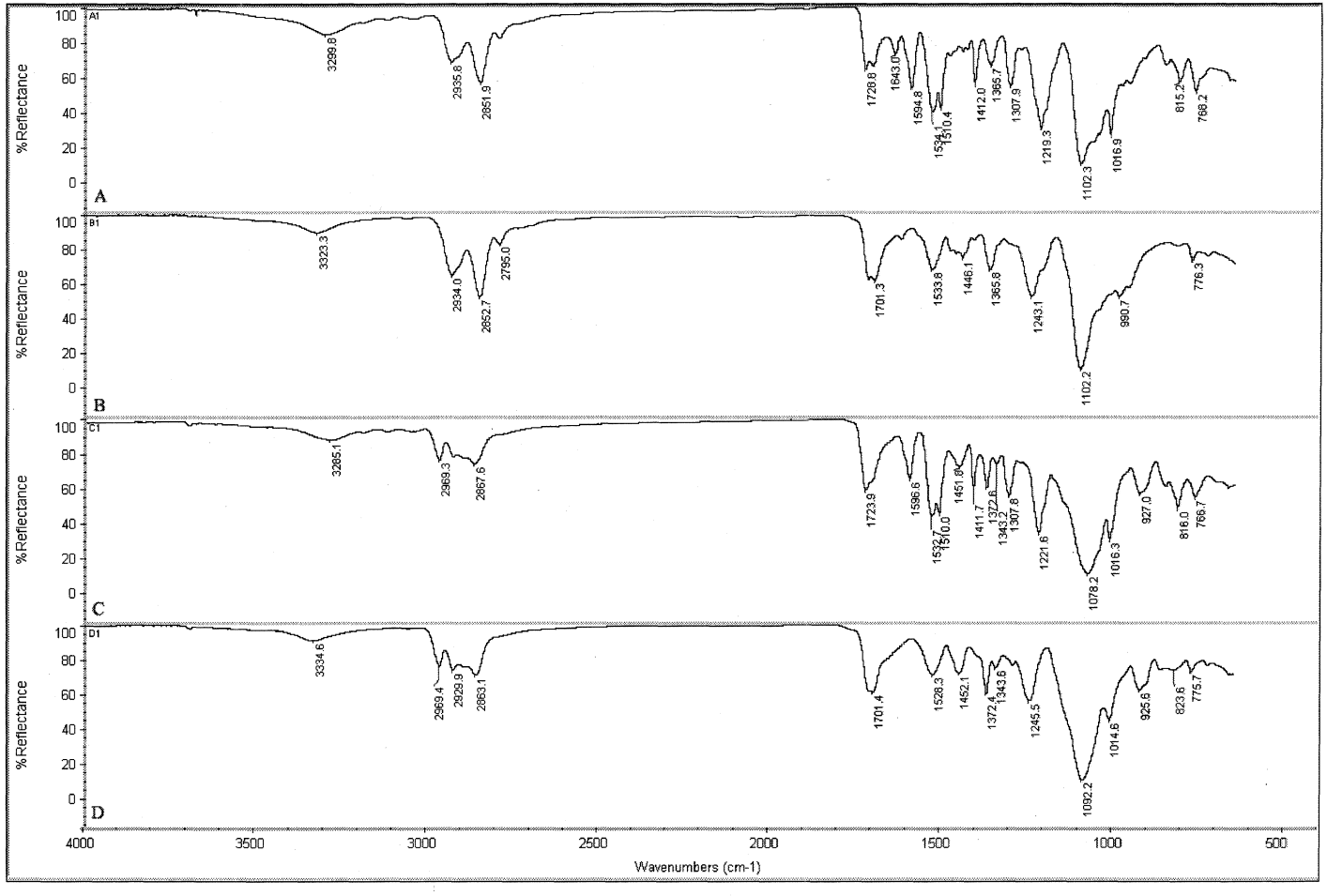

Figure 22: IR spectra of the polyurethanes (aged in PBS) PTMGMDIBOL (A), PTMGHDIBOL (B) and PPGMDIBOL (C) PPGHDIBOL (D)

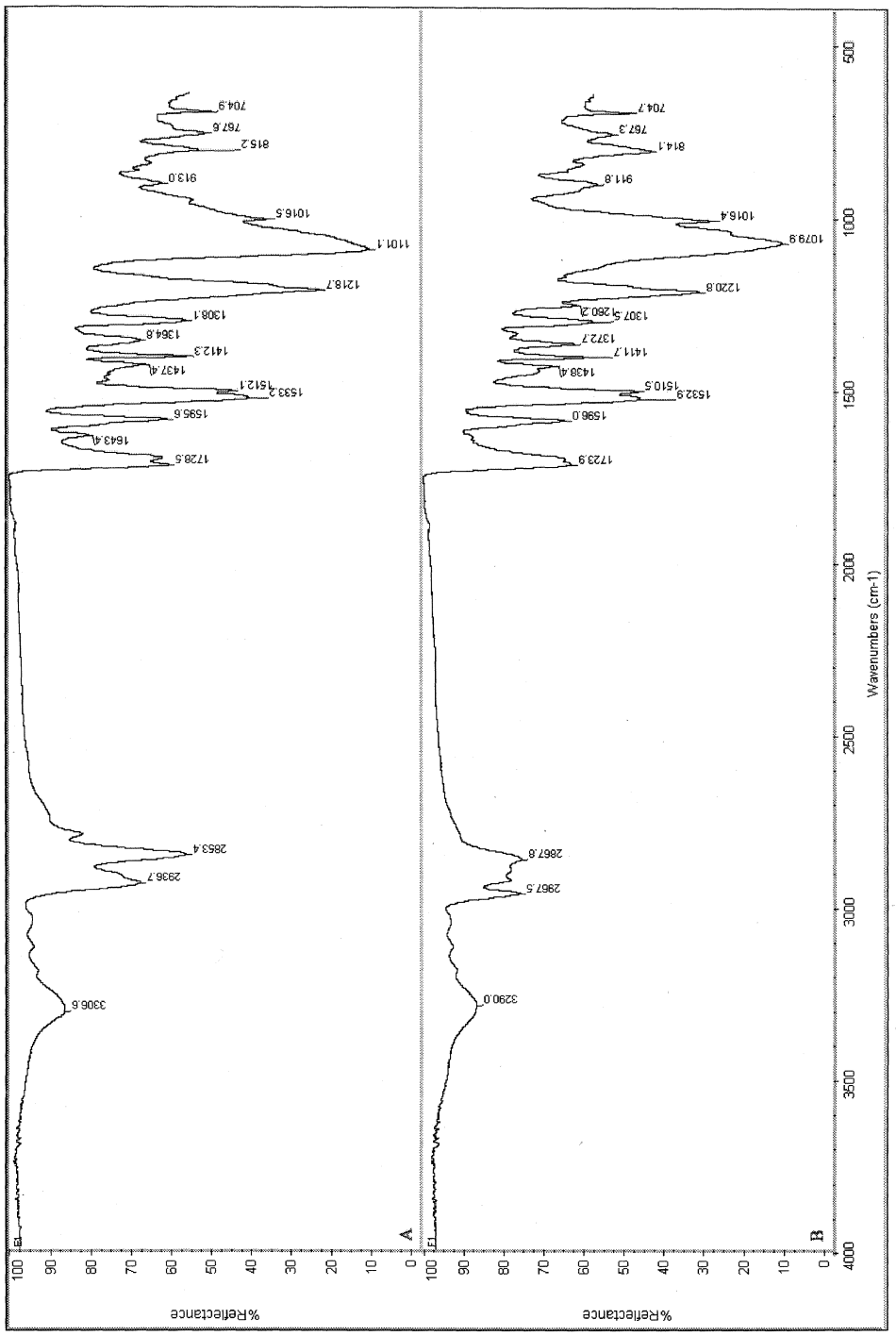

Figure 23: IR spectra of the polyurethanes (aged in PBS) PTMGMDIBPA (A), PPGMDIBPA (B)

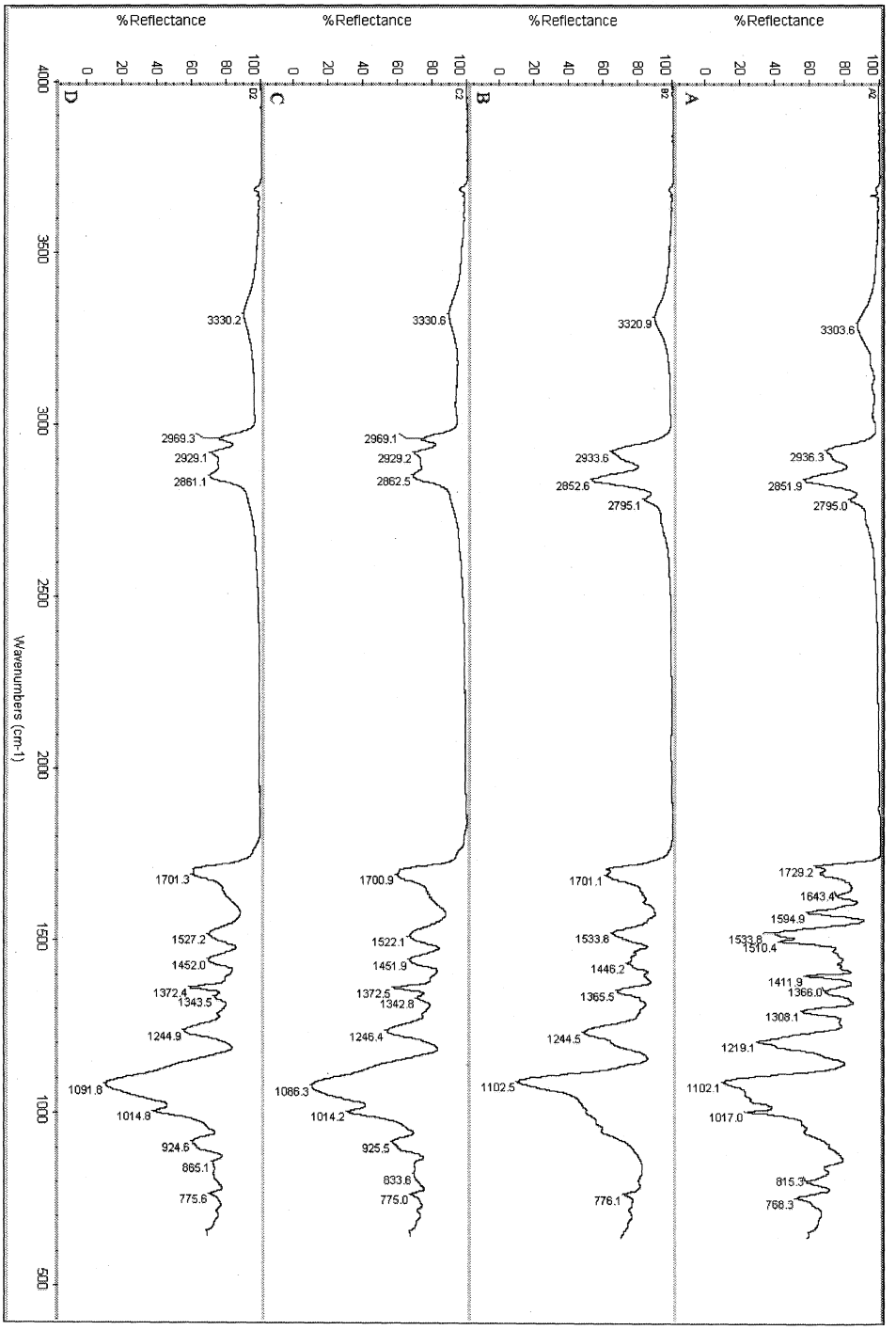

Figure 24: IR spectra of the polyurethanes (aged in Ringer's solution) PTMGMDIBOL (A), PTMGHDIBOL (B), PPGMDIBOL (C) and PPGHDI-BOL (D)

Figure 25: IR spectra of the polyurethanes (aged in Ringer's solution) PTMGMDIBPA (A), PPGMDIBPA (B)

Figure 26: IR spectra of the polyurethanes (control) PTMGMDIBOL (A), PTMGHDIBOL (B), PPGMDIBOL (C) and PPGHDIBOL (D)

Figure 27: IR spectra of the polyurethanes (control) PTMGMDIBPA (A), PPGMDIBPA (B)

4.4.2 Evaluation of hydrolytic stability in hydrolytic enzyme

Significant weight loss due to enzymatic degradation was not observed for both polyurethanes based on IBOL and IBPA (Table 16). The IR spectral response did not reveal any appreciable changes in the aged polymers in comparison with that aged in control buffer media and in deionised water (untreated polyurethane films), as observed in Figures 28 - 31.

Table 16: Gravimetric analysis of polyurethane films aged in hydrolytic enzyme medium

	Weight loss (%)		
Polyurethane	Papain	Buffer	Control
PTMGMDIBOL	0.3 ± 0.1	0.4 ± 0.2	0.5 ± 0.2
PTMGHDIBOL	1.8 ± 0.4	0.9 ± 0.2	0.6 ± 0.1
PPGMDIBOL	0.5 ± 0.1	0.7 ± 0.1	0.8 ± 0.1
PPGHDIBOL	1.9 ± 0.6	1.2 ± 0.7	0.9 ± 0.3
PTMGMDIBPA	0.9 ± 0.1	0.6 ± 0.2	0.4 ± 0.2
PTMGHDIBPA	0.7 ± 0.2	0.4 ± 0.1	0.1 ± 0.1

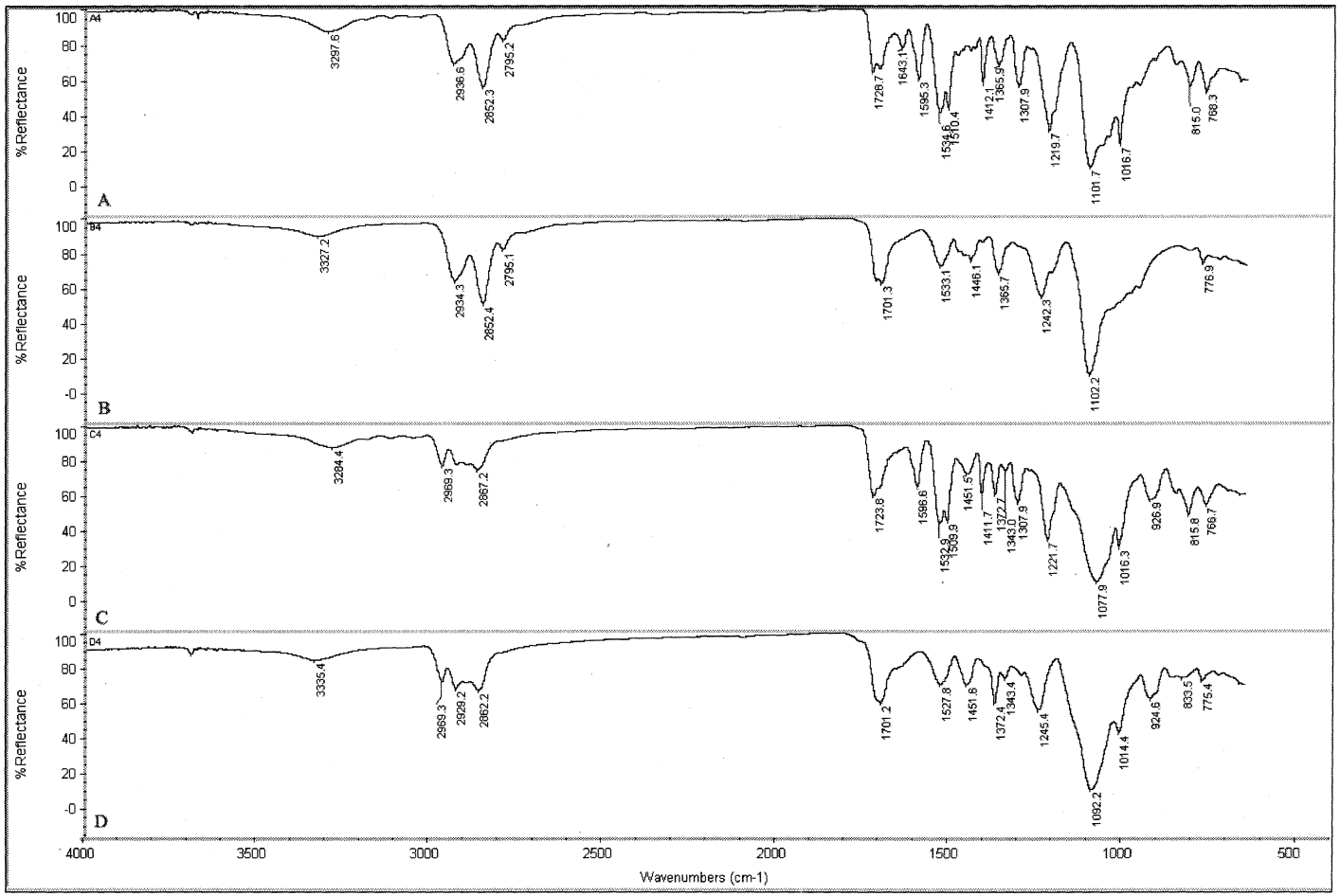

Figure 28: IR spectra of the polyurethanes (aged in presence of papain enzyme) PTMGMDIBOL (A), PTMGHDIBOL (B) and PPGMDIBOL (C) PPGHDIBOL (D)

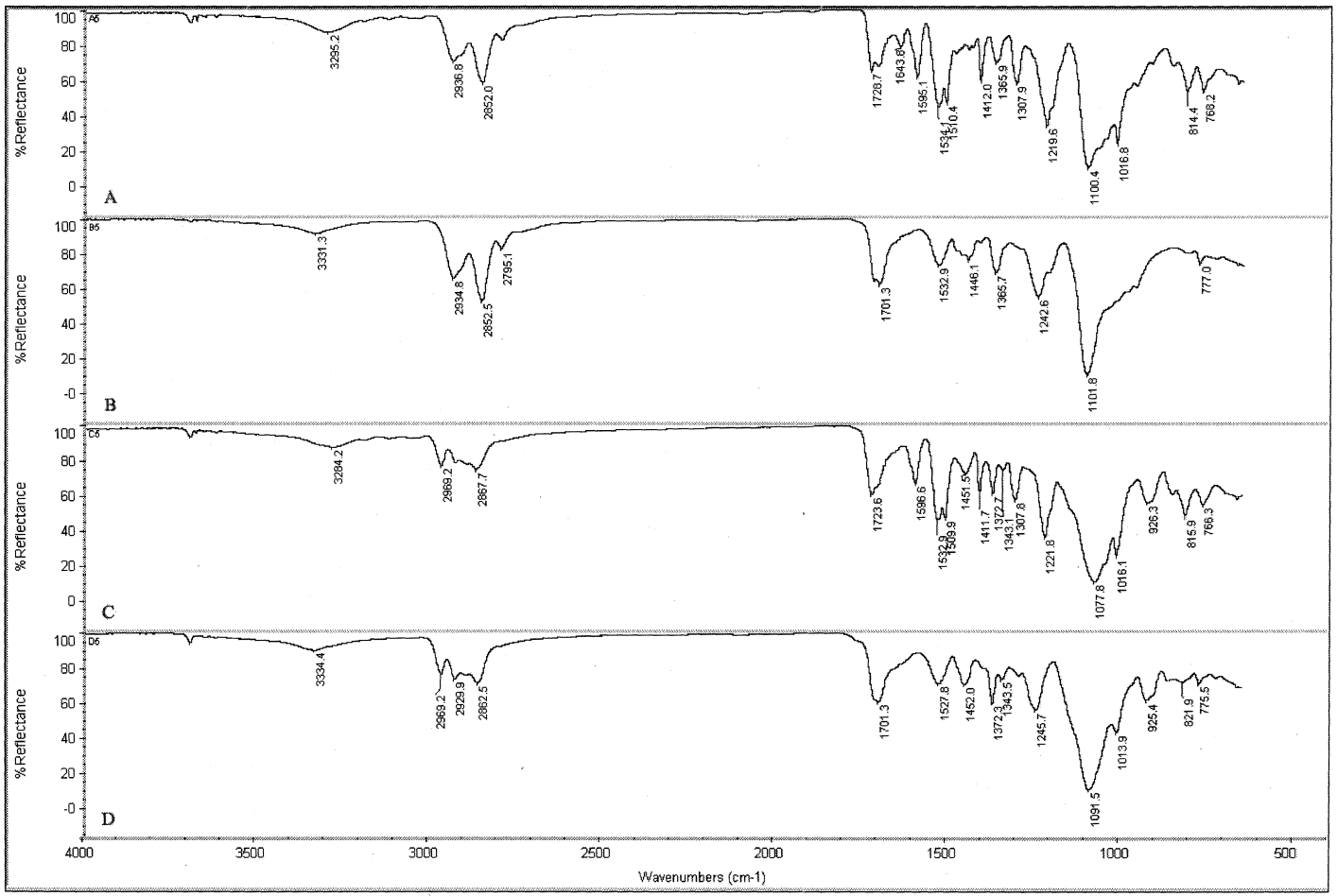

Figure 29: IR spectra of the polyurethanes (aged in HEPES buffer) PTMGMDIBOL (A), PTMGHDIBOL (B) and PPGMDIBOL (C) PPGHDIBOL (D)

