RADIOPAQUE MATERIALS FROM NATURAL POLYMERS:

Special emphasis on chitosan and natural rubber

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Certificate

This is to certify that the research work presented in the thesis entitled **"Radiopaque materials from Natural Polymers: Special emphasis on Chitosan and Natural Rubber"** is an authentic record of research work carried out by Ms. Nisha V.S under my supervision in the Department of Polymer Science and Rubber Technology, Cochin University of Science and Technology, in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Polymer Science and Rubber Technology, Cochin University of Science and Technology. No part of the work reported in this thesis has been presented by her for any other degree from any other institution.

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PREFACE

Polymers are used in every sphere of life now a days. Superior properties such as high strength / weight ratio, low energy requirement for processing, good insulation and excellent water resistance are responsible for replacement of conventional products by these materials. Polymers are used from domestic to industrial, insulation to conduction and water resistance to water absorption applications. Today polymers are being used in agriculture, medical, sports, ablative, automobile, aerospace applications etc.

Degradable polymers are currently being evaluated as medical implants in a wide range of applications, such as orthopaedic bone fixation devices, drug delivery systems, cardiovascular implants and scaffolds for the regeneration / engineering of tissues. Such polymers when used as implants are non-traceable without invasive procedures. A radiopaque polymer would offer the unique advantage of being traceable via routine X-ray imaging. The study of radiopaque polymers has been based on empirical approaches that have led to more systematic investigation in the past few years.

The main aim of the present work is to impart radiopacity in various natural polymers like chitosan, natural rubber and derivatives of chitosan and to characterize it. Also this thesis collated the radiopaque properties of these radiopaque polymers and various technological applications in the medical field.

Contents of the thesis:

The thesis is divided in to six chapters.

Chapter 1: A comprehensive introduction and literature survey of radiopaque materials, chitosan and natural rubber are presented in chapter 1. It also includes a review of the most relevant reports pertaining to the field of work. The scope and objectives of the present investigation is summarized.

Chapter 2: Details of the materials and experimental methods used for the present study are given in chapter 2.

Chapter 3: It is divided in to two parts. Part I gives details on the preparation of chitosan microspheres from different emulsion systems and their characterization. Chitosan microspheres were prepared using different emulsion systems and depending on the morphology of the resulting microspheres, the most desirable one was selected. The microspheres prepared from the optimized system showed better radiopacity by the incorporation of barium sulphate. The radiopaque chitosan microspheres were characterized using IR, X-ray, SEM, XRD etc. Part II of this chapter deals with the preparation of radiopaque microspheres from water soluble derivatives of chitosan and their characterization. Chitosan derivatives like chitosan formate, chitosan acetate and carboxymethyl chitosan were prepared and used as matrices for the preparation of radiopaque microspheres. In order to get good spherical morphology, carboxymethyl chitosan/PVA blend microspheres were prepared. The radiopacity of all systems were studied.

Chapter 4: The iodination of natural rubber to make it radiopaque is presented in the first part of chapter 4. Iodinated NR was compounded at high temperature and its properties like radiopacity, tensile strength, tear strength etc. were studied. In order to retain radiopacity after the curing processes, the room temperature vulcanization of NR was adopted. The radiopaque NR was characterized using UV, X-ray and TGA. The antibacterial properties of iodinated NR was studied by using zone inhibition method. The optical density of INR was also studied using

densitometer. Part II of this chapter comprises the studies on the radiopaque properties of radiopacifier filled NR. Natural Rubber was compounded with barium sulphate and commercial zinc oxide to make it radiopaque. Natural Rubber compounded with 150 phr barium sulphate gave better radiopacity than zinc oxide system. The compounds were characterized using X-ray, TGA, Densitometer etc.The physical properties of this system were also elaborated in this part.

Chapter 5: A detailed study of preparation of a radiopacifier (zinc oxide) in chitosan medium is described in this chapter. This chapter is divided into two parts. The preparation and characterization of zinc oxide prepared from different zinc salts was included in part I. The zinc oxide was prepared from different salts of zinc such as zinc chloride, zinc nitrate and zinc acetate. The zinc oxide formed was studied using SEM and XRD. The effects of respective morphology variation on radiopacity and mechanical properties of the matrix polymer have been evaluated. NR was compounded with zinc oxide precipitated from zinc chloride, zinc nitrate and zinc acetate and the radiopaque properties were evaluated using X-ray and densitometer analysis. Among these systems NR compounded with zinc oxide precipitated from zinc acetate showed excellent radiopacity.

Chapter 6: The comprehensive summary and conclusions of the study are presented in chapter 6.

At the end of each chapter a list of pertinent references is given. A list of abbreviations used in this thesis is also cited.

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

For the last few decades, polymeric materials have emerged as unique class of materials due to their versatility and appeal with outstanding mechanical properties, tailorability, functional properties, environmental stability, ease of processing into customer-desired products and host of other desirable properties. Polymer science and technology in the new millennium are facing new challenges and opportunities. Exhilarating developments are expected in almost all existing areas. The developments in the area of biomedical field are quite amazing. Material science and the new field of nano technology have opened up several possibilities for the engineering of better and smaller devices not only for technological applications, but also for use in humans. Biomedical applications of polymers ranging from diagnostic appliances, prosthetics and stents to engineered biopolymers, is increasing rapidly world over. Polymers when used as implants are non-traceable without invasive procedures. A radiopaque polymer would offer the unique advantage of being traceable via routine X-ray imaging. Radiopaque materials open up a new outlook to various technological applications like biomedical, radiation shielding, toy manufacturing, plastic explosives etc.

1.1 BIOMATERIALS

A biomaterial can be defined as a material intended to interface with biological systems to evaluate, treat, augment or replace any tissue, organ or function of the body[1]. The term biomaterial include all materials used for medical applications that are interfaced with living systems or other systems developed for extra corporeal use. The natural tissues in our body can get damaged due to diseases, trauma or aging. Allografts appears to be the ideal and logical materials for replacement. Shortage of organs for implantation and the need for chronic immunosuppression, however, make them less reliable. Therefore, a variety of other materials have been tried as biomaterials. These include metals, glasses, polymers, ceramics, carbon and composites of various combinations of these[2]. They are used singly and in combination to form most of the implantable devices available today. Metals and alloys have high impact and tensile strength. Stainless steel, gold, titanium and cobalt alloys are the commonly used materials in this group. Ceramics and composites have good biocompatibility and corrosion resistance. Since polymers can be tailor-made to match the mechanical and physical characteristics of many parts of the body, they find maximum applications as biomaterials. Some of the most commmonly used biomaterials and their applications are shown in table 1.1.

Table 1.1: Some of the most commonly used biomaterials and their applications

Field	Applications	Material used
Implants	Cardiovascular	Poly(ethylene terephthalate), Poly(tetrafluoroethylene)
	Facial implants	Collagen, Silicones, Poly(glycolic acid)
	Breast implants	Silicones, Polyurethanes
Dentistry	Dental waxes	Polyethylene, Poly(oxyethylene glycol)
	Dental cements	$Zn_3(PO)_4$, ZnO, Eugenol, Silicates
	Restoratives	Alloys, Resins, Silicates
Devices	Sutures	Polypropylene, Teflon, Dacron
	Pacemaker	Epoxy resins, Dacron, Silicones
	Catheters/tubings	Poly(vinyl chloride), Teflon
	Artificial heart	Polyurethanes, Silicone rubber
Orthopaedic applications	Artificial joints	Ultra high molecular weight poly ethylene (UHMWPE)
	Bone cements	Acrylic resins
	Tendons, ligaments	Polyethylene, Silicones
Ophthalmology	Intraocular lenses	Poly (methyl methacrylate) (PMMA)
	Contact lenses	Poly (hydroxyl ethyl methcrylate) (PHEMA)
	Retinal surgery	Silicone rubber

1.2 POLYMERS AS BIOMATERIALS

The polymers can be of natural origin, (commonly termed biopolymers) and/or synthetic origin, the latter being the most extensively used. They are used in medical equipments as packing materials and as a wide variety of disposable devices. The main reason for the extensive applicability of polymers is the availability of synthetic polymers in a wide variety of chemical compositions and physical properties, their ease of fabrication into complex shapes and structures, their easily tailored surface properties and favorable cost performance ratio[3]. Thus compared to other materials, polymers are advantageous in several ways. They are,

Easy to fabricate: They can easily be fabricated into many forms of final usage, such as fluids, fabrics, films and solids.

Compatible to tissues: Many polymers bear a close resemblance to natural tissues such as collagen, which render them suitable for medical applications.

Available with wide choice: They are available with different properties, transparent ones being suitable for ocular implants, opaque for orthopaedic implants and as adhesive for replacing sutures.

Non-corrosive: Unlike many metals, polymers are non corrosive.

Low in density: The density of most of the polymers are closer to the density of the natural tissues.

Thus polymers constitute, by far, the broadest and most diverse class of biomaterials, making the medical market the fourth largest area of polymer application[4]. The first medical application of polymers made use of commercially available ones, adapted as necessary. Although the science and technology of polymers for biomedical application is at an early stage of development, recent

progress has been dramatic. Polymers penetrate virtually every aspect of medicine, though the science of polymeric biomaterial is much more recent than that of other high molecular weight polymers. Only a few polymers have been specially designed for medical uses, e.g., hydrogels for soft contact lenses, poly (glycolic acid) for absorbable sutures, special ion exchange resins, semipermeable membranes and silicone rubber.

Hundreds of synthetic polymers are available. However, only ten or twenty of them are mainly used in medical device fabrications from disposable to long term implants. This is because, the success of a biomaterial in the body depends on factors such as material properties, design and biocompatibility and hence these aspects should be rigorously satisfied. Some of the polymers commonly used as biomaterials and their applications are shown in table 1.2.

Table 1.2 Commonly used polymers and their medical applications

Polymer	Applications
Polyethylene	Catheter tubes, films for sterile conditioning sacs, syringe pistons, needle covers
Polypropylene	Yarns for surgical sutures, films for sterile conditioning sacs, cast bodies for syringes, rigid nozzles, sterilizable vessels
Poly (vinyl chloride)	Blood bags, medical tubings, dialysis tubings
Polyurethane	Adhesives, emulsions, dental materials, suture materials, blood pumps
Poly(Methyl methacrylate)	Bone cement, intraocular lenses, hard contact lenses
Polycarbonate	Sterilisable feeding bottles, syringes, plasma vials, arterial tubules
Silicones	Dental prostheses, artificial ventricles
Polyamide (Nylon 6,6)	Packaging, hypodermic syringes, inhalator
Chitosan	Coating material, blood anti-coagulant, drug delivery, tissue engineering
Poly (vinyl alcohol) (PVA)	Drug delivery, particulate emboli
Poly (hydroxyl ethyl methacrylate)	Contact lenses, particulate emboli

Search for new biomaterials has expanded rapidly over the last few years[5]. It is important to realize that successful application of a biomaterial is possible only if stringent requirements are met. Some of these are,

Biocompatibility: The material should not induce any undesirable or harmful effect such as blood clotting, allergic reaction, tissue death, inflammation, foreign body reaction etc.

Physical properties: Strength, elasticity, permeability etc. must fit within the application and should be maintained through out the service life of the material.

Manufacture: It should be possible to fabricate, purify and sterilize the part without major hiccups.

Among these properties, the most important requirement of a biomaterial is its biocompatibility. Biocompatibility can be defined as the ability of a material to perform with an appropriate host response in a specific situation[6]. Usually, compatibility of a new material is evaluated as far as possible, through a battery of in vitro tests and a follow-up of in vivo or ex vivo evaluation, using animal models.

Research on new polymeric biomaterials has expanded rapidly over the last couple of decades. It would be very helpful to have a technique for non-invasive evaluation of polymeric implants. This would put the researcher into a position from which it is relatively easy to make observations as a function of time without sacrificing the animal model. X-ray and ultrasound radiographic imaging techniques are the most commonly used non destructive techniques to evaluate materials. The search for a non destructive method of polymer evaluation has ended up to a new area of research, comprising of radiopaque polymers.

1.3 RADIOPAQUE POLYMERS

X-ray and ultra sound depends on variations in density between a specimen and its surroundings[7]. Based on casting shadows, radiographic imaging techniques incorporate the principle of radiopacity, which is the physical property of absorbing X-rays or reflecting ultra sound waves. Light materials are moderately radiopaque while heavy materials strongly absorb X-rays and produce good contrast[8]. The ultra sound imaging approach however suffers from the fact that it has only moderate sensitivity. X-ray imaging being fast, reliable, convenient and non-destructive, is commonly used in clinical practice. A relatively new and perhaps more promising approach for non-invasive evaluation of the performance of a biomaterial is to impart radiopacity to such materials so that they can be monitored for their function and performance in a non-invasive manner.

Radiopacity is now considered as a desirable property of implants used in surgery as it follows the post operative assessment of the fate of the implant using X-radiography[9]. Radiopacity is widely acknowledged as a property of all intra oral materials including denture base materials, denture liners etc. Elastomeric impression materials, endodentic sealers, posts and restorative materials, direct filling restorative materials and resin cement luting agents are all radiopaque[10].

However, until recently, these techniques were not sufficiently sensitive to direct polymers so that the physical changes that occur in polymer implants could be observed. Polymers cannot be detected by imaging techniques because they mainly contain the elements such as carbon, hydrogen, oxygen, nitrogen and in some cases elements like silicon (e.g. silicon rubber). Consequently, polymers exhibit relatively low electron density, which render them radiolucent. Sharp images can be obtained only from materials of high electron density[11,12]. Research into radiopaque polymers explore methods of increasing average electron density

and specific gravity of polymers by incorporating heavy elements into these systems. One of the common practice is to introduce radiopacity via radiopaque fillers. Additives[13-18] such as barium sulphate, zirconium oxide, bismuth halides are incorporated to achieve the necessary X-ray contrast when they are produced by molding, casting, extrusion etc. The incompatibility of inorganics such as barium, bismuth or silver with the polymer matrix often affect the physical and mechanical properties of the implant adversely. Moreover, the possibility of the inorganic ions leaching into the body fluid over long periods of time also causes a threat both from the stand point of the stability of the implant and the toxicity of the metal ions[19].

1.3.1 RADIOPACIFIERS

Radiopacifiers are the substances added to a polymer matrix to impart radiopacity. The following are the commonly used radiopacifiers:

1. Metal inserts such as fine wire, gold gauze or lead foil have been introduced into dental methacrylic resin.

2. Barium sulphate: It is the most widely used compound for dental resins and bone cements. It is very stable, less expensive and can be made in to different colours.

3. Bismuth compounds: It is more expensive than barium sulphate. It has higher density and may produce a brighter and sharper X-ray image than barium sulphate.

4. Tungsten: It is compatible with most polymers. It is more than twice as dense as bismuth and provides a high level of radiopacity. Loading levels of up to 95 % by weight are possible. Host compounds containing tungsten are dark grey in color, which limits coloring option.

One of the most important versatile radiopacifier is triphenyl bismuth. It forms miscible and often optically transparent blends of high opacity with a wide range of polymeric materials including polystyrene, polyvinyl chloride, polyalkenes, polyacrylates and epoxy resins. Low molecular weight iodine compounds in transparent plastic materials and toys provide improved X-ray contrast. Incorporation of elements of high atomic mass to increase the average electron density and specific gravity of polymers is done in many ways.

Based on the preparation, radiopaque polymers are classified into three groups. They are radiopaque polymer blends, radiopaque polymer salt complexes and polymerization products of radiopaque monomers.

1.3.2 RADIOPAQUE POLYMER BLENDS

Radiopaque polymer blends are produced by incorporating the radiopacifying agents as a physical mixture with the polymer. The introduced agent can be a heavy metal, inorganic salt of a heavy element or an organic compound containing a heavy atom substituent. Barium sulphate is an additive commercially used for denture resins and bone cements to make them radiopaque. It does not affect the hardness, solubility or absorption of the resin and tissue implants of the material[20]. But barium sulphate reduces the tensile strength and minimizes the modulus of elasticity. It was observed that polymers containing zirconium dioxide show a high degree of radiopacity than those containing barium sulphate[21]. Metal inserts such as fine wire, gold gauze or lead foil may also be introduced into resins to make them radiopaque. Small quantities of inorganic salts have been added for obtaining radiopacity. Many simple high boiling aromatic and aliphatic halides have been added to the polymerization solution to make them radiopaque[22]. The main drawback of these systems is that the radiopaque additives are not

chemically incorporated into the resin. Many of the metal salts leach into the body fluids over a long time, which makes their radiopacity a temporary phenomenon[23].

1.3.3 RADIOPAQUE POLYMER-SALT COMPLEXES

Radiopaque polymer-salt complex systems are produced by the incorporation of a radiopaque heavy metal into an appropriate polymer ligand via chelation. The resulting systems are homogeneous and posses both polymeric and ionic character X-ray imaging demonstrated that the radiopacity of these systems are high. Cabasso[24] *et al* investigated polymers and monomers that can solubilize heavy metal salts such as barium bromide, bismuth halides, uranyl nitrate and lanthanides. Polymer salt complexes of bismuth tribromide and uranyl nitrate with acrylated polyphosphonates[25] have been synthesized, where the phosphoryl group is believed to provide a strong coordinating site to the metal ion. Similar complexes with polymers containing carbonyl function have also been synthesized[26].

1.3.4 POLYMERIZATION PRODUCTS OF RADIOPAQUE MONOMERS

Polymerization products of radiopaque monomers are produced by the introduction of the radiopacifying element either electrovalently or covalently into the monomer unit prior to polymerization. Barium and zinc acrylates have been reported as radiopacifier and it can be copolymerized with methyl methacrylate (MMA)[27]. However, the ionic nature of these resins leads to significant absorption of water and the slow hydrolysis of poly (zinc acrylates) leading to the loss of the opacifying atoms. 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 new polymeric biomaterials that can exhibit intrinsic radiopacity. Such materials can offer vital advantages since no compromise can be made between the introduction of radiopacity on the one hand

and the preservation of physico-mechanical properties on the other. The disadvantage of radiopaque system formed from covalently bound heavy element is its relatively high cost.

1.4 A REVIEW OF COMMONLY USED RADIOPAQUE SYSTEMS

1. Cyanoacrylic derivatives: Isobutyl 2-cyanoacrylate (IBCA) and N-butyl 2-cyanoacrylate

Isobutyl 2-cyanoacrylate (IBCA) rapidly found acceptance for embolic vascular occlusion, especially for the treatment of arteriovenous malformations (avm's). The main advantage offered by this derivative is its low viscosity and rapid polymerization when in contact with vascular endothelium or ionic solutions such as blood. The injected fluid gets rapidly polymerized by forming a hard intravascular cast trapping blood element.

