# ELUTION CHARACTERISTICS OF GENTAMYCIN FROM BIOACTIVE CALCIUM SULFATE CEMENT FOR THE CONTROL OF OSTEOMYELITIS

**THESIS SUBMITTED BY**

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**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF**

**MASTER OF PHILOSOPHY**

**SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY**
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## DECLARATION

I, **Archana**, hereby declare that the thesis work entitled '**Elution Characteristics of Gentamycin from Bioactive Calcium Sulfate Cement for the Control of Osteomyelitis**' was done by me under the direct guidance of Dr. Manoj Komath, Scientist F, Bioceramics Lab, Biomedical technology wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram, Kerala, India. External help sought are acknowledged.

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2015/M.Phil/08

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## CERTIFICATE

This is to certify that the thesis work entitled '**Elution Characteristics of Gentamycin from Bioactive Calcium Sulfate Cement for the Control Of Osteomyelitis**' submitted by **Archana A (2015/M.Phil/08)** in partial fulfillment for the Degree of Mater of Philosophy in Biomedical Technology was done under my supervision and guidance at Bioceramics lab, Biomedical technology wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram, Kerala, India.

Place: Thiruvananthapuram Dr. Manoj Komath

Date:

The Thesis Entitled

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# ABSTRACT

Chronic osteomyelitis, or bone infection, is a challenge in orthopedics because of the practical difficulties in systemic antibiotic treatment which leads to organ failure and other complications due to very high doses. Local drug delivery is adopted for achieving effective and safe local concentrations of antibiotics. Though acrylic based and ceramic based delivery systems are successfully applied for the purpose, appropriate delivery characteristics, resorption and osteoconductivity are yet to be optimized.

In the present study a calcium sulfate based osteoconductive, degradable system is explored. The material has uniform particle size and hence predictable properties. The release kinetics of drug from the material was evaluated and the influence of geometrical parameters like area and binders to the material was checked to know their effect on the release mechanism. The elution study was done using UV analysis. The observed results were analysed to increase the effectiveness of release for clinical purposes.

The release from the material shows a burst release within 72 hours with 60% release of drug which is slower compared to other reported calcium sulfate materials and it may be due to the micro morphological change shown by the material. The predictable release rate due to change in area can be used effectively for clinical advantages. Study shows that slower releases are shown by bigger pellets and faster release by smaller pellets and this can be used for acute and chronic management of osteomyelitis. A controlled delivery system can be designed with predictable release profile by porosity engineering making the release from a specified area.

# 1. INTRODUCTION

This work deals with the local drug delivery strategy for the control of bone infection, namely *Osteomyelitis*. A new bioactive calcium sulfate material has been suggested for bone grafting. This material is a convenient medium for the local delivery of drugs for bone diseases. In the present work, the drug elution characteristics from the material are investigated so that a model for effective clinical management of osteomyelitis may emerge.

## *1.1 THE THREAT OF OSTEOMYELITIS*

Osteomyelitis refers to bone infection, caused by bacterial exposure during trauma, surgery, orthopedic device implantation, represents a major challenge for clinical orthopedic practice (Liu et al. 2012). Large population around the world suffers from osteomyelitis, which may be attributed to increase in reconstructive orthopedic procedures and higher occurrence of diabetes mellitus along with increased life expectancy (Calhoun, Manring, and Shirtliff 2009).

As the infection extends during the early acute disease, the vascular supply to the bone gets reduced causing the destruction of the bone tissue. In established infection, fibrous tissue and chronic inflammatory cells will form around the granulation tissue and dead bone (Calhoun, Manring, and Shirtliff 2009). Osteomyelitis usually causes edema, vascular congestion, and small vessel thrombosis (Emslie, Ozanne, and Nade 1983).

The infected, nonviable tissues and an ineffective host response lead to chronic osteomyelitis which is intrinsically resistant to antibiotic therapy (Calhoun, Manring, and Shirtliff 2009). *Staphylococcus aureus* is the commonly involved pathogenic micro organism causing chronic device associated osteomyelitis due to its ability to adhere to tissues, undermine host defenses, and invade mammalian cells. *S. aureus* resistant to methicillin were reported and now Methicillin resistant *S. aureus* (MRSA) endemic to India

pose a major threat in device associated osteomyelitis across India (Joshi et al. 2013).

### *1.2 TREATMENT FOR OSTEOMYELITIS*

Acute osteomyelitis is usually successfully treated with intravenous infusion of antibiotics (Lew, Waldvogel, 2004; Darley, MacGowan, 2004).
The treatment of chronic osteomyelitis is done elaborately with multidisciplinary approach in 3 phases: surgical debridement, systemic antibiotic therapy for 4 to 6 weeks and local antibiotic delivery (Sánchez *et al*., 2001; Aslam, Darouiche, 2009; Mouzopoulos, 2011).

Systemic antibiotic therapy for chronic osteomyelitis is very risky because high doses are to be administrated to blood in order to achieve the therapeutic concentration in the infected area, due to the poor local blood supply to the bone (Emslie, Ozanne, and Nade 1983). This can lead to drug related toxicity with organ failure, gastrointestinal side effects and allergic reactions (Tsourvakas 2012). The management has become more difficult with the increasing number of drug resistant bacteria such as MRSA. This necessitates the surgical removal of the infected bone. As an attempt to increase the efficiency of treatment of osteomyelitis, new methods such as local delivery of antibiotics have evolved. The major reason for opting local antibiotic delivery devices is for obtaining a very high local concentration of antibiotics without any associated systemic toxicity.

### *1.3 LOCAL DRUG DELIVERY SYSTEMS FOR OSTEOMYELITIS CONTROL*

For the past fifty years, scientists and physicians were trying to pursue an ideal local drug delivery system for osteomyelitis which could provide high antibiotic levels at the infected site and a safe level in the systemic circulation (Nelson 2004). The choice of material is a very important step while designing a local drug delivery system, and certain factors are to be known like the antibiotic elution curves, the factors that influence elution and the most suitable

local delivery system for the environment into which the material is to be placed (Tsourvakas 2012).

For the application of local antibiotic therapy for bone infections the following factors are to be considered: (Meani et al. 2007)

a) Delivery technique with suitable material.

b) Type of antibiotic that can be used.

c) Pharmacokinetics.

d) Possibility of application.

e) Possibility of combination with osteoconductive and osteoinductive factors.

f) Use as prophylaxis and/or therapy.

g) Drawbacks

Antibiotic impregnated acrylic beads have been used in the treatment of chronic osteomyelitis for nearly 27 years (Nandi et al. 2009). From 1970s the most commonly used local drug delivery system against deep bone infections in orthopedic endoprosthetic surgery in human patients was poly(methyl methacrylate) (PMMA) beads impregnated with antibiotics (Cornell et al. 1993). Since then, local deposition of antibiotic-loaded PMMA has been considered as an efficient method for providing sustained high concentrations of antibiotics locally against bone and soft tissue infections. Experimental and clinical successes have been achieved by PMMA loaded with hydrophilic antibiotics including gentamicin, ceftriaxone, tobramycin and vancomycin (Uskokovic 2015).

Antibiotic-impregnated PMMA is used clinically in two forms as bone cement applied in arthroplasties and as bead chains for musculoskeletal infections. The success of PMMA is on the fact that, it does not usually trigger any immune response from the host and the bead form confers a large surface area, allowing rapid release of the antibiotic.

PMMA cement is available as commercial and non commercial brands and the *in vitro* release of antibiotics varies with brands. Commercially

available beads have consistent diameter of 7mm and are available in strands of 10 to 30 beads whereas non commercial is prepared by the surgeons themselves at the time of use (Frommelt 2001).

In the past decade, the research interests were directed towards biodegradable local drug delivery systems because of the non-bio-degradable nature of PMMA and the additional surgery needed to remove it. Among the biodegradable systems ceramic based bone graft substitutes impregnated with antibiotics against osteomyelitis is of great interest due to their biocompatibility, osteoconductive and osteophilic nature (Kluin et al. 2013).

Hydroxyapatite, bioglass and other calcium phosphates ceramics were extensively studied aiming at their use as implantable materials. Porous blocks of calcium hydroxyapatite have been used as delivery systems for sustained release of antibiotics (Shinto et al. 1992). The release of gentamicin sulfate, cefoperazone sodium and flomoxef sodium was tested *in vitro* and *in vivo* in rats. Beta tricalcium phosphate beads carrying gentamicin and vancomycin were studied as a resorbable bone substitute in rabbits induced with osteomyelitis (Lambotte et al. 1998). Ciprofloxacin loaded tricalcium phosphate ceramic capsules showed a long term release of the antibiotic and proved good biocompatibility with low degradation in *in vitro* studies. Antibiotic impregnated hydroxyapatite has also been used to treat patients with chronic osteomyelitis after removing necrotic tissue. The ceramic material was gradually incorporated into the host bone and uneventful healing was observed within three months with no recurrence of infection (Nandi et al. 2009). Other types of ceramics such as bioglass have also been studied for drug delivery applications (Schlickewei, Yarar, and Rueger 2014).

Self-setting inorganic cements are new generation graft materials which have the properties like biocompatibility, osteoconductivity, and the ability to set in vivo. There are two classes – calcium phosphate cement (CPC) and calcium sulfate cement (CSC). These are aqueous-based setting cements and have a good scope to be used as local drug delivery medium because of the

ease of incorporating antibiotics. Unlike ceramic scaffolds, drugs can be incorporated throughout the whole material volume at the time of making itself, facilitating a sustained drug release (Ginebra et al. 2012).

## *1.4 THE CHALLENGES IN THE LOCAL DRUG DELIVERY FOR OSTEOMYELITIS CONTROL*

Local drug delivery is advisable for better control of osteomyelitis yet there are problems to be solved regarding the selection of materials used for delivery and the proper understanding of elution characteristic of the material.

PMMA represents the current gold standard for local antibiotic delivery and proven to be successful with several antibiotics, there are many challenges faced by this delivery system. The major one is its non biodegradable nature which leads to a second surgery to remove the cement beads which is expensive and painful. The reported elution property of PMMA is poor with slow residual release of antibiotics to undefined periods causing the risk of generating antibiotic resistant bacteria (Kluin et al. 2013). Elution properties of PMMA mainly depend on the type of PMMA, concentration of antibiotic and structural characteristics of the bead. Other problem related to the use of PMMA is the risk of induction of drug resistance due to biofilm formation on the material (Campoccia *et al*., 2010).

Initial burst of antibiotics to the infection site is the major problem with all other alternative materials used as local drug delivery device. Also, there is a lack of standardization and validation. None of these materials has been approved for antibiotic delivery in osteomyelitis treatment by the FDA(Nandi et al. 2009)

## *1.5 THE RATIONALE AND OBJECTIVES OF THE PRESENT WORK*

This work aims for the development of an efficient local drug delivery device for the control of osteomyelitis, taking into account the challenges mentioned in the previous section. The material selected is novel bioactive self-setting calcium sulfate-based cement, which has been developed as a bone filler material. It consist of medical grade low dimensional calcium sulfate and is proven to be a biocompatible, osteoconductive and resorbable material (Sony et al. 2015). Its ability to work as a local antibiotic delivery device with suitable elution characteristics is studied here by *in vitro* release study.

### Hypothesis

It is hypothesized that the release of gentamicin from the material will follow an initial burst with a sustained release, following theoretical models for a porous monolithic matrix. The geometrical parameter like area can have an influence on the release mechanism which can alter the release kinetics of drug from the system.