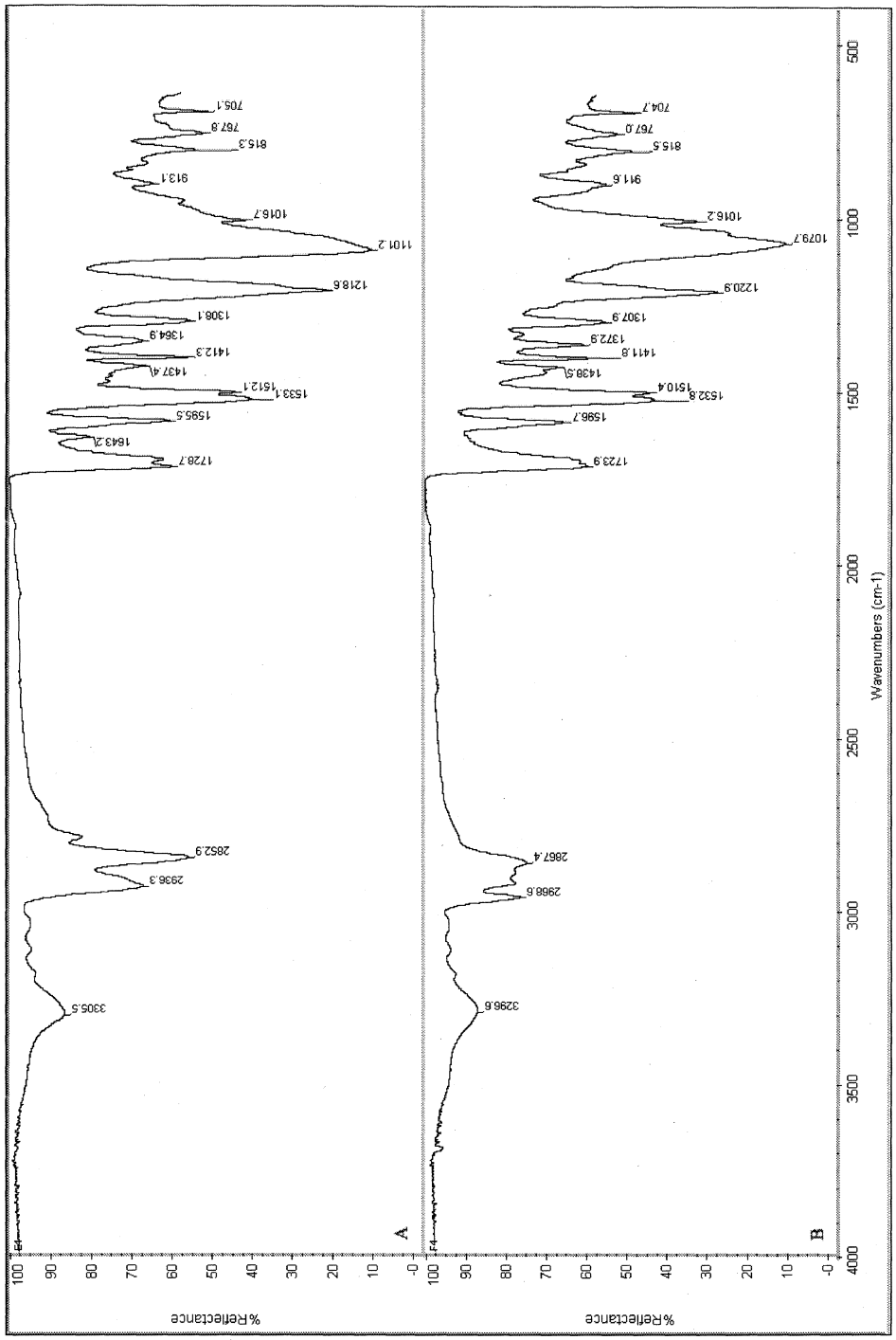

Figure 30: IR spectra of the polyurethanes (aged in papain enzyme) PTMGMDIBPA (A) and PPGMDIBPA (B)

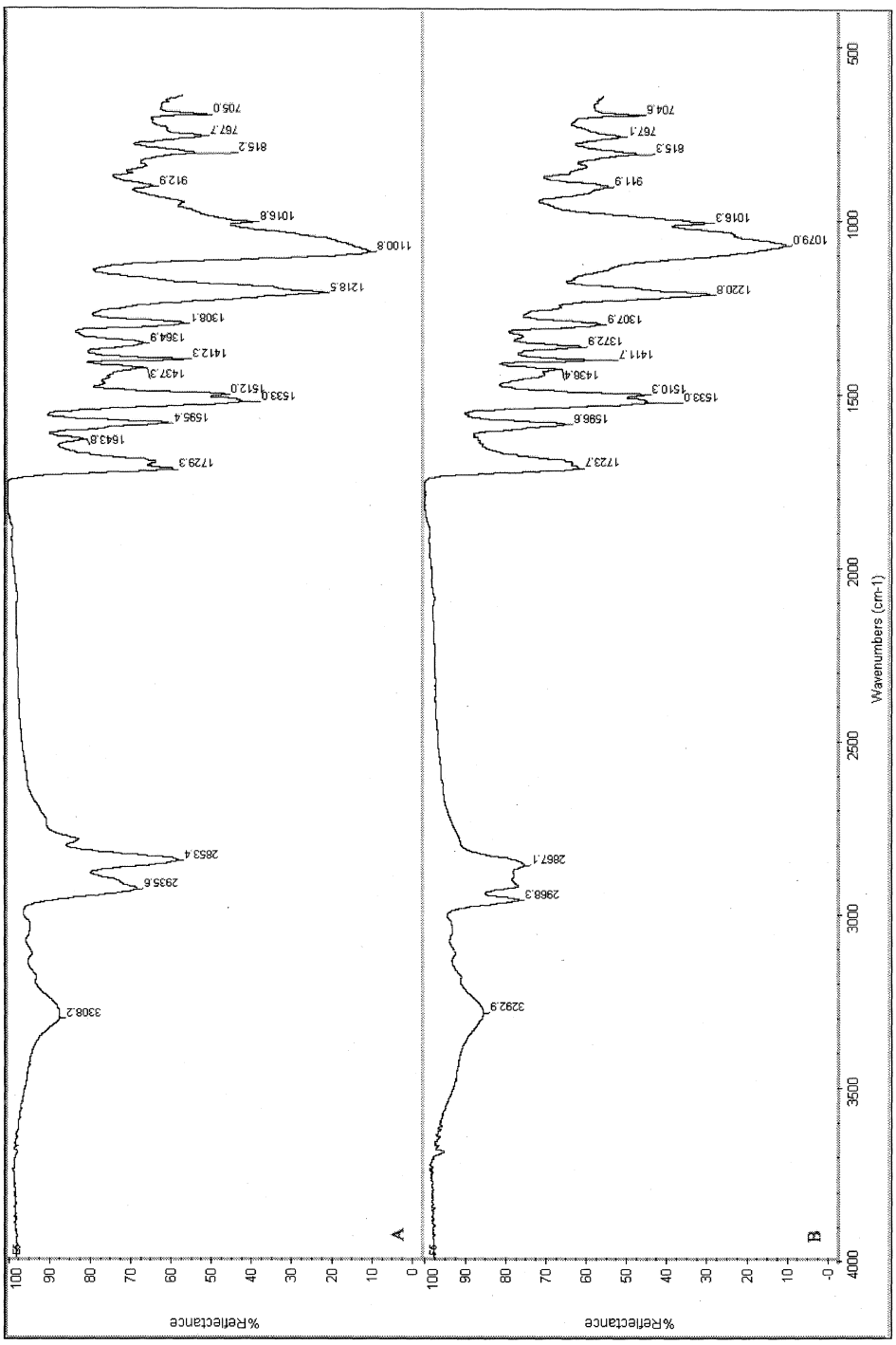

Figure 31: IR spectra of the polyurethanes (aged in HEPES buffer) PTMGMDIBPA (A) and PPGMDIBPA (B)

4.4.3 Evaluation of biostability in oxidative medium

The percentage changes in weight of aged polymers in oxidative medium are given in Table 17. The FTIR spectra revealed no change in the spectral response of the aged polymers in 3% H_2O_2 solution (Figures 32 and 33).

Table 17: Gravimetric analysis of polyurethane films aged in oxidative medium

	Medium	
Polyurethane	H_2O_2	Control
PTMGMDIBOL	0.8 ± 0.2	0.5 ± 0.2
PTMGHDIBOL	2.2 ± 0.1	0.6 ± 0.1
PPGMDIBOL	0.9 ± 0.3	0.8 ± 0.1
PPGHDIBOL	2.4 ± 0.2	0.9 ± 0.3
PTMGMDIBPA	0.7 ± 0.4	0.4 ± 0.2
PPGMDIBPA	0.5 ± 0.3	0.1 ± 0.1

Figure 32: IR spectra of the polyurethanes (aged in oxidative medium) PTMGMDIBOL (A), PTMGHDIBOL (B) and PPGMDIBOL (C) PPGHDIBOL (D)

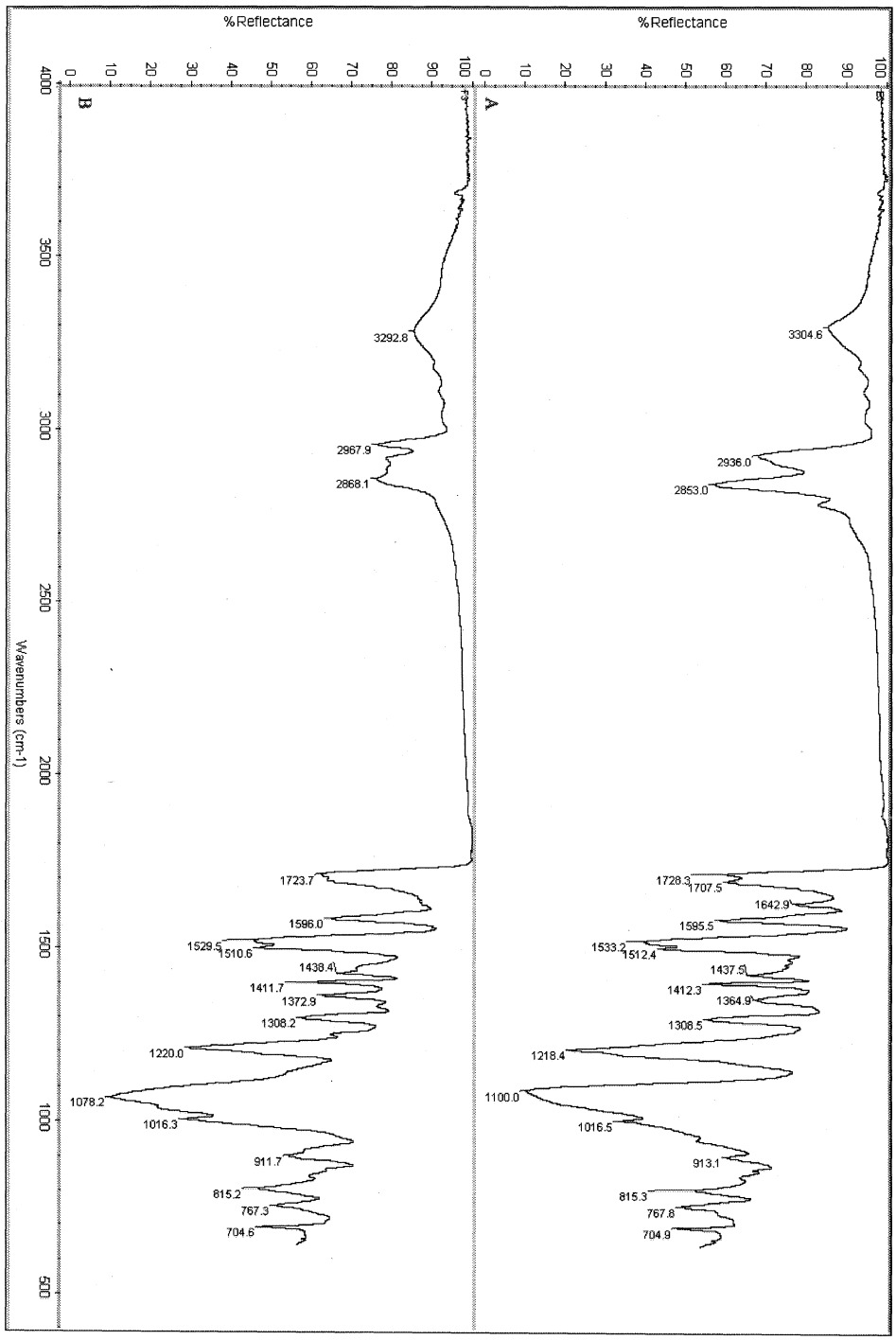

Figure 33: IR spectra of the polyurethanes (aged in oxidative medium) PTMGMDIBPA (A) and PPGMDIBPA (B)

4.4.4 Studies on accelerated chemical degradation of polyurethanes

No degradation has been observed with MDI based samples, but HDI based samples degraded with a significant mass loss and dimensional changes.

4.5 Synthesis and characterization of radiopaque methacrylate copolymers

4.5.1 Synthesis of radiopaque methacrylate copolymers

Random copolymers of GMA with MMA, EMA and BMA were synthesized by free radical polymerizations at 70°C using AIBN as an initiator to give the copolymers P(GMA-co-MMA), P(GMA-co-EMA) and P(GMA-co-BMA) in good yields. Then these copolymers were modified to their radiopaque counter parts by the regioselective ring opening of the pendant epoxy groups using elemental iodine in the presence of a catalytic amount of o-phenylenediamine as shown in Figure 34. Schematic representation of the mechanism of the epoxide ring opening reaction and iodination is as shown in Figure 35. The yields of the non-iodinated and iodinated copolymers are given in Table 18.

Table 18: Percentage yield of copolymers

Copolymers	Yield (%)
P(GMA-co-MMA)	79
P(GMA-co-EMA)	73
P(GMA-co-BMA)	70
IP(GMA-co-MMA)	75
IP(GMA-co-EMA)	71
IP(GMA-co-BMA)	69

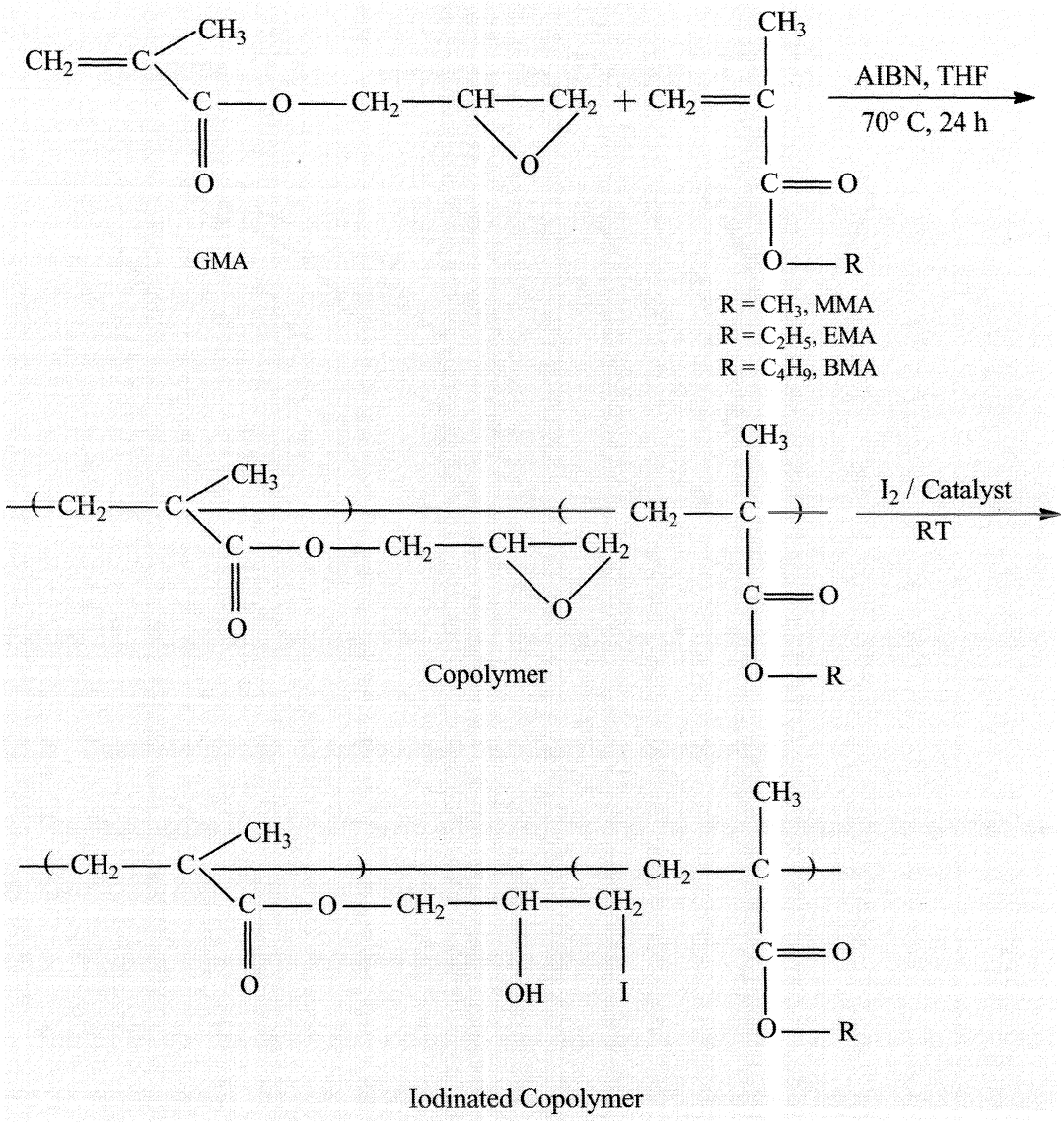

Figure 34: Schematic representation for synthesis of copolymers and iodinated copolymers

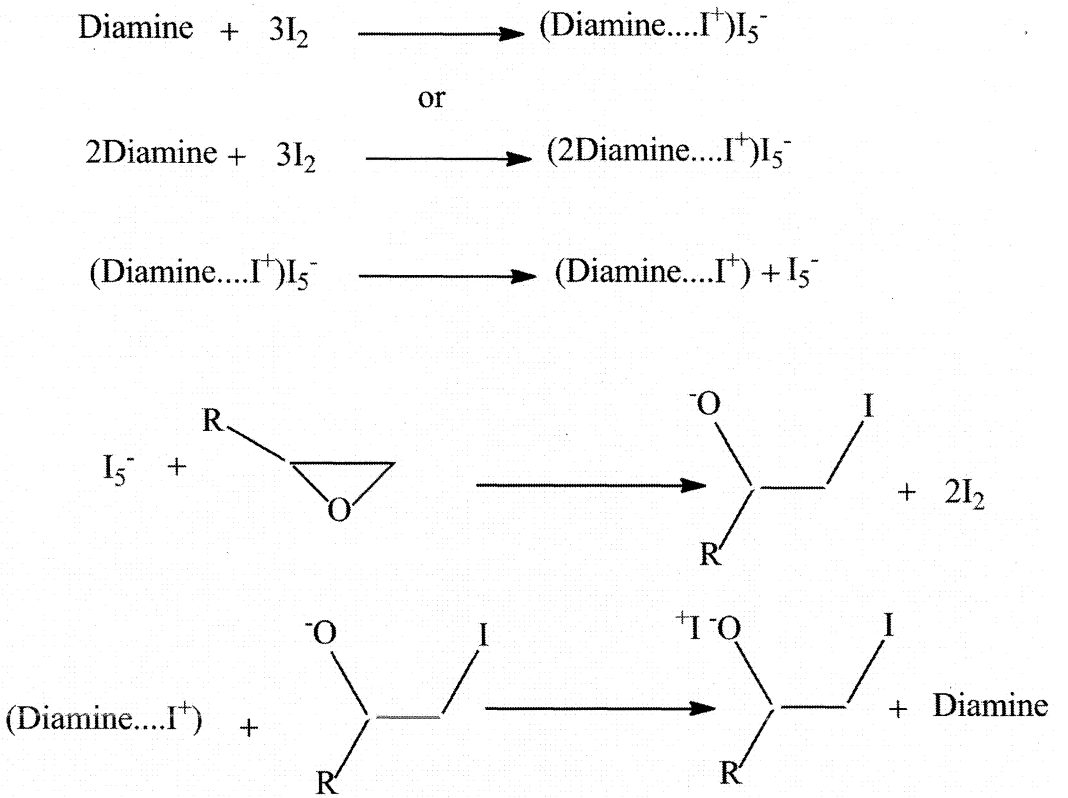

Figure 35: Schematic representation for mechanism of epoxide ring opening reaction

4.5.2 Characterization of radiopaque methacrylate copolymers

The radiopaque copolymers were characterized and the physic-chemical properties were compared with that of non-iodinated copolymers.

4.5.3 Fourier transform infrared spectral analyses

The FT-IR spectra of the non-iodinated and iodinated copolymers are given in Figures 36 and 37, respectively. The peak at 907 cm^{-1} suggested the presence of epoxy functional group in the copolymers and a broad band at 3500 cm^{-1} for hydroxyl functional group indicated the substitution of epoxy ring by iodine.

Figure 36: IR spectra of the copolymers P(GMA-co-MMA) (A), P(GMA-co-EMA) (B) and P(GMA-co-BMA) (C)

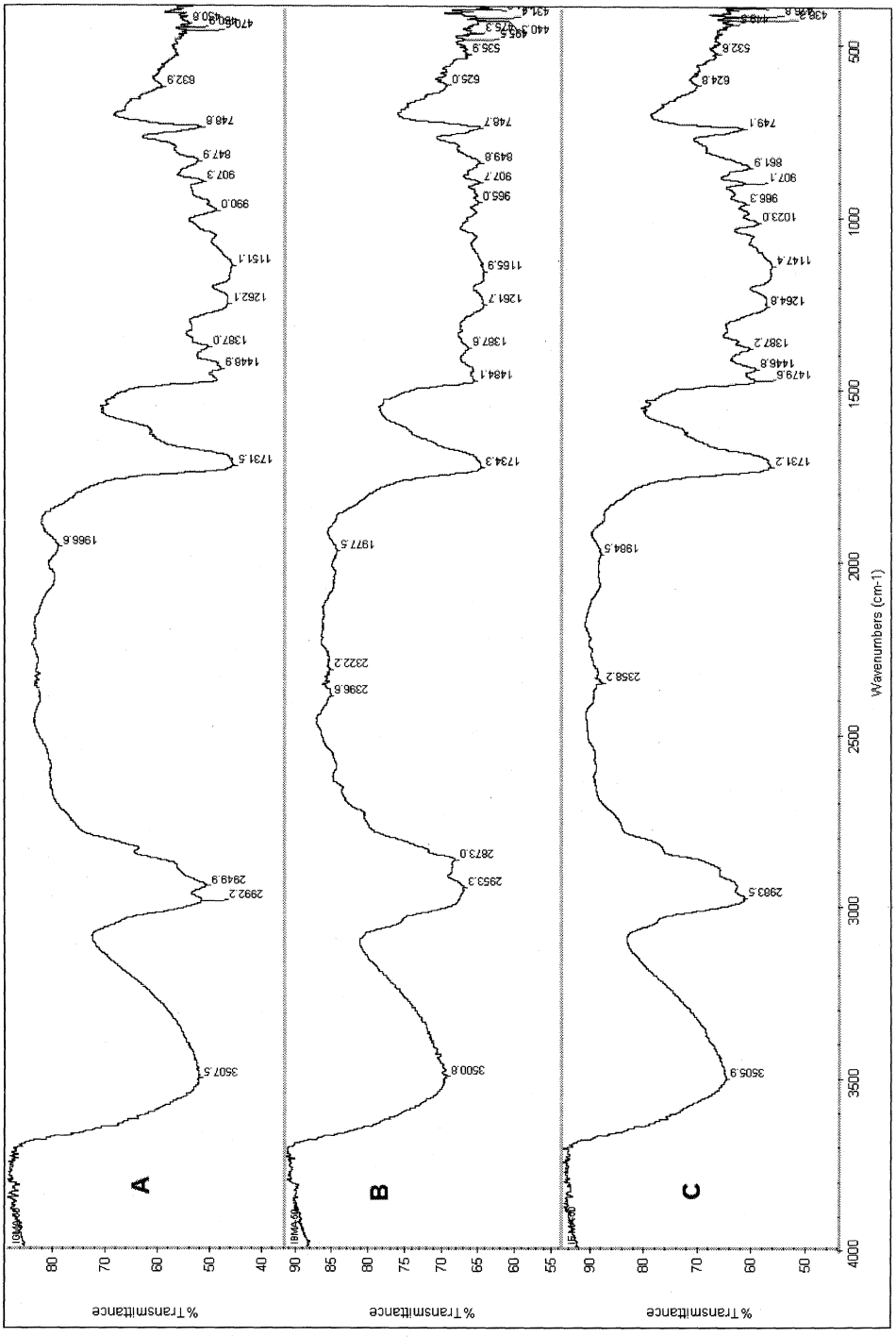

Figure 37: IR Spectra of iodinated copolymers IP(GMA-co-MMA) (A), IP(GMA-co-BMA) (B) and IP(GMA-co-EMA) (C)

4.5.4 NMR spectral analyses

The ^{1}H NMR spectra of the copolymers are given in Figure 38. The actual composition of monomers in the copolymer was determined from the NMR spectra and are given in Table 19.