Besides an uncompleted biocompatibility evaluation for intravascular use, IBCA also exhibits some undesirable characteristics such as an exothermic reaction during polymerization, difficulty to control polymerization time, lack of visibility, possible premature polymerization inside the catheter and rendering control of implantation difficult or hazardous. To avoid premature polymerization, the use of 5 % glucose solution to flush all ionic materials from the system are mandatory, as also modifying polymerization time. A chemically similar monomer (NBCA) was proposed as a fast polymerizing agent for the endovascular treatment of 'avm'. This derivative showed a shorter polymerization time than IBCA by the addition of iophendylate oil or acetic acid.

In-vitro studies showed that the polymerization time was delayed by increasing the proportion of contrast medium ratio, which provided an optimal embolization material with good flow properties. Another acrylic derivative, the ethyl-

cyanoacrylate was patented as an embolic material, but no major advantages were found[28].

A vascular graft catheter comprises highly radiopaque polyolefin compound, where the radiopaque material in the said compound is substantially uniformly dispersed and held within a polymer matrix. During the method, the first step is to heat low density polyethylene to it's melting temperature. The amount of polyolefin is equal to 10 % by weight of the compound. Then an amount of radiopaque metal powder equal to 90 % by weight of the compound is added. The metal powder is preferably tantalum, tungsten, gold or platinum. Thereafter, an amount (at least 0.2 % by weight of the compound) of dispersing agent is added to polyolefin to form a mixture. The dispersing agent is preferably zinc stearate, aluminium stearate or calcium stearate. At last the mixture is mixed and cooled below its melting temperature to form the compound. Once the compound is formed, it can be cut into pellets and then is extruded into a tubular form for making tubular tip.

2. Methyl methacrylate Derivatives

Methyl methacrylate derivatives with an average of twenty two ethylene units were synthesized and chelated with barium bromide. However, permanent radiopacity was not achieved with these derivatives and this limit the potential for their clinical application. Cation-chelating monomers were developed to achieve complete solubilization of heavy salts.

Blends of poly(methyl methacrylate) and heavy metal salts were developed by dissolving bismuth tribromide or sometimes bismuth chloride in MMA up to 40 % by weight. The high solubility of the salt resulted from the interaction between carbonyl group and bismuth because the electron donating monomer would readily interact with radiopacifying heavy metal.

Clear solutions of $BiBr_3$ could also be obtained with other monomer containing a carbonyl functional group. For eg. MMA /$BiBr_3$ mixture was polymerized to form solid resins. The presence of about 40 wt% of the salt decreased the molecular weight of PMMA from 1,20,000 to about 80,000 g/ml and slightly increased the glass transition temperature from 108° C to 123° C. PMMA-$BiBr_3$ resins develop opaqueness on contact with water. The influence of $BiBr_3$ content in PMMA on biocompatibility was tested and no sign to mutagenicity was revealed.

PMMA containing organo bismuth radiopacifying additive has also been reported. The X-ray contrast agent used was triphenylbismuth (PH_3Bi) and it was soluble in PMMA up to 70 %. A minimum of 23 % halogenated derivative was necessary to obtain the same radiopacity as the aluminum standard. Bismuth compound acts as a plasticizer and the glass transition temperature of PMMA was reduced. PH_3Bi is very resistant to moisture and water. Therefore it avoids leaching out in an aqueous environment. It is very stable to heat and air. PH_3Bi also shows lower toxicity as PMMA alone. Radiopaque derivatives could also be prepared using triphenyl bismuth and polystyrene.

Transparent, hard materials were obtained by copolymerizing MMA and styryldiphenylbismuth at 65°C with benzoyl peroxide as initiator. The synthesized products had a glass transition temperature of 100-110° C, close to that of PMMA because the heavy metal was a part of the backbone of the product. Thus, the thermal and mechanical properties of the polymers, in comparison to materials containing heavy metal components as additives only, were improved. 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 copolymerization could be obtained with other monomers such as styrene or other vinyl monomers[29].

Another approach to opacify PMMA has been patented and was achieved by incorporating bromine into the PMMA resin. The synthesis of 2,3-dibromopropyl methacrylate was carried out by refluxing methacrylic acid and 2,3 dibromoproponol in toluene. The product obtained was a colorless liquid with a boiling point 82 – 86° C. It is possible to polymerize the 2, 3-dibromopropyl methacrylate to obtain a homopolymer that possess a high bromine content (55.9 wt%) and hence highly satisfactory radiopacity, but is also highly brittle. To improve the mechanical properties of the brominated polymer, copolymerization of poly (2, 3-dibromopropyl methacrylate) with MMA at 70° C using azo-isobutyronitrile as initiator was employed. The synthesized copolymers had a cross linked structure and their equilibrium water absorption decreased with increasing content of poly (2,3-dibromopropyl methacrylate). The flexural strength decreased continuously while the elastic modulus increased proportionally to the content of the brominated polymer. The loss of tensile strength and impact strength was minimized until 60 % of the bromination.

Synthesis and polymerization of iodine containing methacrylate have been reported. Variable radical polymerization behavior was exhibited when comparing similar methacrylic monomers. For example 2,3,6-triiodophenyl methacrylate showed a poor tendency to homopolymerization and gave only oligomeric product, while 2,3,5-triiodobenzoyloxy alkyl methacrylate yielded polymers with number average molecular weight about 58,000 - 1, 47,000 under similar conditions. The 2,4,6-triiodophenyl methacrylate reduce the MMA polymerization and thus decreased the number average molecular weight of the formed polymers.

1.5 APPLICATION AREAS OF RADIOPAQUE POLYMERS

Manufacturing industries of plastics, biomedical polymers, defense materials etc. explore the properties of radiopaque polymers extensively are indicated below.

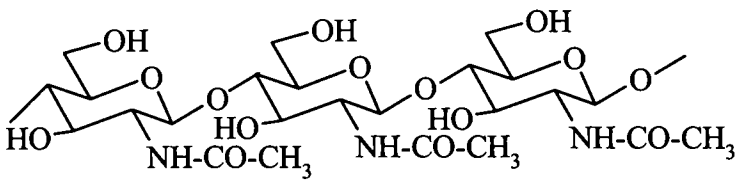
- In biomedical field it is used for the preparation of implants, catheters, medical adhesives and in dentistry for prosthetic applications such as denture or restorative resins
- It is also used for the detection of changes within the body organs such as the kidneys, blood vessels, heart or gastrointestinal system
- Radiopaque compounds are also used to produce shielding components to enclose radiation generating sources
- It is used in toy manufacturing to enable radiographic detection of toys swallowed by children
- Radiopaque polymers are used in plastic explosives, which cannot be detected by conventional X-ray techniques. Incorporation of heavy metal salts into these systems can facilitate their detection for security

1.6 NATURAL POLYMERS USED FOR THE PRESENT STUDY

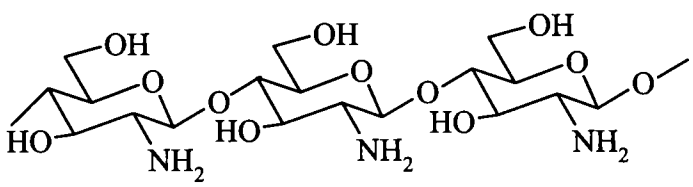
In this thesis an attempt has been made to prepare radiopaque, biocompatible polymers and to explore their radiopaque properties. To this end, we use chitosan and natural rubber as matrix polymers. The chemistry and the applications of these two are reviewed in the following sections.

1.7 CHITIN AND CHITOSAN

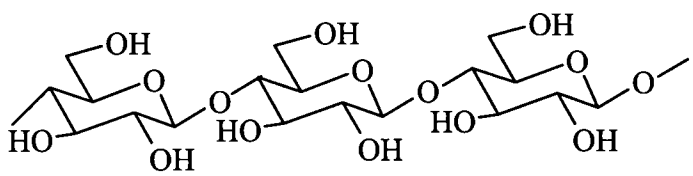
Nature has chosen two different but related polysaccharides to provide structure and integrity to plants and animals like crustaceans and insects. Plants have cellulose in their cell walls while insects and crustaceans have chitin in their shells. Cellulose molecules are large chains of glucose units while chitin molecules are large chains of N-acetyl glucosamine units. Cellulose and chitin are two of the most abundant biopolymers on earth. Chitin is a highly insoluble material resembling cellulose in its solubility and low chemical reactivity. It may be regarded as cellulose with hydroxyl at position C-2 replaced by acetamido groups. The principle derivative of chitin is chitosan[30]. It is formed through N-deacetylation of the chitin molecule. The structures of chitin, chitosan and cellulose are shown in figure1.1.



Chitin



Chitosan



Cellulose

Figure 1.1: Structure of Chitin, Chitosan and Cellulose

Thus chitin is a nitrogenous polysaccharide which is white, hard and inelastic. It is found in the outer skeleton of insects, crab, shrimp and lobsters and in the internal structure of other vertebrates[31]. Chitin has a crystalline structure and it constitutes a network of organized fibers. Chitosan also occurs naturally in some fungi but its occurrence is much less widespread than that of chitin[32].

1.7.2 PROCESSING OF CHITIN AND CHITOSAN

Chitin is widely distributed both in the animal and plant kingdom. In animals, the most readily associated sources are in the shells of crustaceans and mollusks, the backbone of squids and the cuticle of insects. Japan is the major manufacturer of chitin with an annual production of about 500 tones. Serious environmental problems caused by prawn shell waste can be avoided by using it as a raw material for the production of chitin and its derivatives. In addition to control environmental pollution, it is a valuable recourse for more employment and additional income.

In crustaceans chitin is found as a constituent of a complex network with proteins into which calcium carbonate deposits to form the rigid shell. The interaction between chitin and protein is very intimate with covalent bonding, and in essence is a polysaccharide protein complex[33]. The processing of crustacean shells mainly involves removal of proteins and dissolution of calcium carbonate which is present in crab shells in high concentrations. The resulting chitin is deacetylated in 40 % sodium hydroxide at 120 °C for 1-3 h (figure 1.2).

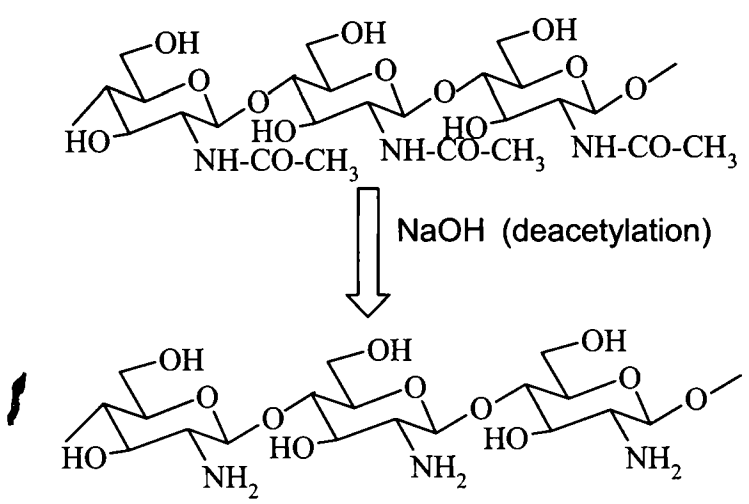


Figure 1.2: Deacetylation of chitin

Deproteinisation is done with dilute alkali and demineralization with dilute acids. Variations in the reagent used and their concentration, as well as the time and temperature of treatment determine the quality and performance of the product[34].

1.7.3 PHYSICOCHEMICAL CHARACTERISTICS OF CHITOSAN

Most of the naturally occurring polysaccharides such as cellulose, dextran, pectin, agar etc. are neutral or acidic in nature, while chitin and chitosan are highly basic polysaccharides. Their unique properties include polyoxysalt formation, ability to form films, chelate metal ions and optical structural characteristics[35].

1.7.3.1 Degree of N-acetylation

Chitosan is characterized by either the degree of acetylation (DA), which corresponds to the N-acetylamine groups or the degree of deacetylation DDA (DDA=100-DA), D-glucosamine groups. The degree of acetylation has an influence on all the physicochemical properties (molecular weight, viscosity, solubility etc.). Many techniques have been tried to determine the degree of acetylation more precisely which include IR spectroscopy, pyrolysis gas chromatography, gel permeation chromatography and UV spectrophotometry[36-45]. The most appropriate technique for rapid characterization seems to be IR spectroscopy.

1.7.3.2 Molecular weight

The knowledge of average molecular weight of chitin and chitosan is very important for industrial uses and for critical applications fields. Although the primary structure of chitosan comprises a backbone of (1-4)-β-D-glucosamine residues randomly acetylated to various extents, the name chitosan is in fact a collective term for deacetylated chitin differing in terms of crystallinity, optical characteristics, degree of deacetylation, impurity content and average molecular

weight. Chitosan molecular weight distribution has been obtained using HPLC[46]. Viscosity measurements are widely used. More recently gel permeation chromatography (GPC) or gel filtration chromatography (GFC) has been applied to study the molecular weight.

1.7.3.3 Solubility

Chitin is highly hydrophobic in nature and is insoluble in common organic solvents as well. It is soluble in hexafluoroisoproponol, hexaflouroacetone, chloroalcohol in conjugation with aqueous solution of mineral acids and dimethyl acetamide containing 5 % lithium chloride[47]. Chitosan, the deacetylated product of chitin, is soluble in dilute acids like acetic acid, formic acids etc. Hydrolysis of chitin with concentrated acids produces relatively pure amino sugars, D-glucosamine. The nitrogen content in chitin varies from 5 to 8 % depending on the extent of deacetylation.

In fact, chitosan is soluble in dilute acids on account of protonation of free amino groups. As in all polyelectrolytes, the dissociation constant of chitosan is not constant but depends on the degree of dissociation at which it is determined. The solubility of chitosan depends on its degree of dissociation.

1.7.3.4 Crystallinity

On the basis of the crystalline structures, chitin is classified into three forms: α,β and γ- chitins (hydrated, anhydrous crystal, and non-crystal). These forms can be examined easily by measuring the X-ray powder diffraction pattern of a chitosan sample[48]. The modified forms of chitosan are less crystalline than pure deacetylated chitosan.

1.7.4 DERIVATIVES OF CHITOSAN

1.7.4.1 Chemical modification of Chitin and Chitosan

Chitosan can carry a large number of amine groups on its chain and thus can form multiple complexes. At higher pH levels (over 4) it can form complexes with colorants and heavy metals. The presence of the pair of free electrons of the amine groups is assumed to be the origin of the dative bonds, an idea confirmed by the observation of a much weaker fixation in chitin. Several chemical modifications can be done on chitin and chitosan. These are acylation, aldimination, carboxymethylation, sulphation, complexation with metal cations and some miscellaneous reactions.

1.7.4.2 N-acetylation

N-acetylation of chitosan leads to fully N- acetylated chitin. Complete N-acetylation may be achieved in 3 minutes at room temperature using a highly swollen chitosan in organic aprotic solvents. Chitosan boiled with large excess of hexanoyl or dodecanoyl chlorides in dry pyridine or chloroform gave fully acetylated derivatives[49]. An aspirin carrier is prepared by the reaction of chitosan with 2-acetoxy benzoic anhydride.

1.7.4.3 Carboxylate derivatives

The insertion of carboxylic functions in chitosan has been widely studied. O-carboxymethylation is achieved with monochloroacetic acid and sodium hydroxide. Carboxymethylation is supposed to proceed preferentially at C-6 as implied from the results of backbone hydrolysis[50]. Crosslinked carboxymethyl chitin or chitosan show high capability of separating bovine serum fibrinogen and albumin. Muzarelli[51] et al demonstrated that N-carboxymethylation could be obtained first, reacting the amino group on chitosan with glyoxylic acid which

yields the intermediate, aldimine. Subsequent reduction gives N-carboxymethyl chitosan that is readily soluble in water for the whole pH range.The structure of Carboxymethyl chitosan is shown in figure 1.3.

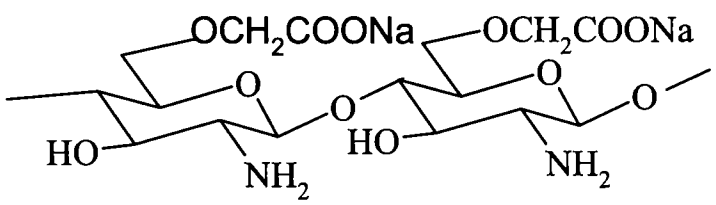


Figure 1.3: Carboxy methyl chitosan

1.7.4.4 Sulphation

Sulphation of chitin and chitosan has been one of the most attractive modifications owing to the possibility of preparing anticoagulant polysaccharide in view of the structural similarity to heparin. For sulfation various reagents have been used including con.H_2SO_4, SO_3/pyridine, SO_3/SO_2 and chloro sulfonic acid. The trityl-chitin/chitosan intermediate has also been used to develop chitin and chitosan sulphates that have been investigated for their anti-HIV activity[52]. In chitin, hydroxyl groups are sulphated, where as with chitosan, sulphation occurs at both hydroxyl and amino groups. The sulphur trioxide-pyridine complex is selective for sulphation of chitosan amino groups[53].

1.7.4.5 Phosphorylation

The insertion of phosphate functions into chitosan has made it possible to develop a wide variety of polymers, soluble and insoluble, which complex and fix metals such as nickel, zinc or cadmium. The phosphorylation reaction of chitin and

chitosan in P_2O_5-methane sulphonic acid system was found to be very efficient. Chitin and chitosan phosphates can easily be made insoluble by the cross linking reaction with adipoyl dichloride. A novel water soluble chitosan derivative carrying phosphonic groups was also synthesized. Chitin and chitosan phosphates adsorb alkaline earth metals and their ions more strongly than that of chitin and chitosan.

1.7.5 APPLICATION AREAS OF CHITIN AND CHITOSAN

The driving force for much of the excitement surrounding chitin and chitosan are the potential applications that the material can be used for. Tabe1.3 lists potential applications for chitin, chitosan and their derivatives[54-67].

Application	Specific use
Water treatment	Coagulating agents for polluted water, removal of metal ions
Agriculture	Plant elicitor, antimicrobial agents, plant seed coating
Textile, paper industry	Fibers for textile and woven fabrics, paper and film
Biotechnology	Chromatography, packing, enzyme immobilizing material
Food/health supplements	Natural thickeners, food additives, Filtration and clarification, Hypocholesteromic agents (slimming agents)
Cosmetics	Ingredients for hair and skin care
Biomedical	Wound dressings, absorbable sutures, anticoagulant or antithrombogenic materials, homeostatic agents, drug delivery, gene delivery

Table 1.3: Potential applications for chitin, chitosan and their derivatives

1.7.5.1 Biomedical applications of chitin

Chitin as a biomaterial can be exploited in two main matters, as a biostable material or as a biodegradable material, chitosan being a safe and friendly substance for the human organism. Medical and pharmaceutical applications can easily be worked out with joint efforts from specialists in various fields. As medical devices, the applications of chitin can be conveniently divided into two classes, external and internal. As an external device, chitin is used for making external communicating devices that come into contact with intact natural channels of the body such as the eye, vagina, and the gastro-intestinal tract and those that breach the body surface or contact blood such as in intravenous catheters or conduit for fluid entry. Examples of chitin applied in external medical devices are contact lenses, wound dressings, haemostatic agents and coating of the inner lumen of blood contacting tubing. Internal devices are normally implants that are targeted for bone, tissue, tissue fluid and blood. Examples of internal medical device applications of chitin include orthopedic implants such as bone pins, plates and cements, tissue engineering scaffolds, systemic anti coagulants, drug delivery components and gene delivery vehicles. Outlined below are some of the biomedical applications of chitin and chitosan.