### Objectives of the Work

- To evaluate the suitability of material as a drug delivery medium in bone by various characterization method.
- To study the release kinetics of gentamicin from the cement by examining the elution characteristics.
- To study the effect of geometrical parameters on elution characteristics, in order to predict the release rate from a defined geometry.
- To study the effect of additives on drug elution characteristics

## 2. PRINCIPLES AND PRACTICES OF LOCAL DRUG DELIVERY FOR BONE DISEASES

### *2.1 CONTROLLED DRUG DELIVERY*

The major advantage of using local drug delivery systems is its ability for 'controlled release' of drugs. The term 'controlled release' not only means prolonged duration of drug delivery but also implies predictability and reproducibility of drug release kinetics. The controlled drug delivery systems can be designed to release drugs in the vicinity of the targeted tissues that require treatment while lowering the exposure of drug to the normal tissues (Chien, Cabana, and Mares 1982). The advantages of such specificity and control of releases are used for the development of antibiotic delivery system for the treatment of osteomyelitis.

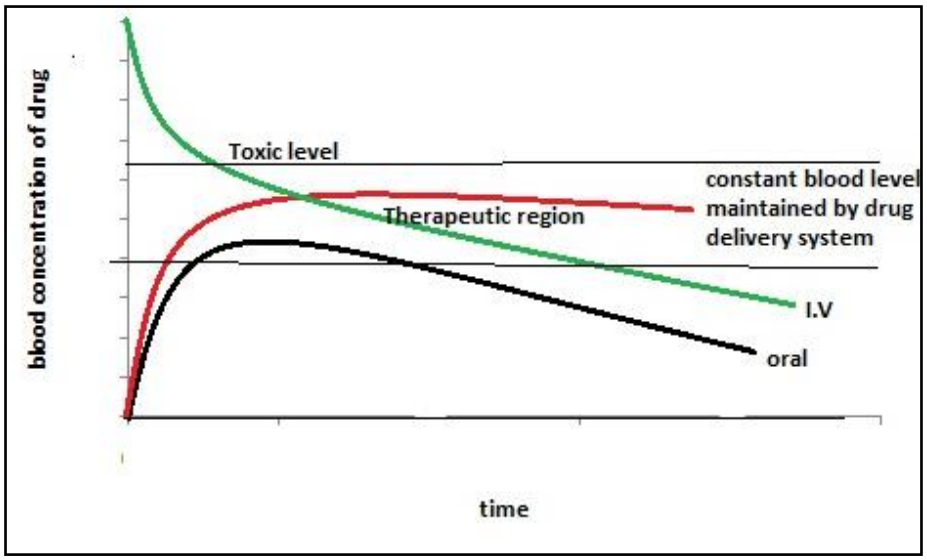



*Fig 2.1: Theoretical illustration of blood drug concentration profiles of long acting controlled drug delivery system and immediate release conventional forms via different route of administration.*

Controlled release systems are generally classified based on their physicochemical, pharmaceutical or clinical aspect. They can also be classified according to their release mechanism and preparation methods as follows (McLaren 2004):

• Physical systems, including diffusion controlled systems such as monolithic porous systems and biodegradable/bioerodable systems.

• Chemical systems, including immobilization of drugs.

• Biological systems, including gene therapy

Depending on the delivery system and the pharmaceutical in use, different release mechanisms are applied. However, there are three major ways by which drugs can be released from a drug delivery system:(i) diffusion, (ii) degradation and (iii) swelling followed by diffusion (McLaren 2004).

Any or all of these mechanisms may occur in a given delivery system. As we are concentrating on a controlled release from porous ceramic based drug delivery device diffusion controlled release is of prime interest.

## *2.2 DIFFUSION CONTROLLED DRUG DELIVERY SYSTEM*

A diffusion controlled release can be obtained by two different systems: reservoir and monolithic or matrix systems. Reservoir system consists of drug depots, which is surrounded by a release rate controlling barrier membrane, where as in a monolithic system there is no local separation between a drug reservoir and a release rate controlling barrier (Siepmann, Siegel, and Rathbone 2011).

In the ceramic based drug delivery system the drug is considered to be dispersed in the matrix hence drug delivery system is assumed as a monolithic or matrix system where the release is controlled by diffusion from the system.

The diffusion controlled drug release in the monolithic system can be described (Fig 2.2) as the sum effect of processes occurring on four different length scales. Firstly, diffusion is driven by the thermal motion of individual molecules on the molecular scale (micro scale). Secondly, the molecular processes will result in certain drug concentrations within the pore system of the matrix (meso scale). Thirdly, on scales significantly larger than the average pore size (macro scale), it is usually possible to describe diffusion using effective diffusion coefficients. Fourthly, the macro scale diffusion will result

in an overall release profile for the system as a whole (global scale) (Schlickewei, Yarar, and Rueger 2014).

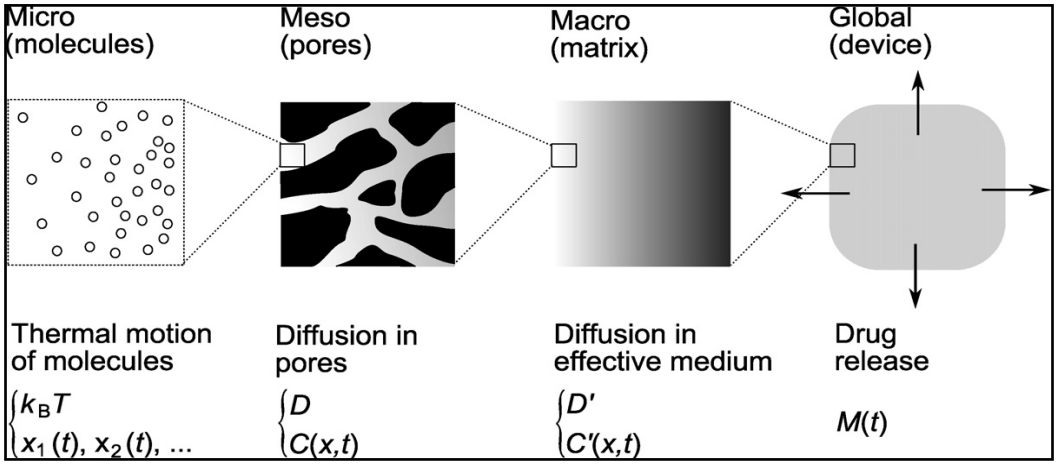



*Fig 2.2 Diffusion explained at four different length scales from a monolithic drug delivery system. (Schlickewei, Yarar, and Rueger 2014).*

The macro to global transition from matrix properties to the release profile of drug is commonly studied in drug-release modeling, and it is the main focus of the present study.

The solubility of a drug and its dissolution rate has a significant influence on the drug release kinetics. If the drug is present in the matrix below its solubility limit it can be dissolved in a matrix and if it is present above its solubility limit, it is dispersed. For a dispersed drug in a matrix it is assumed that the dissolution rate of the drug is minimal compared to the diffusion rate.

## *2.3 MATHEMATICAL MODELS OF DRUG RELEASE FROM A DIFFUSION CONTROLLED DRUG DELIVERY SYSTEM*

During the development of a controlled delivery system mathematical models allow for the quantitative prediction of the effects of formulation and processing parameters on the resulting drug release kinetics. Thus, the required composition, size, shape and preparation procedure of a release system with desired properties become theoretically predictable. In addition, challenges encountered during production are much easier to address when having a clear idea of how the system works.

Mathematical models play an important role in scientific work by helping the extraction of useful information on the crucial properties of materials in particular situations. By analyzing experimental data, the physical and/or chemical mechanisms taking place during an experiment can be qualitatively, and sometimes even quantitatively, accounted for. Modeling can reduce the number of experiments needed in order to make confident predictions of the usefulness of a material in a certain application.

Much effort has been invested in the development of models to elucidate the drug-release processes mentioned. The models can be classified as either mechanistic or empirical/semi-empirical. The purpose of the mechanistic models is to explain the drug release on the basis of the physical and chemical processes that control the release rate (such as diffusion and dissolution), while the empirical/semi-empirical models aim for a description of the release profile without necessarily taking the underlying processes into account. The empirical/semi-empirical models are often used to summarize and evaluate experimental release data when the underlying processes are too complex to be coherently described (Siepmann, Siegel, and Rathbone 2011).

### 2.3.1 Mechanistic Model for Drug Release from diffusion controlled device.

Fick's first law relates the diffusive flux *J* to the concentration *C* by postulating that the flux is proportional to the diffusion coefficient *D* times the spatial concentration gradient

The negative sign in the expression above ensures that the flux goes from regions of high concentration to regions of low concentration.

$$\mathbf{J = -D \frac{\delta c}{\delta x}} \text{ ---------- } \mathbf{(1)}$$

Ficks 2nd law relates the concentration change in a unit volume with time *t* to the second spatial derivative of concentration. Assuming a constant diffusion coefficient, the expression simplifies to:

$$\mathbf{\frac{\delta c}{\delta t} = -D\ \delta^2 C/\delta x^2} \text{ ---------- } \mathbf{(2)}$$

Generally, when solving the equations to obtain information about a drug-release process, it is assumed that the domain in which the drug diffuses (i.e. the pellet) has zero drug concentration on its surface; this is a boundary condition called a perfect sink. The perfect sink condition was assumed, because the drug concentration within the diffusing domain was often much higher than outside. The drug was assumed to be initially homogeneously dispersed throughout the matrix (Frenning 2011).

For diffusion controlled release system drug release is described by equation derived from Fick's second law of diffusion, which is as follows (Crank 1980):

$$\mathbf{M_t/M_0 = 4(Dt/\pi h^2)^{1/2}} \text{---------- (3)}$$

Where, $M_t$ is the amount of drug released at time t, $M_0$ is the total mass of drug loaded into the device, D is the diffusion coefficient of the drug, and h is the thickness of the pellet. This is an early-time approximation which holds for the release of the first 60% of cumulative release, i.e. $0 \leq M_t/M_0 \leq 0.6$.

The late-time approximation, which holds for the final portion of the drug release, i.e. $0.4 \leq M_t/M_0 \leq 1.0$, is described by the following equation:

$$\mathbf{M_t/M_0 = 1 - (8/\pi^2) \exp[(-\pi^2 Dt)/h^2]} \text{---------- (4)}$$

### 2.3.2 Empirical and semi-empirical mathematical models for drug delivery.

Semi-empirical models give an indication for the underlying drug release mechanism under very specific conditions. Empirical/semi-empirical models should generally not be used if the underlying drug release mechanisms are to be elucidated. However, such a descriptive mathematical analysis can be useful for a comparison of different drug release profiles (e.g., for experimental design studies).

A very frequently used and easy-to-apply model to describe drug release is the Korsmeyer-Peppas model, or power law (Korsmeyer et al. 1983).

$$M_t/M_\infty = kt^n \text{ ---------- (5)}$$

Here, $M_t$ and $M_\infty$ are the absolute cumulative amount of drug released at time *t* and infinite time, respectively; *k* is a constant incorporating structural and geometric characteristic of the system and *n* is the release exponent, which might be indicative of the mechanism of drug release. Nicholas Peppas was the first to introduce this equation in the field of drug delivery (Peppas, 1985). The classical Higuchi equation as well as the above described short time approximation (eq.3) represent the special case of the Peppas equation.