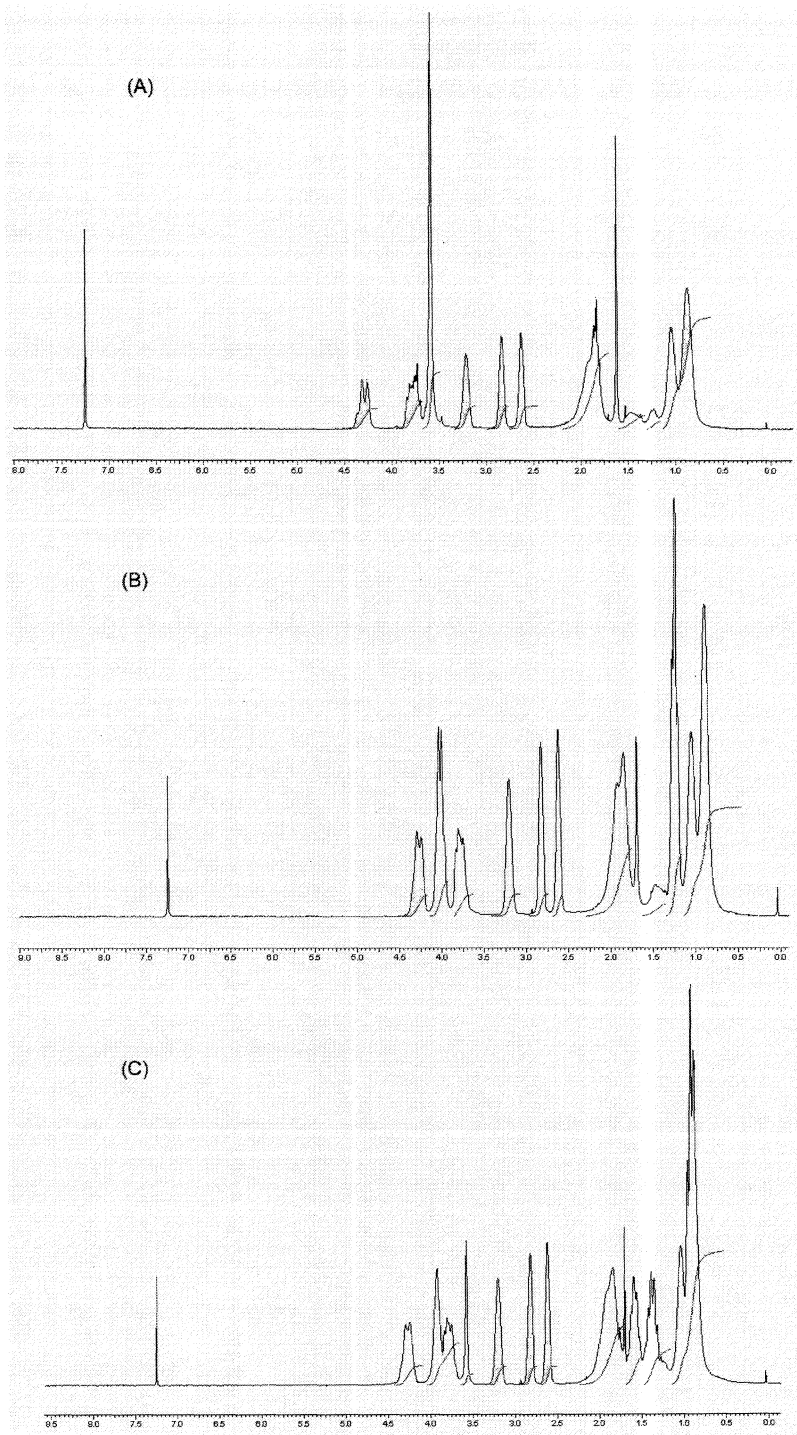

Figure 38: NMR spectra of the copolymers P(GMA-co-MMA) (A), P(GMA-co-EMA) (B) and P(GMA-co-BMA) (C)

Table 19: Composition of copolymers

	Copolymer		
mol - % of	P(GMA-co-MMA)	P(GMA-co-EMA)	P(GMA-co-BMA)
GMA	55	55	48
MMA	45	-	-
EMA	-	45	-
BMA	-	-	52

4.5.5 Gel permeation chromatography analyses

The number and weight average molecular weights of the synthesized copolymers were determined by GPC and are presented in Table 20.

Table 20: Molecular weights and polydispersity of copolymers

Copolymer	M_w	M_n	M_w/M_n
P(GMA-co-MMA)	58861	33993	1.7
P(GMA-co-EMA)	51767	33186	1.5
P(GMA-co-BMA)	70391	47093	1.4
IP(GMA-co-MMA)	89346	42642	2.0
IP(GMA-co-EMA)	65522	42598	1.5
IP(GMA-co-BMA)	86515	64864	1.3

4.5.6 Energy dispersive X-ray analyses

The EDX spectra of the copolymers are given in Figures 39 and 40. EDX were carried out for both iodinated and non-iodinated copolymers to detect the presence of iodine moiety. Only peaks for carbon and oxygen were observed in the case of EDX spectra of noniodinated polymers, where as additional peaks for iodine were seen in the area 3.5 - 5 KeV in EDX spectra of iodinated polymers.

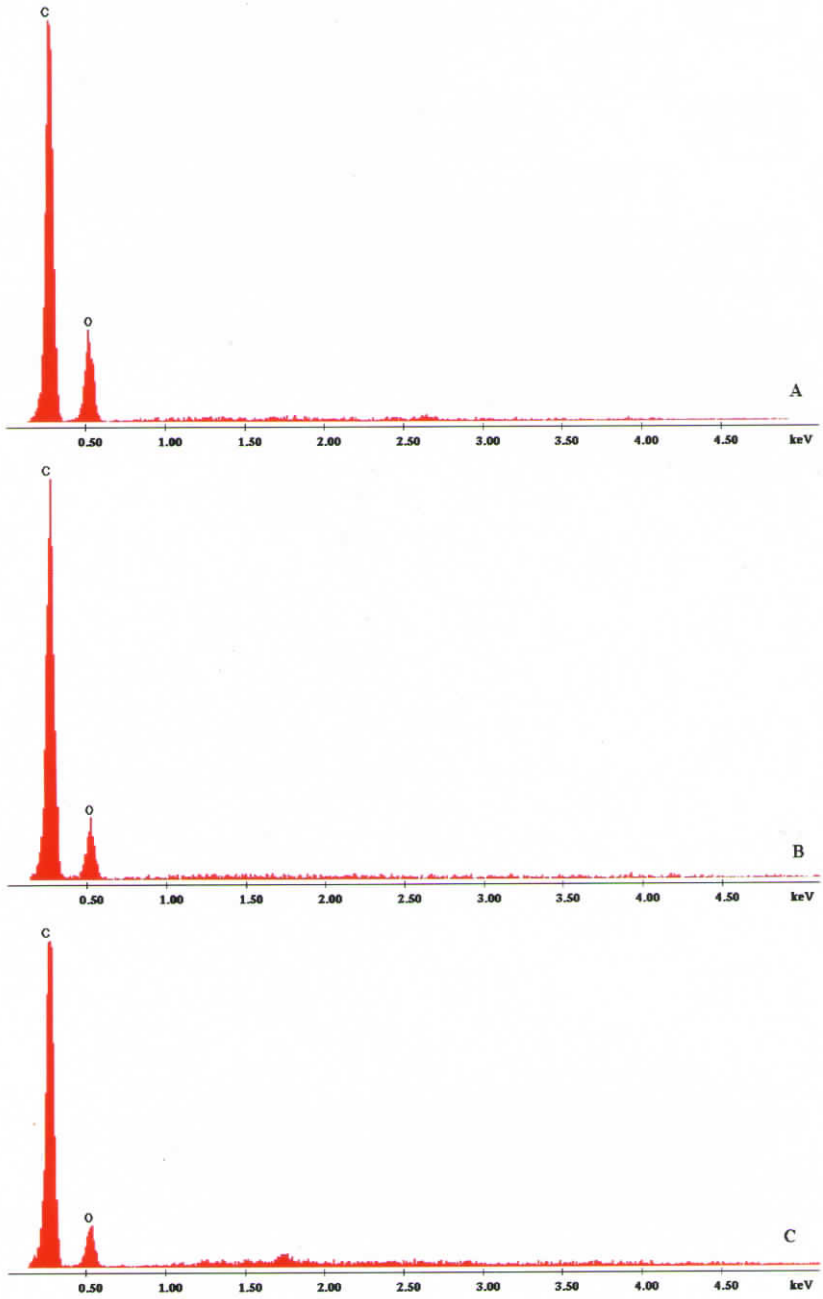

Figure 39: EDX images of copolymers P(GMA-co-MMA) (A), P(GMA-co-EMA) (B) and P(GMA-co-BMA) (C)

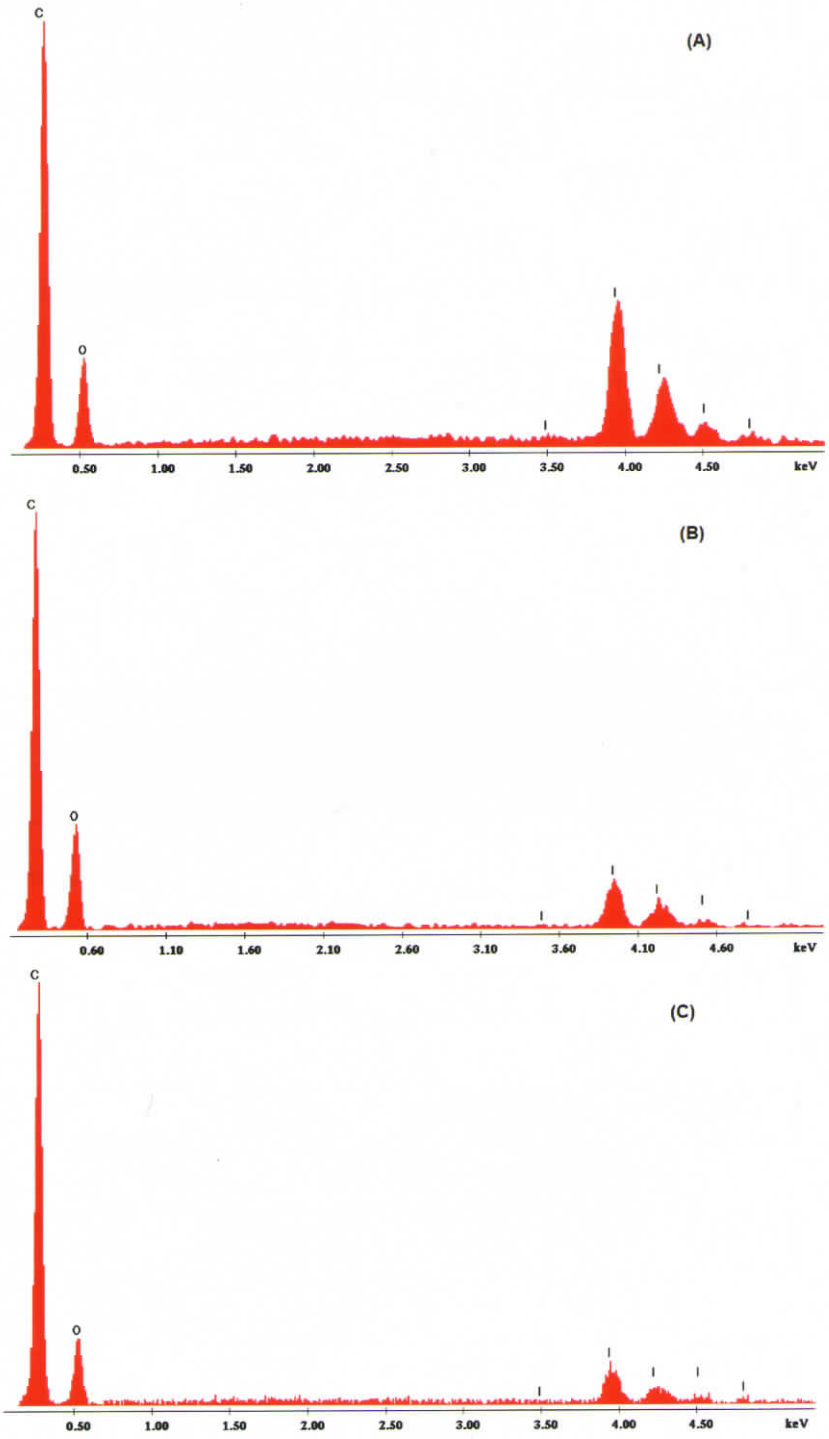

Figure 40: EDX images of copolymers IP(GMA-co-MMA) (A), IP(GMA-co-EMA) (B) and IP(GMA-co-BMA) (C)

4.5.7 Elemental analyses

Iodine content in the copolymers was estimated quantitatively by elemental analyses (Table 21).

Table 21: Quantitative estimation of iodine in copolymers by elemental analysis

Copolymer	Iodine content (%)
IP(GMA-co-MMA)	22.54
IP(GMA-co-EMA)	18.82
IP(GMA-co-BMA)	17.05

4.5.8 Thermal analyses

Thermal characteristics of the copolymers were evaluated by DSC and TGA analysis (Figures 41 - 44). The thermogravimetric analyses data are given in Table 22. The temperature at which first stage of decomposition occurred is given as Td_1 and temperature at 50% decomposition is given as $Td_{1/2}$.

Table 22: Thermal characteristics of the copolymers

Copolymer	T_g (°C)	Td_1 (°C)	Weight remained at Td_1 (%)	$Td_{1/2}$ (°C)
P(GMA-co-MMA)	44	112	98	334
P(GMA-co-EMA)	60	113	98	346
P(GMA-co-BMA)	56	180	95	316
IP(GMA-co-MMA)	99	238	97	316
IP(GMA-co-EMA)	85	231	98	335
IP(GMA-co-BMA)	65	246	98	320

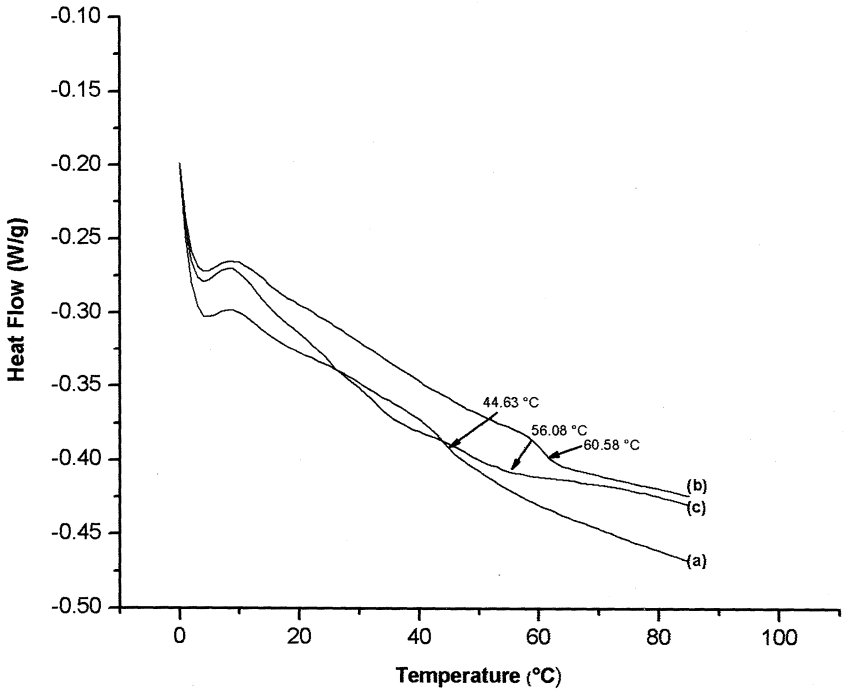

Figure 41: DSC scans of the copolymers P(GMA-co-MMA) (A), P(GMA-co-EMA) (B) and P(GMA-co-BMA) (C)

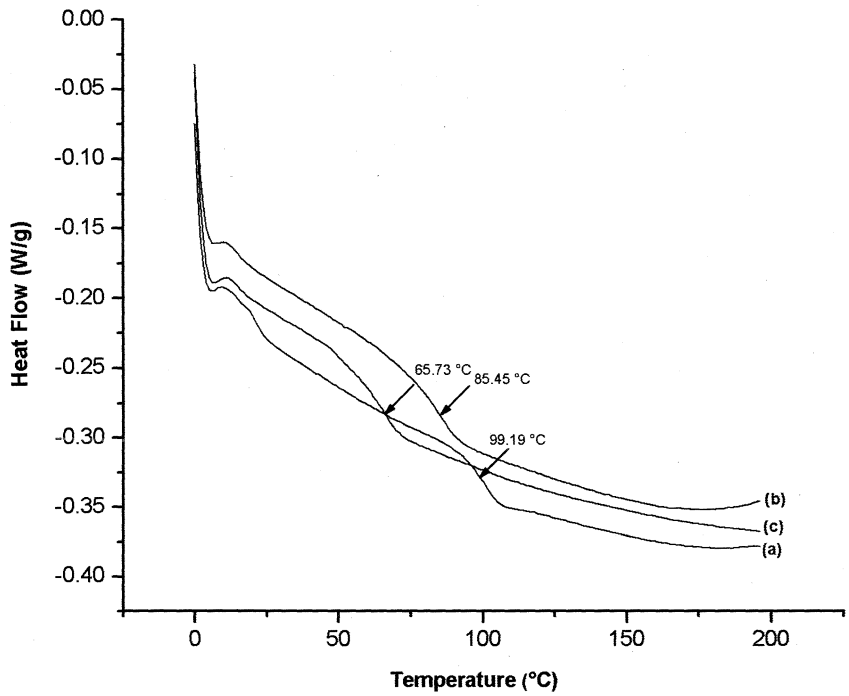

Figure 42: DSC scans of the iodinated copolymers IP(GMA-co-MMA) (A), IP(GMA-co-BMA) (B) and IP(GMA-co-EMA) (C)

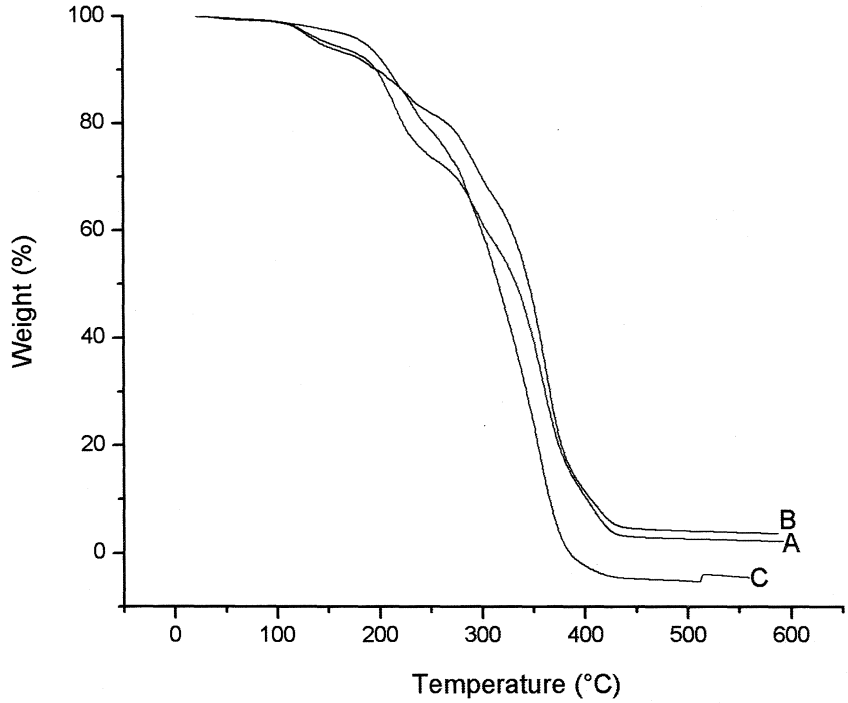

Figure 43: Thermogravimetric traces of P(GMA-co-MMA) (A), P(GMA-co-EMA) (B) and P(GMA-co-BMA) (C)

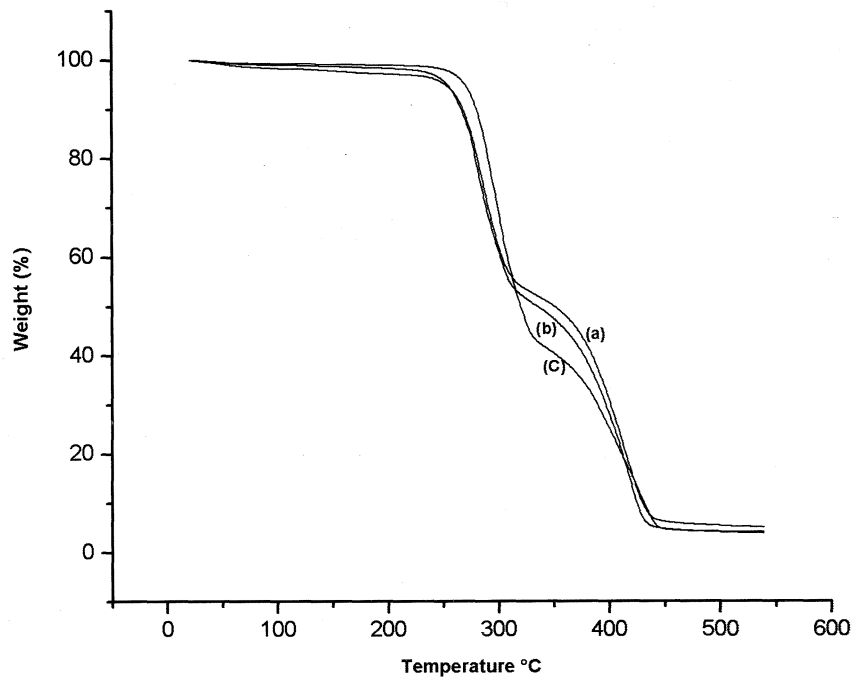

Figure 44: Thermogravimetric traces of IP(GMA-co-MMA) (a), IP(GMA-co-EMA) (b) and IP(GMA-co-BMA) (c)

4.5.9 Evaluation of radiopacity

Radiographic examination of iodinated copolymer showed sharp X-ray images when compared to non-iodinated copolymer, thus indicating radiopacity of the iodinated copolymers (Figure 45). Quantitative evaluation of radiopacity has shown that the radiopacity of iodinated copolymers films having 2mm thickness were higher that of the aluminium wedge having same dimensions (Figure 46).