1.7.5.2 Drug Delivery

Drug delivery is concerned with combining of drugs with other constituents to provide dosage forms suitable for administration to the patient. The non-drug constituents serve roles such as bioprotection of the drug or the body from the drug and absorption enhancement of the drug. The active agent i.e. the drug is combined with a polymeric material. Common requirements for the polymeric material are compatibility with the active agent, non toxicity, stability, sterilizability and biodegradability. An assessment of these factors identifies chitin

as a candidate that fulfills the basic requirements. All interesting properties of chitin and its derivatives, predominantly chitosan, make this natural polymer an ideal candidate for controlled drug release formulations[68–73]. The most popular method of administration by far is oral where micro particulate, liposome, solution, vesicle, film coated, tablet and capsule forms are known. Microspheres and their more recent successor nano spheres are a popular method of effecting drug delivery systems useful in parenteral applications. Hydrogels based on chitin and chitosan have been widely used in controlled release systems. The pH sensitive hydrogels have potential use in site-specific delivery of drugs to specific regions of the gastro intestinal tract and have been prepared for low molecular weight and protein delivery. Chitosan/polyether interpenetrating network (IPN), hydrogel, semi IPN hydrogels of β-chitin/polyethylene glycol, chitosan/gelatin hybrid polymer network etc. were reported in controlled drug delivery.

1.7.5.3 Gene delivery

Mumper et al. were the first to describe the potential of chitosan as a gene carrier. The low toxicity of chitosan and its nature makes it attractive for gene delivery purposes. In early studies, chitosan has been shown to bind nucleic acids, and it is known that chitosan may actually be endocytosed into the cell. The hybrid DNA-chitosan systems can be classified into two categories, they are chitosan-DNA complexes and nanospheres[74-82].

1.7.5.4 Cholesterol and over weight lowering

Chitosan is an effective in lowering total and LDL cholesterol. Chitosan appears to be active in humans at rather low doses, with as little as 1.2 g per day producing significant reductions in serum cholesterol. In vitro studies show that chitosan has been reported to bind bile acids with approximately one-half or equal capacity of cholestyramine, a strong synthetic anion exchanger. Saturated fats are particularly

implicated in raising LDL levels, which increase the risk of developing arthroscleroses, heart attack and stroke. The liver constantly endeavours to clear out the bad cholesterol by dumping it into the intestine.

1.7.5.5 Dressing of wounds

Modified chitin has been administered to humans in the form of dressings for wounded soft and bone tissues[83-87]. Chitin has been found to have an accelerating effect on the wound healing process. The choice of chitosan was to preserve the good antigen affinity property after sterilization. Regenerated chitin fibers, non-woven mats, sponges and films increase the wound healing process by over 30 %. Chitin can also be used as a coating on normal medical materials. Standard silk and catgut sutures coated with regenerated chitin or chitosan show wound healing activities. Surgical gauze coated with regenerated chitin demonstrates a substantially greater amount of activity than uncoated control group. Gel like pastes comprising chitosan blended hydrocolloid materials such as polysaccharide gums has also been described as wound filling compositions[88].

1.7.5.6 Treatment of burns

Chitosan is a very attractive candidate for burn treatment. Chitosan has the ability to form tough, water absorbent and biocompatible films with good oxygen permeability. These films can be formed directly on the burn by application of an aqueous solution of chitosan acetate. The solution although acidic, provides a cool and pleasant soothing effect when applied to the open wounds of burn patients. Additionally chitosan films have the ability to absorb water and are naturally degraded by body enzymes. This means that chitosan need not be removed. Chitin can also be prepared in the water soluble form by carefully deacetylating to about 50 % N-acetyl content[89]. Fluid absorbing chitosan has also been proposed as wound dressing material[90].

1.7.5.7 Ophthalmology

Chitosan possesses optical clarity, mechanical stability, gas permeability (particularly towards oxygen), wettability and immunological compatibility. Contact lenses are made from partially depolymerized and purified squid pen chitosan by spin casting technology. The contact lenses prepared from chitosan are clear, tough and possess other required physical properties such as modulus, tensile strength, tear strength, elongation, water content and oxygen permeability. The antimicrobial and wound healing properties of chitosan along with an excellent film capability make chitosan suitable for development of ocular bandage[91].

1.7.5.8 Chitosan as a fat trapper

Chitosan attaches itself to the fat in the stomach before it is digested, thus trapping fat and preventing its absorption by the digestive tract. Fat, in turn, binds to the chitosan fiber forming a mass which the body can't absorb and is eliminated by the body. Chitosan[92,93] fiber differs from other fibers in that it possesses a positive ionic charge, which gives it the ability to bind chemically with the negatively charged lipids, fats and bile acids.

1.7.5.9 Chitosan as a new haemostatic agent

More recently Malettte[94] *et al* described the use of a new haemostatic agent such as N-hexanoyl and N-octanoyl chitosan which can be used even under most severe conditions of anticoagulation. It is apparently a safe agent which does not adversely affect graft healing.

1.7.5.10 Blood anti-coagulants (heparinoids)

Chitin and chitosan sulphates have blood anticoagulant and lipoprotein lipase (LPL) releasing activities. Chitin 3,6-sulphate showes about two-fold anticoagulant activity and 0.1 fold LPL-releasing activity over those of heparin[95].

1.7.5.11 Anti-bacterial agents

The growth of Escherichia coli was inhibited in the presence of chitosan (more than 0.025 %). Chitosan also inhibits the growth of Fusarium, Alternaria and Helminthosporium. The cationic amino groups of chitosan probably bind to anionic groups of these microorganisms, resulting in growth inhibition[96].

1.7.5.12 Bone substitutes

Bone is largely made up of two components, an intimate combination of collagen and calcium hydroxyapatite. Chitin has been applied both in pure form as well as in combination with calcium compounds in orthopedic applications. Maeda *et al* were one of the first to use chitin in the form of braided filaments, rods and powders. These substitutes are found to be potentially suitable for sutures and temporary artificial ligaments for the knee joint. Borah et al studied the bone induction properties of N-acetyl chitosan. Chitosan was found to be better than the control and concluded that chitosan had osteogenic properties. More recently Chitosan–hydroxyapatite nano composites have been prepared and were found to be mechanically flexible and promoted bone formation.

1.7 5.13 Implants

Implantable devices are expected to be intelligent, nontoxic, nonthrombogenic, non carcinogenic and easily implantable with adequate storage capacity and possess drug stability, biodegradability and sterilizability. Khor and Lim discussed various applications of chitosan implants in a recent review[97]. Chitin and chitosan

have been used in orthopedic and periodontal applications[98,99]. Microspheres based on chitosan implants were prepared by cross linking with genipin and glutaraldehyde[100]. Recently[101] chitosan and sodium hyaluronate implants for controlled release of insulin were studied.

1.8 NATURAL RUBBER

Of all materials provided by nature for man to use as a material of construction, natural rubber is unique. Today there are many man made rubbers but natural rubber still plays a substantial role on the world's industrial stage, a story very different from that of many other natural materials which are challenged by synthetics. Natural rubber[102,103] (NR) (cis, 1, 4-polyisoprene) occurs in over 200 species of plants. The *Hevea brasiliensis* tree accounts for over 99 %of the world's natural rubber production, which in 1986 amounted to over $4x10^6$ tonnes. Historically, rubber as a material was known to and used by man as early as the sixth century, as excavations subsequent to the discovery of America have revealed.

Fresh Hevea latex, from which natural rubber is obtained contains about 25-45 % rubber hydrocarbon and 5-6 % non rubber substances such as amino acids, proteins, carbohydrates, neutral and polar lipids, and inorganic salts; the remainder being water. The high molecular weight and the presence of non rubber substances may give rise to inside reactions; such as cross linking, degradation, cis-trans isomerization during chemical reactions, and finally the reduction in activity. The non rubber substances also prevent the occurrence of certain reactions that can be carried out with synthetic cis-1, 4-polyisoprene. In its chemical reactions it behaves as a simple trialkylethelyne. However the reactions are influenced by two factors compared to the reactions of simple olefins. The first is the polymeric nature of natural rubber, which has a weight average molecular weight of

$1x10^6$ - $2x10^6$. This gives rise to difference in its solubility and viscosity. The second factor is the chemical composition of natural rubber.

1.8.1 DERIVATIVES OF NATURAL RUBBER

Before the 1960's, interest in the chemical modification of natural rubber focused on new materials with unusual properties. In the last 20 years however, more emphasis has been placed on modifying natural rubber in a controlled way without altering its strength properties. A great number of chemical derivatives have been prepared from natural rubber, but only a few have attained commercial significance, mainly because of the high cost of manufacture[104].

The natural rubber derivatives are divided in to four (i) those resulting from bond rearrangements without the introduction of new chemical groups (ii) those resulting from the attachment of pendent functional groups to the natural rubber molecule by olefin addition or substitution reactions (iii) those obtained by grafting of a different polymer at one or more points along the natural rubber molecule and (iv) other derivatives.

1.8.2 Bond Rearrangement Reactions

1. Isomerized rubber

The 1, 4 poly isoprenes occur as isomers with different cis-trans ratios, ranging from the 100 % cis structure of natural rubber to the 100 % trans structure of gutta-percha.

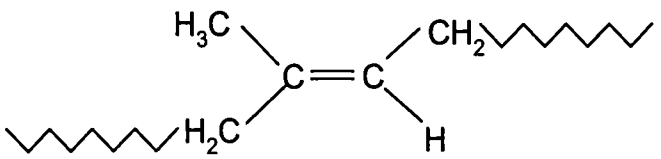


Figure 1.4 : Trans 1, 4-poly isoprene

The first successful cis-trans isomerization of natural rubber was reported in 1959. The transformation can be carried out with the help of catalysts on natural rubber in the form of a solid, solution, or latex. Isomerization takes place when thin film, sheets, or crumbs are heated with sulphur dioxide above 100 °C.

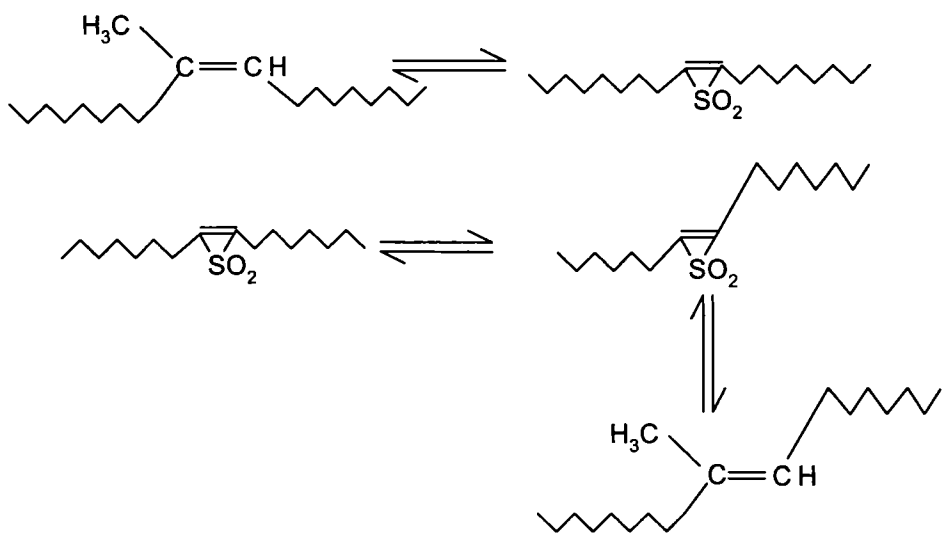


Figure 1.5 : Cis 1, 4-poly isoprene

In commercial practice, butadiene sulfone[105] is used, which produces sulphur dioxide *in situ.* The reaction with sulphur dioxide does not involve free radicals and probably proceeds by an "on-off" reaction at the double bonds.

2. Cyclized Rubber

Cyclized rubber, the first chemically modified derivative of natural rubber, is a hard, resinous product obtained by treating the rubber with strong acids or Lewis acids. Sulphuric acid, titanium chloride, ferric chloride, stannic chloride, p-toluene sulfonic acid and its choride, and boron trifluride etc. have been used. Cyclizations are carried out on solid rubber, solutions or latex, depending on the catalyst. Cyclized rubber has the same empirical formula (C_5H_5) as polyisoprene, but has a lower degree of unsaturation. Cyclization proceeds via the carbenium ion mechanism. The protonated structure may cyclize to one ring, two rings, or more rings before deprotonation. The deprotonation reaction may result in tetra-tri or di substituted double bonds.

Cyclized rubber is also manufactured by adding concentrated sulphuric acid to latex stabilized with nonionic or cationic surfactant. The final concentration of acid in the aqueous phase should be at least 70 % by weight. The mixture is heated at 100° C for 2 h under careful temperature control. The cyclized rubber latex is coagulated by pouring into aqueous alcohol or boiling water, and then filtered, washed and dried. A cyclized master batch can be produced by mixing cyclized and uncyclized lattices before coagulation.

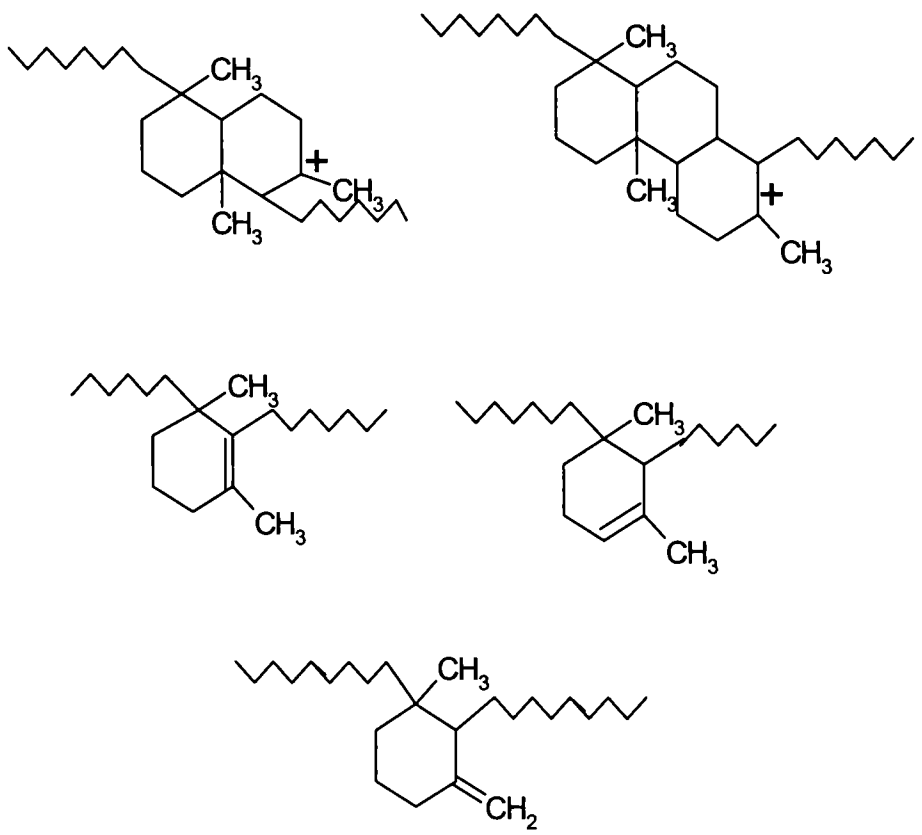


Figure 1.6: Structures of cyclized rubber

3. Hydrogenated Rubber

Complete hydrogenation of natural rubber would give an altering copolymer of ethylene and propylene.

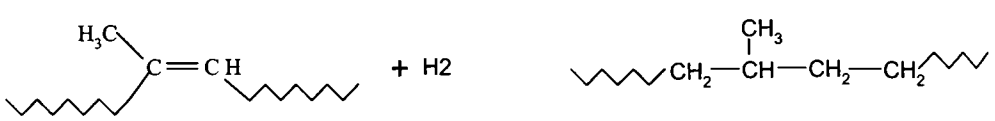


Figure 1.6: Hydrogenation of natural rubber

Hydrogenation without degradation has been accomplished with the help of nickel catalyst on kiselguhr at 170-180° C under a pressure of 1.5 - 2 MPa. However, the catalyst is easily poisoned by impurities and separation of catalyst is difficult. Hydrogenated rubber is more crystalline and has a slightly higher glass transition temperature than natural rubber. It can be vulcanized with the conventional systems. The vulanizates show good ozone resistance at a high degree of hydrogenation.

4. Hydrohalogenated rubber

Hydrogen chloride adds readily to natural rubber to give the derivative rubber hydrochloride. The addition follows Markonikoff's rule but is accompanied by some cyclization. The product has a syndiotactic configuration and has slightly lower chloride content than the theoretical amount.

Rubber hydrochloride is a highly crystalline, tough, semi-elastic and film-forming. Its solubility is similar to that of chlorinated rubber. It loses hydrogen chloride on heating to about 100° C. The addition of hydrogen bromide proceeds like the addition of hydrogen chloride, rubber hydrobromide has been obtained as a powder but it is unstable.

5. Alkyl halogenated Rubber

Polyhalogen derivatives of alkanes containing at least one bromine atom add to rubber in the presence of peroxide. Carbon tetrabromide and trichlorobromomethane show high reactivity. The derivatives are prepared by adding the halogen compounds together with tert-butyl hydro peroxide to the stabilized latex. The reaction is allowed to proceed for about three days at room temperature. The products are elastic and vulcanizable and show some flame resistance. The best combination of physical properties and flame resistance is exhibited by a product containing 15 - 20 % by weight of the halogen compound.

The latex derivative[106] is suitable for the production of rubber-bonded hair pads and flame-resistant latex form for spreading on carpets or fabrics.

6. Epoxidized Rubber

Natural rubber can be epoxidized in solution or in the latex stage by peracids. The reagent may be conveniently prepared *in-situ* to avoid side reactions. The early materials were probably contaminated with products of ring opening reactions. In the presence of strong mineral acids at low epoxidation, diols are formed. In the presence of free acids at high epoxidation and high temperature, the hydroxyl group attaches to an adjacent epoxide group to give a substituted furan. This reaction can continue along the chain to give a polymeric 1,5-disubstituted furan structure of various lengths.