Considering the drug is dispersed in the matrix of the set cement, it is assumed that the drug release kinetics follows Higuchi's law usually at the initial stages (up to 60% release of drug) and it indicates diffusion controlled release of drug from the matrix (Higuchi 1963):

$$M_t = AM_0\sqrt{[DC_s(2C_0 - C_s)t]} \text{ ---------- (6)}$$

Where, $M_t$ is the amount of drug released for time t; $M_0$ is the total amount of drug; A is the surface area of the device; D is the diffusion coefficient of the drug in the matrix; $C_s$ is the solubility of the drug in the matrix; $C_0$ is the initial concentration of the drug in the matrix.

In the case of drug dispersed porous matrix certain parameters like diffusion path-length and the effective volume are considered and the equation is modified to:

$$M_t = AM_0\sqrt{\left[\frac{D\varepsilon}{\tau}C_s(2C_0 - C_s)t\right]} \text{ ---------- (7)}$$

Where, ε is the porosity and τ is the tortuosity of the porous matrix.

### 2.3.3 Mechanism of drug release from the drug delivery device

Since the drug delivery device is considered as a monolithic system the release mechanism can be as that of matrix type delivery system. In this type delivery system it is assumed that the drug crystals or particles cannot delocalize their positions in the matrix and molecules can elute out the matrix only by dissolution in and then by diffusion through the porous structure of the

matrix. The drug crystals in the layer closer to the surface of the device are the first to elute, and when this layer becomes exhausted, the drug crystals in the next layer then begin to deplete. There exists a drug depletion zone with a thickness δp. This thickness becomes greater and greater as more drug solids elute out the device, leading to the inward advancement of the interface of the drug depletion zone further into the core of the device.

There also exists a thin layer of stagnant solution, called the hydrodynamic diffusion layer, on the immediate surface of the device. The thickness δd of this stagnant solution layer varies with the hydrodynamics of the device in the solution in which it is immersed.

The cumulative amount of drug released from a thickness of drug depletion zone δp is defined by

$$\mathbf{Q = (C_0 - Cs/2)\ \delta p \text{ ---------- (8)}}$$

If the depletion layer thickness (δp ) is so small the cumulative amount of drug release become directly proportional to time ( Q = Kt). As the thickness increases the cumulative release from the unit surface area become directly proportional to $t^{1/2}$ ($Q = K\ t^{1/2}$) (Chien, Cabana, and Mares 1982).

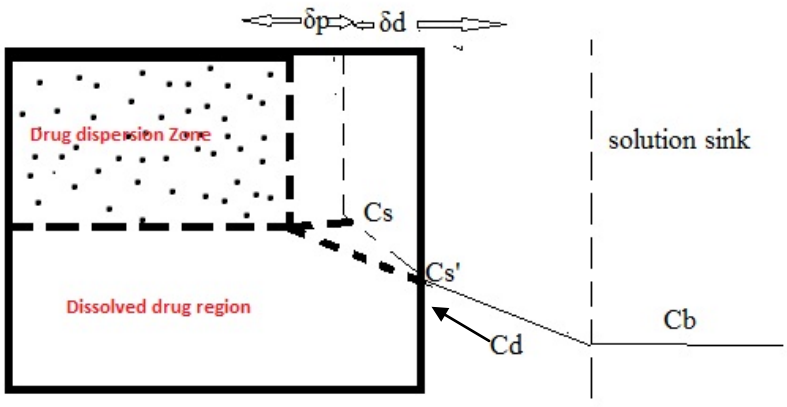



*Fig 2.3: Schematic illustration of controlled release of drug molecules from a monolithic drug delivery system .Here Cs is the solubility of drug in the matrix; Cs'is the concentration of drug at the surface /solution interface;Cd is the concentration at solution/surface interface and Cb is the concentration in the bulk of elution solution.*

## *2.4 MATERIALS USED AS LOCAL DRUG DELIVERY DEVICES FOR BONE DISEASES.*

### 2.4.1 Drug Delivery Devices for the control of osteomyelitis

Drug delivery devices developed for local delivery of antibiotics for bone diseases can be divided into non- biodegradable and biodegradable devices.

**a) Non Biodegradable Devices**

Poly(methyl methacrylate) (PMMA) beads, first clinically applied in 1972, have been the gold standard for the local delivery of antibiotics to bone cavities which is a non biodegradable carrier, their major drawback is the additional surgery required to remove the beads after therapy (Kluin et al. 2013).

The antibiotic loaded PMMA are usually applied as spacers or strings of beads. This can enable for higher local antibiotic concentrations in the infected site while maintaining a low concentration in serum and urine minimizing adverse effects.

PMMA has a compact matrix that is capable of up taking small quantities of dissolution fluid into its outermost layers. In the first hours or days a burst release is observed, as the antibiotic at the cement surface is easily available. More gradually, the dissolution fluid penetrates the bone cement to dissolve antibiotics within superficial regions of the bone cement. It is unable to release drug from the interior of the cement. Often, the total amount of antibiotic released from bone cement is only 10% of the total amount incorporated (Kluin et al. 2013).

Commercially available antibiotic-loaded PMMA for treatment purposes has better release kinetics than hand-mixed preparations due to a more uniform antibiotic distribution in the PMMA matrix (Anagnostakos and Schröder 2012). Antibiotic-loaded bone cements for clinical use are prepared, ensuring prolonged release from both superficial and deeper layers within the PMMA matrix. This is developed by increasing the cement porosity by the incorporation of soluble glycin in beads, changing its composition or increasing the antibiotic loading (Bistolfi et al. 2011). Despite their modified release

kinetics, it has been found that even it did not release all of their antibiotic content and after 2 weeks in situ, only 20 - 70% of all incorporated gentamicin was released, followed by a decrease in gentamicin concentration. Thus increasing porosity and antibiotic loading of PMMA carriers increased the burst release as surface PMMA-based antibiotic carriers are frequently used for the treatment of osteomyelitis. These beads are routinely used against osteomyelitis without understanding the infecting bacterial strains and their sensitivity for antibiotics ). Growth of gentamicin-resistant staphylococci on retrieved gentamicin loaded PMMA beads has been demonstrated, raising question about the efficacy of this treatment method (Kluin et al. 2013).

**b) Biodegradable drug delivery devices.**

The disadvantages of the non-biodegradable antibiotic-loaded system paved way for the development of biodegradable drug delivery devices for the treatment of osteomyelitis.

Idea of biodegradable local drug delivery device is that, it should deliver antibiotics at appropriate levels and lengths of time based on application, and then degrade thereby obviating the need for a second surgical procedure for the carrier removal. Use of a biodegradable bone substitute as an antibiotic carrier is a suitable choice which reduces systemic side effects, and shortens the period of treatment.

These materials cover up dead space originating from surgical debridement and release their entire antibiotic load after degradation, leaving no substratum for bacterial colonization. Biodegradable devices have been classified into three groups: natural polymers, inorganic osteoconductive materials, and synthetic degradable polymers.

Like non-degradable devices, antibiotic release from degradable devices is affected by surface area and diffusion through the carrier matrix, and is additionally influenced by factors like mode and rate of degradation, swelling, creation of pores and osmotic/pH variations. This causes different release mechanisms operating at the same time.

### 2.4.2 Osteoconductive Materials as Drug Delivery Devices

Incorporation of antibiotics within osteoconductive biomaterials (calcium sulfate, calcium phosphate and hydroxyapatite or tricalcium phosphate) has been proposed for local treatment of osteomyelitis and to aid bone void management (Kawanabe et al, 1998; Makinen et al, 2005; Nelson et al, 2005). All these implants show a rapid release of the antibiotic in a controlled manner, more or less (McLaren, 2004). One of the advantages of this class of materials is that it provides the opportunity to release local antibiotics at high concentrations and eventually induce the bone regeneration process during the time period of material degradation.

Bone grafting in chronic osteomyelitis is sometimes required after removal of large amounts of necrotized bone, in situation of infected prostheses or large bone defects in infected trauma wounds. In these cases, the defect can be filled with bone graft material loaded with antibiotics (Beardmore et al. 2005). Common bone graft materials are autologous bone, allografts and bone graft substitutes.

Hydroxyapetite (HA) is a biocompatible ceramic in bone mineral composition, used widely as synthetic bone graft due to its osteoconductive nature. Bioactive glass (which combines silica, sodium oxide, calcium oxide and phosphates) is a man-made material which has osteoinductive property. This class of biomaterial gives the additional advantage of tuning the mechanical and biological properties and resorption characteristics according to need. Therefore bioactive glass is preferred for several specific bone graft applications (Schlickewei, Yarar, and Rueger 2014)

## *2.5 CALCIUM SULFATE AS DRUG DELIVERY MATERIAL*

One of the most successful bone graft substitutes used as a degradable carrier is calcium sulfate dihydrate (CSD of Gypsum mineral) used since 1892 (Tsourvakas 2012). The hemihydrate form ($CaSO_4 \cdot 0.5H_2O$), better known as plaster of Paris, are used that are stronger and harder, making the material

suitable as a bone void filler. Calcium sulfate can be loaded with water-soluble antibiotics, and hence the use of aminoglycosides, vancomycin, daptomycin and teicoplanin has been widely explored (Wichelhaus et al. 2001). Antibiotic-loaded calcium sulfate generally tends to release its content at an uncontrollable rate. Overall, approximately 45 - 80% of the antibiotic content is released within the first 24h (Aiken et al. 2015).

Gypsum consists of calcium sulfate dihydrate ($CaSO_4 \cdot 2H_2O$) or CSD, when heated to $1108^0$C, calcination process loses water and gets converted to calcium sulfate hemihydrate,:

$$\mathbf{2CaSO_4 \cdot 2H_2O \rightarrow (CaSO_4)_2 \cdot H_2O + 3H_2O}$$

Calcium sulfate hemihydrate exists in two forms, α and β, which differ in crystal size, surface area, and lattice with different physical properties. The α-hemihydrate form is hard and relatively insoluble when compared with the β-hemihydrate (Thomas and Puleo 2009).

When the β-hemihydrate (Plaster of Paris) is mixed with water, CSD is formed in a mild exothermic reaction:

$$\mathbf{(CaSO_4)_2 \cdot H_2O + 3H_2O \rightarrow 2CaSO_4 \cdot 2H_2O + Heat}$$

As the hemihydrate dissolves, a two-phase suspension of hemihydrate particles in saturated aqueous solution is formed. When the solution becomes supersaturated with dihydrate, crystals nucleate in the suspension and form a precipitate. Nucleation and crystal growth continue until the solution is under-saturated, leading to further dissolution of the hemihydrate. Alternating dissolution and precipitation continues, with growth of existing crystals or nucleation of new crystals (Anusavice 2003).

Dreesman in 1892 first implanted calcium sulfate (Plaster of Paris cement) in humans as a void filler of tubercolous osteomyelitis (Campana et al. 2014). Calcium sulfate is a biocompatible, biodegradable material with osteoconductive properties which makes it a good bone substitute. In different studies, calcium sulfate showed high resorption rates and good biocompatibility (Kelly et al. 2001; Klemm 2001).

Calcium sulfate has been used as an antibiotic carrier, which exhibits a burst release followed by an extended drug release from several hours to weeks depending on the formulation, thereby verifying its clinical effectiveness and its reliability as an antibiotic carrier (Cornell et al. 1993). $CaSO_4$ has been used to deliver vancomycin, gentamycin, tobramycin, lincomycin, cefazolin, teicoplanin, and fucidin (Parker et al. 2011).