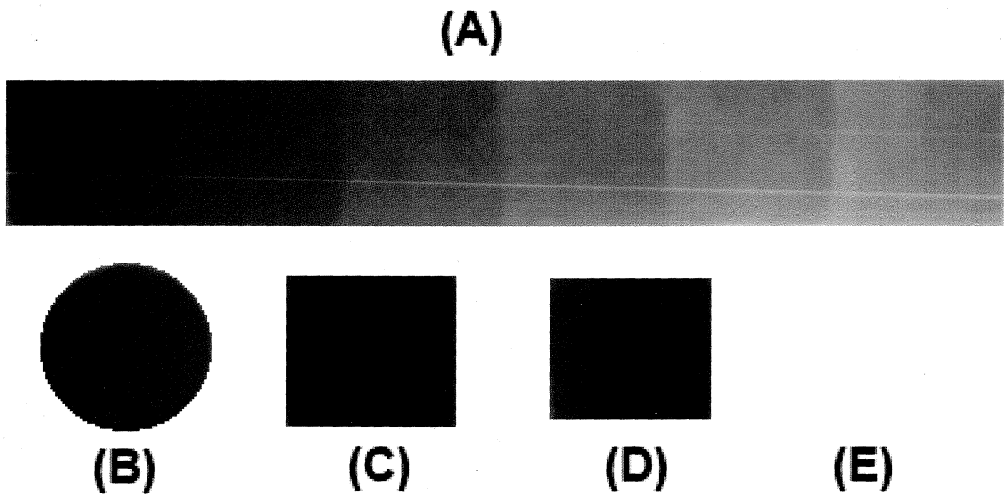

Figure 45: Positive print of a radiograph showing: an aluminium step wedge 0.5-3 mm thick in 0.5 mm steps (right to left) (A), IP(GMA-co-MMA) (B), IP(GMA-co-EMA) (C), IP(GMA-co-BMA) (D), P(GMA-co-MMA) (not visible in print) (E)

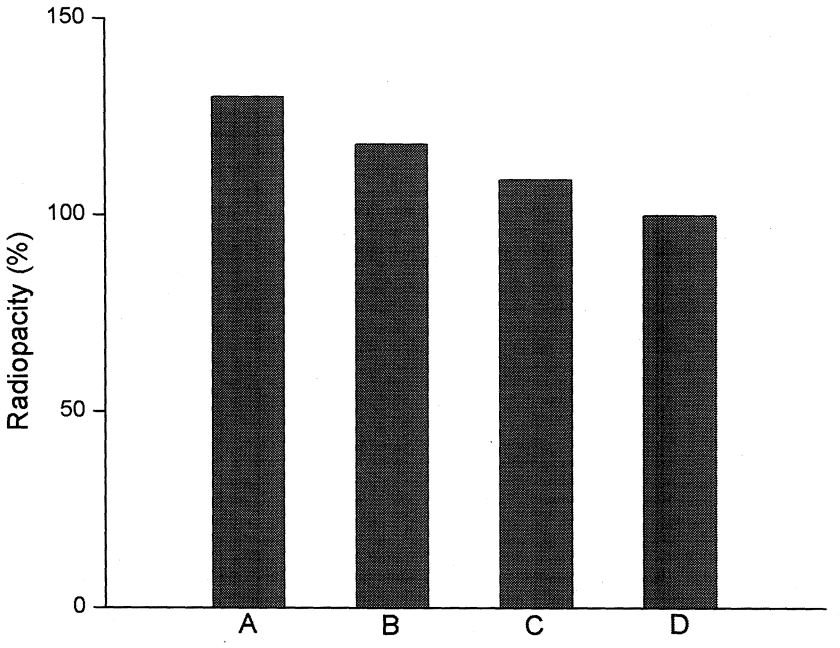

Figure 46: Graph illustrating the radiopacity of IP(GMA-co-MMA) (A), IP(GMA-co-EMA) (B), IP(GMA-co-BMA) (C) evaluated as ratio of absorption relative to standard 2mm thick Al wedge (D)

4.6 Studies on biocompatibility of radiopaque polymers

The vital aim of the work was to evaluate the material for biomedical applications. With this intention, cytoxicity, blood and tissue compatibility of the radiopaque polymers have been examined.

4.6.1 Evaluation of cytoxicity *in vitro*

Preliminary cytotoxicity evaluation was done to assess the potential of the iodinated polymers for biomedical applications. The polymers were found to be non-cytotoxic in nature. Representative microphotographs of fibroblasts cells around iodinated polyurethanes and iodinated copolymer films (scored as zero) are given in Figures 47 - 49. In order to test the cytotoxicity of the polymers quantitatively, MTT assay was performed. Quantitative assessment of the cytotoxicity to cells after contact with the material extracts showed more than 70% metabolic activity by all polymers when compared to cells without the material for 24 h of contact (Figures 50 and 51).

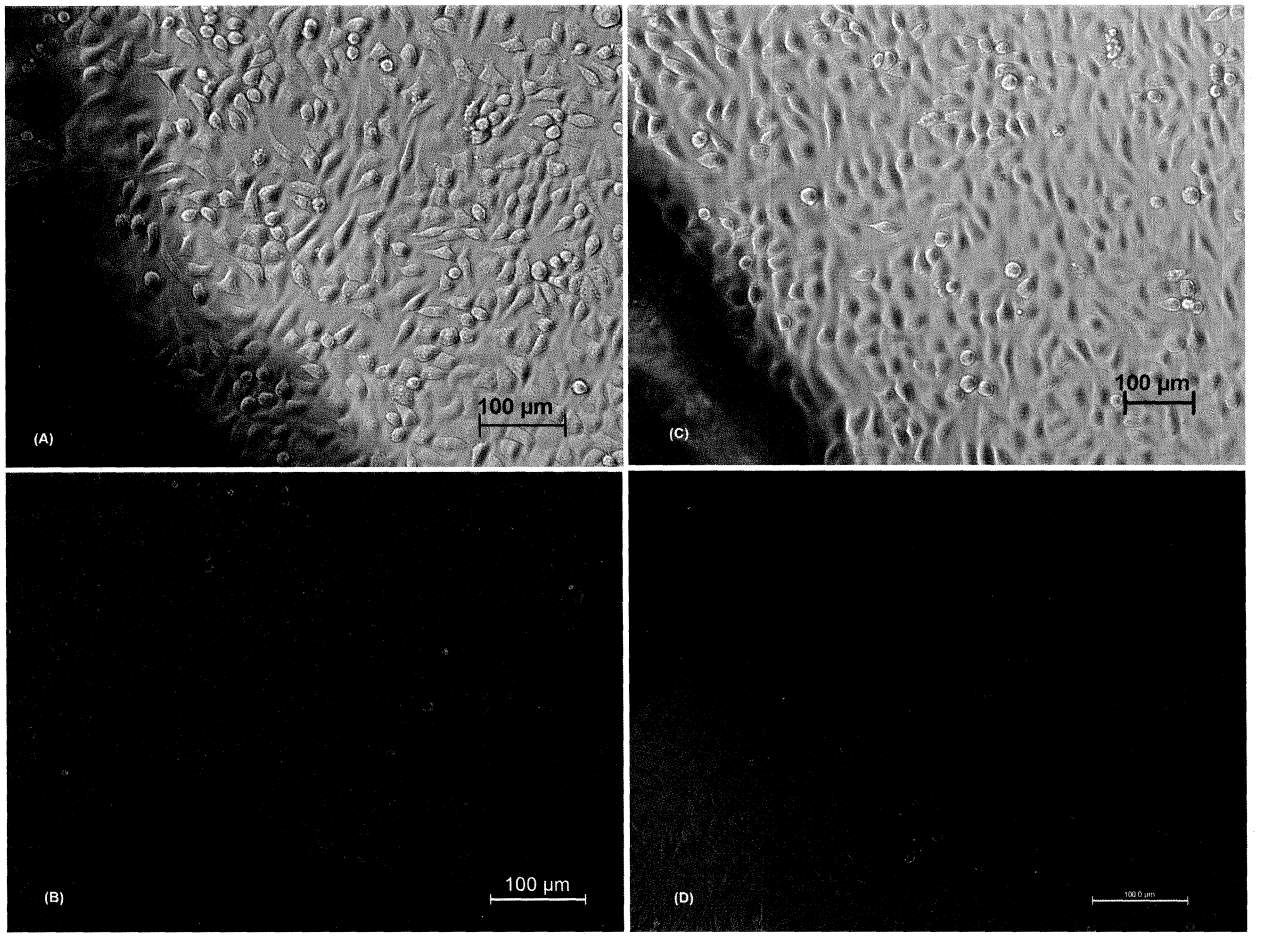

Figure 47: Representative microphotograph of fibroblast cells around PTMGMDIBOL (A), PTMGHDIBOL (B), PPGMDIBOL (C), PPGHDIBOL (D)

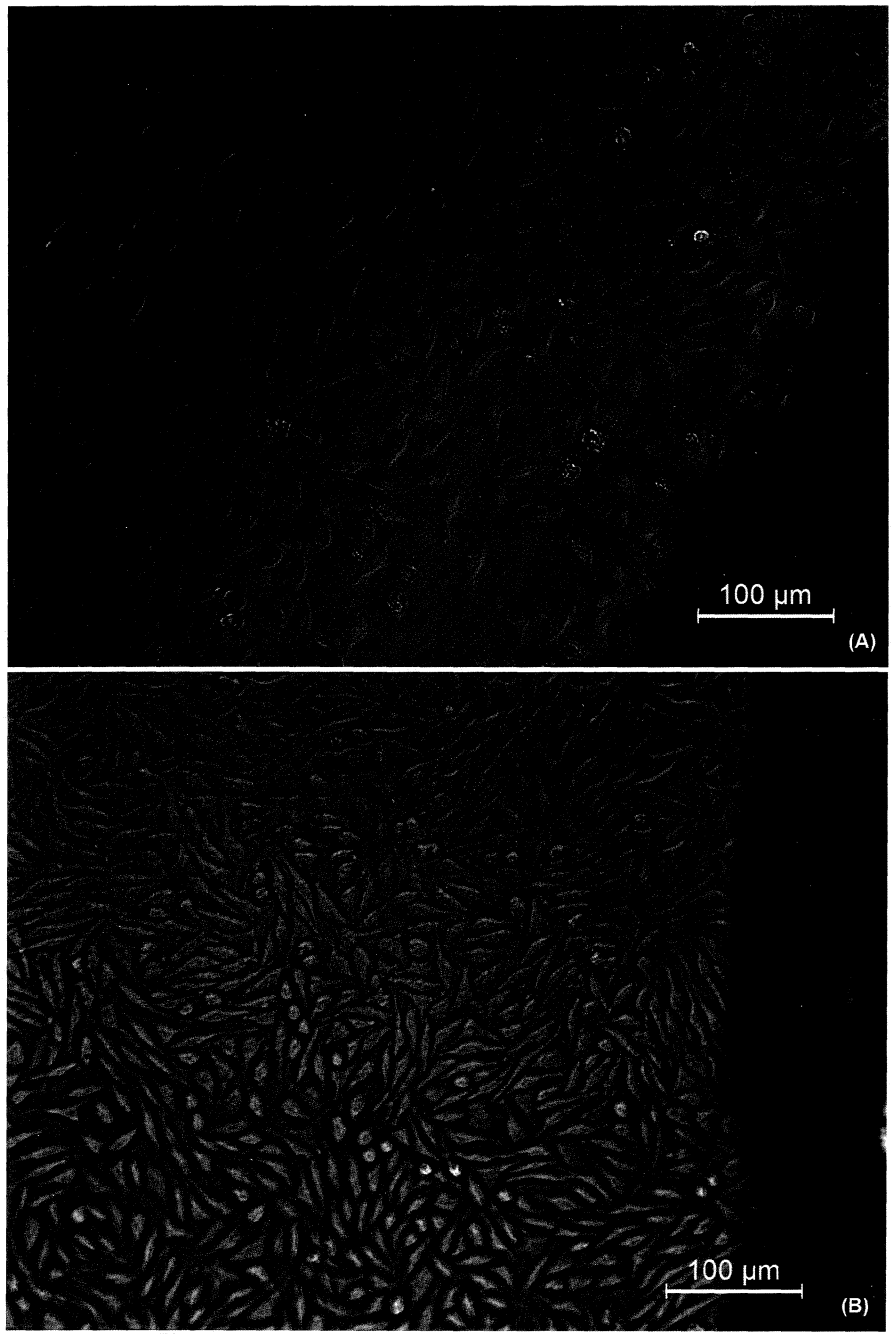

Figure 48: Representative microphotograph of fibroblast cells around PTMGMDIBPA (A), PPGMDIBPA (B)

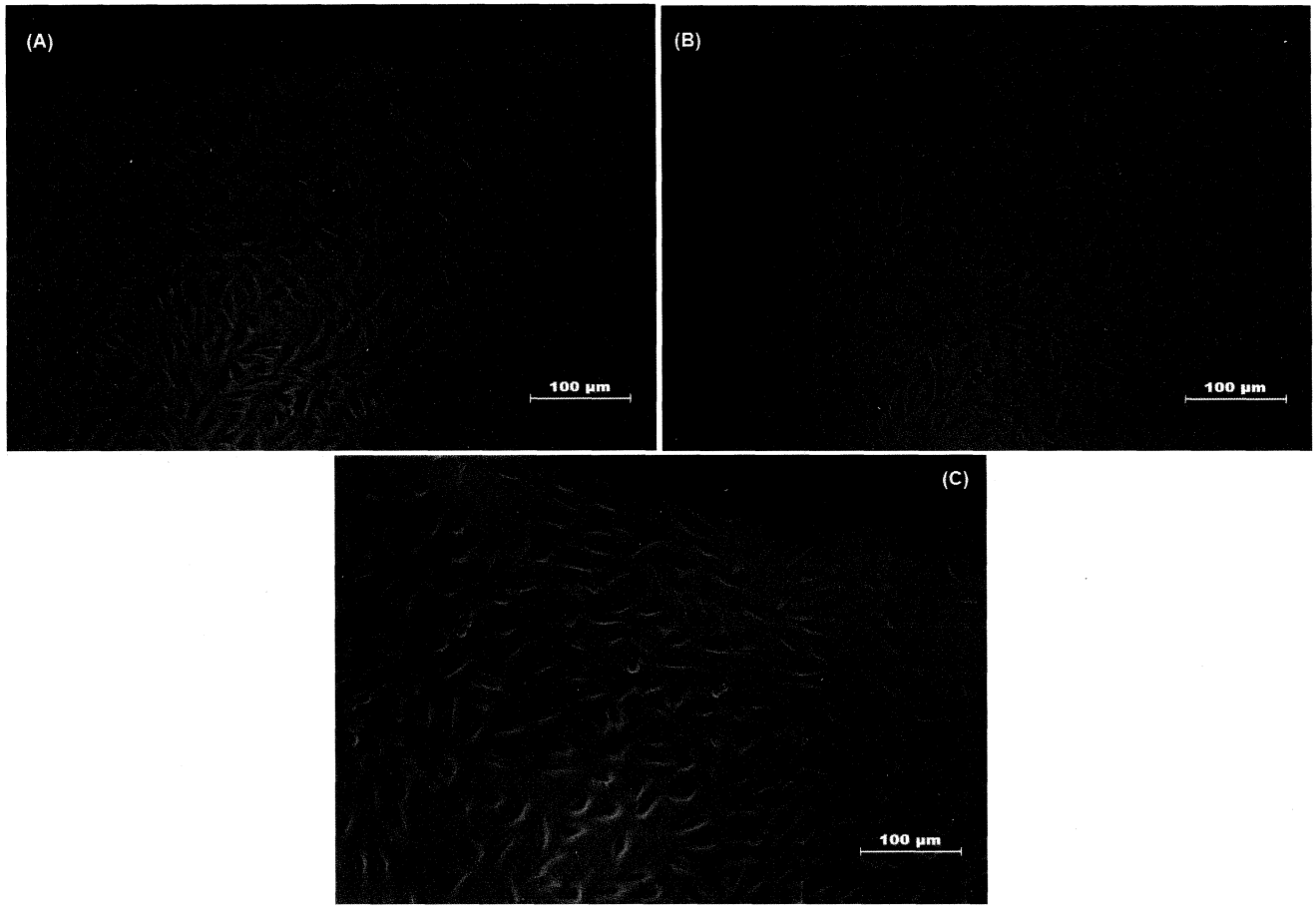

Figure 49: Representative microphotograph of L929 mouse fibroblast cells around iodinated copolymers IP(GMA-co-MMA) (A), IP(GMA-co-EMA) (B), IP(GMA-co-BMA) (C)

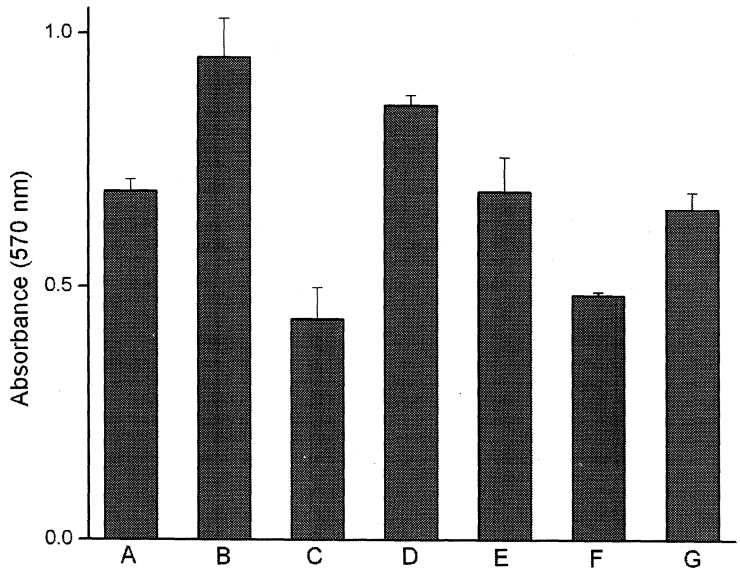

Figure 50: MTT reduction of mouse fibroblast cells with polyurethane extracts PTMG-MDIBOL (A), PTMGHDIBOL (B), PPGMDIBOL (C), PPGHDIBOL (D), PTMG-MDIBPA (E), PPGMDIBPA (F) in comparison with negative control (high density polyethylene-PE) (G)

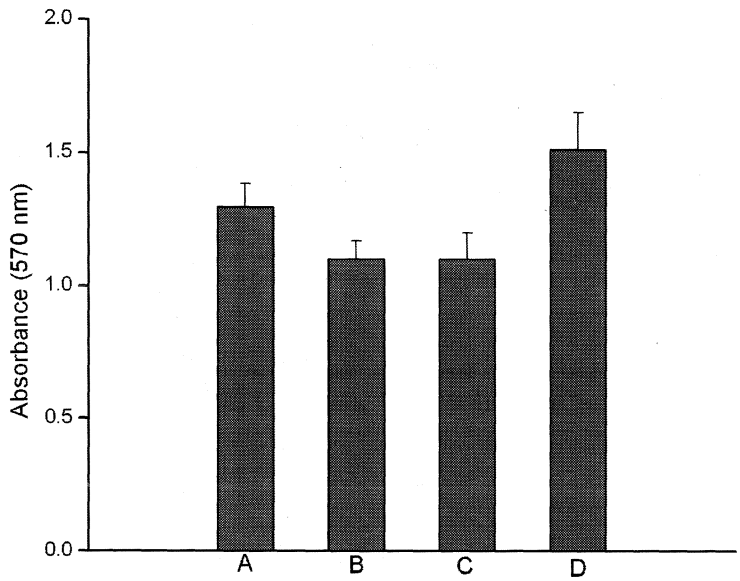

Figure 51: MTT reduction of mouse fibroblast cells with iodinated copolymer extracts IP(GMA-co-MMA) (A), IP(GMA-co-EMA) (B), IP(GMA-co-BMA) (C) in comparison with negative control (high density polyethylene-PE) (D)

4.6.2 Evaluation of blood compatibility *in vitro*

In vitro screening of the radiopaque polyurethanes, PTMGMDIBOL and PTMGMDIBPA for blood-compatibility showed that there was no significant difference in count for RBC,WBC and platelets immediately and after 30 min of contact (Table 23). Determination of fibrinogen content showed that there was no change in fibrinogen concentration when evaluated after 30 min exposure of blood to the iodinated polyurethanes tested (Table 24). Partial thromboplastin time indicated that the intrinsic coagulation pathway has not been adversely affected. The values obtained for the copolymer were within accuracy limits.