Epoxidized natural rubber is prepared from latex with performic acid generated *in-situ*. The latex is stabilized with a nonionic stabilizer and formic acid and hydrogen peroxide are added. The reaction is carried out at 30-65° C for different periods of time, depending on the degree of epoxidation required. The epoxidized latex is washed and dried. The acid is neutralized with base before or after coagulation.

7. Halogenated rubber

Natural rubber has been halogenated (e.g. chlorinated); the reaction is complex. Fully chlorinated natural rubber contains about 65 % chlorine, the empirical formula of which is $C_5H_8Cl_{3.5}$. This suggests that the chlorination reaction involves more than one isoprene unit and since the products are soluble, cyclization rather than cross-linking is indicated. Studies by Bloomfield[107] showed that light, oxygen and peroxides did not affect the rate of reaction, which would, therefore, seem not to be of the free radical type. He also showed that the reaction occurred in three stages represented empirically by the following equation.

$$C_{10}H_{16} + Cl_2 \longrightarrow C_{10}H_{14}Cl_2 + ClH$$

$$C_{10}H_{14}Cl_2 + Cl_2 \longrightarrow C_{10}H_{13}Cl_5 + ClH$$

$$C_{10}H_{13}Cl_5 + Cl_2 \longrightarrow C_{10}H_{11}Cl_7 + ClH$$

Figure 1.7: Chlorination of natural rubber

In the first stage it was shown that one molecule of hydrochloric acid was liberated for each molecule of chlorine absorbed, and there was a considerable drop in the unsaturation of the rubber. Since a simple substitution reaction should not reduce unsaturation, this suggests that at least one rearrangement process is occurring such as cyclization. The ultimate structure of chlorinated natural rubber is not known, but one suggested structure is as follows.

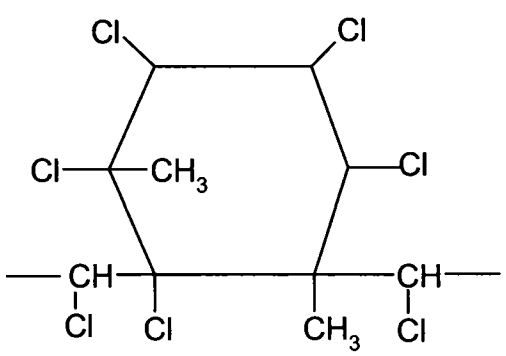


Figure 1.8: Chlorinated natural rubber

The intrinsic viscosity of chlorinated rubber is much lower than that of NR for a given molecular weight. This suggests a more compact molecule, may be a cyclized structure rather than a linear one. Chlorinated rubber requires plasticizer to reduce the brittleness of the film. Choice of plasticizer gives the possibility of

matching the protective quality of the chlorinated rubber finish. The resistance of chlorinated rubber decreases as the temperature is increased. Chlorinated rubber finds various applications in paint and lacquers, moisture proofing, adhesives and heat insulation.

Bromination of polyisoprene appears to be less complicated. If bromine is passed into a solution of rubber in chloroform in the temperature range of 0 -40 °C the reaction is largely additive to give $(C_5H_8Br_2)_n$ although some substitution may occur. Different results are obtained by the use of N-bromosuccinimide. This reagent is used for the specific purpose of brominating alkenes at the allylic position. In natural rubber this type of bromination proceeds via free radical intermediates to give products substituted in the allylic position. Side reactions are said to lead to cyclization or cross linking.

Fluorine reacts very violently and destructively with rubber and product containing 30 % of fluorine has been obtained and these are quite rubbery and can be used for gaskets in fluorine generators and fluorine lines.

1.8.3 APPLICATION AREAS OF NATURAL RUBBER

With its wide range of properties, natural rubber can be used in a large variety of applications. Despite this the share of natural rubber in the elastomer market has decreased progressively since World War II. This is partly due to the higher price of natural rubber relative to SBR and partly due to inadequate supplies. The increase in the share of natural rubber in the last few years is due to the large switch to radial tires in the United States and elsewhere.

1. Tires

In passenger-car-radial-ply tires, natural rubber is used in the carcass as well as the sidewall; the latter due to the superior fatigue resistance and low heat build up of

natural rubber. In commercial vehicles, the amount of natural rubber used increases with the size of the tyre. In large earthmover tires, for example, almost 100 % natural rubber is used due to the requirements of low heat generation and high cutting resistance. Natural rubber is used in blends with halo butyl rubbers in the inner liner of tubeless tires.

2. Mechanical Goods

These include a large variety of products such as hose, conveyor belts, rubber linings, gaskets, seals, rubber rolls, rubberized fabrics etc. In these products, the choice of elastomer is made on the best compromise between price and performance. Natural rubber is used in some products only because it has certain properties that cannot be matched by any other rubber.

3. Engineering Products

Rubber is a unique engineering material because, unlike other engineering solids, it has high elastic deformability and an almost theoretical value for Poisson's ratio. The stiffness of a natural rubber component in different directions may be varied independently by the judicious use of shape effects. In dynamic applications such as springs, anti-vibration mountings, bushings, and so forth, high fatigue resistance, good strength, and durability are additional points in favor of natural rubber. Natural rubber is now accepted as suitable for use in bridge bearings, in place of neoprene.

4. Latex goods

Natural rubber latex has now been largely replaced by polyurethane in foam for upholstery and bedding. The main uses of latex are in dipped goods, foam, carpet backing, thread and adhesives. Natural rubber is extremely suitable for rubber footwear manufacturing.

Important trends in rubber chemistry have taken place in every decade for the past hundred years. The future of rubber chemistry is certainly challenging but may well take on different directions.

Keeping in view, the ample potentials of radiopaque polymers, an attempt has been made in the present investigation to develop radiopaque systems from natural polymers, which are promising to be applicable in the medical field. Most of the conventional radiopaque systems are based on synthetic polymers. Radiopaque systems based on natural polymers are found scarcely. In such a scenario, owing to their excellent performance characteristics, chitosan and natural rubber were selected for present work.

1.9 SCOPE AND OBJECTIVES OF THE WORK

The work devoted to investigate the properties and applications of radiopaque polymers is meagre when compared with the volume of literature available on polymers for biomedical applications. The primary objective of the work is to impart radiopacity in selected natural polymers and to highlight their applications in medical field.

The specific objectives of the work are:

1) To select a suitable emulsion system for the preparation of chitosan microspheres and to study the effect of emulsion systems on the morphology of chitosan microspheres.

2) To introduce radiopacity in chitosan microspheres by the encapsulation of barium sulphate.

3) To prepare and characterize water soluble derivatives of chitosan like carboxy methyl chitosan, chitosan acetate and chitosan formate and to prepare radiopaque microspheres using these derivatives.

4) To prepare and characterize radiopaque natural rubber (i) by the incorporation of radiopaque fillers like zinc oxide and barium sulphate and (ii) by the iodination of natural rubber in the latex stage.

5) To study the radiopacity, physico-chemical and morphological characteristics of the polymer systems used for the investigations.

6) To prepare zinc oxide having different surface morphology using chitosan medium by an *in-situ* precipitation method and to study its effects on radiopacity in NR.

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CHAPTER 2

EXPERIMENTAL TECHNIQUES

The materials used and the experimental procedures adopted in the present investigation are detailed in this chapter. The techniques used for characterization of materials are Scanning electron microscopy, Thermo gravimetric analysis, Infrared spectroscopy, UV-Visible spectroscopy and X-ray Diffraction spectroscopy. Radiopaque natures of the materials are confirmed using general clinical X-ray analyzer and Densitometer. Invitro analysis of antibacterial properties are done using Zone of inhibition method.

2.1 MATERIALS AND METHODS

2.1.1 PREPARATION OF MICROSPHERES BASED ON CHITOSAN

- **Chitosan and its derivatives**

Chitosan was obtained from India Sea Foods, Cochin, India. Chitosan samples having viscosities 55 cps and 330 cps were used for the studies. The specifications of samples are given below.

Ash (%)	-	0.45
Protein	-	Nil
Moisture (%)	-	8.35
Degree of Deacetylation (%)	-	84.72
pH	-	7.41
Particle size (mesh)	-	24

- **Liquid paraffin**

Light liquid paraffin was purchased from Merck India Ltd. Heavy Liquid Paraffin was obtained from Reidel (India) chemicals. Specifications of the samples are as follows:

	Light liquid paraffin	Heavy liquid paraffin
Density (g/cc)	0.83-0.86	0.86-0.89
Viscosity (cps)	$\geq$ 30	$\geq$ 64

- **Glutaraldehyde**

Glutaraldehyde (assay 25 %, density 1.059-1.063 g/cc) was collected from Loba chemic.

- **Sorbitan sesqueoleate**

Sorbitan sesqueoleate was obtained from Sigma Aldrich.

- **Naphthenic oil**

Naphthenic oil was supplied by Hindustan Organic Chemicals, Cochin; the sample had a viscosity gravity constant of 0.87.

- **Silicone oil**

Silicone oil used was commercial grade obtained from Merck India Ltd.

- **Oleic acid**

Oleic acid used for the preparation was obtained from s.d fine chemicals, Mumbai.

- **Barium sulphate**

Barium sulphate used for the preparation of radiopaque systems was obtained from Merck India Ltd.

- **Solvents**

Solvents like acetone, heaxane, methanol, toluene etc were supplied by s.d fine chemicals Chemicals Ltd., Mumbai, India. Acetic acid and Ammonium hydroxide were purchased from Nice Chemicals, India.

➢ ***Preparation***

Chemical cross linking method was used for preparation of chitosan microspheres. It involves the emulsification followed by cross-linking with a suitable reagent. Different emulsion systems like naphthenic oil/ammonium oleate, silicone oil/ammonium oleate and liquid paraffin/sorbitan sesqueoleate were used for preparation of chitosan microspheres. Glutaraldehyde was used as the cross linking agent and barium sulphate was used to impart radiopacity.

Microspheres of water soluble derivatives of chitosan were prepared using liquid paraffin /sorbitan sesqueoleate emulsion system.

2.1.2 PREPARATION OF FILLED RADIOPAQUE NATURAL RUBBER

Barium sulphate ($BaSO_4$) and zinc oxide (ZnO) were used as radiopacifying fillers in natural rubber. The rubber chemicals used for the preparation of radiopaque natural rubber are given below.

- **Natural rubber (NR)**

Natural rubber used was solid block rubber (ISNR-5) obtained from Rubber Research Institute of India, Kottayam, having the Mooney viscosity [ML (1+4) 100 °C = 85.3]. The Bureau of Indian standard (BIS) specifications for this grade of rubber is given below.

Volatile matter (% max)	-	1.00
Nitrogen content (% max)	-	0.70
Ash content (%)	-	0.60
Initial Plasticity index P_0 (min)	-	30.00
Plasticity Retention Index, PRI (min)	-	60.00

- **Zinc oxide**

Zinc Oxide was obtained from Meta Zinc Ltd., Bombay. It has the following specifications:

Specific gravity	-	55
Purity (%)	-	99.85
Heat loss (2 h at 100 °C) (% max)	-	0.5

- **Stearic acid**

Stearic acid used was supplied by Godrej soaps Ltd., Bombay and has the following specifications:

Melting point (°C)	-	50 - 69
Acid number	-	185 - 210

- **Tetra methyl thiuram disulphide (TMTD)**

TMTD was supplied by NOCIL India Ltd, Bombay, having the following specifications:

Melting point (°C)	-	136
Specific gravity	-	1.4

- **N-cyclo hexyl-2-benzthiazyl sulphenamide (CBS)**

N-cyclo hexyl-2-benzthiazyl sulphenamide was supplied by Polyolefin's Industries, Bombay; having the following specifications:

Moisture (%)	-	0.5
Specific gravity	-	1.27

- **Sulphur**

Sulphur was supplied by Standard Chemical Company Pvt. Ltd., Chennai and had the following specifications:

Specific gravity	-	2.05
Ash (% max)	-	0.01

Solubility in CS_2 (% max) - 98

2.1.3 IN SITU PRECIPITATION OF ZINC OXIDE

Zinc chloride having purity of 97 % was supplied by Universal Laboratories, Mumbai. Zinc acetate (purity 98.5 %) and Zinc nitrate (purity 96 %) were supplied by s.d Fine Chem. Ltd, Mumbai. Chitosan having viscosity of 330cps was used.

➢ ***Preparation***

Zinc oxide was precipitated from zinc chloride, zinc nitrate and zinc acetate, in chitosan medium. The zinc oxides formed were calcined.

2.1.4 PREPARATION OF IODINE DOPED RADIOPAQUE NATURAL RUBBER

- **Natural rubber latex**

High ammonia type 60 % centrifuged latex was obtained from Njavallil Latex Ltd. The specifications are,

Dry rubber content (%) - 60.04

Total solid content (%) - 61.05

Alkalinity as ammonia (%) - 0.73

- **Hydrochloric acid**

Hydrochloric acid having an assay 37-38 %, supplied by s.d. fine-chem. Ltd, Mumbai.

- **Potassium iodide**

Potassium iodide having an assay 99.8 % was obtained from s.d. fine-chem. Ltd., Mumbai, India.

- **Iodine**

Iodine was supplied by Qualigens fine chemicals, Mumbai, India.

- **Vulcastab VL**

Vulcastab VL was obtained from ICI Kolkata, India.

- **Potassium oleate**

Potassium oleate was prepared by the reaction between oleic acid and potassium hydroxide. 10 % solution of potassium oleate was prepared by warming a stoichiometric mixture of oleic acid and potassium hydroxide solution.

- **Preparation of INR**

Centrifuged latex was stabilized using potassium oleate and vulcastab VL. It was then acidified with 1N HCl. The acidified latex was iodinated and the coagulam of iodinated rubber was washed several times with water and was dried at room temperature. The iodinated natural rubber (INR) was cured at high temperature and at low temperature.

- **Preparation of INR vulcanizates**

The mixing was done using a laboratory size two roll mixing mill at a friction ratio of 1:1.25 as per ASTM D 3184 (1980) and ASTM D 3182 (1982). After complete mixing, the stock was passed six times through the tight nip of the mill and finally sheeted out at a fixed nip gap. The samples were kept overnight for maturation.

- **Cure time determination**

Cure characteristics has been studied using a Rubber Process Analyzer (RPA2000, Alpha Technologies). The die type used was biconical and the die gap was 0.487.

The cure times of the samples were determined at 150° C at a frequency of 50.0 and a strain of 0.20 deg.

- **Compression moulding**

Blanks cut from unvulcanised sheets marked with the machine direction were vulcanized at a temperature of 150 ± 2° C and at a pressure of 200 kg/cm^2 in an electrically heated hydraulic press to their respective optimum cure times. Rectangular moldings were cooled quickly in water at the end of each curing cycle and were used for subsequent property measurements.

- **Mechanical properties**

Dumb bell shaped tensile specimens were punched out from the vulcanized sheets and the mechanical properties were studied using a Shimadzu Universal Testing Machine (Model-AGI) with a load cell of 10 kN capacity as per ASTM D 421-68. The gauge length between the jaws at the start of each test was adjusted to 30 mm and the measurements were carried out at a cross head speed of 500 mm/min.

2.2 CHARACTERIZATION TECHNIQUES

2.2.1 SCANNING EECTRON MICROSCOPY (SEM)

Scanning electron microscopic studies were found to be a very powerful tool in polymer research for studying the morphology[2,3]. Scanning electron microscopy allows the imaging of the topography of a solid surface by using a back scattered or secondary electrons with good resolution of about 5 nm. In this technique, a fine probe of electron is scanned over the sample surface using deflection coils. The interaction between the primary beam and the specimen produces various signals, which are detected, amplified and displayed on a cathode ray tube screened synchronously with the beam. They can also be conveniently deflected and focused by electronic or magnetic field so that magnified real-space images

can be formed. This makes the technique suitable to produce very impressive, in-focus images from highly irregular structures. In the present investigation, a Philips Scanning Electron Microscope (model XL30 ESEM FEG) was used to study the morphology of different chitosan microspheres and zinc oxide particles. The samples were sputtered with gold.

2.2.2 POWDER X-RAY DIFFRACTION (XRD)

Powder X-ray diffraction is a standard method for the characterization and has been used to determine the crystalline phases, solid solutions, that are present and to measure the particle size and shape. Monochromatic X-rays, incident on a crystalline solid, are diffracted owing to the crystal structure of the solid[4]. For a maximum to occur in the diffraction pattern at a particular angle of incidence θ (with respect to lattice planes (hkl), the Bragg equation must be satisfied:

$$n\lambda = 2d\sin\theta \qquad (1)$$

Where; d -interplanar distance between (hkl) planes, n -order of diffraction and λ wavelength of incident X-rays.

Crystallite size was calculated using the Scherrer equation,

$$L = (0.9\lambda 180)/(\pi FWHM \cos\theta) \qquad (2)$$

Where, $FWHM_{hkl}$ is the full width at half-maximum of an *hkl* peak at θ value.

Powder X-ray diffraction data were recorded using Rigaku D-Max Ni filtered Cu K_α radiation (λ=1.54 A°) diffractometer equipped with a monochromatic diffracted beam at a scan rate of 4 °/min. Peak width at half-height was used to assess the relative crystallinity of the materials.

2.2.3 THERMAL ANALYSIS

Thermal analysis includes a group of techniques in which specific physical properties of a material are measured as a function of temperature. Thermo gravimetric analysis (TGA) provides a quantitative measurement of weight changes associated with thermally induced transitions. It can directly record the loss in weight as a function of temperature or time[5]. In TGA, the weight of sample is continuously recorded as the temperature increased. Samples were placed in a crucible that is positioned in a furnace on a quartz beam attached to an automatic recording balance. The horizontal quartz beam is maintained in the null position by the current flowing through the transducer coil of an electromagnetic balance. Any change in the weight of the sample causes a deflection of the beam, which is sensed by one of the photodiodes connected to act as a position sensor to determine the movement of the beam. The beam is then restored to the original null position by a feedback current sent from the photodiodes to the coil of the balance and the current is proportional to the change in weight of the sample.

TGA/DTG were done on a Thermo gravimetric analyzer (TGA Q 50, TA Instruments) under nitrogen atmosphere at a heating rate of 20° C/ min from room temperature to 800° C.

2.2.4 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FT-IR)

Infrared spectroscopy is considered as the first and the most important of the modern spectroscopic techniques that has found general acceptance in polymer structure analysis. Vibrations in molecules or in solid lattices are excited by the absorption of photons, which is the basic principle of infrared spectroscopy. Infrared spectroscopic investigations can be used to characterize active centers on catalysts surfaces and chemisorbed molecules. It involves examination of the twisting, bending, rotating and vibrational motions of atoms in a molecule. Upon

interaction with infrared radiation, portions of the incident radiation are absorbed at specific wavelengths. The infrared spectrum of a compound is essentially the superposition of absorption bonds of specific functional groups, yet subtle interactions with the surrounding atom of the molecule impose the stamp individually on the spectrum of each compound[6]. Infrared spectra were recorded with KBr pellets on a Bruker FTIR (model- Tensor 27) in the range 400-4000 cm^{-1}.