In the present study we selected a new calcium sulfate having low-dimensional uniform crystals made out of a patented chemical process. This material is medical grade and proved to be osteoconductive and resorbing in animal experiments.

The uniform low dimensional (3-5μm) crystals offer higher surface area and uniform porosity and it is expected to have a better drug release characteristics compared to conventional materials.

# 3. EXPERMENTAL

## *3.1 PREPARATION AND CHARACTERIZATION*

### 3.1.1 Preparation of Calcium Sulfate Cement

In order to prepare the powder, calcium sulfate dihydrate was milled and then heated at $125^0$C up to 6 hrs to get converted into calcium sulfate hemihydrate and it was sieved to get a particle size below $125\mu$.

The cement was prepared by wetting calcium sulfate hemihydrate powder with water in a specific ratio. The preparation was done at room temperature ($27^0$C) by adding the wetting medium drop-wise from a syringe and kneading thoroughly and uniformly using a spatula. The cement paste was then molded into discs with diameter and thickness according to need. The wetting ratio was kept as 0.5 ml/g throughout the samples so has to have the appropriate consistency and to maintain a constant drug concentration for the elution study.

Gentamicin loaded cement was prepared by adding gentamicin sulfate solution as the wetting medium.

### 3.1.2 Characterization

The prepared calcium sulfate cement was characterized using following techniques.

**(i) X-ray powder diffraction**

Phase of the set cement was determined using X-ray diffraction analysis (Bruker D8 Advance X-Ray diffractometer). X-ray powder diffraction is a non-destructive analytical technique that is based on the observation that the scattered intensity of a monochromatic X-ray beam hitting a sample varies as a function of incident and scattered angle $2\theta$. For crystalline materials, the positions of the intensity peaks are described by Bragg's law,

$$\mathbf{n\lambda = 2d\sin\theta}$$

Where λ is the incident radiation wavelength, *n* is a positive integer and *d* the interplanar distance between consecutive lattice planes in the crystal structure, generating constructive interference of the reflected radiation. Powder diffraction data was compared by matching the data to a database maintained by the International Center for Diffraction Data (ICDD).

**(ii) Fourier Transform Infrared Spectroscopy**

The powdered cement was pressed into pellets after mixing with potassium bromide [1 wt % of KBr] in a uni-axial press (SPEX-3630 X-PRESS) at a pressure of 3 tons with 1 minute holding time and 1 minute releasing time. Potassium bromide [KBr] reference pellets were also made. The FTIR spectrum of each sample pellet was recorded in the absorbance mode in Thermo-Nicolet 5700 FTIR spectrophotometer. Data were collected in the range 500 $cm^{-1}$ to 4000 $cm^{-1}$ at a resolution of 4 $cm^{-1}$ (64 scans) after subtracting the background (64 scans in the reference pellet). Phase conversion was confirmed by comparing the peaks with that of standard CSD peaks.

**(iii) Scanning Electron Microscopy**

The micro morphological analysis of the samples was done using SEM. This technique is based on the interaction of the specimen surface with the high-energy incident electron beam which reveals the surface microstructure of the specimen. The microstructures were studied using 30 kV Environmental Scanning Electron Microscope (ESEM-Quanta 200, Germany).

### *3.2 DRUG ELUTION FROM CALCIUM SULFATE CEMENT*

The elution studies from the cement were carried out to understand the release mechanisms and the factors that affecting the release of drugs from the cement.

**3.2.1 Spectrophotometric Method**: The gentamicin release study was conducted using UV visible spectrophotometer (Shimadzu-SPD-20A, Japan).

Gentamicin analysis was done by following Sampath and Robinson's (1990) procedure. o-Phthaldialdehyde reagent was formulated by adding 0.5g o-phthaldialdehyde, 12.5 ml methanol and 0.6 ml 2-mercaptalmethanol to 112 ml 0.04 M sodium borate in distilled water solution. The reagent was stored in a brown bottle in a dark chamber and settled for at least 24h before use. One milliliter gentamicin solution, 5ml ***iso***propanol and 1 ml o-phthaldialdehyde reagent were reacted for 45 min at room temperature (24°C). The absorbance, which corresponds to the gentamicin concentration, was measured at 333nm (Zhang et al. 1994).

### 3.2.2 Determination of the Elution characteristic

The general elution characteristics of the drug loaded cement were studied using *in vitro* drug release. Exactly 2g of cement was mixed with 1 ml gentamicin sulfate solution (Genticyn, Abbot, Mumbai) which contain 40g of gentamicin. The drug loaded cement was molded in Teflon moulds into disc with 30mm diameter. Each set was placed in 5ml phosphate buffer solution (PBS) (pH 7.2) in a static condition at room temperature ($27^0$C). Entire volumes of PBS were replaced with fresh PBS after every 24 hour time interval. Samples were stored in refrigerator until analysis using UV as mentioned above. The experiments were done in triplicate.

### 3.2.3 Elution characteristics variation with respect to surface area

In actual clinical application, the cement will be custom-shaped as beads and used at the infected site. The area-to-volume ratio is the deciding factor in drug release rate (P N Raju 2010). In the present study, the samples are made into regular geometric shape so as to understand the relation of release rate and surface area. Samples were made into discs of equal volume and only certain areas were exposed, by masking the rest so as to get different area-to-volume ratio. This disc geometry with masking enables to load uniform drug concentration provides wide range of area to volume ratio to experiment with and also keep other parameters (porosity and tortuosity) affecting the release rate a constant.

Cement loaded with 20mg/g gentamicin was molded into 15mm diameter discs. Antibiotic loaded pellets were masked with cyanoacrylate in such a way that specific circular surface area of the pellet gets exposed to the solution. The areas and the surface area/volume ratio of different samples are given in Table 1.The *in vitro* elution studies were conducted same way as mentioned above.

*Table 3.1: Pellets made with different surface area by volume ratio*

| | Samples | Exposed surface area ($cm^2$) | Surface area/volume ($cm^2/cm^3$) |
|---|---|---|---|
| 1 | $A_1$ | 0.2 | 0.013 |
| 2 | $A_2$ | 0.79 | 0.053 |
| 3 | $A_3$ | 1.77 | 0.119 |
| 4 | $A_4$ | 3.54 | 0.24 |

### 3.2.4 Effect of Chitosan on elution characteristic

It is already known that additives to drug delivery systems alter the delivery characteristics. Chitosan is one such material which is proven to be biocompatible and implantable in hard tissue sites (Parker et al. 2011). Therefore it is incorporated in to the cement along with antibiotic with varying concentration and its effects on the release were studied.

Chitosan was prepared in 0.5%, 0.25%, 0.125% and 0.06%. Cement powder was mixed with 0.5ml chitosan solution containing the pre-determined quantity of chitosan and 0.5ml gentamicin sulfate solution which contain 20mg gentamicin. Discs with diameter 30 mm weighing 2.3g were made by mixing the chitosan antibiotic solution as the wetting medium with the cement powder. *In vitro* elution of the samples was carried out.

# 4. RESULT AND DISCUSSION

## *4.1 MATERIAL CHARACTERIZATION*

### 4.1.1 X-ray Powder Diffraction

X-Ray Diffractometry (XRD) was done on the sample using Siemens D5005 Diffractometer with Cu Kα radiation generated at a voltage of 40kV and a current 30mA. The spectra are taken in the 2-theta range of 10-60 º at a rate of 2º/min with 0.02º step size. The diffraction data is compared with the standard ICDD data cards to identify the phases present. The XRD of the dried cement powder is shown in figure 4.1. All the major peaks match with the reported standard peaks of calcium sulfate dihydrate (ICDD 21-0816).

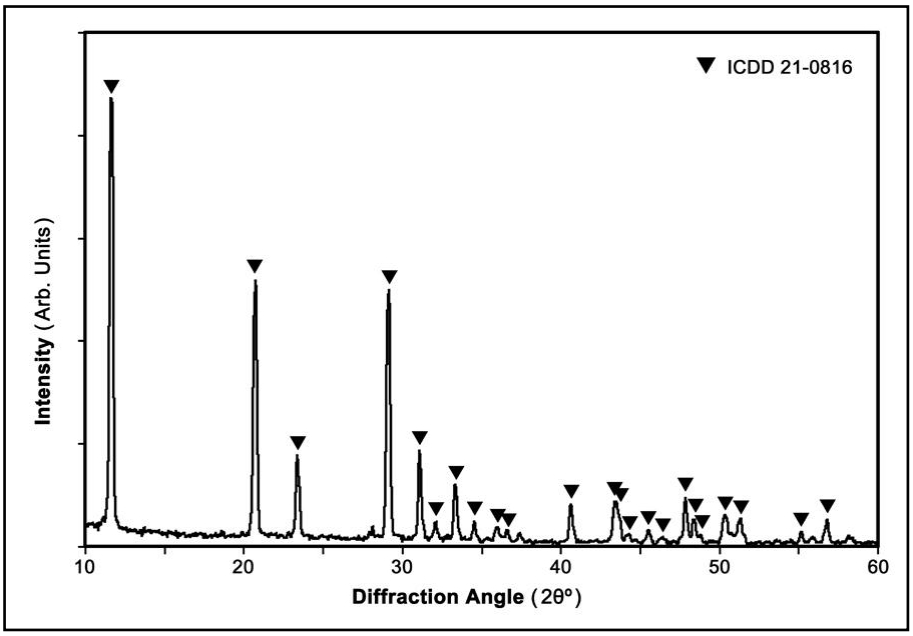



*Fig 4.1 X-Ray diffraction spectrum of the cement. Peak positions of standard calcium sulfate dihydrate (ICDD 21-0816) are marked.*

### 4.1.2 Fourier Transform Infrared Spectroscopy

The Fourier Transform Infrared absorption analysis (FTIR) of the dried cement powder is done using Thermo-Nicolet 5700 spectrometer. The dried powder is thoroughly mixed with spectroscopic grade KBr powder and made in the form of thin transparent pellets. Spectrum is recorded as a superimposition

of 64 scans in the range from 4000 cm-1 to 400 cm-1 at resolution of 4 cm-1. Pellet of KBr alone is used to record the background spectra.

The FTIR spectrum of the dried cement powder (figure 4.2) contains all the typical absorptions of calcium sulfate dihydrate. The band in between 1100-1300 cm-1 (with peak at 1120 cm-1) corresponds to S-O stretch. The peaks at 463, 601 and 669 cm-1 correspond to $SO_4$ anti symmetric bend. Overtones of $SO_4$ ($2\upsilon_3$) are present as shallow absorptions around 2238 and 2116 $cm^{-1}$. The $\upsilon_1$ bending mode of $H_2O$ gives a peak at 3406 $cm^{-1}$ and the $\upsilon_3$ mode could be seen at 3492 and 3550 $cm^{-1}$. The peak at 3243 $cm^{-1}$ is additional $\upsilon_2$ bend of $H_2O$. The deformation vibrations of the OH groups manifest as doublet at 1621 and 1686 $cm^{-1}$.