Table 23: RBC, WBC and Platelet Counts of human blood immediately and after 30 min exposure to polyurethanes films

	RBC count ($10^6/mm^3$)	
Material	before	after
PTMGMDIBOL	7.45 $\pm$ 0.03	7.65 $\pm$ 0.01
Reference	7.42 $\pm$ 0.05	7.39 $\pm$ 0.04
PTMGMDIBPA	6.33 $\pm$ 0.1	6.33 $\pm$ 0.1
Reference	6.21 $\pm$ 0.1	6.26 $\pm$ 0.2

	WBC count ($10^6/mm^3$)	
Material	before	after
PTMGMDIBOL	8.45 $\pm$ 0.05	8.3 $\pm$ 0.05
Reference	8.45 $\pm$ 0.05	8.6 $\pm$ 0.1
PTMGMDIBPA	8.40 $\pm$ 0	8.1 $\pm$ 0.3
Reference	8.25 $\pm$ 0	8.05 $\pm$ 0.05

	Platelet count ($10^6/mm^3$)	
Material	before	after
PTMGMDIBOL	305 $\pm$ 4	285 $\pm$ 3
Reference	306 $\pm$ 3	302 $\pm$ 5
PTMGMDIBPA	250 $\pm$ 2	228 $\pm$ 3
Reference	243 $\pm$ 3	240 $\pm$ 4

Table 24: Data on plasma coagulation assay with polyurethanes

	Fibrinogen concentration (g/dL)		PTT (s)	
Material	before	after	before	after
PTMGMDIBOL	2.88 ± 0.03	2.83 ± 0.03	100 ± 2	102 ± 4
Reference	2.89 ± 0.05	2.86 ± 0.01	158 ± 1	160 ± 3
PTMGMDIBPA	4.00 ± 0.1	3.74 ± 0.1	273.2 ± 3	275.6 ± 4
Reference	3.97 ± 0.02	3.82 ± 0.02	228.7 ± 1	231.7 ± 1

4.6.3 Hemolysis assay

The percentage hemolysis of blood in contact with radiopaque polyurethane films at 37 °C for 60 min are as shown in Table 25. Both the samples were found to be non-hemolytic, the extent of haemolysis being lower than the permissible level of 5%.

Table 25: Hemolytic potential of polyurethanes

Sample	Hemolysis (%)
PTMGMDIBOL	0.14 ± 0.01
Reference	0.06 ± 0.01
PTMGMDIBPA	0.04 ± 0.01
Reference	0.03 ± 0.01

4.6.4 *In vivo* evaluation of intracutaneous irritation in rabbit

The intracutaneous irritation test was done to assess the potential of radiopaque polymers, PTMGMDIBPA and IP(GMA-co-MMA) to produce irritation following intradermal injection of material extracts. The test results indicated that the PS and CSO extracts of the test materials produced an average irritation score of zero in PS and zero in CSO extract, following intradermal injection (Table 26). Hence the test materials met the requirements of the ISO protocol as the difference between the test sample mean scores and the control mean scores were less than 1.

Table 26: Evaluation of intracutaneous irritation in rabbits for radiopaque polymers

	Irritation Score			
	Erythema		Oedema	
Polymer	PS	CSO	PS	CSO
Control	0	0	0	0
PTMGMDIBPA	0	0	0	0
Control	0	0	0	0
IP(GMA-co-MMA)	0	0	0	0

4.6.5 Evaluation of *in vivo* biocompatibility - subcutaneous implantation

In vivo biocompatibility was studied by the subcutaneous implantation of the radiopaque polymers, PTMGMDIBPA and IP(GMA-co-MMA) in rat animal models for 30, 60 and 90 days. Gross examination revealed that all the animals were in good health condition throughout the experimental period. The consumption of feed and water were normal during the observation period. When the animals were sacrificed at the end of 90 days, none of the implantation site (both test and control) showed any macroscopic abnormalities such as haemorrhage, necrosis, discolouration or infection. Representative X-ray images of the rats with the test specimens are shown in Figures 52 and 53. The microphotographs of tissue sites for negative control, UHMWPE and test material for post-implantation period of 4, 8 and 12 weeks for PTMGMDIBPA and IP(GMA-co-MMA) are given in Figures 54 and 55.

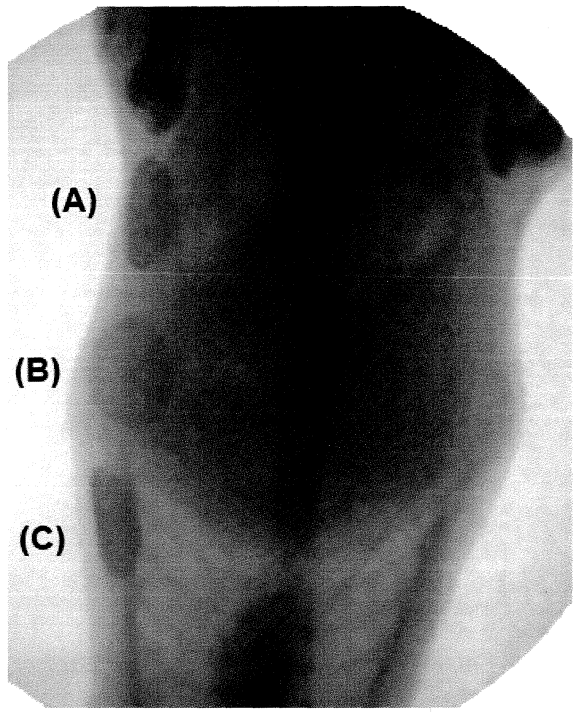

Figure 52: Representative X-ray image of a rat bearing three test specimens PTMG-MDIBPA (A), (B) and (C)

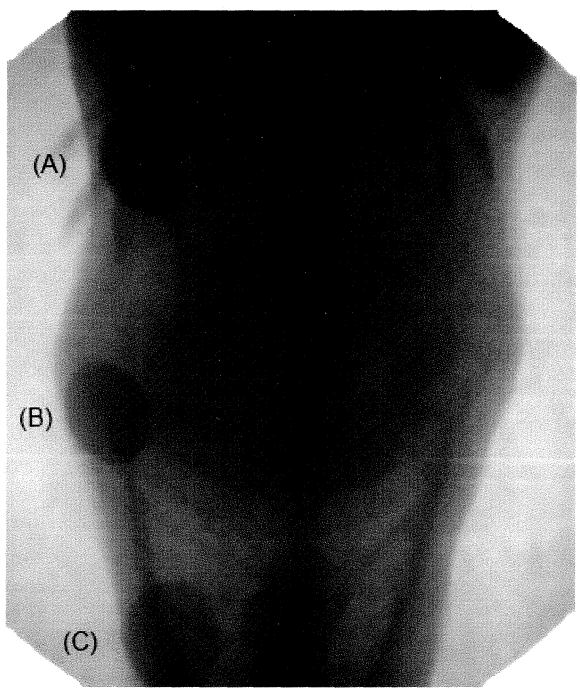

Figure 53: Representative X-ray image of a rat bearing three test specimens (IP(GMA-co-MMA) (A), (B) and (C)

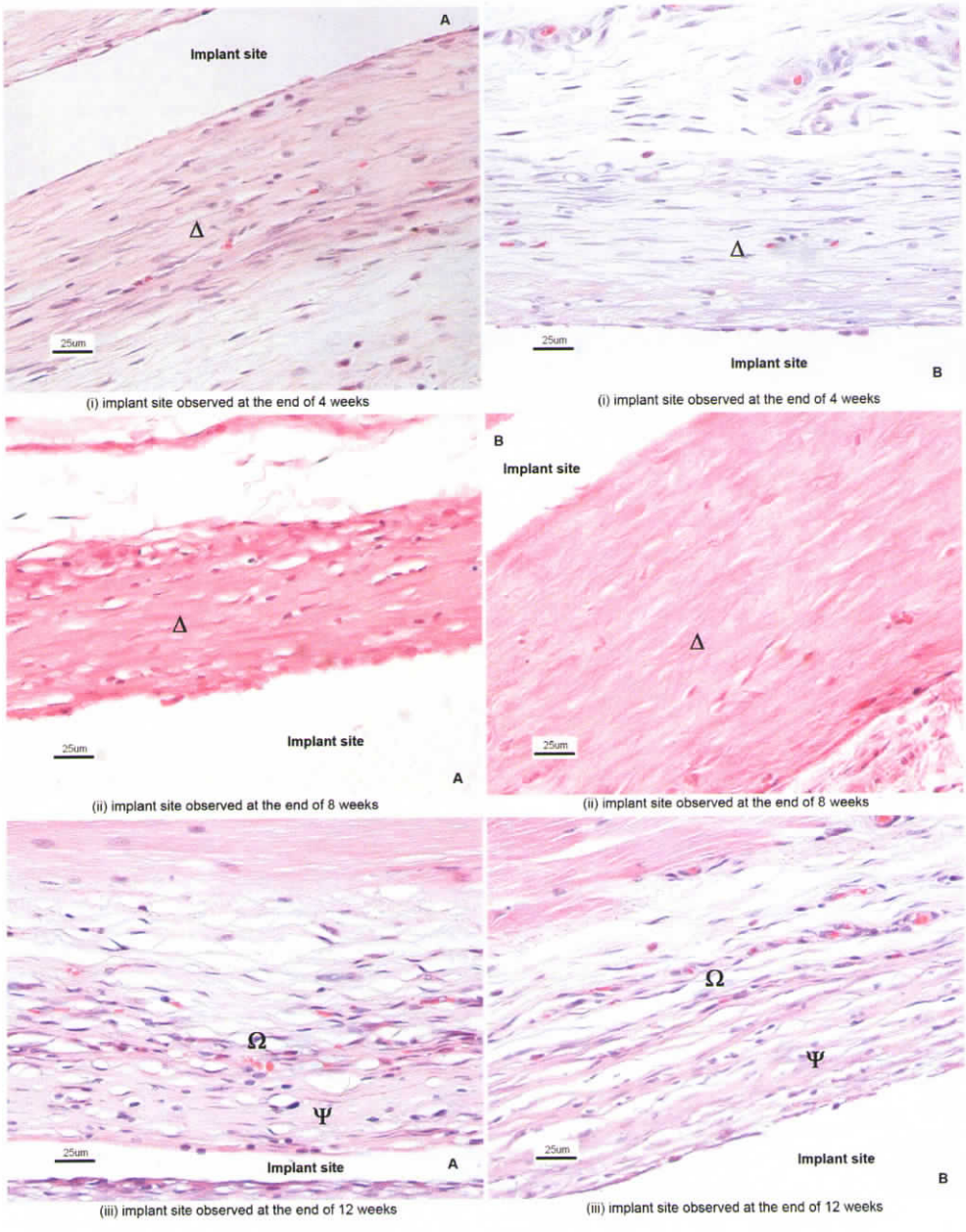

Figure 54: Optical photomicrograph showing tissue response of test material PTMG-MDIBPA (A) and control UHMWPE (B)

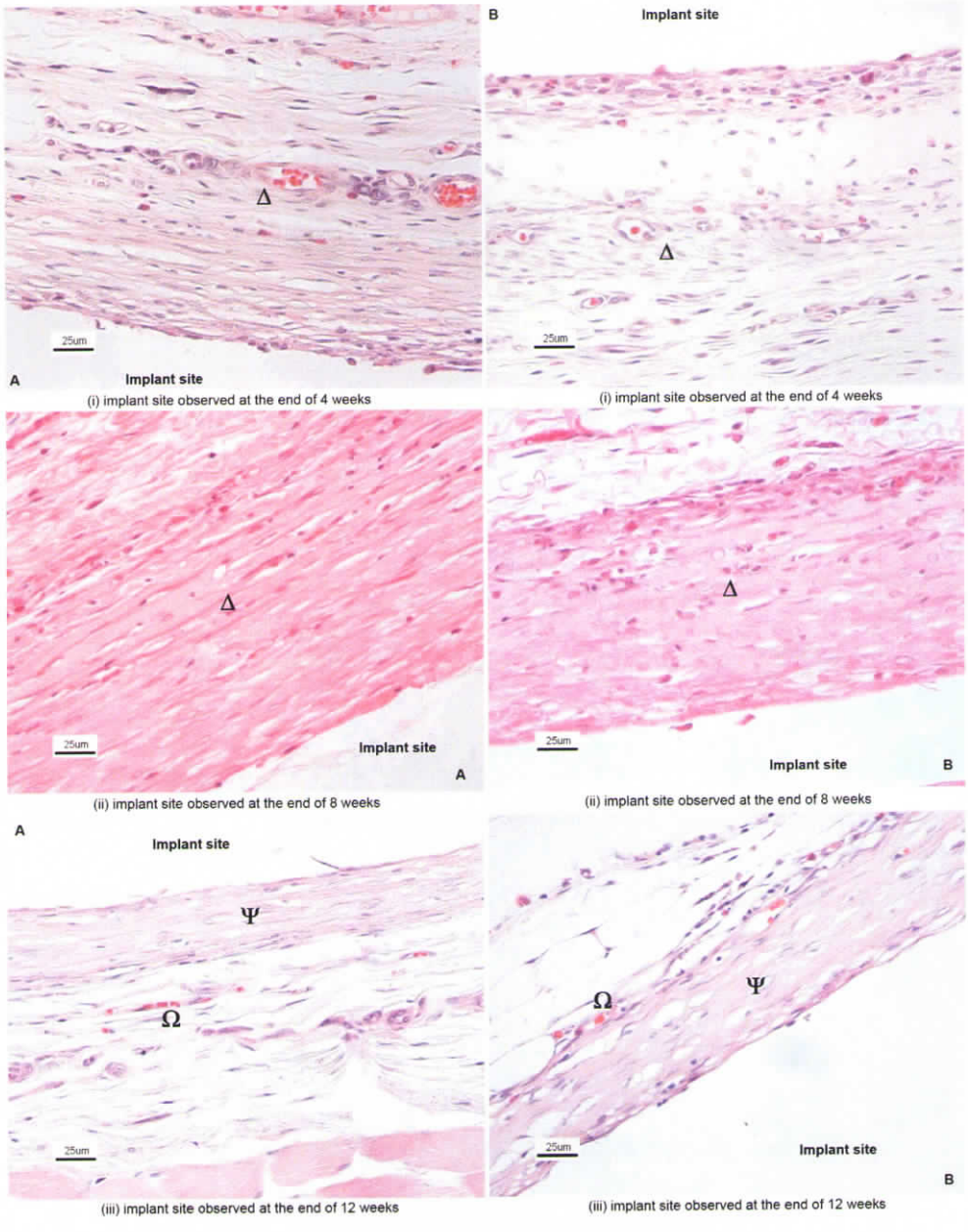

Figure 55: Optical photomicrograph showing tissue response of test material IP(GMA-co-MMA) (A) and control UHMWPE (B)

DISCUSSION

5 DISCUSSION

5.1 Synthesis & characterization of iodinated chain extenders

Chain extenders are compounds with active hydrogen atoms such as aliphatic and aromatic diols. The properties of the polyurethanes can be drastically improved by the addition of chain extender. The role of the chain extender is to produce an "extended" sequence in the copolymer consisting of alternating chain extenders and diisocyanates. These extended sequences, or hard segments, act both as filler particles and physical crosslink sites to increase mechanical strength. Chain extenders are also used to increase the hydrogen bond density and the molecular weight of the polyurethane.

Polyurethanes having intrinsic radiopacity could be prepared by employing chain extenders containing radiopacifying elements like iodine. It was possible to introduce sufficient concentration of the radiopacifying elements by using polyiodinated chain extenders. The few reports on radiopaque polyurethanes employed brominated compounds to induce radiopacity. When using brominated chain extenders, the polymer must have desired bromine content (about 15% of the weight of polyurethane) to impart useful radiopaque properties. Adequate bromine content could be achieved only by increasing the amount of chain extender. This resulted in a relatively large hard segment ratio leading to stiffness and toxicity (Markush & Sarpeshkar 1993, 1994).

5.1.1 Synthesis and characterization of aliphatic chain extender 2,3 diiodo-2-butene-1,4 diol

The chain extender IBOL was synthesized by the iodination of 2-butyne-1,4 diol (Figure 56). The synthesized IBOL was characterized by ^{1}H and ^{13}C NMR spectroscopy. In the NMR spectra, the peak at δ 4.22 (4H, d) corresponded to protons in -CH2 groups. The peak at δ 5.55 (2H, s) indicated the presence of protons in hydroxyl groups. With ^{13}C NMR spectra, the peak at δ 73.88 indicated the presence of two carbons in -CH2 groups. The peak at δ 105.15 suggested the presence of two olefinic carbon atoms. From all these data, the structure of the compound was assigned to be that of IBOL.

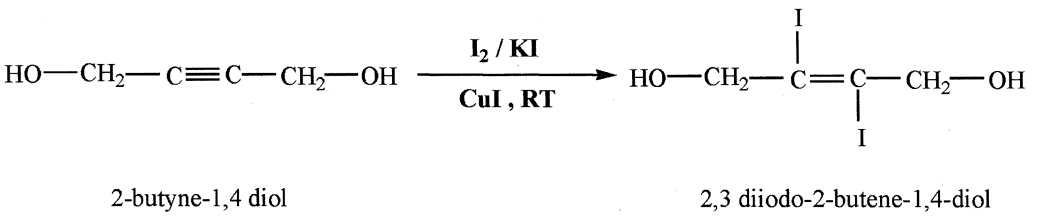

Figure 56: Synthesis of chain extender 2,3 diiodo-2-butene-1,4-diol

5.1.2 Synthesis and characterization of aromatic chain extender bis hydroxyethyl ether of tetraiodo bisphenol A

Initially iodination of bisphenol-A was carried out by the method reported in literature for preparation of iodophenols, where phenols could be iodinated by the *in situ* oxidation of sodium iodide using sodium hypochlorite with methanol as solvent (Edgar & Falling 1990). To add flexible spacers to reduce the rigidity of the system, iodinated bisphenol-A was coupled with 2-chloro ethanol to obtain bis hydroxyethyl ether of tetraiodo bisphenol A. The iodinated aromatic chain extender, IBPA was synthesized using bisphenol A as the starting material as shown in Figure 57. The chain extender was purified by column chromatography using hexane and ethyl acetate as solvent.

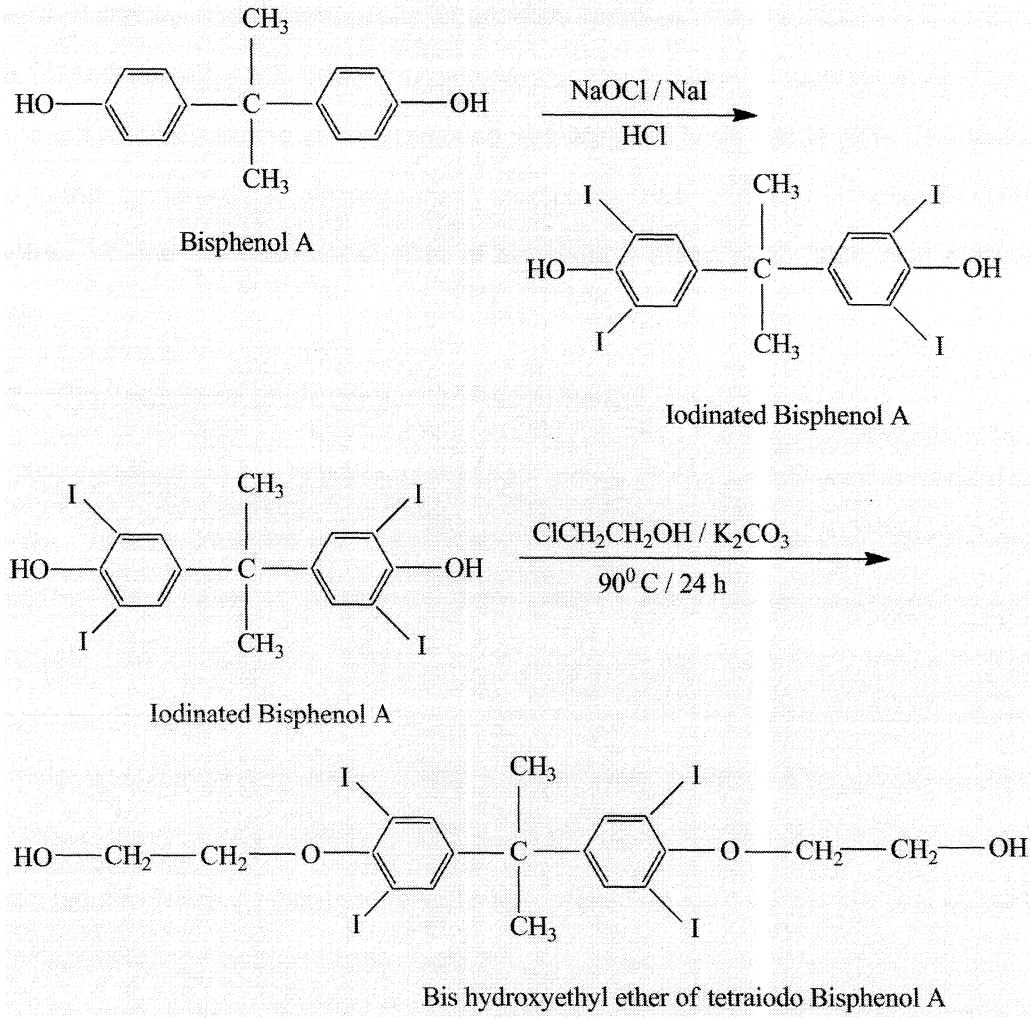

Figure 57: Synthesis of chain extender bis hydroxyethyl ether of tetraiodo bisphenol A

NMR spectra of IBPA showed chemical shift due to six methyl protons at δ 1.57 and a singlet at δ 2.4 which corresponded to two protons of -OH group. Chemical shift due to the symmetrical protons meta to the -OH group in each ring could be seen as a singlet at δ 7.55. The singlet signal indicated the absence of protons on the adjacent carbon as a result of the substitution of the protons by iodine. The downfield shift in comparison to that in the bis hydroxyethyl ether of bisphenol A confirmed the presence of four electron withdrawing iodine atoms *ortho* to -OH group. Of the carbon signals recorded in ^{13}C NMR spectra, the peak at δ 30.62 indicated the presence of two methyl carbons in [$C(CH_3)_2$] groups. The peak at δ 41.45 suggested the presence of carbon atom attached to methyl groups [$C(CH_3)_2$]. The peaks at δ 62.13 and δ 74.27 corresponded to ethylether carbon atoms in [-O-CH_2-CH_2-OH] groups.

The NMR spectra also showed peaks for aromatic carbon atoms at δ 90.84 [Ar-C (ortho)], δ 138.15 [Ar-C (meta)], δ 149.12 [Ar-C (quarternary)] and δ 155.31[Ar-C (quarternary)]. From the spectral data, the structure of the compound was assigned to be that of IBPA. The structure was further confirmed by comparison with the spectral data of similar compounds such as Bisphenol A and bis hydroxyethyl ether of bisphenol A (Kiran et al. 2009, Hait & Sivaram 1998).