2.2.5 UV-VISIBLE SPECTROSCOPY

UV-Visible spectrum was taken using a HITACHI-330 model spectrophotometer. The absorption of ultra violet or visible radiation by polymers leads to transitions among the electronic energy levels of the macro molecules, and as a result of this, a typical electronic absorption spectrum is obtained. The ultra violet and visible spectroscopy yield information on multiple bonds and aromatic conjugation within the macromolecules. The non bonding electrons on oxygen, nitrogen and sulphur may also be involved in extending conjugation of multiple bond systems in polymers[7].

2.2.6 CONDUCTIVITY MEASUREMENTS

Room temperature D.C. electrical conductivity of the samples was measured using keithley nanovoltmeter (model 2400) using four probe electrode configuration. The samples had the dimensions 60 x 20 x 3 mm.

2.2.7 IN-VITRO ANALYSIS OF ANTIBACTERIAL PROPERTY

The micro organism needed for the experiment was sub cultured on to agar plates and incubated overnight at 37°C. Antimicrobial properties were assessed using zone inhibition method. Small pieces of iodinated coagulum and control compound were plated separately onto agar plates streaked with E.coli. Plates

were incubated at 37° C for 24 h and were examined for a zone of inhibition around the pieces.

2.2.8 RADIOPACITY STUDIES

Radiopacity of different systems were studied using a general clinical X-ray instrument of 40 kV energy and 2 MAs current. In this technique, the radiation from X-ray tube is transmitted through the material and reaches the film. After processing the film (the radiograph) the negative image is obtained[7]. The filled radiopaque NR vulcanizates were compression molded to 1 cm thick and 60 mm diameter for radiopacity studies. X-ray films of the samples are using Collimex general X-ray instrument.

2.2.9 OPTICAL DENSITY MEASUREMENTS

The optical density was measured using densitometer. It is a device for determining the degree of darkening of X-ray film or photographic film and hence the amount of radiation received. This instrument consists of a light source, a tiny aperture through which the light is directed and alight detector (photocell) to measure the light intensity transmitted through the film. The term optical density refers to the degree of blackening of the film. The degree of blackness is directly related to the intensity of radiation. In other words the measurement of blackness is called photographic density or optical density (OD)[8]. The optical density of X-ray films were measured using a Calden densitometer (CDIT). The optical desity is directly proportional to radiation exposure. Figure 2.1 shows the densitometer used for the study.

Figure 2.1: Densitometer used for optical density measurements

Optical density was calculated using equation,

$OD = \log_{10} (I_0/I_t)$,

Where I_0 is the intensity of light incident on the film and I_t is the intensity of the light transmitted through the film. I_0/I_t is the light stopping effect and opacity.

A densitometer gives a direct reading of optical density. The transmittance is the fraction of I_t / I_0 of the incident light passing through the film. As the optical density OD increases, transmittance decreases[9].

2.2.10 CALCULATION OF EFFECTIVE ATOMIC NUMBER (EAN)

Effective atomic number is denoted by $\overline{Z}$.

It is defined as the atomic number of an element with which photons interact the same way as with the given composite material.

$\overline{Z} = (a_1 z_1^{2.94} + a_2 z_2^{2.94} + \ldots\ldots\ldots\ldots + a_n z_n^{2.94})^{1/2.94}$, Where, a_1, a_2..... are fractional contents of each element to the total number of electrons in the mixture; Z_1, Z_2, ... refers to the atomic number of the corresponding element[9].

2.2.11 DETERMINATION OF ATTEUNATION COEFFECIENT

For the determination of attenuation coefficient, the X-ray photographs are taken using cone in order to reduce scattering. The atteunation coeffecient is calculated using the equation

$$I/Io = e^{-\mu x}$$

Where I_0 is the value of optical density of background of the film and I_{is} the optical density of the sample 'x' the thickness of the sample and 'μ' the attenuation coefficient.

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CHAPTER 3

PREPARATION AND CHARACTERIZATION OF RADIOPAQUE CHITOSAN MICROSPHERES

3.1 INTRODUCTION

Polymeric beads with diameter in the micrometer range find use in various medical applications, such as bone cements[1], in local delivery of drugs[2] and in treatment of female stress urinary incontinence.[3,4] Medical microspheres usually consists of poly (methyl methacrylate), poly (lactic acid-co-glycolic acid), poly (tetra fluoroethylene) or silicone rubbers. A particularly important application of microspheres relates to their use as so-called bulking agents; microspheres are injected via a syringe, usually as a suspension. The practical utility of microspheres with X-ray visibility lies in the fact that the clinician can assess possible migration in a direct manner. Polymers encapsulated with radiopacifying agents have recently been proposed as particulate emboli in endovascular embolization and for investigation of a variety of gastrointestinal disorders. Radiopaque hydrogel microspheres derived from many natural and synthetic

polymers such as collagen, gelatin, cellulose, silicones and acrylates have been used as embolization agents[5]. Non-biodegradable hydrogel microspheres, which possess high hydrophilicity, compressibility, swelling ability and biocompatibility have recently been shown to perform better in vascular occlusion[6,7]. Radiopaque hydrogel microspheres were commonly prepared by the encapsulation of barium sulphate. Barium sulphate encapsulated poly vinyl alcohol[8], poly (methyl mathacrylate), poly (hydroxyethyl methacrylate)[9,10] and poly (ethylene) were reported.

During the past few decades, there has been a significant increase of interest in using natural polymers for various medical applications. Different types of polysaccharides such as agar, alginate, carrageenan, chitin and chitosan have been used in different fields of medicine. Chitin is the second most abundant natural polymer and is found as a structural component of crustacean shells and fungal cell walls. Chitosan is derived from chitin via deacetylation with an alkali. Chitosan has been reported as a rather imperative matrix in a variety of pharmaceutical, environmental and biotechnological applications due to its excellent properties like biocompatibility, low toxicity, and chemical inertness, good film forming properties, high mechanical strength and hydrophilicity. Chitosan is a copolymer of linked β (1-4), 2-amino-2-deoxy-D-glucan and 2-acetamidodeoxy-D-glucan. Chitosan has been extensively examined for its potential in the development of controlled release drug delivery systems in the form of chitosan gels, tablets, capsules, microcapsules and microspheres[11].

In this chapter the studies on the preparation, characterization and radiopacity of chitosan microspheres are reported. The chitosan microspheres are prepared using different emulsion systems and are converted in to radiopaque by the incorporation of barium sulphate. The synthesis and radiopacity of chitosan derivatives and chitosan / PVA blends are also reported.

PART I

3.2 STUDIES ON RADIOPAQUE CHITOSAN MICROSPHERES

3.2.1 INTRODUCTION

Microcapsule is defined as a spherical particle with size varying from 50 nm to 2nm, containing a core substance. Microspheres are in a strict sense, spherical empty particles. However, the terms microcapsules and microspheres are often used synonymously. In addition, some related terms like microbeads and beads are used alternatively. Recently Yao[12] *et al* highlighted the preparation and properties of chitosan microspheres and microcapsules. Chitosan microspheres have been prepared by chemical denaturtion, ion-induced coagulation spray drying methods and multiple emulsion techniques.

Spray drying includes four sequential stages: atomization through a spray nozzle, contact of sprayed feed with warm air, drying of the droplets and collection of the solid chitosan. In drug delivery, chitosan solutions with drug can fed to a spray drier at a slightly acidic pH. The size of the particle is influenced by various process parameters such as size of the nozzle, rate of feeding and inlet air temperature. The inlet air temperature is measured prior to flowing into the drying chamber and may be set at 160° C or higher; however the gradient between the wet surface and unsaturated gas actually leads to evaporation at much lower temperatures. The spray drying technique has been applied to chitosan suspensions, chitosan salts, chitosan (gelatin–ethylene oxide) and chitosan-ethyl cellulose mixture.[13,14] These microspheres are most suitable as a drug carrier.

The multiple emulsion techniques include three steps: Preparation of primary oil/water emulsion in which the oil dispersed phase is constituted of CH_2Cl_2 and

the aqueous continuous phase is a mixture of 2 % v/v acetic acid solution: methanol (4/1) containing Chitosan (1.6 %) and tween (1.6 w/v); (2) Multiple emulsion formation with mineral oil (oil outer phase) containing span 20 (2 % w/v); (3) Evaporation of aqueous solvents under reduced pressure.[15] Chemical cross linking is an option of this method if the cross linking agent is added just after the emulsion formation; enzymatic cross linking can also be performed.[16] Physical cross linking may take place to a certain extent if chitosan is exposed to high temperature. The emulsion technique is convenient when the drug is particularly sensitive to certain parameters connected to the spray drying. The emulsion technique may associate to cross linking or other treatments of the microspheres.

Microspheres of chitosan, cross linked with glutaraldehyde, sulfuric acid or heat treatment, have been prepared to encapsulate diclophenac sodium by Kumbar et al[17]. In many studies chitosan has been crosslinked with glutaraldehyde to make it a rigid polymer to be used as a core material in controlled drug delivery.[18] Chitosan microspheres were produced in water-in-oil emulsion followed by cross linking in the water phase. The cross linking of Chitosan took place at the free amino groups in all cases and lead to the formation of imine groups or ionic bond. Polymer crystallinity also increases after cross linking. Micro spheres have smooth surfaces with size in the range of 40-230 μm.

Of these methods the most common method used to prepare chitosan microspheres is the chemical denaturation method. Chemical denaturation involves denaturation of chitosan in the inner phase of water/oil (w/o) emulsion. Denaturation is usually carried out using glutaraldehyde with continues stirring. The chemical cross linking method for the preparation of chitosan microspheres involves emulsification followed by cross linking.

In this part, a method for the preparation of radiopaque chitosan micro spheres encapsulated with $BaSO_4$ is reported. An attempt is made to prepare radiopaque chitosan microspheres using different emulsion systems. Three emulsion systems, namely, silicone oil/ammonium oleate, naphthenic oil ammonium oleate and liquid paraffin/sorbitan sesqueoleate are used for the study. The micro spheres are characterized by SEM, XRD and IR spectroscopy. The radiopaque nature of the micro spheres is confirmed by their X-ray images.

3.2.2 EXPERIMENTAL

- ***Materials Used***

1. Chitosan
2. Barium Sulphate
3. Silicone oil
4. Naphthenic oil
5. Liquid paraffin
6. Ammonium oleate
7. Sorbitan sesqueoleate
8. Glutaraldehyde

- ***Preparation of Chitosan encapsulated Barium sulphate microspheres***

Chitosan solution in acetic acid was used for the preparation of chitosan microspheres. The cross linking reaction of chitosan with glutaraldehyde is an instantaneous one. 3 % solution chitosan having a viscosity of 55 cps is used for this study.

1. Using silicone oil/ammonium oleate and naphthenic oil/ ammonium oleate systems

The dispersion medium was prepared by mixing 80 ml silicone oil, 20 ml water, 0.38g ammonia solution and oleic acid (3.2 g). The mixture was stirred for 30 minutes. A paste of chitosan containing barium sulphate was added. It was vigorously stirred for 2 min. Then glutaraldehyde saturated with toluene was added and vigorously stirred for another 2 min. Aqueous glutaraldehyde solution was added at every half an hour interval and the reaction was continued for a total of 3 h. After the reaction, it was filtered, washed with acetone, water and then dried in vacuum at room temperature.

Microspheres were also prepared in naphthenic oil using the same method.

2. Using liquid paraffin/sorbitan sesqueoleate system

Equal volumes of heavy liquid paraffin and light liquid paraffin were mixed and the mixture was taken in a plastic beaker. 1 ml of sorbitan sesqueoleate was added and stirred well. Then a free flowing paste of 3 % chitosan solution containing $BaSO_4$ was added while stirring. Glutaraldehyde saturated with toluene was added and the stirring was continued at the same speed for 5 min. After 5 min the stirring speed was reduced. Aqueous glutaraldehyde solution was then added at every 30 min intervals and the stirring was continued at room temperature for 3 h. After the reaction the spheres were filtered off, washed several times with hexane and water. The spheres were again washed with plenty of ice-cold water to remove excess acetic acid and glutaraldehyde. The microspheres were then dried in vacuum at room temperature.

3. Radiopaque microspheres from the blend of Poly vinyl alcohol (PVA) and Chitosan

A 4 % solution of 55cps chitosan in acetic acid was blended with 4 % solution of Poly vinyl alcohol (PVA). A paste of barium sulphate and this blend was added to liquid paraffin/sorbitan sesqueoleate system. It was then cross linked with glutaraldehyde. The reaction was continued for 3 h. The microspheres obtained were washed several times with hexane and with plenty of ice-cold water to remove excess acetic acid and glutaraldehyde. The spheres were then dried in an air oven at 50° C.

3.2.3 RESULTS AND DISCUSSION

3.2.3.1 Studies on Chitosan microspheres prepared in Silicone oil/ ammonium oleate and Naphthenic oil/ammonium oleate emulsion system

The SEM photographs of microspheres prepared from silicone oil/ammonium oleate emulsion systems, washed with diethyl ether and acetone are shown in figures 3.1 and 3.2 respectively. It is clear from the photographs that the microspheres prepared from naphthenic oil/ammonium oleate emulsion system do not possess good spherical geometry and surface smoothness. It may be due to the instantaneous cross linking reaction. It is also clear from the figure 3.1 that the diethyl ether washing give only irregular micro particles.

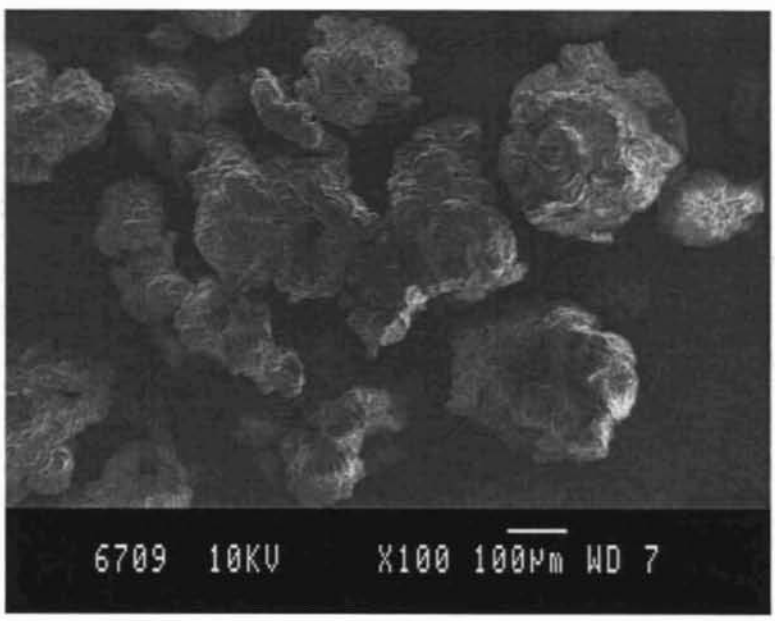


Figure 3.1: SEM photograph of microspheres prepared from Silicone oil/ammonium oleate system with diethyl ether washing (Sil/DEE)

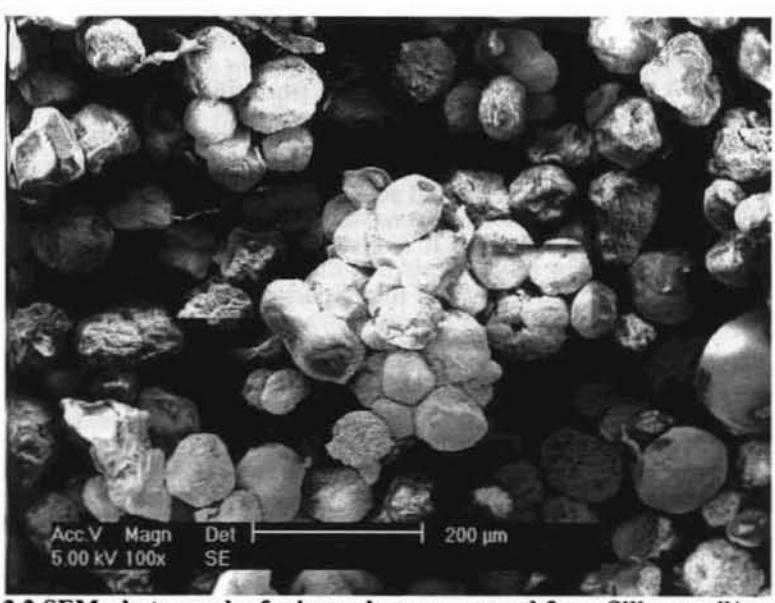


Figure 3.2 SEM photograph of microspheres prepared from Silicone oil/ammonium oleate system with acetone washing (Sil/ACT)

In acetone washing the particles formed are found to be non spherical. They are of non-uniform size and are not smooth as evident from the figure 3.2. Large clumping is observed in these spheres.

Figures 3.3 and 3.4 shows the SEM micrographs of microspheres obtained from Naphthenic oil/ammonium oleate system with diethyl ether washing and acetone washing respectively. In Naphthenic oil/Ammonium oleate system after washing in diethyl ether, the particles lost their spherical shape as shown in figure 3.3. Perfectly spherical microspheres are obtained after acetone washing, but large clumping is observed in these spheres as in the case of silicone oil/water emulsion system.