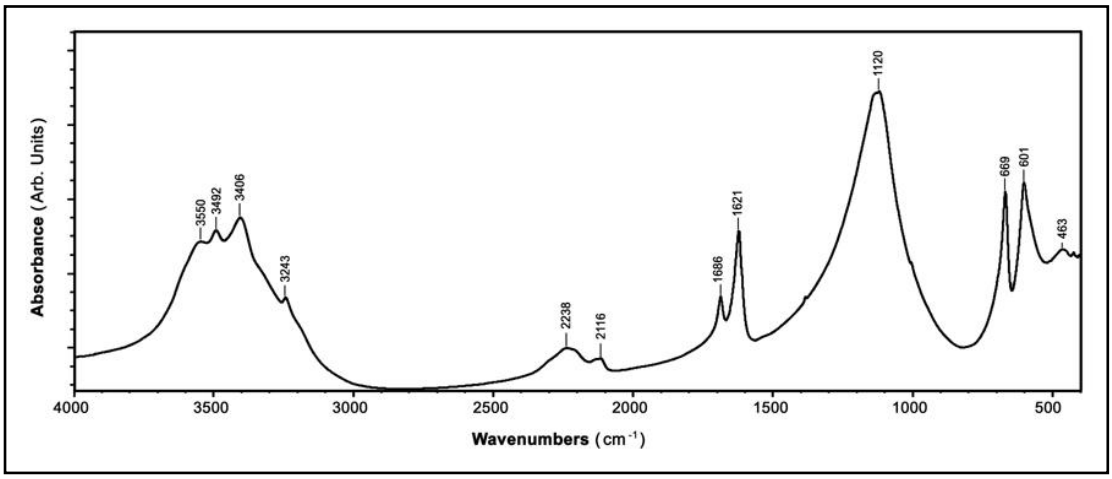



*Fig 4.2 FTIR spectrum of the cement sample.*

The phase analysis done using XRD and FTIR shows that the material is pure phase of CSD, which has undergone complete conversion during setting. This material is implantable grade bone graft material as per earlier report (Sony et al. 2015).

## *4.2 ELUTION STUDY*

### 4.2.1 Elution Characteristic Analysis

The release rate from the cement was plotted as a function of time as shown in Figure 4.3. The elution profile from the material shows a prominent initial burst release followed by a sustained release. The initial burst lasts for less than 72 hours and represents the cumulative release around 60% of the drug.

The initial burst release fits with the Higuchi model where, the amount of drug released exhibits a linear relationship against the square root of time (Figure 4.4). After the initial burst, release of drug shows a late time approximation (corresponding to Eq.4) with exponential release (figure 4.5) (Anderson, Rosenholm, and Linden 2008).

In this analysis, the release profile of the burst region (within 60% release) fits linearly into square root of time graph, which indicates the diffusion to be rate limiting. After the bust region, the elution is exponential as evident from the result, which again matches with the approximated theoretical

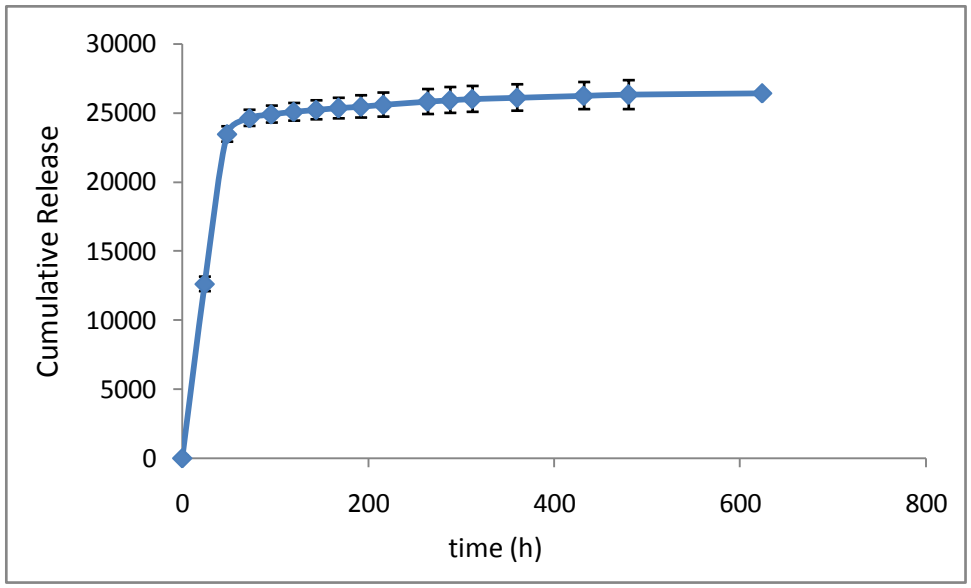



*Fig 4.3: Elution profile of gentamicin from cement pellet*

equation (Eq. 4). This proves that the release of the drug from the cement matrix is diffusion controlled.

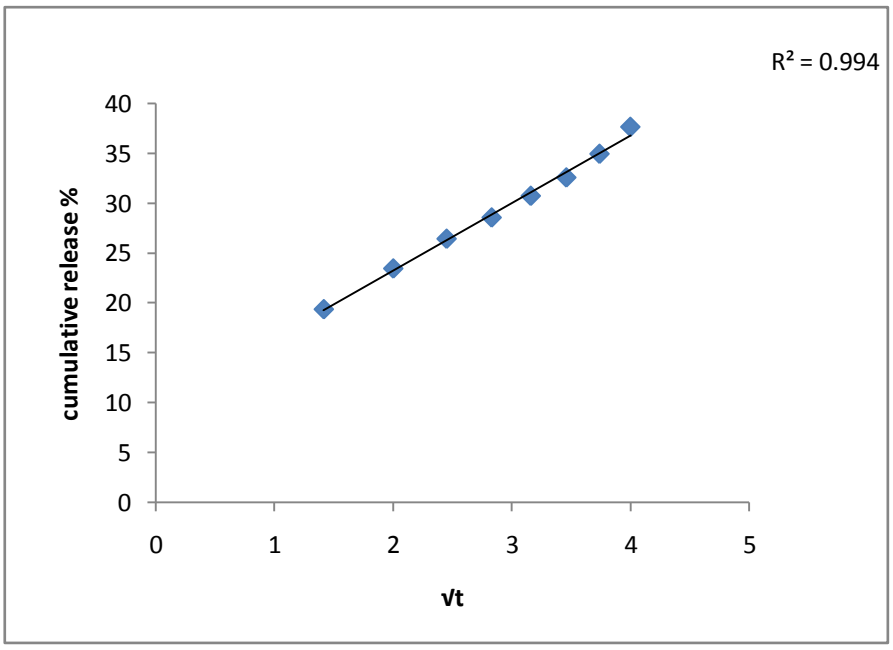



*Fig4.4: Higuchi Square root time plot for initial burst release.*

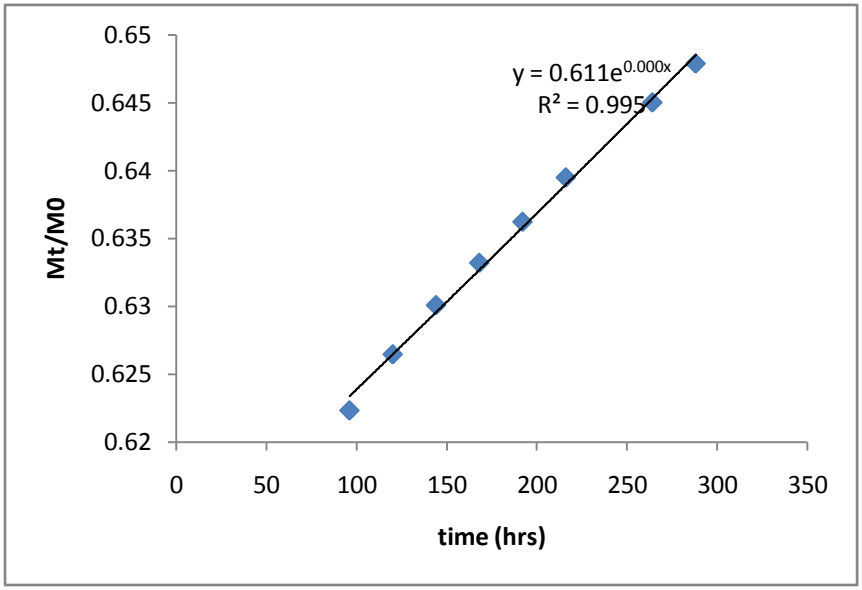



*Fig 4.5: Material showing exponential release after the initial burst*

### 4.2.2 Internal Micro morphology in Scanning Electron Microscopy

The present elution study shows a general decrease in the release rate compared to the release rates reported for other cements in similar cases of drug elution (Parker et al. 2011). In those reports, the initial burst occurred within 24 hrs. This variation indicates that certain micro-morphological features in the cement may be influencing the release. To investigate this possibility, the cement pellet containing drug has been subjected to SEM study after cracking across the thickness. The images of the cross section of the cement are shown in figure 4.6 A & B. In the inner part (figure 4.6A), uniform crystals of 2-3 micron crystals are seen evenly distributed, with porosities in between. The outer surface of the set cement (figure 4.6B) shows a dense structure throughout. This layer with very less porosity may be impeding the fast release of the drug.

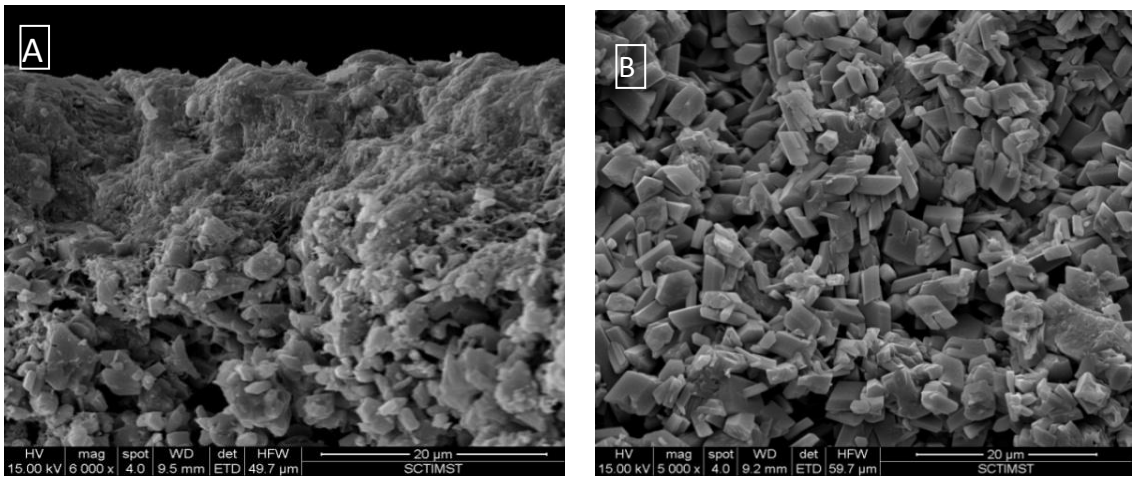



*Fig 4.6 ESEM image of cross section of the set calcium sulfate cement A) near the top surface B) inside the pellet.*

### *4.3 SURFACE AREA AND RELEASE KINETICS*

The previous section on the release kinetics provides clear evidence that the drug release is diffusion controlled and follows the diffusion dependent mathematical model. In the related theory, surface area of the drug delivery medium is a crucial parameter influencing the release (Eq.6). Therefore, it is relevant to explore the nature of release from different surface areas by keeping the substrate size the same and masking the rest of the area.