5.2 Synthesis of radiopaque polyurethanes

Polyurethanes are one of the most important groups of biomaterials used in medical technology. They are polymers with the characteristic urethane linkage in their chemical repeat structure. As a family of biomaterials, polyurethanes are most frequently synthesized as segmented block copolymers. Segmented polyurethanes are mainly linear elastomeric block copolymers consisting of soft and hard segments derived from a polyol and diisocyanate-chain extender combination respectively. These polymers typically exhibit a two-phase microstructure due to the phase separation of the hard and soft segments. The segmented block copolymeric nature makes the polymer versatile with respect to their unique physical properties, biocompatibility and biodegradation character.

Polyurethanes are composed of short, alternating polydisperse blocks of soft and hard segments. The soft segment is typically a low glass transition temperature (T_g) polyester, polyether, polyetherester, polyalkene, or polyalkyl diol, generally of molecular weight 400-5000. Due to their aliphatic structure and low intermolecular interaction, particularly the abundant ether bonds, polyol molecules rotate and bend easily and are therefore soft materials. Consequently, the polyol sequence of polyurethane-segmented block copolymers is referred to as the soft segment. Mechanical properties of polyurethane are driven by the ability of the polyol moieties to pack themselves in closer molecular arrangement. Poly(tetramethylene glycol) (PTMG) and Poly(propylene glycol) (PPG) are the most commonly used macrodiols in conventional medical formulations.

The hard segment is usually a high glass transition temperature, possibly semicrystalline aromatic diisocyanate, linked with a low molecular weight chain extender. Because of the structure of the diisocyanates and the strong intermolecular interactions such as hydrogen bonding

among urethane groups that form following the reaction of isocyanate with chain extender, the segments that contain isocyanate and chain extender are more rigid than polyol, are typically glassy at room temperature and therefore are called hard segments. Most of the commercially available polyurethanes are based on aromatic diisocyanates 4,4'-methylenediphenyl diisocyanate (MDI) or toluene diisocyanate. Aromatic-based diisocyanates present a fairly rigid molecular structure due to delocalization of the π electrons throughout the aromatic rings, therefore impeding rotation of the C-C bonds. The presence of an aromatic isocyanate in the hard segment produces a stiffer polymer chain with a higher melting point. Although there are a vast number of potential combinations of diisocyanates and chain extenders to form the hard segment of polyurethanes, only relatively few have been used in medical implants. Due to ease of handling, its symmetric structure, high reactivity and purity, MDI is the most frequently used diisocyanate for medical applications along with the chain extender 1,4-butanediol. Typical aliphatic isocyanates used in biomedical polyurethanes are 1,6-hexane diisocyanate (HDI) and methylene bis(p-cyclohexyl isocyanate).

Segmented polyurethanes consist of alternating hard segments and soft chain segments. The microphase separation of these two chemically distinct components gives rise to the unusual and useful physical and mechanical properties of polyurethanes. The driving force behind the phase separation into hard and soft domains is the hard segment mobility and hard segment interactions.Thermodynamically, phase separation is more complete with aromatic hard segments because of increased thermodynamic incompatibility between hard segments and aliphatic soft segments. However, from a kinetic viewpoint phase separation becomes more complete with aliphatic hard segments because of increased mobility. The size of the structural units and their properties and the extent of phase separation, may have strong effect on the final properties of the polymer.

When used as biomedical implants, it would be advantageous if polyurethanes were made radiopaque to facilitate their assessment in a non invasive manner, such as by X-ray imaging. Numerous approaches have been tried to modify polyurethanes so as to make it radiographically opaque. The most preferred method to impart radiopacity to polyurethanes is to covalently link the radiopaque component as a structural unit of the polymer. Radiopaque

polyurethanes were prepared by employing polyols, diisocyanates or chain extenders containing radiopacifying elements like iodine

5.2.1 Synthesis of polyurethanes using iodinated chain extenders, 2,3 diiodo-2-butene-1,4 diol and bis hydroxyethyl ether of tetraiodo bisphenol A

Aromatic and aliphatic radiopaque polyurethanes based on PTMG, PPG and IBOL have been synthesized from MDI and HDI by the conventional two-step solution polymerization reactions. Solution polymerization by prepolymer method is known to give materials with better properties than one shot process.

The first step involved the formation of pre-polymer, followed by chain extension with IBOL to yield segmented polyurethanes, PTMGMDIBOL, PPGMDIBOL, PTMGHDIBOL and PPGHDIBOL. The reactions were carried out at 1:1 and 1.2:1 molar ratio of isocyanate and hydroxyl groups for MDI and HDI respectively. Relatively higher isocyanate index and chain extension at 80 °C for 18 h has been used in the case of HDI based polyurethanes, since the reactivity of aliphatic diisocyanates and the polyols are low in comparison with the aromatic counterparts. Polyurethanes were also synthesized by reacting MDI and polyol PTMG or PPG, followed by chain extension with IBPA to yield segmented polyurethanes PTMGMDIBPA and PPGMDIBPA. The isocyanate index used for the synthesis of polyurethanes was maintained as one so as to restrict the occurrence of side reactions. The polymerization reaction for polyurethane synthesis has features of both addition and condensation polymerization and hence referred to as polyaddition or rearrangement polymerization (Brydson 1999). Process optimisation is important for designing polyurethanes as the process variables would affect the required properties of the polymer. The process variables viz. concentration of reactants, temperature and duration of reaction were optimised in the present syntheses. The low value of isocyanate index and low temperature restrict the occurrence of side reactions leading to the formation of allophanate or biuret. Further, the DBTDL is a specific catalyst for urethane bond formation rather than allophanate or biuret. Therefore, the present formulation (isocyanate index) and reaction conditions for pre-polymer formation and chain extension have resulted invariably in linear polyurethanes without any side chains or cross links.

5.3 Characterization of radiopaque polyurethanes

5.3.1 Fourier transform infrared spectral analyses

The properties of the polyurethanes are strongly dependent on their hydrogen bonded structure and phase separated microphase morphology. The Fourier transform infrared-attenuated total reflectance (FTIR - ATR) spectral analyses were used to characterize polyurethanes. Absorption bands in the carbonyl and N-H stretching in the ATR-IR Spectra indicated the presence of hydrogen bonds. All the polyurethanes exhibited absorption bands between 3302 cm^{-1} and 3319 cm^{-1} due to hydrogen bonded N-H groups. It has been reported that one of the most important factor for the microphase separation in polyurethanes is the strong hydrogen bonding between the hard segments (Yilgor et al. 2006). The presence of hydrogen bonded carbonyl absorption peak, (1688 cm^{-1} and 1700 cm^{-1}) in HDI based polyurethanes revealed the inter urethane hydrogen bonding of the hard segment domains which may lead to a higher degree of micro-phase separation in the surface.

The shift of carbonyl peak towards lower wave number region is an indication of the extensive hydrogen bonding between the carbonyl and the -N-H unit of another or the same polymer chains. Micro-phase separation is usually obtained for phase separable polyurethanes prepared in solution under homogeneous conditions. Moreover, urethane carbonyl at 1688 cm^{-1} and 1700 cm^{-1} reveal the presence of crystalline region in HDI based polyurethanes (Corish 1959). The absence of peak at 1702-1708 cm^{-1} for C=O urethane carbonyl stretching (bonded) and presence of peak between 3302 cm^{-1} and 3319 cm^{-1} for N-H stretching (bonded) for MDI based polyurethanes reveal hydrogen bonding interactions between ether groups of the soft segments and the urethane amide in the hard segments. Such segmental interaction leads to lesser degree of phase segregation in MDI based polyurethanes. The peaks at 813 cm^{-1} - 815 cm^{-1} in MDI based polyurethanes represent aromatic rings; thus reveal the presence of phase-mixed hard segment in the surface.

The absence of the band at 2270 cm^{-1} in the polyurethanes suggested the absence of unreacted free isocyanate group as reported by (Kwok et al. 2000). All the synthesized polyurethanes were linear polymers. Therefore, none of the polymers showed peaks for allophanate linkages due to crosslinking. Allophanates are formed due to the reaction be-

tween free isocyanates and the initial urethane bonds formed during the pre-polymer stage of polyurethane synthesis when the isocyanate index is high. Allophanate linkages are normally characterized by a triplet of intense IR bands centered at 1220, 1280 and 1310 cm^{-1} as well as unique N-H absorption peaks at 3298, 3267 and 3233 cm^{-1} (Stovbun et al. 1996). The absence of these characteristic bands in the IR spectra of present polyurethanes demonstrated that undesirable side reactions had not occurred during the syntheses of the polymers.

5.3.2 Gel permeation chromatography

Molecular weights of polyurethanes were determined by GPC. High molecular weight polyurethanes were obtained with MDI based polyurethanes when compared to analogous HDI based polyurethanes even though the isocyanate indexes were lower in the case of former. This is attributed to effective condensation of MDI and polyol during pre-polymer formation and chain extension of growing polyurethane chains. It has been reported that polyurethane prepared using aromatic chain extender 4,4'-isopropylidinedi(2,6-diiodophenol) as chain extender had lesser number average (30, 700) and weight average molecular (68, 700) weight due to the presence of bulky iodine atoms and the resultant decrease in chain extension (Kiran et al. 2009). However, the present polyurethanes synthesized using bis hydroxyethyl ether of tetraiodo bisphenol A had high number average and weight average molecular weight due to the presence of flexible spacers between the iodine atoms, thereby reducing the steric hindrance during chain extension.

5.3.3 X-Ray diffraction studies

The diffraction patterns for PTMGHDIBOL, PPGMDIBOL PPGHDIBOL and PPGMDIBPA showed two main crystalline regions. X-ray diffractograms showed sharper and more intense peaks at around 2θ = 20° and 28.5° for a partially crystalline character which is due to hydrogen bonding interaction among hard segment domains and phase segregation of the hard and soft segments. The peak at 2θ = 20° in PTMGHDIBOL corresponds to PTMG lattice and the other peaks of the polyurethanes in the diffractograms can be due to the hard segments (Mallakpour & Rafiemanzelat 2008). The crystallinity and the resultant phase separation for PPGMDIBOL and PPGMDIBPA are somewhat different from the observation of the ATR-IR

studies, which pointed to a phase mixed morphology for the polyurethane. This observation could be due to the different penetration depth of the surface region studied with ATR-IR and XRD. Studies on surface properties of polyurethanes based on PPG by ATR-IR have revealed that more phase mixing occurs in the near-surface region than in the bulk (Wang et al. 1999). The partially crystalline nature of PPG based polyurethanes is due to the incompatibility of hard and soft segments. The incompatibility of segments due to the side chain methyl groups in PPG resulted in the phase separated morphology. Only diffuse scattering with maximum intensity near 20° (2θ) was observed for PTMGMDIBOL and PTMGMDIBPA. This is attributed to an amorphous arrangement of chain segment in the polyurethane (Miller et al. 1985). It could be due to the constraint on hard segment mobility caused by the covalent joints between the hard and soft segments, and the hard segment-soft segment hydrogen bonding. Therefore, absence of sharp peaks at 2θ = 20° and 28.5° in PTMGMDIBOL is due to poor phase separation and steric hindrance of the hard segment (Fernández et al. 2008).

5.3.4 Energy dispersive X-ray analyses

EDX analyses of polyurethanes revealed the presence of iodine atoms. The peaks for iodine moiety in the spectra between 3.5 and 5keV confirmed the chain extension of polyurethanes with IBOL and IBPA. The peak intensity for iodine in HDI based polyurethanes were more than MDI based counterparts suggesting the presence of more iodine atoms in the former. This is due to lower molecular weight of HDI though the concentration of chain extender is same in both the polyurethanes. The peak intensity for iodine in polyurethanes prepared using IBPA was much higher than IBOL based polyurethanes due to the tetra iodination of the former. Sharp peaks were obtained for iodine moiety in the range 3.5 and 5 KeV where as no peaks were seen in that area for non-iodinated polyurethane prepared using bis hyroxyethyl ether of bisphenol A.

5.3.5 Elemental analyses

The quantitative iodine estimation by elemental analyses in polyurethanes was found to be between 3% and 16% with respect to total mass. The iodine concentration in these polyurethanes is sufficient for visibility under normal X-ray operating conditions as reported

elsewhere (Zhao 2008). It has been reported that if the iodine content is sufficiently high (greater than or equal to 3-5% by total mass) in a biomaterial, then the addition of a contrast agent is not required for clinical monitoring (Aldenhoff et al. 2002). Radiopaque acrylic cement prepared from 2,5-diiodo-8-quinolyl methacrylate having a concentration of the radiopacifying agent as low as 1.6% with respect to the total mass is reported to have exhibited good radiopacity (Vázquez et al. 1999). The iodinated polyurethanes prepared with chain extenders, IBOL and IBPA acquired radiopacity with 3% - 16% iodine content in contrast to the iodinated polyurethane prepared with aromatic chain extender 4,4'-isopropylidinedi(2,6-diiodophenol) having an iodine content of 23% (Kiran et al. 2009).

5.3.6 Thermal analyses

Thermal characterization of polyurethanes was performed using TGA. It has been reported that thermal degradation occurs through a concerted reaction mechanism and first stage of degradation may be at C-NH bond, the weakest bond in polyurethane (Kurian et al. 2000). The last stage of degradation is the oxidative degradation of the backbone structure of polyurethane. By varying the concentration of the soft segment, it was also observed that degradation starts in the hard segment while the apparent weight loss is correlated with the soft segment (Ferguson & Petrovic 1976). It is also claimed that polyurethanes based on PTMG degrade easily in air by oxidation on the carbon atom (α carbon) next to the oxygen (Xiao et al. 1994) while breakage of the C-O bond and subsequent unzipping was proposed as the mechanism in an inert atmosphere (Petrovic et al. 1994). Moreover, it has been proven previously that the polyether soft segment and the hard segment are more stable when mixed in a urethane copolymer, indicating that there is a mutual stabilization effect in segmented polyurethanes (Ferguson & Petrovic 1976).

As observed in the traces for polyurethanes, in nitrogen atmosphere all TGA curves display a slower initial and then a more rapid degradation process, suggesting a two-step mechanism for the degradation. From the TGA curves, it was found that degradation was comparatively slow in the polyurethanes, indicating that they are reasonably stable up to their melting points. The TGA of the polyurethanes showed thermal stability up to 260 $^{\circ}\mathrm{C}$ for polyurethanes with IBOL as chain extender and 270 $^{\circ}\mathrm{C}$ for polyurethanes with IBPA as chain extender. PTMGMDI-

BOL was thermally more stable than PTMGHDIBOL. Weight loss started at lower temperatures and was more rapid in HDI based polyurethane.

It is postulated that the degree of phase separation plays a major role in the decomposition of polyurethane copolymers. The urethane groups can behave as anti oxidants and have a stabilizing effect on the soft segment (Wang & Hsieh 1997). Therefore, the presence of hard segments can increase the stability of the soft segment, while the soft segment may have a protection function on the hard segment and hence increase the stability of segmented polyurethanes. The above facts can be interpreted as the mutual stabilization effect of segmented polyurethanes. Combining the above results, it is generally concluded that the degree of phase separation has a tremendous effect on the thermal stability of polyurethane copolymers and there is a mutual stabilization effect between the soft and hard segments. When polymer samples degrade in air, lower decomposition temperatures manifest the importance of the extent of interurethane hydrogen bonding. Consequently, stronger interurethane hydrogen bondings arising from sharper domain/matrix interfaces reduce degradation rates. It means that a higher thermal stability correlates with a higher degree of phase separation in this case. However, in the case of degradation in a nitrogen atmosphere, more phase mixing favors the thermal stability of polyurethanes after the dissociation of interurethane hydrogen bonding at a higher decomposition temperature (Wang & Hsieh 1997). More compact domains in PTMGHDIBOL would have lead to a better phase separated system than PTMGMDIBOL.

The high thermal stability of polyurethanes, PPGMDIBOL & PPGHDIBOL could possibly be due to hindrance of methyl side chain in the polymeric chain and the resultant decreased chain flexibility. The thermal stability of polyurethanes, PTMGMDIBPA and PPGMDIBPA could be attributed to the presence of MDI. From the viewpoint of chemical structures, MDI-based polyurethanes were expected to have higher thermal stability because they contain benzene rings, which have high cohesive energy and bulkiness (Liaw 1997).

Aromatic isocyanate based polyurethanes are so far considered as very sensitive to processing conditions (Schollenberger & Stewart 1971, 1976). Aromatic polyurethanes are known to produce methylene dianiline (MDA), a suspected carcinogen under thermal or themohydrolytic conditions (Szycher & Valdez 1983). With the present MDI based polyurethanes, the

thermal stability was significantly higher which is ascribed to the greater intermolecular bond strength.

5.3.7 Dynamic mechanical analyses

The low T_g shown by HDI based polyurethanes is obviously due to maximum flexibility imparted by methylene sequences (Pandya et al. 1986). Polyurethanes based on MDI are believed to possess significant chain rigidity because of the high cohesive energy and bulkiness of the benzene ring (Kim & Lee 1992). Therefore, MDI based polyurethanes with its rigid hard domains and phase-mixed morphology would have decreased the chain flexibility of soft segments leading to a higher T_g. The performance of polyurethane elastomers at elevated temperature is dependent upon the structure of the rigid segments and their ability to remain coherent at a higher temperature. PPG based polyurethanes showed higher T_g than their analogous PTMG based polyurethanes due to the hindrance of the methyl side chain in the former case and the resultant decreased mobility. It has been documented in literature that the T_g value of pure PPG (-70 °C) is higher than that of pure PTMG (-79.8 °C) (Tang et al. 1996). The T_g of PPGMDIBPA was higher than that of PTMGMDIBPA. This result also may be accounted for by the decrease of the chain flexibility caused by increased steric hindrance brought about by methyl side chain in polymeric backbone of PPG based polyurethane.

The storage modulus versus temperature plot shows a drop in stiffness for each polymer near their respective T_g. All series of samples exhibited a major relaxation and one rubbery plateau corresponding to the two phase in the material. This is characterized by significant decrease in the storage modulus, which corresponds to the T_g of the soft segments. It is rather well established that reduced internal mobility of polymer chains increases onset of storage modulus decline. MDI based polyurethanes exhibited a relatively higher storage modulus decline temperature than its analogous HDI based polyurethanes. The increase in temperature of storage modulus decline could be due to the presence of hard segments in the soft-segment phase and the restrictions introduced by the hard-segment domains where the two ends of the soft-segment chains were anchored. PPG based polyurethanes also showed decrease in storage modulus at a higher temperature than PTMG based polyurethanes due to the reduced flexibility of the polymeric backbone with methyl side groups. Higher storage modulus was

seen in the case of PPGMDIBPA than PTMGMDIBPA due to decreased flexibility of the chains in the former case.

5.3.8 Determination of mechanical properties

PTMGMDIBOL showed considerably higher tensile strength than the PTMGHDIBOL; MDI based polyurethane had an ultimate strength of the order of 35 MPa with an elongation at break of 752%. Higher mechanical properties of PTMGMDIBOL were attributed to the aromatic unit of the diisocyanate and to an increase in concentration of the urethane and phenyl groups. Urethane and phenyl groups have improved cohesiveness and function as constituents for the secondary inter chain hydrogen bonding (Liaw 1997). The cohesive forces participate in inter-molecular hydrogen bonding and restrict the rotation of the polymer segments, thereby resulting in enhanced mechanical properties. The higher Young's modulus of polyurethanes are attributed to the greater order in the hard-segment domains and the resultant degree of crystallinity. Evaluation of mechanical properties indicated that the polyurethanes based on PPG have lower tensile strengths than PTMGMDIBOL. PPG based polyurethanes have the disadvantage of reduction in the polymer symmetry, thereby resulting in a lower tensile strength. Analysis showed that the polyurethanes derived from IBPA & MDI have lower hardness and tensile strength when compared to PTMGMDIBPA. The decrease in such mechanical properties of polyurethanes based on IBPA, can be accounted for by an increase of free volume caused by tetra iodination.

5.3.9 Evaluation of radiopacity

All the polyurethanes exhibited opacity equivalent to that of an aluminum wedge (Figure 17). It was found that HDI based polyurethanes were markedly radiopaque than its MDI based counterparts owing to its higher iodine content: even thin objects (¡0.5 mm) could be easily visualized using X-ray fluoroscopic techniques as are routinely used in the clinic, e.g., during coronary angiography. X-ray images of polyurethanes synthesized using IBPA were much sharper than the image of aluminium wedge, a widely used radiographic standard. Polyurethanes prepared with non-iodinated chain extenders were not at all visible in the radiographic films. Quantitative evaluation of radiopacity revealed that the radiopacity of IBOL

based polyurethanes were comparable and IBPA based polyurethanes were much higher than 2 mm thick aluminum wedge. The X-ray opacity of these polyurethanes is sufficient for clinical monitoring when used as biomedical implants.

5.3.10 Contact angle measurement

Surface water contact angle is a measure of the surface wettability of the polymer. Since a biomaterial which interfaces with blood has a surface tension close to that of water, measurement of contact angles in water phase is desirable (Kaelble & Moacanin 1977). The lower the water contact angle, the higher is the tendency of water to spread over the surface (Rabek 1980). Thus hydrophilic materials are expected to have lower contact angles than hydrophobic materials. It is observed that all the present polyurethanes are hydrophilic in nature. As the polyurethane biomaterial is placed into contact with a physiological medium, such as blood or tissue, its surface layers will undergo motions in order to accommodate the new interfacial situation. In contact with aqueous environments, it is obviously favorable for hydrophilic constituents of the polymer to become enriched at the interface (Macocinschi et al. 2009).

5.4 Biostability of radiopaque polyurethanes

Polyurethanes are used in many biomedical applications where the long-term stability of the material is mandatory. Conventional polyurethanes are among biomaterials not intended to degrade but are susceptible to hydrolytic, oxidative and enzymatic degradation *in vivo* (Tatai et al. 2007). The susceptibility of polyurethanes to such degradation is a problem for long lasting biomedical implants. Differences in the degree of degradation reported and in the mechanisms proposed for the degradation may have resulted from the various definitions assigned to biodegradation. Lemm et al. (1980) considered changes in surface proper ties or loss of mechanical strength to be biodegradation. Potts defined biodegradable materials as those which, because of their chemical structure, are susceptible to be assimilated by microorganisms such as fungi and bacteria (Potts et al. 1973). Vert has stated that biodegradable polymeric implant systems and devices can be attacked by biological elements so that the integrity of the system is affected and, in some cases not necessarily, gives fragments or other degradation products (Vert et al. 1992). Huang et al. (1979) defined biodegradation as enzyme-induced

degradation. Marchant et al. (1984) presented biodegradation as "occurring on many different structural levels, i.e., molecular, macromolecular, microscopic, and macroscopic, depending on the mechanism." Ratner et al. (1988) adopted the classical polymer science definition of degradation that requires backbone chain breakage and a reduction in average molecular weight and developed an *in vitro* model to study the polyurethane susceptibility to degradation by exposing the polymer to enzymatic and oxidative treatments.