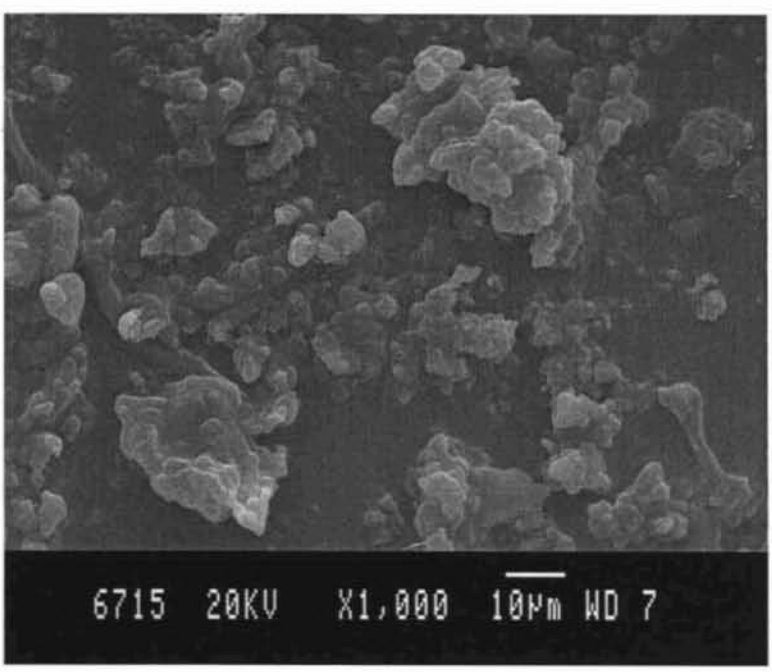


Figure 3.3: SEM photograph of microspheres obtained from naphthenic oil/ammonium oleate system with diethyl ether washing (NO/DEE)

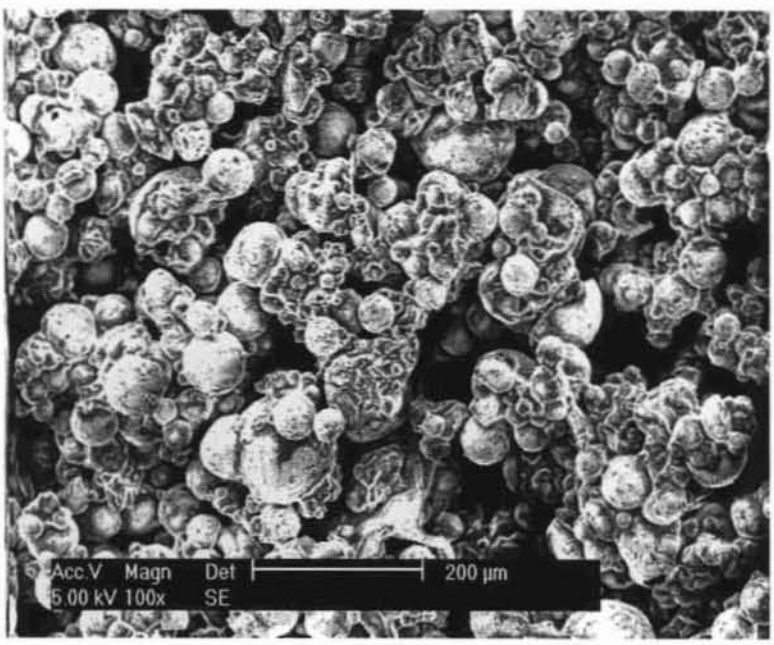


Figure 3.4: SEM photograph of microspheres obtained from naphthenic oil/ammonium oleate system with acetone washing (NO/ACT)

Since the naphthenic oil/ammonium oleate and silicone oil/ammonium oleate systems does not give smooth spherical microspheres of chitosan, these microspheres are not taken for further detailed studies.

3.2.3.2 Studies on Chitosan microspheres prepared in liquid paraffin oil/ sorbitan sesqueoleate system

a. Characterization

- **SEM Analysis**

The SEM micrographs of chitosan microspheres prepared from liquid paraffin / sorbiton sesqueoleate system is shown in figure 3.5 (a and b). The microspheres

formed have smooth surfaces, with sizes in the range of 30-240 μm. In liquid paraffin/sorbitan sesqueoleate system cross linking reaction takes place in a slow and uniform manner in order to generate microspheres of good spherical geometry and non-agglomeratory in nature. The degree of stirring (i.e., time and speed of stirring during emulsification) affects the size of dispersed droplets. The particle size depends on the viscosity of the dispersant and the dispersion medium, concentration of the stabilizing agent and stirring speed. It is also observed that the barium sulphate is firmly trapped inside the microspheres, as it did not leach out on prolonged standing in water on sonication. Chitosan microspheres obtained from liquid paraffin/sorbitan sesqueoleate shows better surface morphology than other two systems. Hence for further detailed studies we have chosen microspheres prepared from liquid paraffin/sorbitan sesqueoleate system.

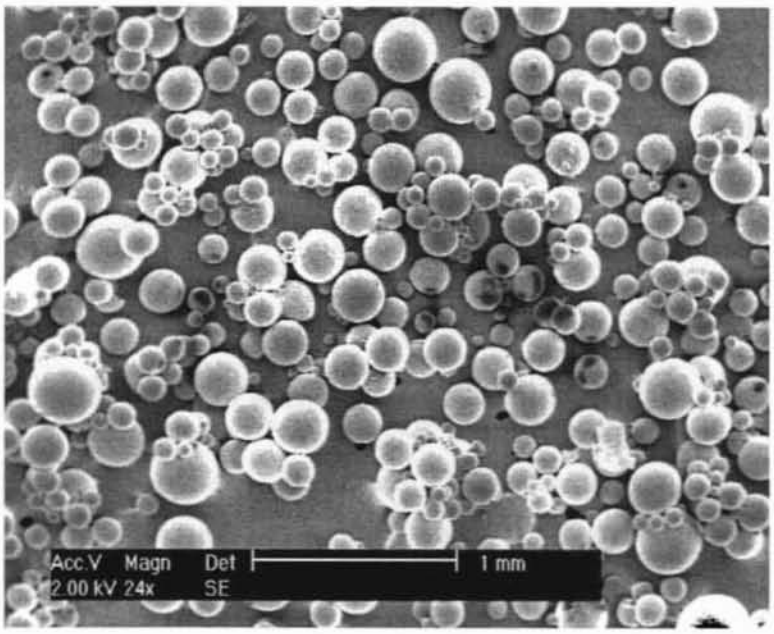


Figure 3.5 a: SEM photograph of microspheres obtained from paraffin oil/sorbitan sesqueoleate system

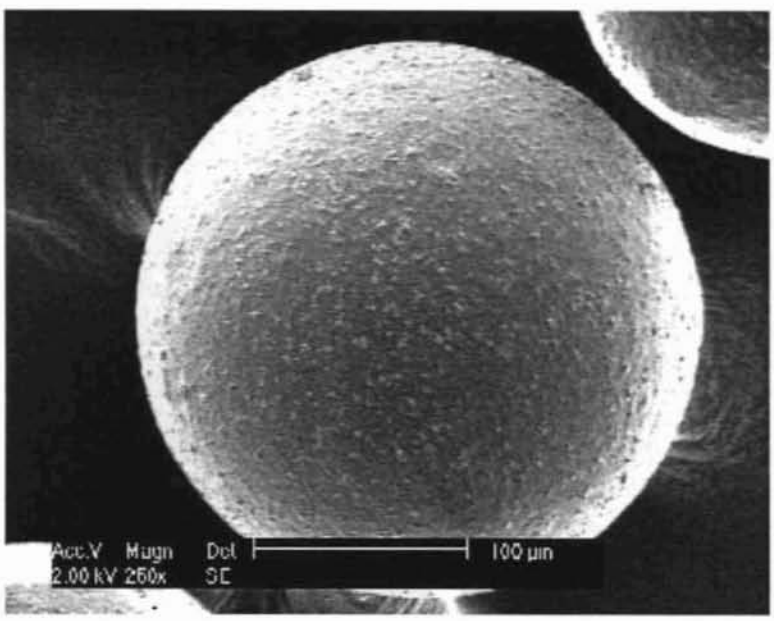


Figure 3.5 b: SEM photograph of Single microsphere obtained from liquid paraffin oil/sorbitan sesqueoleate system

- **Infrared Spectroscopy**

Figures 3.6 and 3.7 show the IR spectra of chitosan microspheres and chitosan/barium sulphate microspheres respectively. In figure 3.6, a broad peak in the range 3350 to 3300 cm^{-1} is observed. The peaks are assigned to an -OH stretching, indicating inter molecular H-bonding. The spectra also overlapped in the same region of a -NH stretching[19]. Also a peak at 1647 cm^{-1} representing the stretching vibrations of C=N bond, confirms the formation of chitosan – glutaraldehyde crosslinks. The NH_2 stretching peak at 1600 cm^{-1} indicates the presence of glucosamine functional group and the characteristic bands at 2879 cm^{-1} and 1300 cm^{-1} represents the protonated amine stretch and deformation vibrations.

Figure 3.7 shows a peak position of OH stretching at 3000 cm^{-1}. This low frequency shift is due to the interaction of barium sulphate and chitosan chain. The IR data suggested that there is an association between chitosan and barium sulphate ions and that may be link the chitosan chains.

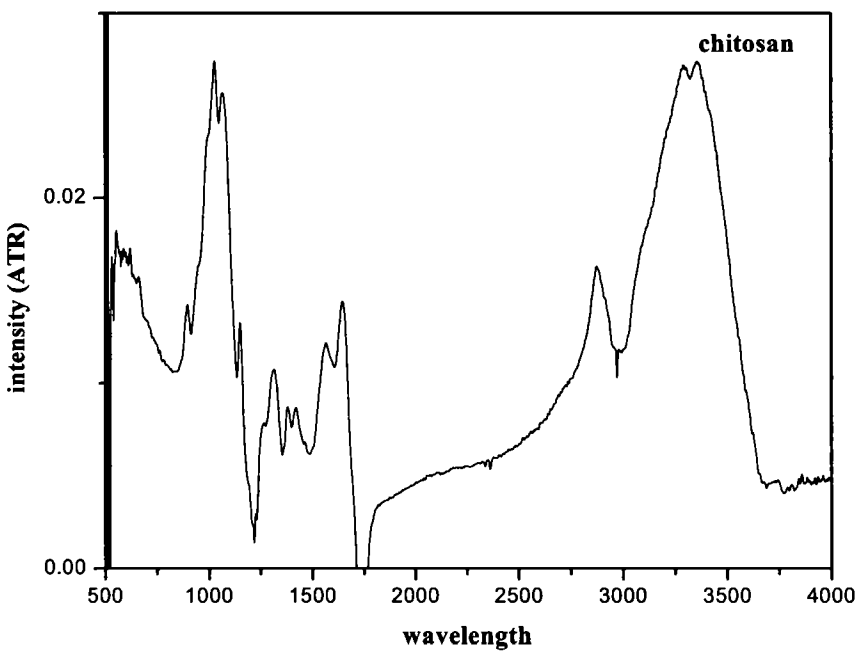


Figure 3.6: IR spectrum of chitosan microsphere alone

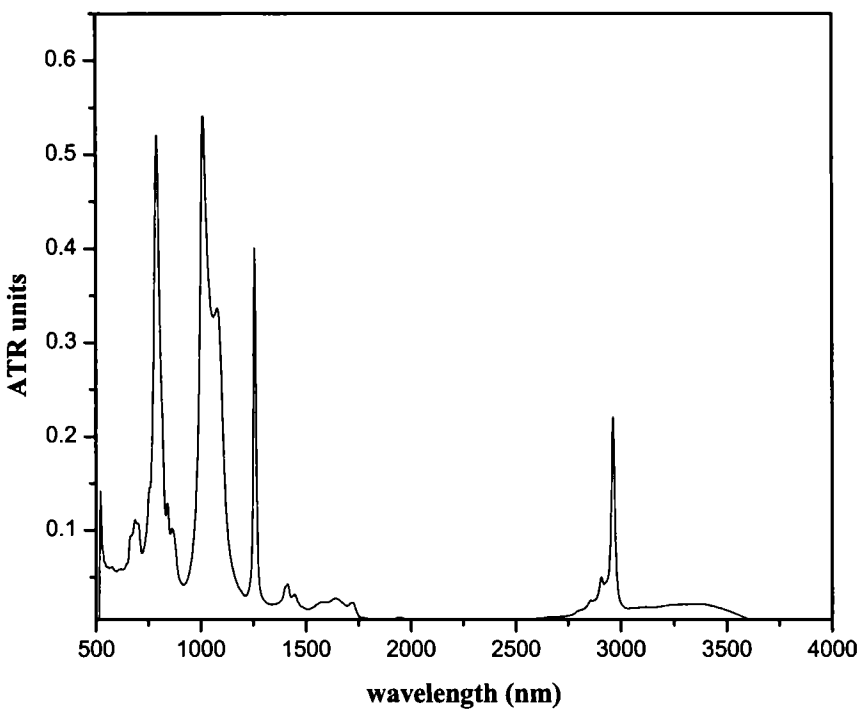


Figure 3.7: Infrared spectrum of chitosan microspheres encapsulated with barium sulphate.

From the IR spectra the cross linking reaction of chitosan-glutaraldehyde can be explained as follows.

Glutaraldehyde crosslinking occurs through a Schiff's base reaction between aldehyde ends of the crosslinking agent and the amine moieties of chitosan to form imine functions as shown in figure 3.8. The crosslinking of chitosan took place at the free amino group in all cases and lead to the formation of imine groups or ionic bonds.

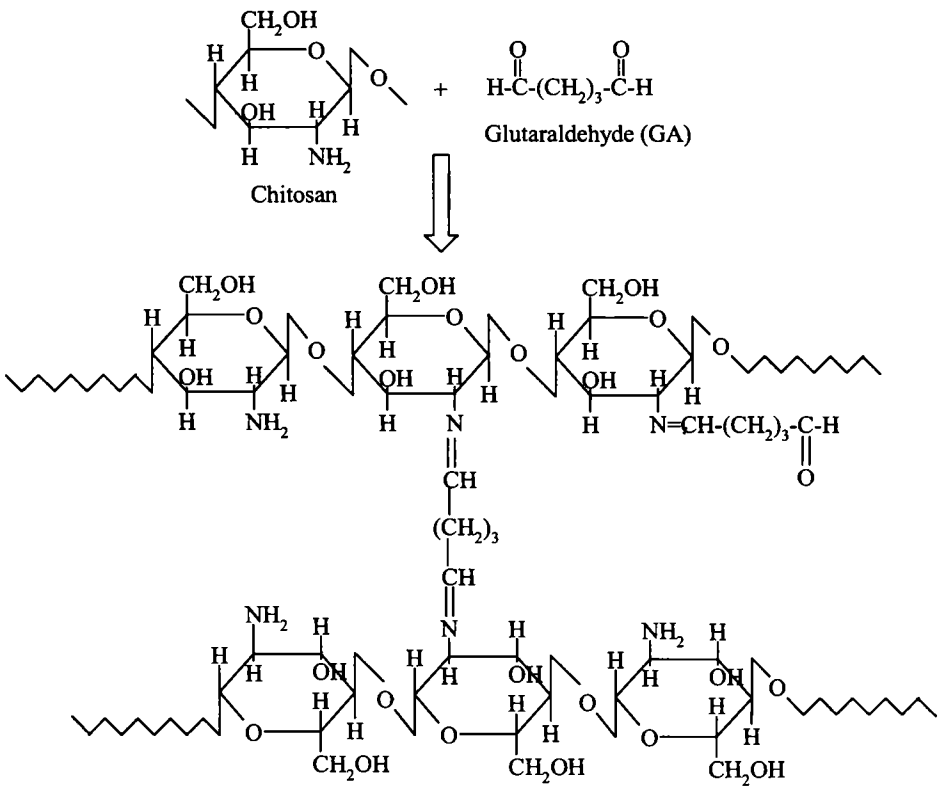


Figure 3.8: Crosslinked chitosan

- **X-ray Diffraction Studies (XRD)**

Figure 3.9 shows the XRD analysis of chitosan (CHN) and chitosan/barium sulphate (CHN/BS) microspheres. It is clear from the figure that the broad peak at $2\theta = 20.03$ in chitosan is shifted to a sharp narrow peak at $2\theta = 25.63$ in CHN/BS. Also the narrow peaks at $2\theta = 28.65$, 32.58 and 42.41 indicates the improvement of crystallinity due to the incorporation of barium sulphate inside chitosan microspheres.

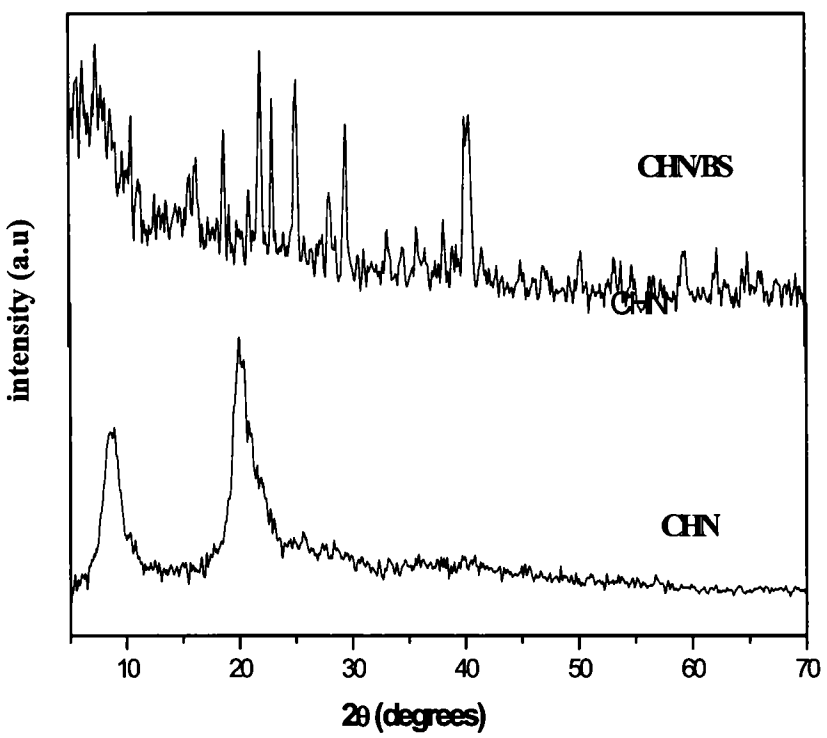


Figure 3.9: XRD patterns of chitosan and chitosan microspheres containing barium sulphate

- **Radiopacity studies**

X-ray photograph of the chitosan microspheres is shown in figure 3.10. Chitosan is radiolucent in nature. By the incorporation of barium sulphate, its electron density increases and it becomes radiopaque. The microspheres show intense radiopacity due to the presence of barium sulphate inside these spheres.

Figure 3.10: A X-ray photograph of chitosan microspheres containing barium sulphate (filled in polyethylene tube)

3.2.3.3 Studies on Chitosan / PVA blend microspheres prepared in Liquid paraffin/ Sorbitan sesqueoleate system

- **SEM analysis**

The surface morphology of the microspheres prepared from blend of Chitosan/PVA are studied using scanning electron microscopy and is shown in figure 3.11 (a and b). It is clear from the figure that the chitosan/ PVA blend gives smooth, spherical particles in liquid paraffin/ sorbitan sesqueoleate system.