The elution profiles (figure 4.7) of the same quantity of drug from different exposed areas (as specified Table 3.1) show a significant change in their release kinetics. When the release area is restricted, the burst release is affected and gets prolonged. The less the available area the slower is the release rate. Slowly it approaches zero-order kinetics (Q inversely proportional to 't'as described by Chien, 1982

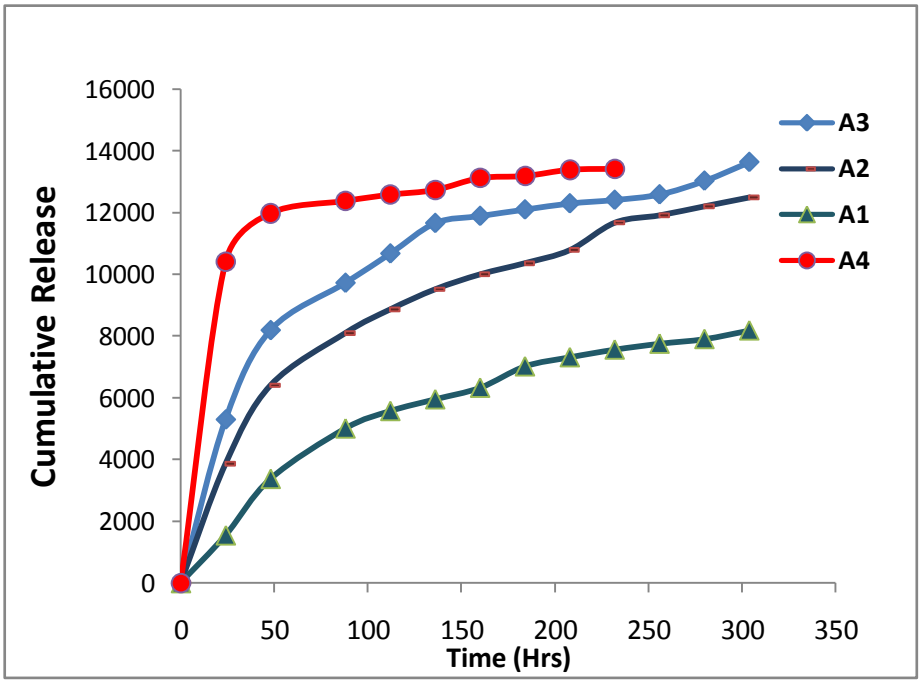



*Fig 4.7: Change in release kinetics with varying surface area*

The dependence of the release rate (Q/t) to the exposed area is plotted separately (figure 4.8a). It shows a linear relationship, indicating that the nature of drug elution could be brought to zero order just by reducing the area, in a constant volume. The system tends to reservoir-type release system.

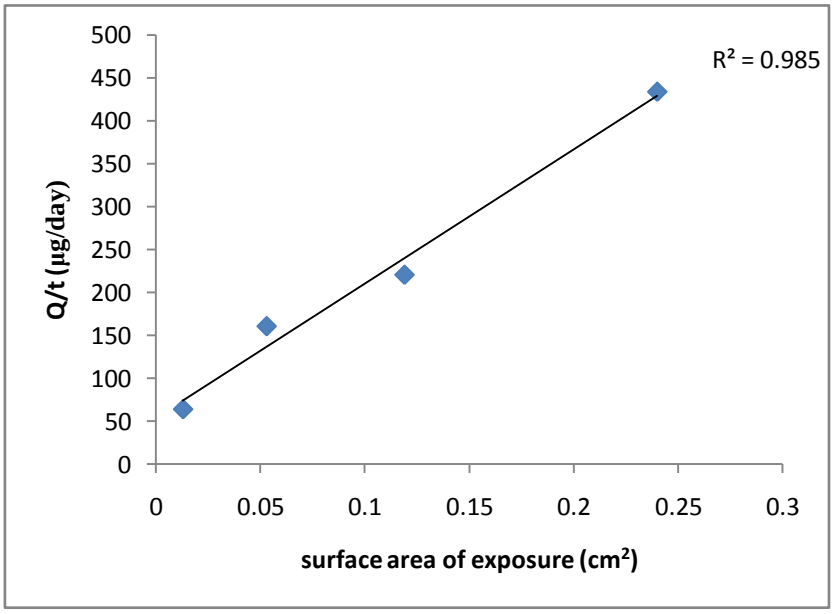



*Fig 4.8 a) Dependence of the release rate (Q/t) on area of exposure*

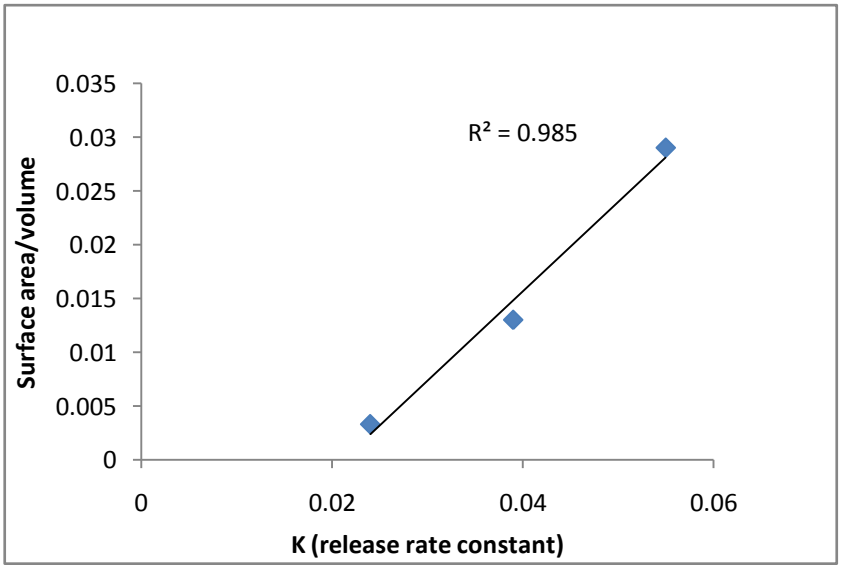



*Fig. 4.8: b) Change in release rate constant with respect to surface area by volume of the pellet*

The data is converted to area/volume ratio versus release rate constant (figure 4.8b) to identify the rate parameter relation of total drug content getting released through the exposed area. The linear regression coefficient ($R^2$ ) is 0.985 (for both) which is rather a high value, indicating a strong relation between area and drug release kinetics.

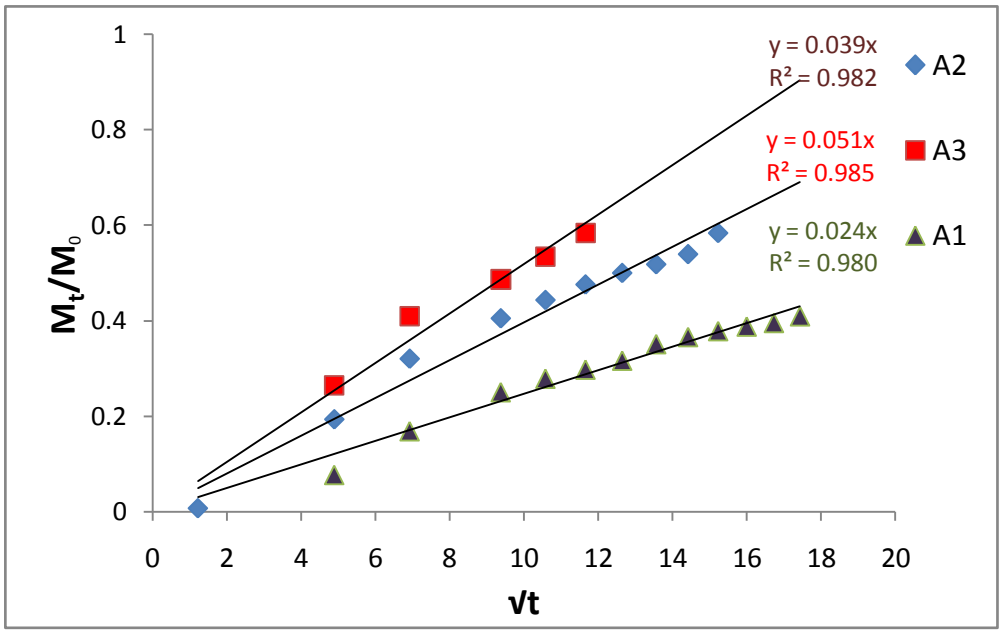



*Fig 4. 9: Square root of time relation with release from different area*

| **Area** | **Slope** | **Diffusivity ($cm^2$/min)** |
|---|---|---|
| A3 | 0.051 | 0.005403 |
| A2 | 0.039 | 0.003159 |
| A1 | 0.029 | 0.001747 |

*Table 4.1: Dependence of surface area on diffusivity of the drug*

The square root time relation with different area is further explored (figure 4.9) to find the rate controlling parameter corresponding to Higuchi relation. The graph shows a linear pattern with high linear regression coefficients. The diffusion coefficient of the drug were calculated using Equation (3) (Crank, 1975) and were obtained from a linear regression of $Mt/M_0$ versus $t^{0.5}$.

The change in the slope of the graph reveals that there is a change in diffusion coefficient corresponding to each area (table 4.1). Since all other parameters (including porosity and tortuosity) were assumed to be constants, the change in release area should affect only the release rate. The area variation should not affect the diffusion coefficient, by theory. This anomaly observed in the data could be explained by correlating to the diffusion layer formed on the immediate surface of the device. The change in the diffusion layer thickness formed on the immediate surface of the device may affect the diffusion coefficient. The thickness of the diffusion layer is smaller than that of the available surface area of elution. On reducing the area, lesser quantity of the drug will be released. Consequently, the thickness of the diffusion layer reduces, thereby reducing the drug depletion layer thickness. Then a condition occurs when the δp becomes proportional to time and a ‘Q versus t’ pattern of drug release is observed.

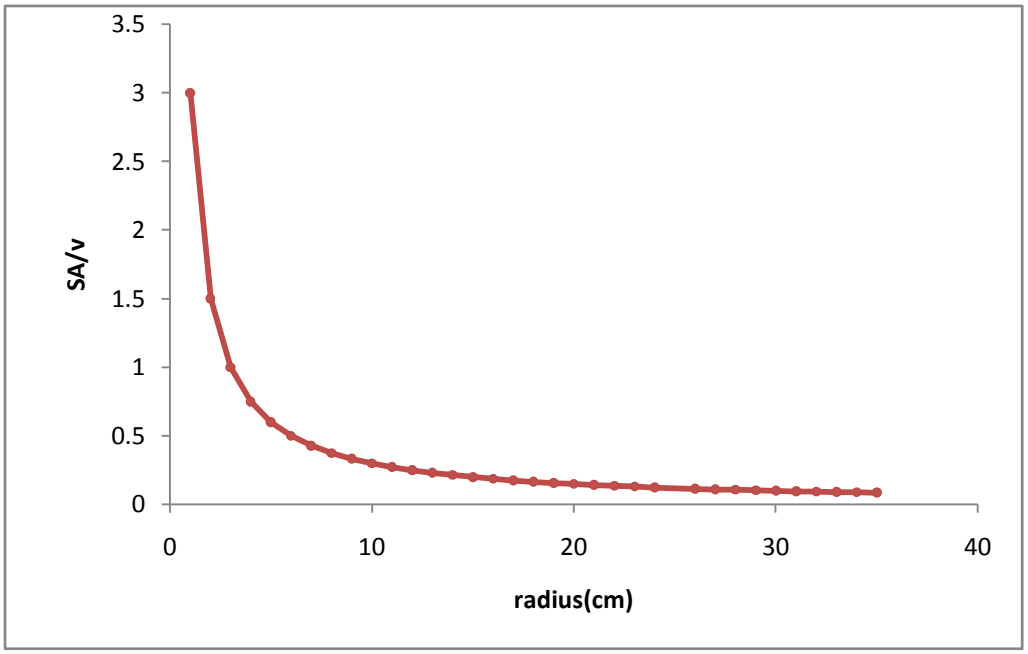



*Fig 4.10: Theoretical relation between surface area to volume ratio with the radius of a sphere*

Understanding this mechanism enables to design a drug delivery device with suitable geometry to reduce the burst release.