Degradation can lead to significant changes in the polymer mechanical properties, surface chemistry and structure leading to malfunction and implant failure. The major underlying causes of degradation in polyurethanes are hydrolysis and oxidation. Hydrolytic and oxidative stability of the various segments and sequences in a polymeric material is important, as is the hard-soft segment composition ratio. Chemical composition of the polyurethane is primarily attributed as the chief determinant of biostability.Investigations have shown that hydrolytic and enzymatic degradation increased by substituting aromatic diisocyanate with aliphatic diisocyanate (Kim & Kim 1998). However, synthesis, processing and fabrication methods as well as storage and sterilization conditions can also play a role.

Polyether-based polyurethanes are resistant to hydrolysis than poly ester-based polyurethanes due to greater phase separation in them. However, *in vivo* as well as *in vitro* experiments (Tang et al. 1996) have demonstrated that Poly (ether urethanes) are susceptible to biodegradation. A number of hypotheses including two chemical mechanisms, oxidation and enzymatic hydrolysis, have been proposed to explain the nature of biodegradation. Theoretically, H_2O_2 and .OH produced by macrophages and other phagocytes during respiratory burst may initiate the oxidation by free radical reactions (Zhao et al. 1991); lysosomal enzymes that are released from inflammatory cells could catalyze the hydrolysis (Williams 1980).

The present polyurethanes contain ether linkages in soft segments and urethane functionality in hard segments, which can be attacked by hydrolytic, oxidative or enzymatic agents.

5.4.1 Evaluation of hydrolytic stability in ionic medium

Generally in polyurethanes, the urethane or carbamate linkage is susceptible to hydrolysis similar to that of an ester linkage of a substituted carbamic acid. Unlike in the hydrolytic degradation of carboxylic ester linkage, the hydrolytic degradation occurs less readily in ure-

thane groups (Schollenberger & Stewart 1971). The urethane groups are more resistant to hydrolytic cleavage, and are not considered susceptible to hydrolysis under normal implant conditions. However, under extraneous polymer processing conditions such as injection moulding at high temperature and extrusion in the presence of water, urethane linkage is prone to undergo thermohydrolytic cleavage (Stokes et al. 1995). Relatively, the hydrolytic degradation of polyurethanes in pure water is low; however the presence of anions and cations has a strong catalytic effect.

Aging studies on the present polymers in hydrolytic media *viz.* Ringer's solution and PBS revealed the hydrolytic stability of poly (ether urethanes). The polyurethane films were removed from the treatment solution and vacuum-dried for 24 h prior to analysis. Polyurethanes aged in hydrolytic media revealed no significant change in weight when compared to control (untreated polyurethane films- aged in deionised water). No new peaks were observed in any of the spectra after treatment in PBS or Ringer's solution. The films incubated in PBS and Ringer's solution retained all the characteristic peaks of the base polyurethane. The aging studies confirmed that the present polyurethanes are appreciably stable in hydrolytic ionic medium.

5.4.2 Evaluation of hydrolytic stability in hydrolytic enzyme

Since medical implants survive in predominantly hydrolytic environment *in vivo*, the importance of hydrolytic enzymes has been recognized in the mechanism of degradation of segmented polyurethanes (Williams 1992). Degranulation of neutrophils (PMN) which results in the release of reactive oxygen species and hydrolytic enzymes may be responsible for the biodegradation of implanted biomaterials (Anderson 1993). It has been reported that organic synthetic polymers such as polyamides, polyurethanes and polyesters undergo enzymatic degradation (Williams 1992, Vondracek & Dolezel 1984). Enzymatic reactions are specific in nature. Major sites for enzymatic attack in polyurethanes are urethane linkages in hard segments. Poly(ether urethanes) are susceptible to degradation *in vivo* due to hydrolytic enzymes, mainly through the cleavage of urethane linkages.

Even though the enzymes are designed for highly specific interactions with particular biological substrates, some appear capable of recognizing and acting upon "unnatural" substrates such as poly(ether urethanes). Papain is a thiol endopeptidase enzyme, which exhibits a sim-

ilar enzymatic activity of lysosomal enzymes. Papain has the specificity to peptide bond (-NH-CO-) degradation. Since urethane (-NH-CO-O-) linkage in poly(ether urethanes) is more or less similar to that of peptide (amide) in protein, studies on papain-induced hydrolytic degradation is more appropriate. Susceptibility of poly(ether urethanes) to hydrolytic degradation by papain enzyme after a limited exposure to free radicals have been reported (Hsu & Huang 2000).

However, the data on aging studies of present poly(ether urethanes) in hydrolytic enzyme revealed hydrolytic stability. There was no significant weight loss due to enzymatic degradation for both polyurethanes based on IBOL and IBPA. The IR spectral response did not reveal any appreciable changes in the aged polymers in comparison with that aged in control buffer media and untreated polyurethane films. The peaks for enzymatically hydrolysable urethane linkages have appeared intact in aged polymers. It has been reported that the degree of hard segment micro domain formation in polyurethane materials as well as its structure influences the ability of enzymes to degrade the polymers (Tang et al. 2001). It is hypothesized that the enzymatic stability of present polyurethanes (having 75% of hard segments) is due to their ability to form hard segment micro domain. It may have contributed to the formation of a protective structure for the hydrolysable hard segment linkages located within the micro domains as reported elsewhere (Santerre & Labow 1997).

5.4.3 Evaluation of biostability in oxidative medium

Degradation of poly(ether urethanes) is more associated with oxidative process than hydrolysis. It appears that oxidation occurs in the polyether soft segment at the α-methylene position. Catastrophic failure of polyether urethane (Pellethane) coated cardiac pacing lead was the first manifestation of clinical failure of polyurethane device (Stokes et al. 1989). Treatment with an oxidative solution are known to reproduce the chemical and physical characteristics of polyurethanes *in vivo* degradation at an accelerated rate. Based on the principle that H_2O_2 produced by macrophages and other phagocytes during respiratory burst may initiate the oxidation by free radical reactions, aging studies were carried out in 3% H_2O_2 as per ISO standards.

The *in vitro* stability of polyurethanes in oxidative medium was assessed from mass loss

of aged polymers and also from IR spectra. None of the polymers showed significant weight loss due to oxidative degradation. The weights of aged samples were same as that of control polyurethanes films. The FTIR spectra revealed no change in the spectral response of the aged polymers. The aging studies confirmed that the polyurethanes were appreciably stable in oxidation medium. The resistance of polyurethanes against oxidation is possibly by the protection of the soft segments by the hard segment domains.

It should be pointed out here that all of the degradation phenomena afore mentioned are closely related to the surface organization of polyurethanes. In polyurethane-based materials, a microphase segregation process leads to the formation of a two-microphase structure with regions enriched in either hard or soft segments. Because of the mobility of the soft segments, the surface composition of segmented polyurethane varies in order to find the composition (or the hard/soft segments ratio) that will minimize the interfacial free energy. This particular behaviour of polyurethanes has significant importance as the host response is largely determined by the surface composition of the material.

5.4.4 Studies on accelerated chemical degradation of polyurethanes

Accelerated degradation media like concentrated nitric acid (HNO_3) or alkaline sodium hypochlorite (NaClO) have been used to evaluate degradation of polyurethanes even though such adverse conditions are not prevalent in the human body (Fare et al. 1999). The present studies on accelerated aging in boiling deionized water for 100 h and boiling potassium hydroxide (0.5 M) for 4 h was carried out as per the method reported elsewhere (Thomas & Muthu 2008). The accelerated aging studies revealed dimensional changes and weight loss in polyurethanes, PTMGHDIBOL and PPGHDIBOL. However, no degradation (weight loss) or dimensional changes were observed in all the other polyurethanes. The accelerated test in harsh and aggressive chemical conditions clearly indicated that the present poly(ether urethanes) would be stable in physiological conditions.

5.5 *Synthesis and characterization of radiopaque methacrylate copolymers*

5.5.1 Synthesis of radiopaque methacrylate copolymers

Methacrylate polymers are widely used for medical applications such as contact lenses, bone cements for partial or total joint replacement, embolic materials and in dentistry as orthodontic and denture base materials (Vert 2007, Yang 1997, Horak et al. 1997, Saralidze et al. 2003). Imparting radiopacity to methacrylates would be an added advantage as it enables the non-invasive evaluation of the polymeric biomaterials, when used as implants. Methacrylate polymers are radiolucent due to the absence of high electron density elements in their polymeric backbone. Several approaches have been made to introduce radiopacifying elements to polymeric chain, however, physical and mechanical properties of the polymers were adversely affected making it clinically unacceptable. One of the earliest methods for imparting radiopacity to polymeric systems involved the incorporation of radiopaque additives. Barium salts were frequently used as an additive commercially for methacrylate based dental resins and bone cements, though it reduced the mechanical strength and fracture toughness of bone cement (Molino & Timmie Topoleski 1996, Lewis et al. 2005). Radiopaque additives such as tantalum or tungsten salts have been incorporated with Poly(methyl methacrylate) to be used as percutaneous biomaterial for vertebroplasty (Jensen et al. 1997, Cotten et al. 1998). The side effects observed were inflammatory reaction, heat related damage and embolic potential associated with leakage of monomer into perivertebral spaces and draining veins. Poly(methyl methacrylate) containing an organobismuth radiopacifying additive such as triphenyl bismuth have also been reported (Rawls et al. 1996, Smid et al. 1993). A minimum of 23% weight of halogenated derivative was necessary to obtain the same radiopacity as the aluminium standard. The bismuth compound acted as a plasticizer reducing the glass transition temperature and a slight elevation in cytotoxicity was described due to a reduction in monomer conversion. Radiopaque miscible polymer coordination complex of poly(methyl methacrylate) and uranyl nitrate were developed for biomedical applications (Cabasso et al. 1989). However, the polymer does not degrade through the main chain and the biocompatibility has not been evaluated, nor has the long-term stability of the complex *in vivo* been established.

Polymeric biomaterials with inherent radiopacity were developed by covalently binding the radiopacifying elements with the monomers prior to their polymerization or carrying out a post-polymerization halogenation of reactive groups. Physico-mechanical and radiocontrast properties of the polymer were not compromised by this method and the secondary release of radiopacifying agent was also prevented.

2,3-Epoxypropyl methacrylate (GMA) is an interesting monomer exhibiting polymerizable methacrylic unsaturation and an epoxide function of potential reactivity. This monomer has been homopolymerized and copolymerized by means of free radical initiators (azo-catalysts) known to selectively attack methacrylic double bonds. Free-radical random copolymerizations of GMA with conventional monomers have been widely investigated. The interest in these copolymers is largely due to the ability of the pendant epoxy group to enter into a large number of chemical reactions. GMA copolymers that contain epoxide groups have become increasingly important for biomedical applications such as binding enzymes and other biologically active species.

Copolymer based on GMA and co-monomers such MMA, EMA and BMA were synthesized by free radical polymerization. The copolymer was made radiopaque by the epoxide ring opening of GMA using the catalyst o-phenylenediamine and the subsequent covalent attachment of elemental iodine.

Epoxides are well known carbon electrophiles capable of reacting with various nucleophiles and they undergo regioselective ring opening reactions to form vicinal halohydrins (Eshghi et al. 2004). The regioselective ring opening of epoxides using elemental iodine in the presence of a catalytic amount of o-phenylenediamine affords vicinal iodo alcohols. The major nucleophile in the course of the reaction is the pentaiodide ion (I_5^-) and this bulky nucleophile plays a fundamental role in the high regioselectivity observed. It is due to the attack on the less sterically hindered epoxide carbon (Eshghi et al. 2004).

Halogenative cleavage of epoxides occurs by the following four-step mechanism: Initial step involves the formation of a 1:3 or 2:3 molecular complex between diamine and elemental iodine, in which I_5^- exists as an ion pair. This complex is further decomposed to release I_5^- in the second step and the molecular halogen is converted to a nucleophilic halogen species

in the presence of a suitable diamine. In the third step ion participates in the ring opening of epoxides and ultimately the catalyst is regenerated. These steps occur continuously until all of the epoxides and halogen are consumed.

5.5.2 Characterization of radiopaque methacrylate copolymers

Physico-chemical characterizations of the iodinated copolymers were carried out and the properties were compared with analogous non-iodinated copolymers.

5.5.3 Fourier transform infrared spectral analyses

IR absorption bands in the range 2962-2950 cm^{-1} corresponded to asymmetrical and symmetrical stretching of the methyl and methylene groups in the copolymers. The peak at 1718-1737 cm^{-1} in the spectra is attributed to the ester carbonyl stretching of GMA and other co-monomer units. The signal at 907-908 cm^{-1} revealed the presence of epoxy groups in the copolymers. The FT-IR spectra of the copolymers after iodination showed marked reduction in intensity of absorbance of the epoxy groups at 905 cm^{-1} where as broad band for hydroxyl functional groups appeared at 3500 cm^{-1} indicating the halogenative cleavage of epoxy ring to form iodohydrin. The peaks at 905 cm^{-1} in iodinated copolymers suggested the presence of residual epoxy groups and the partial iodination of the copolymers could be due to the close packing of the voluminous iodine moiety around the polymeric backbone resulting in a diminished reaction rate at higher graft ratios (Navarro-Rodriguez et al. 1998).

5.5.4 NMR spectral analyses

The average composition of the copolymers was determined from the ^1H NMR spectra. The H^1 NMR spectra of the copolymers showed two signals at 4.3 and 3.7 ppm due to splitting of methylene protons in the CH_2O- group attached to the carbonyl group of the GMA unit by the methyne proton of the epoxy group. The peak at 3.2 ppm corresponded to the methyne proton of epoxy group. The peaks at 2.6 and 2.8 ppm were assigned to the methylene protons of the epoxy group. The resonance signals at 4.03 and 3.92 ppm were attributed to two methylene protons of -$COOCH_2$ in copolymers P(GMA-co-EMA) and P(GMA-co-BMA) respectively and three methyl protons of -$COOCH_3$ in P(GMA-co-MMA) appeared at 3.59 ppm. The peaks at 0.9-2.6 ppm were due to the methylene groups in the polymeric chain and other alkyl

groups. Absence of signals around 5.00 and 5.30 ppm indicated the absence of protons corresponding to the methacrylic unsaturation. Thus, H^1 NMR data confirmed the incorporation of both monomeric units in the copolymer and the stability of the epoxy groups to free radical polymerization. The assignment of the resonance peaks in the H^1 NMR spectra resulted in the accurate evaluation of the content of each kind of monomeric units incorporated into the copolymeric chains as reported elsewhere (Safa & Nasirtabrizi 2006). The mol fraction of GMA in the copolymers were calculated from measuring the integrated peak area of the three resonances of epoxide protons of the GMA unit and methoxyl or methylenoxyl protons of other monomer units. The following expression is used to determine the composition of copolymer P(GMA-co-MMA). Let m_1 be the mole fraction of GMA and $1 - m_1$ is that of MMA monomer. The proton resonances of the methoxyl group in MMA at 3.59 ppm and those of the epoxide group in GMA at 3.22, 2.84 and 2.63 ppm are clearly resolved. The GMA contains 3 epoxide protons and MMA contain 3 methoxyl protons:

$$\begin{aligned} A &= \frac{\text{Integrated peak area of } 2.63 - 3.22}{\text{Integrated peak area of } 3.59} \\ &= \frac{3m_1}{3(1-m_1)} \end{aligned} \tag{4}$$

This on simplification gives:

$$m_1 = \frac{A}{1+A} \tag{5}$$

Therefore, the mole fraction of GMA in copolymer P(GMA-co-MMA) was determined from the above equation (5). A similar method was used to calculate of the mole compositions of the other copolymers. The values of the corresponding mol fractions of GMA and related comonomer in the copolymers which more or less match to the experimental 50:50 ratio.

5.5.5 Gel permeation chromatography analyses

The weight average molecular weights, number average molecular weights and polydispersity were determined using GPC. The GPC analyses showed that the iodinated copolymers had higher molecular weights than their analogous copolymers due to the incorporation of iodine.

5.5.6 Energy dispersive X-ray analyses

Iodination of copolymers were further confirmed by Energy Dispersive X-ray analysis (EDX). In the case of non-iodinated copolymers, only two peaks were obtained, one of carbon and the other of oxygen and in iodinated co-polymers, additional peaks were detected in the area 3.5-5 keV. The peaks around 3.5- 5 keV in EDX images were assigned to the presence of iodine atoms in the iodinated co-polymeric chains.

5.5.7 Elemental analyses

The percentage iodine in the copolymers was estimated quantitatively by iodine estimation. It has been reported that for clinically relevant X-ray visibility the biomaterial should contain at least 3-5 wt % of iodine (Vázquez et al. 1999). The percentage weight of iodine in the present iodinated copolymers was found to be as high as 23% in comparison with 13.5% for the iodinated copolymer prepared with GMA and MMA in the weight ratio of 70:30 (Dawlee et al. 2009).

5.5.8 Thermal analyses

Thermal characteristics of the copolymers were determined by DSC and TGA analysis. The DSC scans showed that the T_g of the modified copolymers increased significantly after iodination. We have reported that the T_g of iodinated copolymer prepared with GMA and MMA in the weight ratio of 70:30 has shifted from 58 to 105 °C (Dawlee et al. 2009); however with the present iodinated copolymer IP(GMA-co-MMA) [GMA and MMA in the mol ratio of 50:50] the T_g has shifted from 44 to 99 °C. The incorporation of iodine moiety into the copolymeric chains and the presence of hydroxyl groups are believed to have increased the rigidity of the chains resulting in higher T_g values. The copolymers were found to be stable only up to 180 °C. However the iodinated copolymers showed higher thermal stability than their non-iodinated counter parts. The iodinated copolymers were stable up to 250 °C and grafting with highly sterically hindered iodine atoms decreased the free volume of the polymeric chains thereby reducing the thermal decomposition (Safa & Nasirtabrizi 2006). These thermal characteristics enable the copolymers to be processed by any processing techniques that rely upon plastic flow at elevated temperature, thereby making them good candidate materials for biomedical

applications.

5.5.9 Evaluation of radiopacity

X-ray analysis of iodinated copolymeric films showed excellent radiopacity when compared to the standard aluminum wedge. The X-ray visibility of the iodinated copolymer films are compared with an aluminum step wedge and a non-iodinated copolymer. It can be seen from the graph that the radipacity of iodinated copolymers were much higher than 2 mm thick aluminum wedge. The X-ray opacity of these copolymers is sufficient for clinical monitoring when used as biomedical implants.

5.6 Studies on biocompatibility of radiopaque polymers

Most medical devices use synthetic biomaterials as their principal component. A biomaterial is a non-drug substance for inclusion in a physiological system that augments or replaces the functions of a bodily tissue or organ. Polymeric biomaterials must be compatible and inert, must interact with the assorted tissues and organs in a non-toxic manner, and must not destroy the cellular constituents of the body fluid with which they interface. The ability of biomaterials to fulfill their role in medical devices depends on their degree of biocompatibility.

5.6.1 Evaluation of cytoxicity *in vitro*

In vitro cytotoxicity testing evaluates lysis, growth inhibition, and other impacts on cells by polymers, component materials, and leachates using morphological, biochemical and metabolic criteria. The cell culture methods take advantage of the sensitivity of cells cultured *in vitro* towards toxic compounds. Impurities (e.g. catalysts, solvents, monomers and oligomers) introduced in a polymeric biomaterial during synthesis affect the polymer-cell interaction. Impurities can also be deposited on the surface in the later stages, e.g. air-borne particulate, dust, mould release agents etc. The presence of impurities on the surface makes it highly thrombogenic and cytotoxic. It has been reported that leachable components or residual solvents in polyurethane may cause necrosis of the neointima, while contamination could kill the cells (Bruck 1979).

Standard cell culture tests were developed for the biocompatibility evaluation of biomedical devices. One of the most sensitive toxicity testing protocols is based on direct contact of

the sample with a cell culture. Fibroblast cell lines (L929) are frequently used in cytotoxicity tests. Tests based on the medical end uses of materials are conducted with cell lines that are as similar as possible to the cells or tissues that will be in contact with the polymers *in vivo*. The polymeric films were placed in direct contact with a monolayer of fibroblast cells for 24 h. The fibroblasts are spindle shaped cells. The cells were evaluated for the general morphology, vacuolization, detachment, cell lysis and membrane disintegration. Comparison of these properties of the cells before and after direct contact with the samples is the basis for the evaluation of a possible toxic effect of the samples. Samples showing greater than 60% cell death were considered to be toxic. The studies on cytocompatibility of the polyurethanes and acrylate copolymers showed non-cytotoxic cellular response. The cells retained their original spindle shaped morphology and no detectable zone of cell lysis, vacuolization, detachment or membrane disintegration was observed around or under the specimens even after 24 h of contact.

Cell viability was also determined by a tetrazolium-based colorimetric assay (MTT assay) to quantitatively confirm the results. The MTT assay is often used to evaluate polymer cytotoxicity, or more precisely the metabolic activity of cells in contact with the material. The MTT assay is simple, reproducible, and does not use radioisotopes. Quantitative assessment of the cytotoxicity to cells after contact with the polyurethane extracts showed 83.9% , 111%, 70% and 100% metabolic activity by PTMGMDIBOL, PTMGHDIBOL, PPGMDIBOL & PPGHDIBOL, respectively and extract of test materials PTMGMDIBPA and PPGMDIBPA showed 85.9% and 77%, respectively when compared to the control. The MTT assay of cells after contact with extracts of iodinated copolymer IP(GMA-co-MMA), IP(GMA-co-EMA) and IP(GMA-co-BMA) showed 82%, 71% and 70% metabolic activity, respectively. The studies revealed that the polymers did not possess any leachants capable of inducing cell toxicity. The cell viability and morphology tests provide the first screening for cytotoxicity. Thus, their non-cytotoxic nature makes these polymers potential candidates as biomedical implants.