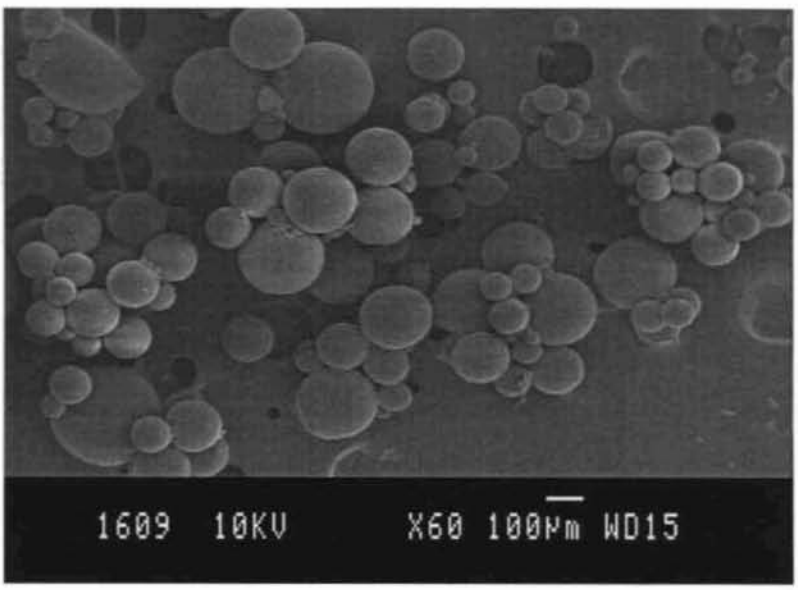


Figure 3.11 a: Scanning electron micrograph of radiopaque microspheres of chitosan/PVA blend

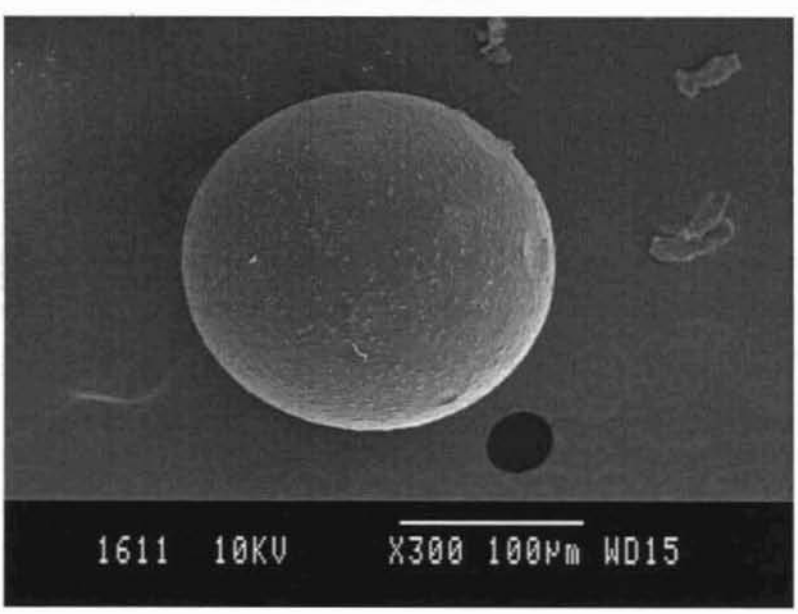
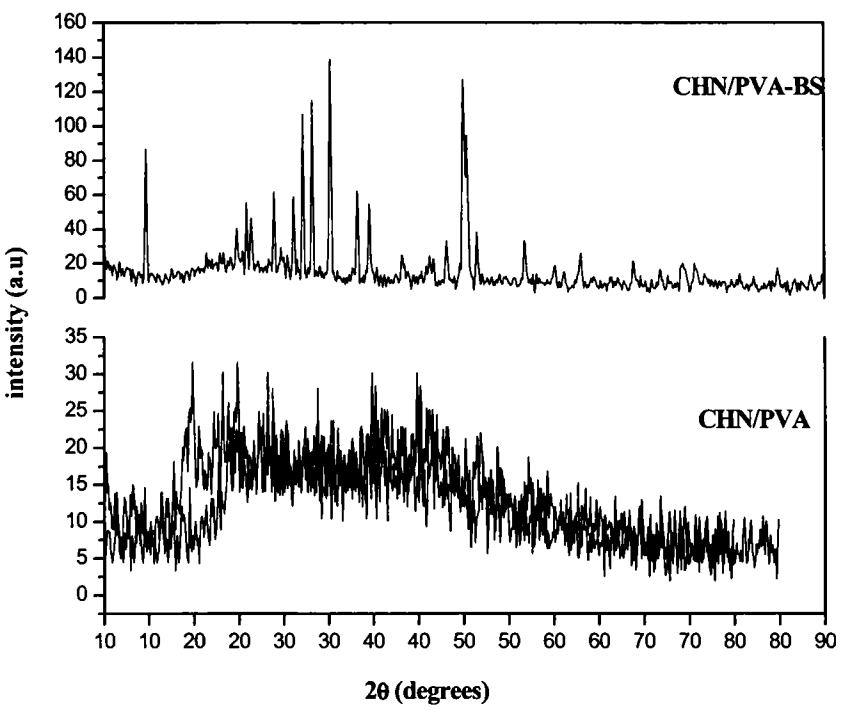


Figure 3.11 b: Scanning electron micrograph of single microspheres of chitosan/PVA blend

- **XRD analysis**

Figure 3.12 shows the XRD analysis of chitosan/PVA and chitosan/PVA/barium sulphate microspheres.



3.12: XRD patterns of blend of chitosan/PVA and chitosan/PVA microspheres containing barium sulphate

It is clear from the figure that the CHN/PVA microspheres give broad peak at 2θ in the range of 20° C to 50° C and it indicates that the blend is highly amorphous in nature. But when it is encapsulated with barium sulphate, it becomes more crystalline as evidenced by the sharp narrow peaks of CHN/PVA/BS microspheres.

- **Radiopacity studies**

X-ray photograph of the blend of chitosan/PVA microspheres is shown in figure 3.13. The microspheres show intense radiopacity due to the presence of barium sulphate inside these spheres, as a result of increase in electron density.

Figure 3.13: A X-ray photograph of chitosan/PVA microspheres containing barium sulphate (filled in polyethylene tube)

PART II

3.3 RADIOPAQUE MICROSPHERES FROM THE DERIVATIVES OF CHITOSAN

3.3.1 INTRODUCTION

Chitosan and its derivatives have been studied extensively for various biomedical applications. The poor intractability of chitin is due to the presence of strong inter and intra molecular hydrogen bonding. Chemical manipulation was seen as one

route to overcoming the intractability of chitin to make chitin more accessible. In chitin, the C-6, and C-3 positions of the monomer contain hydroxyl groups and in chitosan, there is an additional N-2 amino functionality that can participate in chemical reaction. All three sites are available for chemical reaction and therefore, the chemistry of chitin and chitosan has been principally one of chemical derivatization of the functional groups.

Historically, the intractability of chitin dictated heterogeneous chemical reactions as the starting point for scientists of the day to commence unraveling the chemistry of chitin. Concurrently, homogeneous reactions were conducted beginning with strong acids culminating in the introduction of homogeneous reactions with the chitin solvent 5 % LiCl/dimethyl acetamide (DMAc). All this effort has led to a better understanding of the chemical modification reactions of chitin. Though chitosan is water insoluble, it is readily soluble in dilute organic acids such as acetic acid, citric acid, malic acid and hydrochloric acid.[20] Many acids have been used to prepare chitosan base controlled release drug delivery systems. Chitosan was used as a vehicle for sustained release tablets,[21,22] a direct compressible diluents,[23,24] a tablet disintegrent[25] and as a tablet binder. Chitosan derivative such as glutamate, aspirate and hydrochloride salts have been used for colon-specific drug delivery and to enhance the delivery of therapeutic peptide across intestinal epithelial.[26] Spray dried chitosan microspheres using acetic acid as a solvent, loaded with insulin for protein delivery and chitosan microspheres loaded with dexamethasone as well as spray dried lactose composite particles containing an ion complex of alginate-chitosan were studied.

In this part, the preparation, characterization and radiopacity studies of chitosan derivatives are reported.

3.3.2 EXPERIMENTAL

1. Preparation of chitosan formate

Chitosan formate was prepared from chitosan of DA 86 % having viscosity of 330cps. Reaction was carried out at room temperature using formic acid in ethyl acetate. The product obtained was washed with ethanol and dried in an air oven at 50 °C.

2. Preparation of chitosan acetate

Chitosan acetate was prepared using glacial acetic acid in ethyl acetate. Chitosan having degree of deacetylation 86 % was used for the study. The product obtained was washed with ethanol and dried in an air oven at 50° C.

3. Preparation of O-Carboxy methyl chitosan (O-CMC).

chitosan (15 g) and 9 g monochloroacetic acid were suspended in 150 ml sodium hydroxide solution (42 % by weight).[27] The system was reacted at 0° C for 48 h and then the pH is adjusted to 1 with hydrochloric acid. After filtration, the solid product was washed with methanol for two times. The O-Carboxy methyl chitosan yielded was dried in an oven at 60° C.

4. Preparation of O-Carboxy methyl chitosan (O-CMC)/PVA blend

5 % solution of carboxymethyl chitosan was mixed with 5 % solution of PVA and this blend was used for the preparation of microspheres.

5. Preparation of microspheres from chitosan formate, chitosan acetate, carboxy methyl chitosan

Sorbitan sesqueoleate was mixed with paraffin oil. Then a free flowing paste of 3 % solution of derivative of chitosan containing $BaSO_4$ was added while stirring. Glutaraldehyde saturated with toluene was added and the stirring was continued at

the same speed for 5 min. After 5 min the stirring speed was reduced. Aqueous glutaraldehyde solution was then added every 30 min intervals and the stirring was continued at room temperature for 3 h. After the reaction the spheres were filtered off, washed several times with hexane and water. The spheres were again washed with plenty of ice-cold water to remove acetic acid and glutaraldehyde. The spheres were then dried in an air oven at 50 °C.

3.3.3 RESULTS AND DISCUSSION

3.3.3.1 Studies on chitosan formate

- **Infrared spectroscopy**

Figure 3.14 shows the IR spectrum of chitosan formate. A broad peak in the range 3150 to 3600 cm^{-1} is assigned to an -OH stretching, indicating inter molecular hydrogen bonding. The peak at 1631 cm^{-1} representing the NH_3+ band and the peak at 1548 cm^{-1} represents the carboxylate band of $-COO^-$. These characteristic peaks indicate the presence of electrostatic attractions between the chitosan and the formic acid. A formate ion stretching vibration is observed at 1413 cm^{-1}, confirm the formation of chitosan formate.

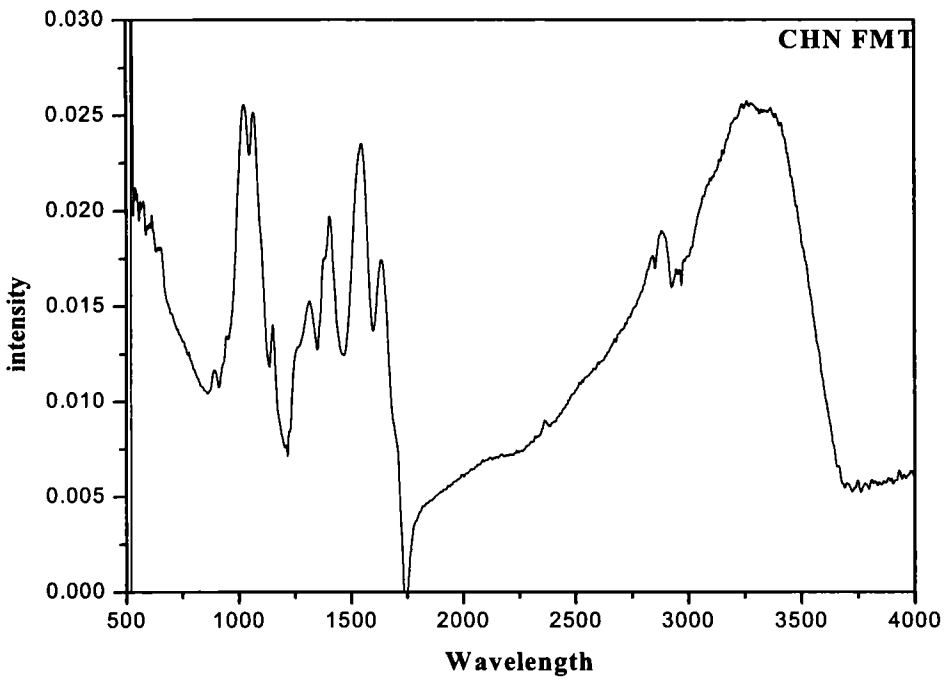


Figure 3.14: IR spectrum of chitosan formate

- **SEM Analysis**

The microspheres prepared from chitosan formate with barium sulphate show good surface morphology, by cross linking with glutaraldehyde as shown in figure 3.15. Free flowing microspheres of different diameters are obtained.

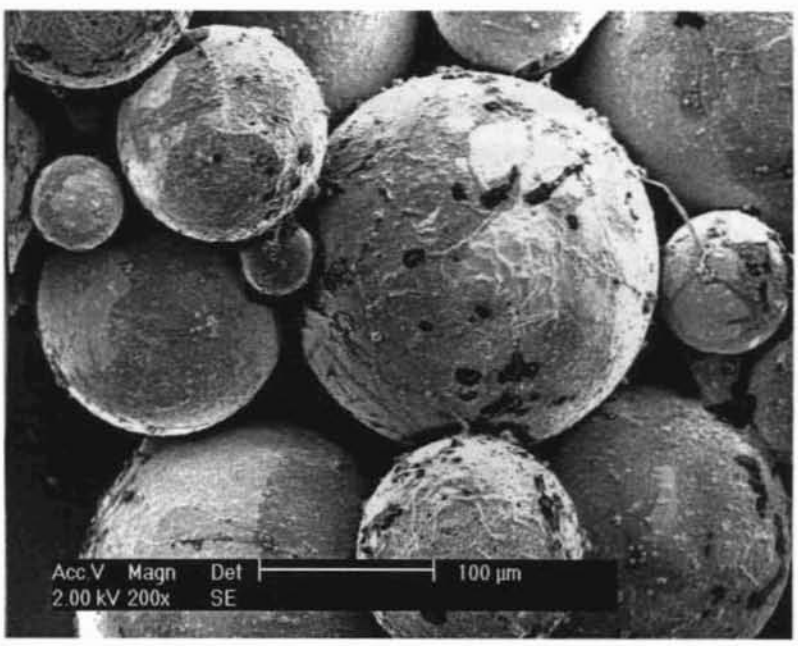


Figure 3.15: SEM photograph of chitosan formate/barium sulphate microspheres

- **XRD studies**

The presence of barium sulphate inside the microspheres is confirmed by XRD patterns of these microspheres. The XRD patterns of chitosan formate with and without barium sulphate is shown in figure 3.16.

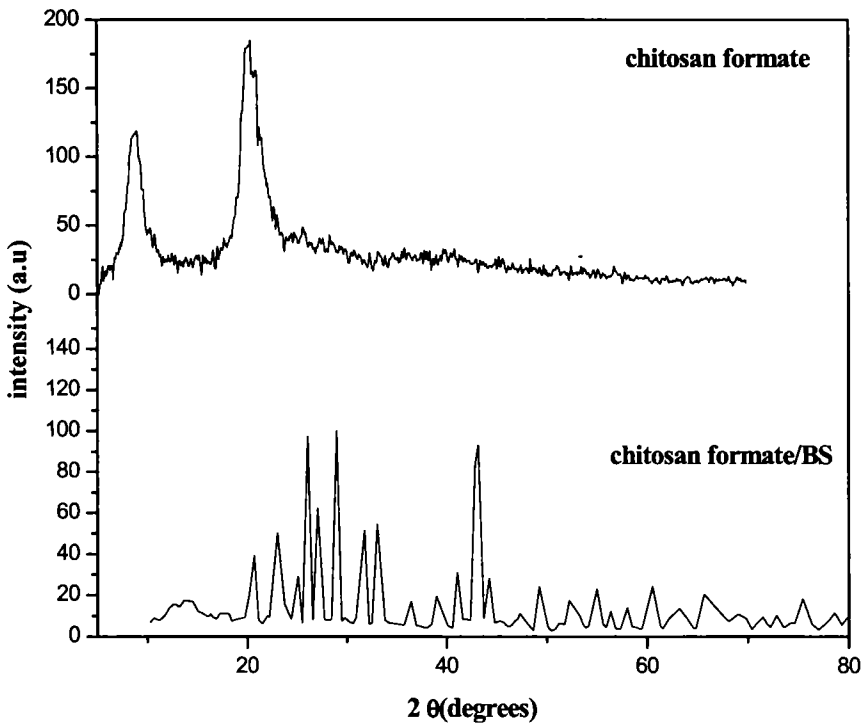


Figure 3.16: XRD patterns of Chitosan formate and chitosan formate/barium sulphate microspheres

The polymer crystallinity increases after cross linking and after the incorporation of barium sulphate inside the chitosan microspheres. It is clear from the figure that the broad peak at 2θ=19.82 in chitosan is shifted to a sharp narrow peak at 2θ = 26.10 in chitosan formate/barium sulphate. Also the narrow peaks at 2θ = 28.93, 33 and 43.13 in the case of chitosan formate/barium sulphate microspheres indicates the enhancement of crystallinity due to the incorporation of barium sulphate inside chitosan microspheres.

- **Radiopacity studies**

Figure 3.17 shows the X-ray photographs of chitosan formate microspheres containing barium sulphate. Due to the incorporation of barium sulphate the electron density of chitosan formate increases and it shows intense X-ray images as in the case of chitosan/barium sulphate microspheres.

Figure 3.17: X-ray photograph of chitosan formate microspheres containing barium sulphate (filled in polyethylene tube)

3.3.3.2 Studies on chitosan acetate

- **IR spectroscopy**

Figure 3.18 shows the IR spectrum of chitosan acetate.

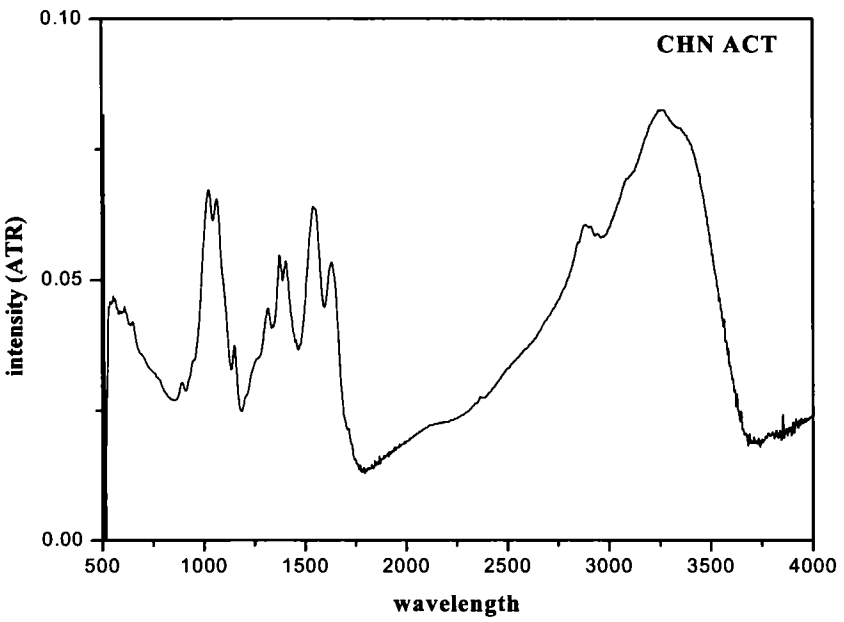


Figure 3.18: IR spectrum of chitosan acetate

The spectrum exhibits characteristic peaks at 2970 cm^{-1} and 1380 cm^{-1} due to the protonated amine stretch and deformation vibrations. The peak at 1556 cm^{-1} indicates the presence of $-COO^-$ due to the interaction between the chitosan chain and acetic acid. A peak at 1650 cm^{-1} indicates the presence of an amide band. The peak around 1400 cm^{-1} indicates that the symmetric stretching vibrations of carboxylate anion present and it confirms the formation of chitosan acetate.