This geometrical relation could be extended to beads (spherical shape) as this is the shape applicable clinical situation. Generally, it is easier to make beads of the cement in a surgery setting and the release parameters of beads have more relevance in actual application. An attempt is done to translate the release characteristics corresponding to the area to volume ratio obtained in the present study into that of a sphere. Figure 4.10 shows the theoretical relation between surface area to volume ratio and the corresponding radius of a sphere.

The release rates corresponding to the area to volume ratio of the cement pellets is compared with the respective radius of the sphere shape (having the same area to volume ratio) and plotted in Figure 4.11.

A logarithmic plot is obtained, indicating that the release rate from the sphere drastically changes at lower radius values. At higher radius, the release

rates are slower and controllable, but the radius values are impractically high (50cm to 250 cm). From the study the predictable release rate from practically possible radius (0.5 -5 cm) is in between 500 – 400 µg/day.

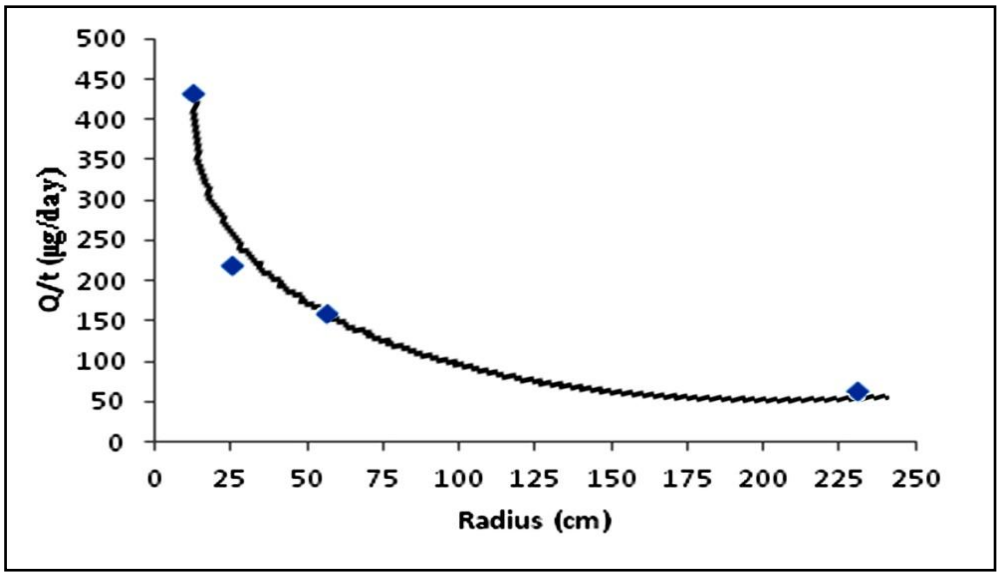
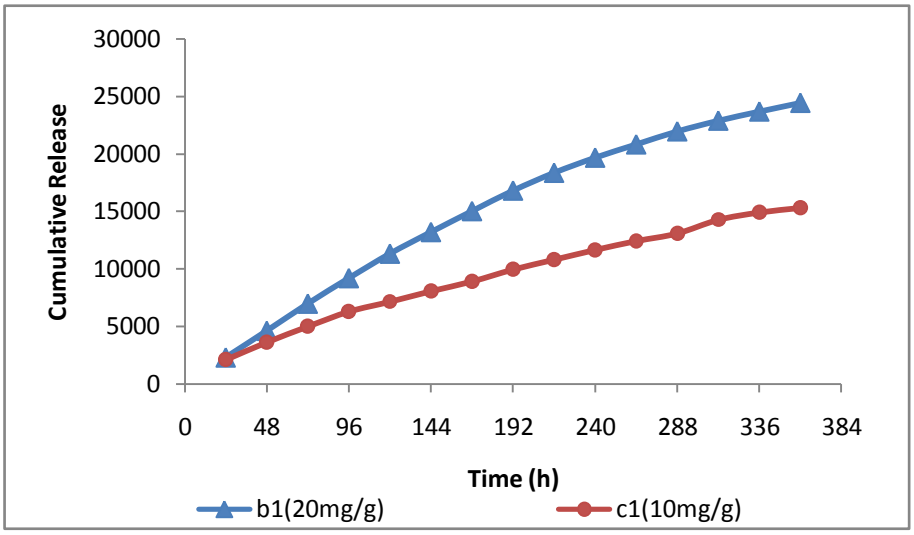



*Fig 4.11: Relation between release rate and radius of sphere.*

The release kinetics from specific area with varying concentration was studied to understand the effect of concentration on drug release. The release patterns (fig 4.12) with different concentration show no significant variation in the release rate. However, the ultimate cumulative value is affected by the concentration. The effect of change in surface area was prominent in the release plots.

*a)*




*b)*

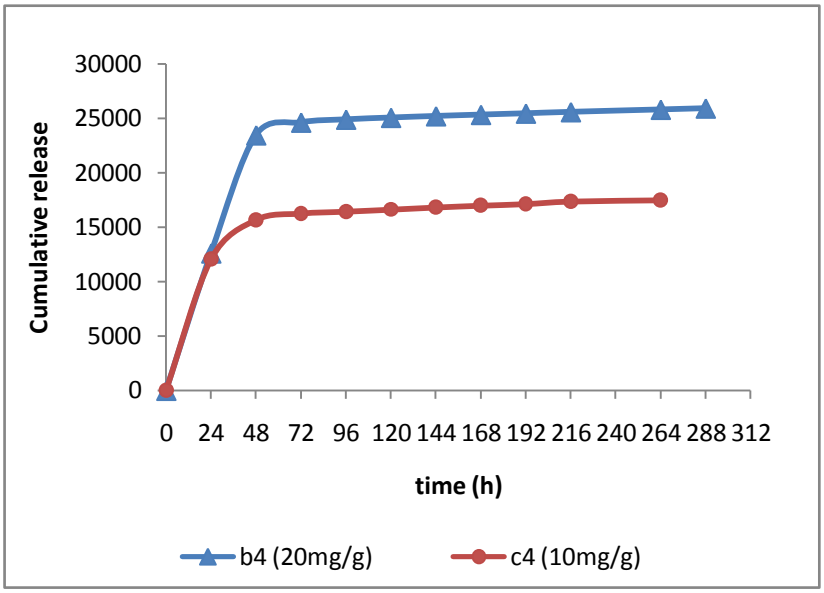



*Fig 4.12 a)&b): Elution from two specific exposed surface areas with different concentration of drug*

### *4.4 EFFECT OF ADDITIVES*

The elution characteristics of cement with chitosan as an additive show no significant variation in the release kinetics n the present study (figure 4.13). The addition chitosan is done (along with antibiotic) with an assumption that the drug molecules will bind with chitosan and lower the release rate. The result shows that the elution characteristic is similar to that of cement pellet without chitosan. The amount of chitosan that can be added directly to the cement is limited since it can alter the setting time of cement. Therefore no further experiments were carried out by increasing the concentration of chitosan.

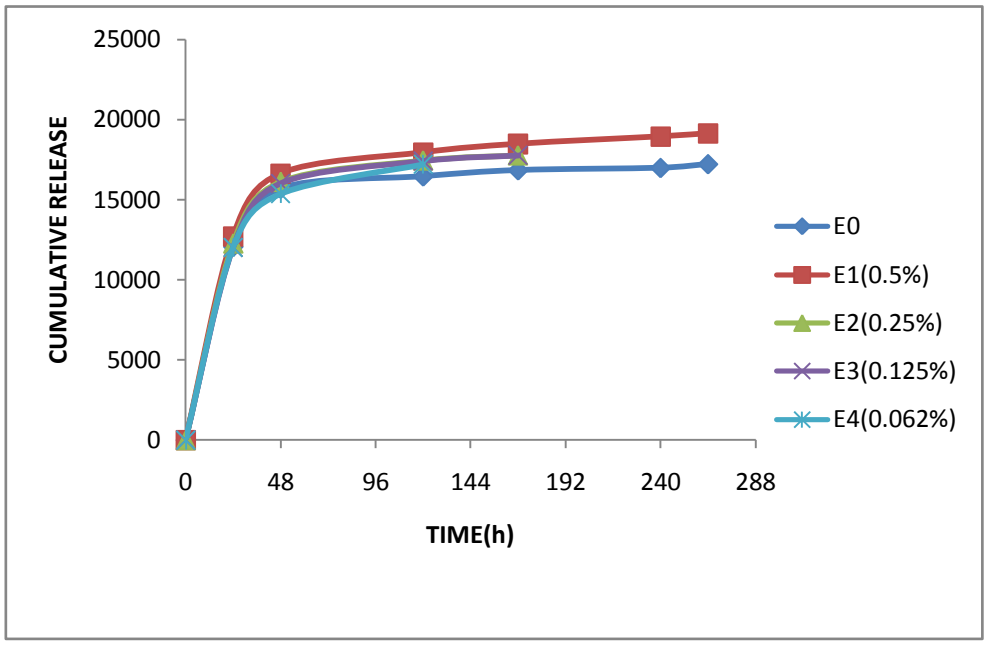



*Fig 4.13: Elution characteristics of gentamicin from pellets with varying chitosan concentration.*

## 5. CONCLUSION

The present study investigated the performance of new calcium sulfate based bone filler cement for drug elution application in osteomyelitis. It revealed the essential features of gentamicin elution from the cement mass which could be helpful in correlating with clinical needs.

The outcome of the study reveals the elution characteristic of gentamicin from the bioactive calcium sulfate cement, *in vitro*. Mathematical models of elution kinetics have been used to analyze the experimental data of elution obtained through spectroscopic estimation. The material showed a diffusion controlled release mechanism. From the model, the parameters that could control the diffusion rate were obtained. The standard quantity of the drug loaded cement gave a burst release of about 72 hours followed by sustained release above the minimum inhibitory concentration for more than 20 days.

The study done by masking the area of a fixed volume cement pellet, helped to elucidate the relation of surface area on release rate. This helped in predicting the elution rate for a given geometry. It was possible to translate this information to understand the release rate from a spherical geometry of the cement. The spherical beads are more convenient geometry to be used for clinical application of drug delivery.

The observed release rate from the cement makes it a good candidate for drug delivery in the case of bone diseases compared to the data of other osteoconductive materials. The study also indicates that the clinically convenient sizes of drug loaded spheres (up to 1 cm in diameter) or beads provide a faster release than sustained behavior. This will be particularly useful in the acute management of osteomyelitis. In order to achieve sustained release, the geometry of the drug eluting mass is to be altered to obtain lower area-to-volume ratio.

The use of binders like chitosan in the cement shows no significant change in the release kinetics of the loaded drug and it does not serve the intended purpose in the present case.

The understanding of the release mechanism shows that reducing the area of exposure can slow down the release rate. Theoretically, for a slow release system, the cement beads could be coated with a biocompatible gel so as to expose a small area in order to obtain a near-zero-order release.