5.6.2 Evaluation of blood compatibility *in vitro*

Only materials in prolonged or permanent contact with blood pathways are subjected to hemocompatibility testing. Acrylic biomaterials are expected to be in contact with body through

tissue, bone or dentin since the end use of the materials is for orthopedic and dental applications. Therefore, blood interaction assays were confined to polyurethanes, PTMGMDIBOL and PTMGMDIBPA as they are intended to be used as implants and external communicating devices, and thus will be in contact with blood. Materials used for blood contacting applications require characterization for blood compatibility to confirm their safety. Most of the materials in contact with blood are incompatible to a greater or lesser extent due to their physical and chemical structure which either disrupts the cellular elements of blood or activates several of the plasmatic protein system. The blood material interaction is a highly complex process. The first event is the adhesion of proteins on the surface, followed by adhesion and activation of platelets, activation of leukocytes, complement systems etc., activation of plasmatic proteins triggering the coagulation cascade leading to the formation of blood clot. Clotting is initiated by platelet activation as well as by extrinsic and intrinsic pathways. Exposure of blood to biomaterial can lead to platelet adhesion, activation and aggregation. Adenosine diphosphate, in platelets, is believed to trigger the platelet reactions and cause its degranulation. Eventually, activated platelets generate thrombin which results in the formation of a fibrin clot. The extrinsic pathway is usually not activated by contact with materials and involves a tissue factor. The intrinsic pathway on the other hand is initiated by the exposure of blood to nonendothelial surfaces and hence very important for biomaterials. The adsorption of Factor XII (Hageman Factor) upon a biomaterial surface initiates the intrinsic pathway. Factor XII is activated by the adsorption of coagulation proteins including Factor XII itself, Factor XI, prekallikrein, and high molecular weight kininogen (Kaplan et al. 1982). After the activation of cofactors, the coagulation cascade will proceed to produce fibrin. So evaluation of blood compatibility of materials is a very complex process and hence a multiplicity of test procedures is required to establish the relative thrombogenicity.

Blood compatibility of polyurethanes is known to be influenced by their microstructure and surface properties (Hearn et al. 1988, Garrett et al. 2001). The morphology of polyurethanes in biological medium result in a complex surface microenvironment that differentially mediates biological interactions with the material surfaces. *In vitro* evaluation of hemocompatibility of PTMGMDIBOL and PTMGMDIBPA showed no significant difference in counts for RBC and

WBC immediately and after 30 min of exposure to human blood. The results confirmed that there was no significant cell lysis of RBC. The absence of significant reduction of WBC suggested that the elastomers would not induce complement activation. The degree of interaction between the blood platelets and the material determines the thrombogenicity of the polymer. The polyurethanes showed reduced platelet consumption comparable to that of the reference. The segmented morphology in polyurethanes have resulted in the preferential surface segregation of the soft segment block in the polymer in the biological medium and enhanced the anti-thrombogenic properties as reported in literature (Liu et al. 2005, Jayabalan et al. 2000). Sa Da Costa et al. (1980, 1981) reported the relationship of surface composition of segmented polyurethanes and platelet retention. Those polyurethanes (aromatic diisocyanate/ polyether polyols based) which contained highest fraction of ethereal carbon in the surface were found to have the lowest platelet retention. Lelah et al. (1983) has also suggested that material having a higher surface soft segment concentration was more thromboresistant. The polyurethanes were found to exhibit low propensity to activate contacting blood platelets. The studies on dynamic contact angle support the surface segregation in biological medium to form hydrophilic moiety on the surface of polyurethanes which promotes anti-thrombogenic character. It has also been reported that hydrophilic surfaces retard blood coagulation by decreasing the platelet adhesion (Merrill et al. 1982).

Adsorption of plasma proteins on to artificial surface is the first event to occur when blood contacts a surface and the adsorbed protein layer is known to influence the blood compatibility (Szycher & Valdez 1983). While albumin passivates a surface, fibrinogen activates the surfaces. Determination of fibrinogen content showed that there was no change in fibrinogen concentration when evaluated after 30 min exposure of blood to the samples tested. The result suggested that the adsorption of fibrinogen on to the present polyurethane surface is significantly low. Partial thromboplastin time (PTT) is a performance indicator measuring the efficacy of both the intrinsic (contact activation pathway) and the common coagulation pathways. Normal results for human PTT assay for bleeding disorders are between 60-70 seconds. Assay on PTT values for the reference materials indicated prolonged coagulation. Similarly PTT assay with the polyurethanes also indicate prolonged coagulation suggesting blood com-

patibility. This may be due to passivation of polyurethane surfaces via adsorption of plasma proteins such as albumin. There is no significant difference in PTT values for samples analysed immediately and after 30 min incubation in blood plasma. The data also suggests that the polyurethanes do not have any leachable activator for coagulation pathways.

5.6.3 Hemolysis assay

Hemolysis is the premature destruction of red blood cells and the destruction of red blood cells release their free hemoglobin into the plasma (Thompson et al. 1977). The hemolysis assay is designed to evaluate the hemolytic properties of the materials used in the fabrication of medical devices that may contact blood. In most of the medical applications of polyurethanes, as implantable devices or external communicating devices, it comes in contact with blood. So the evaluation of the hemolytic potential of polyurethanes assumes great importance. The haemolysis assay showed that the polyurethanes, PTMGMDIBOL and PTMGMDIBPA were non-hemolytic in nature. The hemolytic potential of the material is defined as the measure of the extent of hemolysis that may be caused by the material when it comes in contact with blood.

5.6.4 *In vivo* evaluation of intracutaneous irritation in rabbit

The chemicals released from device materials that contact the body may produce skin irritation. In general terms, such irritation is a local tissue response characterized by the usual signs of inflammation-redness and swelling-and sometimes accompanied by heat and pain. Numerous chemicals are capable of causing irritation, either immediate or delayed, and some of these may be present in materials as additives, processing or manufacturing aids, or inadvertent contaminants. For example, residual concentrations of ethylene oxide present in gas-sterilized devices can produce an irritant response if they are not reduced to acceptable levels before the device is used; and residues of such contaminants as catalysts in a particular batch of materials can cause unexpected irritation responses in users or patients. The intracutaneous irritation test is the *in vivo*, nonclinical test commonly used to evaluate materials for possible contact hazards. This test in the protocol of biomaterial evaluation is to assess the allergic responses produced by the extract of material when applied through intracutaneous

injection in rabbits. For medical device materials, saline and vegetable oil are used to ensure extraction of both water-soluble and fat-soluble chemicals. The intracutaneous reactivity test is aggressive in that it makes use of extracts prepared under exaggerated conditions and places them directly into the skin of the test animal, thereby maximizing the chance of finding irritant chemicals if they are present. At 24, 48, and 72 hours after injection, the test and control sites are observed and scores are given for the severity of any redness (erythema) or swelling (edema). The *in vivo* intracutaneous irritation test with extracts of polyurethane, PTMGMDIBPA and acrylate copolymer, IP(GMA-co-MMA) in both the media (PS and CSO) did not reveal adverse local responses in rabbits such as erythema or edema. The test results clearly indicated that the polymers were free from extractable low molecular weight fractions or catalysts. The *in vivo* toxicological screening studies revealed biocompatibility of the polymers.

5.6.5 Evaluation of *in vivo* biocompatibility - subcutaneous implantation

All polymeric biomaterials act as foreign bodies when they are implanted, leading to an acute inflammatory response and the accumulation of phagocytes. Histocompatibility deals with biocompatibility in terms of whether the implant or its degradation products, if any, initiate adverse tissue responses in the host, or conversely whether deleterious changes in the chemical, physical and mechanical properties of the implant material are caused by the host environment. The tissue response to an implant is a specialized version of inflammation and repair or the mammalian reaction to local injury.

Polymeric biomaterials, like any other material implanted in body elicit initial inflammatory reactions following implantation. Inflammation begins when surgical injury causes migration of cells from the circulating blood to the site of polymer implantation, where a transient inflammatory exudate is formed. Monocytes that migrate from the vasculature to the implant site may adhere to the surface of the materials. Over the period of time, these adherent monocytes differentiate into macrophages, which can then fuse together to foreign body giant cells (Kao et al. 1994, Zhao et al. 1992). After some time, fibrous capsules may be observed around the implant. Signs of non-biocompatibility are usually associated with necrosis, calcification and tumorigenesis (Rihova 1996). The rats with implanted iodinated polymers, PTMGMDIBPA & IP(GMA-co-MMA) specimens were radiographed after 12 weeks of implantation. X-ray images

of all rats clearly showed the position of the polymeric films where as the control materials (UHMWPE films) were not visible. The biocompatible control material was selected as per the ISO 10993 protocol. The shape and location of the polymeric films were clearly seen in the X-ray images implying that no loss of radiopacifier occurred during the implantation period. *In vivo* subcutaneous implantation of the candidate iodinated polymers, PTMGMDIBPA & IP(GMA-co-MMA) in rat model and histopathological analyses of the tissues surrounding the implants confirmed the *in vivo* biocompatibility. The histological studies revealed no encapsulation, hemorrhage, infection or necrosis around the test materials. The general physical conditions of all the experimental animals were normal during the experimental period. All animals recovered quickly and showed no signs of infection, and no premature death occurred. The increase in body weight and feed intake were normal and none of the animals showed any abnormality or behavioral changes during the experimental period. The histopathological studies revealed that the tissue response of test materials was similar to that of control material at the end of 4, 8 and 12 weeks. At 4 weeks post implantation, mild to moderate inflammatory response (represented as Δ in figures) was noticed in test and control groups of both the polymers. The inflammatory response was decreased (shown as Δ in figures) after 8 weeks post implantation, in both the polymers. Evidence of repair, was noted with fibrous tissue capsule and neovascularisation in both groups. The histopathological analysis at 12 weeks post implantation also revealed evidence of repair with fibrous tissue capsule consisting of fibrocytes and collagen (designated as Ψ in figures) around the implant site and neovascularisation (angiogenesis, represented as Ω in figures) in both groups of the polymers. Fatty infiltration was absent. The histopathological analyses confirmed the *in vivo* biocompatibility of the iodinated polymers, PTMGMDIBPA & IP(GMA-co-MMA).

SUMMARY AND CONCLUSION

6 SUMMARY AND CONCLUSION

Polymeric biomaterials having radiopacity are being used for medical applications such as cardiovascular implants, prostheses, orthopedic implants and controlled drug release devices since it allows post-operative assessment of the fate of the device using X-radiography. Often these devices are made radiopaque by incorporating metal powder such as tantalum or metal salts of barium, bismuth, uranium into the systems. Conventional polymers cannot be detected by X-ray and ultrasound because they contain elements such as C, H, O and N, which exhibit low electron density and low specific gravity. Hence, strategies to develop radiopaque polymers focus on ways to increase the average electron density and specific gravity of polymers. The incompatibility of inorganic additives with polymer matrix often affects the physical and mechanical properties of the implants or prostheses adversely. Moreover, the possibility of inorganic ions leaching into the body fluid in the case of long term applications makes radioapacity a temporary phenomenon apart from producing systemic toxicity. The second approach towards achieving radiopacity is to prepare radiopaque polymer-salt complexes. This method is popular for dental implants. But the ionic nature of these systems cause absorption of water leading to hydrolysis resulting in the loss of the radiopacifying atom. A better approach to make radiopaque polymers is to synthesize monomers having covalently bound heavy halogen atoms such as iodine and use these monomers as building blocks of new polymers that show intrinsic radiopacity or graft radiopacifying agents onto preformed high molecular weight polymers. In the present investigation, we have made use of the former strategy for the preparation radiopaque polyurethanes, where the radiopacity is due to the covalently bound iodine on the chain extenders and radiopaque acrylic copolymers based on GMA were synthesized by the latter strategy such as post-polymerization halogenation of reactive groups.

Novel segmented polyurethane elastomers possessing inherent radiopacity were synthesized using MDI, HDI, PTMG, PPG and iodinated chain extenders, IBOL & IBPA. The iodinated chain extenders were synthesized by simpler and economical procedures. The physicochemical and mechanical characterizations of the polyurerthanes were carried out. High molecular weight radiopaque polyurethanes possessing adequate visibility under normal X-ray operating conditions were obtained. The FTIR and XRD analyses revealed hydrogen bonding

interaction in PTMGHDIBOL leading to a phase-separated surface morphology. However, PTMGMDIBOL exhibited a phase-mixed surface morphology which undergoes dynamic surface reorganization in aqueous medium to a phase-separated surface morphology. The partially crystalline nature of PPG based polyurethanes is due to the incompatibility of hard and soft segments. The incompatibility of segments due to the side chain methyl groups in PPG resulted in the phase separated morphology. Only diffuse X-ray scattering was observed for PTMGMDIBOL and PTMGMDIBPA and this is attributed to an amorphous arrangement of chain segments in the polyurethanes. PTMGMDIBOL showed considerably higher tensile strength than all other polyurethane. All the polyurethanes possessed excellent thermal stability. Surface properties by contact angle studies have shown that the polyurethanes were hydrophilic.

In vitro biostability of radiopaque polyurethanes were evaluated by aging the samples in different medium for 6 months at 37 °C. There was neither significant change in weight nor evidence for bond breaking at the degradation-susceptible urethane or ether groups in the IR spectra of aged polyurethanes in simulated physiological conditions. The studies with accelerated chemical degradation revealed degradation of HDI based polyurethanes. However, no degradation (weight loss) or dimensional change was observed with other polyurethanes.

Radiopaque copolymers of GMA with MMA, EMA and BMA were synthesized by introducing iodine atoms via the regioselective ring opening reactions of epoxide groups. The percentage weight of iodine in the present copolymers was found to be as high as 23%. The iodinated copolymers showed higher glass transition temperature and thermal stability in comparison with unmodified polymers. The presence of bulky iodine atoms in the polymer backbone decreased the flexibility of the macromolecules and created modified polymers with novel properties. Radiographic analysis showed that the copolymers possessed excellent radiopacity. *In vitro* and *in vivo* biocompatibility studies were carried out on radiopaque polyurethanes and acrylic copolymers in order to establish the compatibility of the present polymeric materials with living system. All the iodinated polymers were cytocompatible with L929 fibroblast cells. The normal cellular morphology of fibroblast cells was retained when compared to positive control (cytotoxic material). Quantitative assessment of cytoxicity to cells by MTT assay after exposure with material extracts have shown more than 70% cell viability for all radiopaque

polymers. The studies indicated that the present polymers were free of harmful leachants that can induce adverse responses in body system.

In vitro screening of candidate polyurethanes, PTMGMDIBOL and PTMGMDIBPA for blood compatibility has shown that the materials were blood compatible. There was no significant difference in count for RBC, WBC and platelets immediately and after 30 min of contact. PTT and fibrinogen content values were also within accuracy limits, indicating that the intrinsic coagulation pathway has not been adversely affected. The polyurethanes were also found to be non-haemolytic, the extent of haemolysis being lower than the permissible level of 5%. The toxicological studies and *in vivo* subcutaneous implantation of radiopaque polymers, PTMGMDIBPA and IP(GMA-co-MMA) were carried out to assess the *in vivo* biocompatibility. Intracutaneous irritation tests in rabbit animal models revealed that the materials possessed no leachants capable of producing an allergic reaction. *In vivo* subcutaneous implantation of the materials in rats followed by histopathological analyses of the tissues surrounding the implant confirmed the biocompatibility of the radiopaque polymers. X-ray images of all rats after 12 weeks of implantation showed that the polymers were radiopaque. The shape and location of the polymeric films were clearly seen in the X-ray images implying that no loss of radiopacifier occurred during the implantation period. The newly synthesized polyurethanes with adequate iodine content offer elastomeric and radiopaque character, which is a technical advance over the existing reports (Drewes Jr & Parker 1994, Kiran et al. 2009). Drewes Jr & Parker (1994) have reported the development of flexible, radiopaque polyether block amide plastic material catheter with a moderately radiopaque proximal tubular member portion and a highly radiopaque distal tubular member portion which contains greater than 75 weight percent of a radiopaque agent. The favorable properties of the synthesized polyurethane suggest that these polyurethanes could be used as medical-surgical tubings, cannulas, guiding catheters, angioplasty balloon catheters, drainage catheters, and any other medical catheter that desirably includes a highly radiopaque portion and as a coating material for several biomedical implants and devices, *viz.* endovascular stents and also as film-forming materials for visualization of the gastrointestinal tract.

Acrylate polymers are widely used for dental and orthopaedic applications. A number of

different formulations of acrylates have been employed in dentistry as orthodontic and denture base materials, while fixation of orthopaedic implants to bone was revolutionized by the introduction of acrylate bone cement as a fixation material. A major drawback of acrylate polymers is that they are radiolucent, since polymers hardly absorb X-ray radiation due to the absence of heavy elements within their structure. Iodinated copolymers of GMA with other methacrylate monomers could be used as dental or bone cements in place of barium or zirconium particles, which are usually added to provide X-ray opacity. It can also be used in vertebroplasty for the augmentation of osteoporosis induced vertebral compression fractures. Almost invariably, an injectable PMMA bone cement that contains a large amount of $BaSO_4$ particles is used normally in these procedures. The deleterious effects of this radiopacifier have been detailed in the literature. Another concern arising from both laboratory studies and clinical observations of cements has been the lack of toughness of PMMA, as it exhibits brittle characteristics upon failure. Therefore, it is intuitively reasonable to suggest that the iodinated copolymers of GMA with MMA, EMA and BMA would exhibit permanent radiopacity and be a tougher and less brittle material when used for medical applications.

The radiopaque polyurethanes possessed good *in vitro* biostability when aged in physiological medium for 6 months. Extensive studies on biostability of the materials such as long term *in vitro* study (12 months) and *in vivo* study need to be be investigated further. Stability of acrylate copolymers *in vitro* and *in vivo* could be explored in detail. Since preliminary *in vitro* screening of radiopaque polyurethanes showed blood compatibility, further detailed studies like protein adsorption, interaction of various cellular components etc. could be investigated. Investigations on systemic toxicity for delayed hyper sensitivity and long term implantation for tissue compatibility of the radiopaque polymers need to be done. Radiopaque polymeric microspheres based on iodinated polyurethanes and iodinated copolymers could be investigated for their potential applications as embolic materials.

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Original papers

1. **S. Dawlee**, A. Sugandhi, B. Balakrishnan and A. Jayakrishnan, "Oxidized Chondroitin Sulfate-Cross-Linked Gelatin Matrixes: A New Class of Hydrogels," in *Biomacromolecules*, vol 6, 2005, 2040.

2. **S. Dawlee**, A. Jayakrishnan and M. Jayabalan, "Studies on novel radiopaque methyl methacrylate: glycidyl methacrylate based polymer for biomedical applications," in *J Mater Sci: Mater Med*, vol 20, 2009, 243.

3. **S. Dawlee** and M. Jayabalan, "Development of segmented polyurethane elastomers with low iodine content exhibiting radiopacity and blood compatibility," in *Biomedical Materials*, vol 6, 2011, 055002.

4. **S. Dawlee** and M. Jayabalan, "Studies on inherently radiopaque acrylate copolymers for biomedical applications," in *J. of Applied Polymer Science*, Accepted for publication.

5. **S. Dawlee** and M. Jayabalan, "Iodinated glycidyl methacrylate copolymer as a radiopaque material for biomedical applications," in *J. of Biomaterials Applications*, Accepted for publication.

6. **S. Dawlee** and M. Jayabalan, "Intrinsically radiopaque polyurethanes with potential biomedical application as catheters," in *Acta Biomaterialia*, Under Review.

Patents

- **S. Dawlee** and M. Jayabalan, "Polyurethane Elastomer With Adaptable Surface Properties," *(Applied for Indian Patent)*.

Conference Proceedings

1. **S. Dawlee** and M. Jayabalan, "Inherently radiopaque methyl methacrylate- glycidyl methacrylate based polymers for biomedical applications," *International Conference on Advanced Materials*, 8^{th} to 13^{th} October 2007, Bangalore, India.

2. **S. Dawlee** and M. Jayabalan, "Studies on novel radiopaque polymers for biomedical applications," *International Conference on Functional Materials*, 27^{th} to 29^{th} November 2009, IIT Madras, Chennai, India (Poster presentation).

3. **S. Dawlee** and M. Jayabalan,, "Synthesis and Characterization of Radiopaque Polyurethanes for Medical Applications," 21^{st} *Kerala Science Congress*, 28^{th} January to 31^{st} January 2009, Kerala, India.

4. **S. Dawlee** and M. Jayabalan,, "Polyurethanes possessing intrinsic radiopacity for Medical Applications," *MACRO 2009*, 9^{th} March to 11^{th} March 2009, IIT Madras, Chennai, India (Poster presentation).

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Education

04/2006 - present	**Ph. D. Scholar** at Sree Chitra Tirunal Institute for Medical Sciences and Technology. Biomedical Technology Wing, Trivandrum, Kerala, India. Advisor: Dr. M. Jayabalan
8/2000 - 8/2002	**Master of Science in Applied Chemistry**, National Institute of Technology, Trichy, Tamil Nadu, India.
07/1997 - 04/2000	**Bachelor of Science in Chemistry**, University of Kerala, Kerala, India.

Professional experience

05/2003 - 12/2005	Sree Chitra Tirunal Institute for Medical Sciences and Technology. Biomedical Technology Wing, Trivandrum, Kerala, India. Project advisor: Dr. A. Jayakrishnan, Scientist G & Head, Polymer Chemistry Division (SCTIMST).
2/2002 - 07/2002	National Institute for Interdisciplinary Science and Technology (NIIST), CSIR, India. Project advisor: Dr. Mangalam Nair, Scientist, Organic Chemistry Division (NIIST).

Special achievements

03/2006	Senior Research fellowship from Council of Scientific and Industrial Research, New Delhi, India.
01/2009	Young Scientist Award from Kerala State Council for Science, Technology and Environment, Trivandrum, India

APPENDICES

APPENDICES

Composition of buffers

Phosphate Buffered Saline (0.1 M, pH 7.4)

Disodium hydrogen phosphate	17.927 g
Monosodium hydrogen phosphate	5.73 g
Sodium chloride	9 g
Distilled water	1000 mL

Ringer's Solution (pH 7.4)

Sodium chloride	9 g
Sodium hydrogen carbonate	0.42 g
Calcium chloride	0.24 g
Glucose	1 g
Distilled water	1000 mL

HEPES Buffer Solution (pH 6.8)

HEPES	23.83 g
Sodium azide	0.20%
Mercaptoethanol	4.6878 g
EDTA	3.7224 g
Distilled water	1000 mL

ISO Standards

1. Plastics - Determination of tensile properties - Part 5: Test conditions for unidirectional fibre-reinforced plastic composites. ISO 527-5, 1997

2. Biological evaluation of medical devices. Part 13. Identification and quantification of degradation products from polymeric medical devices. ISO 10993-13, 1998

3. Biological evaluation of medical devices. Part 5. Tests for cytotoxicity: *In vitro* methods. ISO-10993-5, 2009.

4. Biological evaluation of medical devices. Part 4.Selection of tests for interaction with blood. ISO-10993-4, 2002.

5. Biological evaluation of medical devices. Part 10. Test for irritation and delayed type hypersensitivity: Intracutaneous Reactivity Test. ISO-10993-10,2002

6. Biological evaluation of medical devices. Part 6. Tests for local effects after implantation. ISO-10993-6, 2007