This work could be taken forward by continuing studies to confirm the elution analytically and assess the inhibitory concentrations microbiologically. The study in whole has been conducted by measuring the UV absorbance, after derivatizing the drug molecule. This may introduce errors in estimation. HPLC is the more accurate than UV analysis, so the elution study has to be done further using HPLC. To check actual effective concentration of drug, microbiological detection of minimum inhibitory concentration is to be done.

## REFERENCES


Aiken, Sean S., John J. Cooper, Hannah Florance, Matthew T. Robinson, and Stephen Michell. 2015. "Local Release of Antibiotics for Surgical Site Infection Management Using High-Purity Calcium Sulfate: An In Vitro Elution Study." *Surgical Infections* 16 (1): 54–61. doi:10.1089/sur.2013.162.

Anagnostakos, Konstantinos, and Katrin Schröder. 2012. "Antibiotic-Impregnated Bone Grafts in Orthopaedic and Trauma Surgery: A Systematic Review of the Literature." *International Journal of Biomaterials* 2012. doi:10.1155/2012/538061.

Anderson, J., J. Rosenholm, and M. Linden. 2008. "Mesoporous Silica: An Alternative Diffusion Controlled Drug Delivery System." *Topics in Multifunctional Biomaterials and Devices*.

Anusavice, Kenneth J. 2003. *Phillips' Science of Dental Materials*. Elsevier Health Sciences.

Beardmore, Anthony A., Daniel E. Brooks, Joseph C. Wenke, and Darryl B. Thomas. 2005. "Effectiveness of Local Antibiotic Delivery with an Osteoinductive and Osteoconductive Bone-Graft Substitute." *The Journal of Bone and Joint Surgery. American Volume* 87 (1): 107–12. doi:10.2106/JBJS.C.01670.

Bistolfi, Alessandro, Giuseppe Massazza, Enrica Verné, Alessandro Massè, Davide Deledda, Sara Ferraris, Marta Miola, Fabrizio Galetto, and Maurizio Crova. 2011. "Antibiotic-Loaded Cement in Orthopedic Surgery: A Review." *ISRN Orthopedics* 2011 (August). doi:10.5402/2011/290851.

Calhoun, Jason H., M.M. Manring, and Mark Shirtliff. 2009. "Osteomyelitis of the Long Bones." *Seminars in Plastic Surgery* 23 (2): 59–72. doi:10.1055/s-0029-1214158.

Campana, V., G. Milano, E. Pagano, M. Barba, C. Cicione, G. Salonna, W. Lattanzi, and G. Logroscino. 2014. "Bone Substitutes in Orthopaedic Surgery: From Basic Science to Clinical Practice." *Journal of Materials Science. Materials in Medicine* 25 (10): 2445–61. doi:10.1007/s10856-014-5240-2.

Chien, Yie W., Bernard E. Cabana, and Stanley E. Mares. 1982. *Novel Drug Delivery Systems: Fundamentals, Developmental Concepts, Biomedical Assessments*. M. Dekker.

Cornell, C. N., D. Tyndall, S. Waller, J. M. Lane, and B. D. Brause. 1993. "Treatment of Experimental Osteomyelitis with Antibiotic-Impregnated Bone Graft Substitute." *Journal of Orthopaedic Research: Official Publication of the Orthopaedic Research Society* 11 (5): 619–26. doi:10.1002/jor.1100110502.

Crank, John. 1980. *The Mathematics of Diffusion*. 2nd Revised ed. edition. Oxford Eng: Oxford University Press.

Emslie, K. R., N. R. Ozanne, and S. M. Nade. 1983. "Acute Haematogenous Osteomyelitis: An Experimental Model." *The Journal of Pathology* 141 (2): 157–67. doi:10.1002/path.1711410206.

Frenning, Göran. 2011. "Modelling Drug Release from Inert Matrix Systems: From Moving-Boundary to Continuous-Field Descriptions." *International Journal of Pharmaceutics* 418 (1): 88–99. doi:10.1016/j.ijpharm.2010.11.030.

Frommelt, Lars. 2001. “Gentamicin Release from PMMA Bone Cement: Mechanism and Action on Bacteria.” In *Bone Cements and Cementing Technique*, edited by G. H. I. M. Walenkamp, D. W. Murray, U. Henze, and H.-J. Kock, 119–25. Springer Berlin Heidelberg. http://link.springer.com/chapter/10.1007/978-3-642-59478-6_10.

Ginebra, Maria-Pau, Cristina Canal, Montserrat Espanol, David Pastorino, and Edgar B. Montufar. 2012. “Calcium Phosphate Cements as Drug Delivery Materials.” *Advanced Drug Delivery Reviews* 64 (12): 1090–1110. doi:10.1016/j.addr.2012.01.008.

Higuchi, T. 1963. “Mechanism of Sustained-Action Medication. Theoretical Analysis of Rate of Release of Solid Drugs Dispersed in Solid Matrices.” *Journal of Pharmaceutical Sciences* 52 (12): 1145–49. doi:10.1002/jps.2600521210.

Joshi, Sangeeta, Pallab Ray, Vikas Manchanda, Jyoti Bajaj, D.S. Chitnis, Vikas Gautam, Parijath Goswami, et al. 2013. “Methicillin Resistant Staphylococcus Aureus (MRSA) in India: Prevalence & Susceptibility Pattern.” *The Indian Journal of Medical Research* 137 (2): 363–69.

Kelly, C. M., R. M. Wilkins, S. Gitelis, C. Hartjen, J. T. Watson, and P. T. Kim. 2001. “The Use of a Surgical Grade Calcium Sulfate as a Bone Graft Substitute: Results of a Multicenter Trial.” *Clinical Orthopaedics and Related Research*, no. 382(January): 42–50.

Klemm, K. 2001. “The Use of Antibiotic-Containing Bead Chains in the Treatment of Chronic Bone Infections.” *Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases* 7 (1): 28–31.

Kluin, Otto S., Henny C. van der Mei, Henk J. Busscher, and Daniëlle Neut. 2013. “Biodegradable vs Non-Biodegradable Antibiotic Delivery Devices in the Treatment of Osteomyelitis.” *Expert Opinion on Drug Delivery* 10 (3): 341–51. doi:10.1517/17425247.2013.751371.

Korsmeyer, Richard W., Robert Gurny, Eric Doelker, Pierre Buri, and Nikolaos A. Peppas. 1983. “Mechanisms of Solute Release from Porous Hydrophilic Polymers.” *International Journal of Pharmaceutics* 15 (1): 25–35. doi:10.1016/0378-5173(83)90064-9.

Lambotte, J. C., H. Thomazeau, G. Cathelineau, G. Lancien, J. Minet, and F. Langlais. 1998. “[Tricalcium phosphate, an antibiotic carrier: a study focused on experimental osteomyelitis in rabbits].” *Chirurgie; Mémoires De l’Académie De Chirurgie* 123 (6): 572–79.

Liu, Xin-Ming, Yijia Zhang, Fu Chen, Irine Khutsishvili, Edward V. Fehringer, Luis A. Marky, Kenneth W. Bayles, and Dong Wang. 2012. “Prevention of Orthopedic Device-Associated Osteomyelitis Using Oxacillin-Containing Biomineral-Binding Liposomes.” *Pharmaceutical Research* 29 (11): 3169–79. doi:10.1007/s11095-012-0812-7.

McLaren, Alex C. 2004. “Alternative Materials to Acrylic Bone Cement for Delivery of Depot Antibiotics in Orthopaedic Infections.” *Clinical Orthopaedics and Related Research*, no. 427(October): 101–6.

Meani, E., C. Romanò, L. Crosby, and G. Hofmann. 2007. *Infection and Local Treatment in Orthopedic Surgery*. Springer Science & Business Media.

Nandi, Samit Kumar, Prasenjit Mukherjee, Subhasis Roy, Biswanath Kundu, Dipak Kumar De, and Debabrata Basu. 2009. "Local Antibiotic Delivery Systems for the Treatment of Osteomyelitis – A Review." *Materials Science and Engineering: C* 29 (8): 2478–85. doi:10.1016/j.msec.2009.07.014.

Nelson, Carl L. 2004. "The Current Status of Material Used for Depot Delivery of Drugs." *Clinical Orthopaedics and Related Research*, no. 427(October): 72–78.

P N Raju, K. Prakash. 2010. "Effect of Tablet Surface Area and Surface Area/Volume on Drug Release from Lamivudine Extended Release Matrix Tablets." *Int. J. Pharm. Sci. Nanotech.* 3: 872–76.

Parker, Ashley C., J. Keaton Smith, Harry S. Courtney, and Warren O. Haggard. 2011. "Evaluation of Two Sources of Calcium Sulfate for a Local Drug Delivery System: A Pilot Study." *Clinical Orthopaedics and Related Research* 469 (11): 3008–15. doi:10.1007/s11999-011-1911-1.

Schlickewei, Carsten W., Sinef Yarar, and Johannes M. Rueger. 2014. "Eluting Antibiotic Bone Graft Substitutes for the Treatment of Osteomyelitis in Long Bones. A Review: Evidence for Their Use?" *Orthopedic Research and Reviews*, September, 71. doi:10.2147/ORR.S44747.

Shinto, Y., A. Uchida, F. Korkusuz, N. Araki, and K. Ono. 1992. "Calcium Hydroxyapatite Ceramic Used as a Delivery System for Antibiotics." *The Journal of Bone and Joint Surgery. British Volume* 74 (4): 600–604.

Siepmann, Juergen, Ronald A. Siegel, and Michael J. Rathbone. 2011. *Fundamentals and Applications of Controlled Release Drug Delivery*. Springer Science & Business Media.

Sony, Sandhya, S. Suresh Babu, K. V. Nishad, Harikrishna Varma, and Manoj Komath. 2015. "Development of an Injectable Bioactive Bone Filler Cement with Hydrogen Orthophosphate Incorporated Calcium Sulfate." *Journal of Materials Science. Materials in Medicine* 26 (1): 5355. doi:10.1007/s10856-014-5355-5.

Thomas, Mark V., and David A. Puleo. 2009. "Calcium Sulfate: Properties and Clinical Applications." *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 88B (2): 597–610. doi:10.1002/jbm.b.31269.

Tsourvakas, Stefanos. 2012. "Local Antibiotic Therapy in the Treatment of Bone and Soft Tissue Infections." In *Selected Topics in Plastic Reconstructive Surgery*, edited by Stefan Danilla. InTech. http://www.intechopen.com/books/selected-topics-in-plastic-reconstructive-surgery/local-antibiotic-therapy-in-the-treatment-of-bone-and-soft-tissue-infections.

Uskokovic, Vuk. 2015. "Nanostructured Platforms for the Sustained and Local Delivery of Antibiotics in the Treatment of Osteomyelitis." *Critical Reviews in Therapeutic Drug Carrier Systems* 32 (1): 1–59.

Wichelhaus, T. A., E. Dingeldein, M. Rauschmann, S. Kluge, R. Dieterich, V. Schäfer, and V. Brade. 2001. "Elution Characteristics of Vancomycin, Teicoplanin, Gentamicin and Clindamycin from Calcium Sulphate Beads." *Journal of Antimicrobial Chemotherapy* 48 (1): 117–19. doi:10.1093/jac/48.1.117.

Zhang, X., U. P. Wyss, D. Pichora, and M. F. Goosen. 1994. “Biodegradable Controlled Antibiotic Release Devices for Osteomyelitis: Optimization of Release Properties.” *The Journal of Pharmacy and Pharmacology* 46 (9): 718–24.