- **SEM Analysis**

The SEM micrograph of the chitosan acetate microspheres are shown in figure 3.19. It is clear from the figure that the microspheres obtained from chitosan acetate do not exhibit good spherical geometry and surface smoothness. This may be due to the presence of bulky acetate group in chitosan, which will affects the

cross linking reaction. Hence further radiopacity studies are not carried in chitosan acetate.

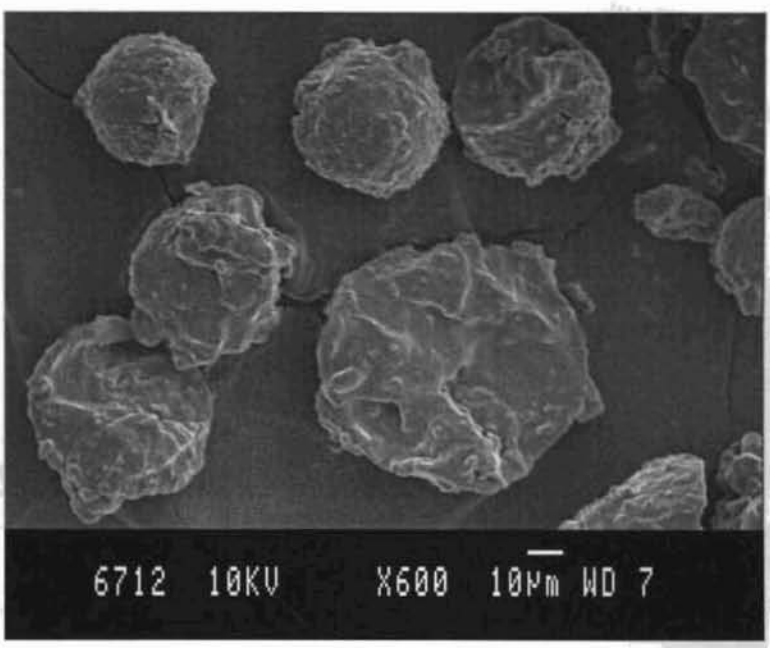


Figure 3.19 : SEM photograph of chitosan acetate/barium sulphate microspheres

3.3.3.3 Studies on carboxy methyl chitosan

- **IR spectroscopy**

The infrared spectroscopy of carboxy methyl chitosan is shown in figure 3.20. A broad peak in the range 3360 cm^{-1} is assigned to an -OH stretching, indicating inter molecular H-bonding. The spectrum also overlapped in the near by region of a -NH stretching (i.e at 2900 cm^{-1}). NH_2 deformation peaks are observed at 1580 cm^{-1} and around 1300 cm^{-1}. The peak at 1416 cm^{-1} indicates the symmetric

stretching vibrations of carboxylate anion, which confirms the formation of carboxy methyl chitosan.

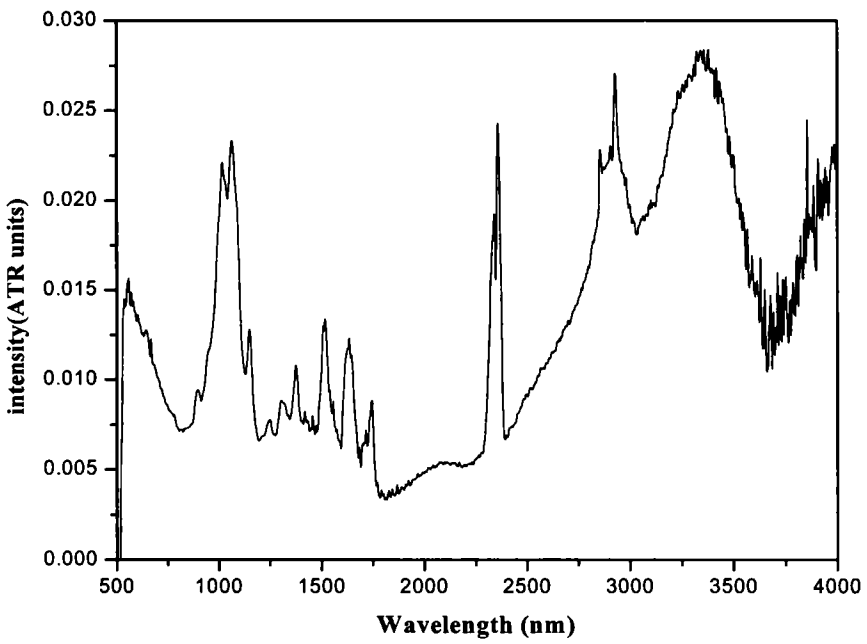


Figure 3.20:IR Spectrum of carboxy methyl chitosan

Since the carboxy methyl chitosan does not give spherical microspheres, a blend of carboxy methyl chitosan/PVA is used for further studies.

3.3.3.4 Studies on CMC/PVA blend

- **SEM Analysis**

SEM micrographs of carboxy methyl chitosan/PVA blend microspheres with barium sulphate is shown in figure 3.18 (a and b). The figure shows that the blend gives free flowing, smooth spheres as in the case of chitosan.

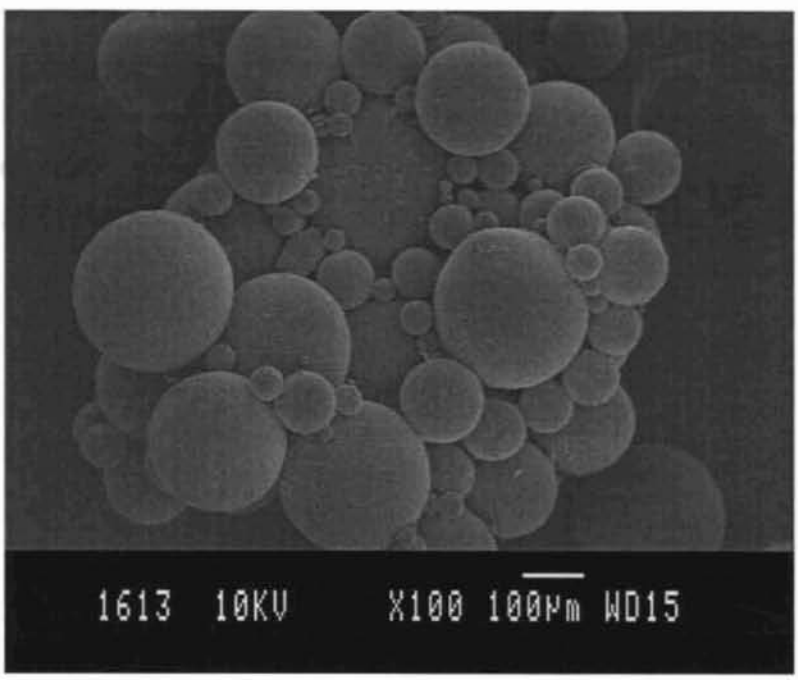


Figure 3.21 a: Scanning electron micrographs of radiopaque microspheres of Carboxy methyl chitosan /PVA blend

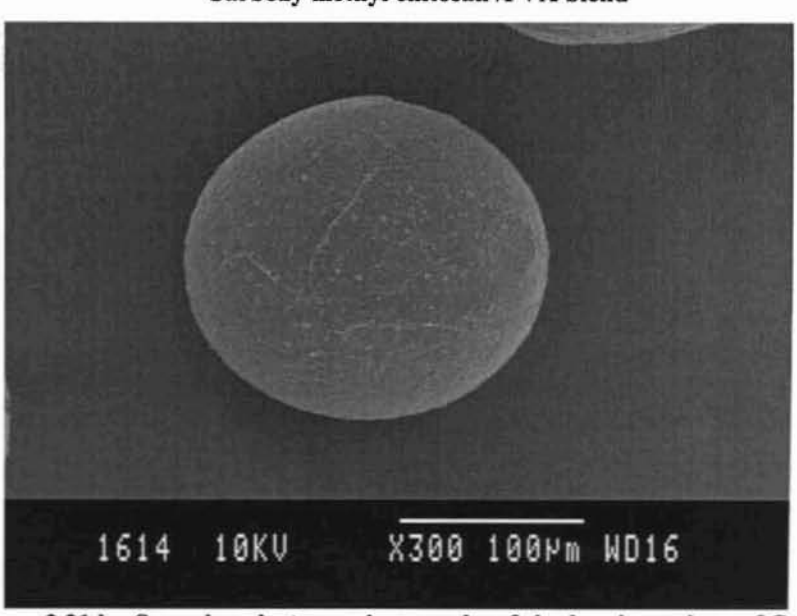


Figure 3.21 b: Scanning electron micrographs of single microsphere of Carboxy methyl chitosan /PVA blend

- **XRD studies**

The polymer crystallinity increased after cross linking and due to the incorporation of barium sulphate inside the microspheres of CMC/PVA blend, as shown in the figure 3.22. The broad peak at $2\theta = 20^{\circ}$ obtained is changed to sharp narrow peaks in CMC/PVA/BS.

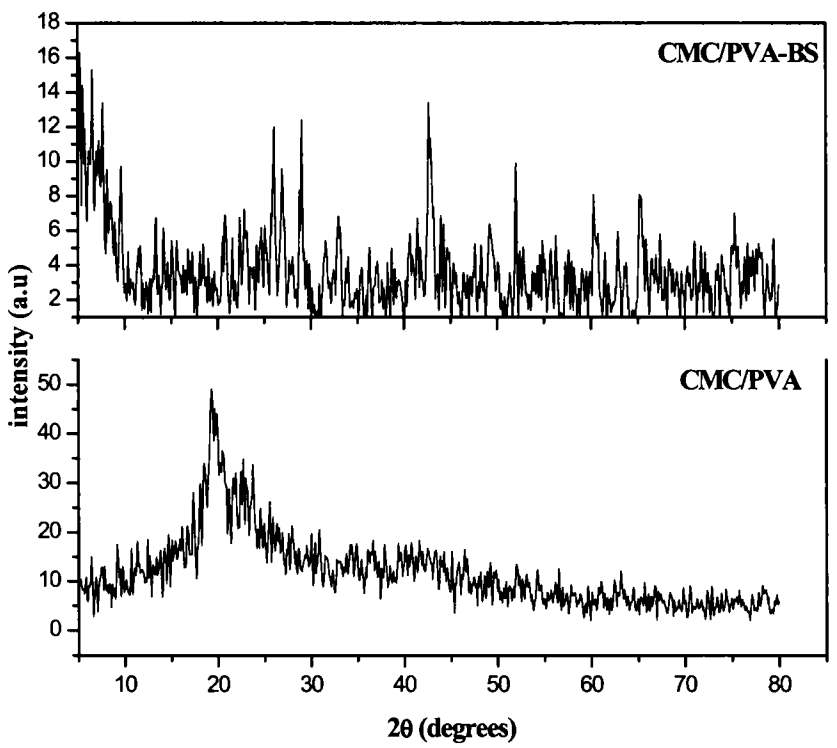


Figure 3.22 XRD patterns of blend of CMC/PVA and $BaSO_4$ encapsulated CMC/PVA microspheres

- **Radiopacity studies**

X-ray photographs of the blend of CMC/PVA microspheres is shown in figure 3.23. The blend of CMC/PVA contains carbon, hydrogen, oxygen and nitrogen

and is radiolucent in nature as in the case of chitosan. By the incorporation of barium sulphate its electron density increases and it becomes radiopaque. The microspheres show intense radiopacity due to the presence of barium sulphate inside these spheres.

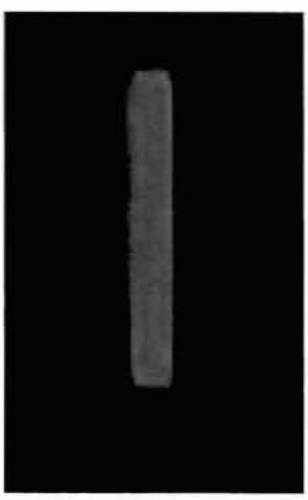


Figure 3.23: X-ray photograph of CMC/PVA microspheres containing barium sulphate (filled in polyethylene tube)

3.4 CONCLUSIONS

- Chitosan microspheres can be prepared by the *in situ* cross linking reaction with glutaraldehyde
- Liquid paraffin / sorbitan sesqueoleate emulsion system gives perfect spherical chitosan microspheres
- Radiopacity is imparted on chitosan microspheres by the incorporation of barium sulphate
- Excellent radiopaque microspheres of chitosan/PVA/BS blend is prepared using liquid paraffin/ sorbitan sesqueoleate system
- Chitosan derivatives like chitosan formate, chitosan acetate, carboxy methyl chitosan have been prepared
- Chitosan formate and blend of CMC/PVA is found to give microspheres with perfect spherical geometry using liquid paraffin/sorbitan sesqueoleate system
- Chitosan formate/BS, CMC/PVA/BS microspheres prepared from liquid paraffin/sorbitan sesqueoleate system show intense radiopacity

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CHAPTER 4

PREPARATION AND CHARACTERIZATION OF RADIOPAQUE NATURAL RUBBER

4.1 INTRODUCTION

Currently radiopaque polymers are of great interest to medicine and dentistry because of their wide area of applications such as catheters, surgical tools, dental products and radiation shielders. Radiopacity is a desirable property of implants used in medicine and dentistry. When used to produce medical devices for insertion into the body for diagnostic or surgical purposes, radiopaque materials should render the devices visible under fluoroscopy or X-ray imaging[1]. The additives or radiopacifiers attenuate energy differently than surrounding body tissues, providing the contrast in X-ray image. Because they can resist X-ray penetration, radiopaque compounds are also used to produce shielding components to enclose radiation generating sources. Since the impermeability of a shielding material is proportionate to its density, lead has historically been used, but not without health and environmental concerns. Lead is toxic to many of our

tissues and enzymes. Children particularly are susceptible to lead poisoning because it can get accumulated in their nervous system as their bodies grow and develop. Death by lead poisoning is uncommon, but dangerous levels of lead in children may cause serious health problems including lower intelligence, poor school performance, seizures, unconsciousness and possibly death. Although lead poisoning is especially vulnerable with children, it may cause health hazards in adults too. High levels of lead in adults may lead to complications such as high blood pressure, digestive problems, nerve disorders, memory and concentration problems, muscle and joint pain and cataracts etc.

Radiopaque polymers also offer similar radiation-shielding properties and density as compared to that of lead but less toxic. Ease of their production also render them attractive. Research into radiopaque polymers explores methods of increasing average electron density and the specific gravity of polymers by incorporating heavy elements into these systems.

Radiopaque polymers can be prepared by several ways. Based on the mode of preparation, radiopaque polymer systems are grouped into three. They are radiopaque polymer blends, radiopaque polymer salt complexes and polymers of radiopaque monomers.[2-4] But all these methods exhibit specific disadvantages which have precluded their use. The best method to produce radiopaque polymers is to synthesize reactive monomers having covalently bonded heavy atoms and use these monomers as building blocks for new polymeric biomaterials that can exhibit intrinsic radiopacity. Such materials could offer important advantages since no conciliation has to be struck between introduction of radiopacity on the one hand and preservation of physico-mechanical properties on the other.

The radiopaque compounds can be fabricated using a variety of thermoplastic polymers and additives to customize product's specific gravity and physical

properties (such as flexural modulus, tensile strength, and impact strength) for specific end-use requirements. The type and amount of additives depends on the base resin and on the thickness, surface smoothness, color and other required features of the product.

Several covalently bound halogen-containing polymers have been synthesized to impart radiopacity to polymers. Most commonly used halogen atom is iodine. Iodine has a very diverse number of applications in a wide variety of industries, including in x-ray contrast media (25 %), followed by biocides (20 %), catalysts (10 %) and feed additives (10 %).

In this chapter, the studies on the preparation, charcterization and radiopacity studies of radiopaque natural rubber are reported. The natural rubber is proposed to be made into radiopaque by its iodination and using radiopacifiers as fillers. Optical density of the radiopaque natural rubber is also proposed to be elaborated. The radiopaque natural rubber is proposed to be characterized using UV-VIS spectroscopy, thermo gravimetric analysis and clinical X-ray techniques. Natural rubber (NR) is proposed to be chosen as a base matrix owing to its excellent properties such as better mechanical properties, low cost and renewable source. These features make NR dominant in many industrial, automotive, engineering and medical applications. In medical field it finds various applications such as urinary catheters, medical tubings, gloves etc.

PART I

4.2 RADIOPAQUE NATURAL RUBBER THROUGH IODINATION

4.2.1 INTRODUCTION

In view of the fact that iodine is a heavier atom compared to bromine and since iodine containing dyes are routinely used in interventional radiology, attempts were made to synthesize iodine containing compounds as well as monomers.[5-6] Several iodine containing compounds like iopanoic acid {3-(3-amino-2,4,6-triiodophenyl)-2-ethyl propanoic acid} and iothalamic acid(5-acetamido-2,4,6-triiodo-N-methyl isophthalamic acid) are clinically used as non-toxic radio contrast materials.

The first attempt in this direction was carried out by Jayakrishnan[7] *et al.* They synthesized triiodophenyl methacrylate and iothalamic ester of hydroxyethylmethacrylate (HEMA). But the monomers were found to be highly resistant to homopolymerization and copolymerization with monomers such as 2-hydroxyethylmethacrylate and methyl mathacrylate under the conditions of polymerization was tried. Jayakrishnan[10] *et al* also reported a new iodine monomer based on 3, 4, 5-triiodobenzoic acid and its copolymerization with monomers such as MMA and HEMA. Kruft[8] *et al* synthesized p-iodobenzoyl methacrylate and studied the copolymerization with MMA and HEMA. They synthesized genuine polymers with satisfactory low thrombogenicity. Kruft[9] *et al* also studied the synthesis and copolymerization of several iodine containing monomers described above as well as 2(o-iodobenzoyl)-ethyl methacrylate. Benzina[10] *et al* synthesized another iodine containing monomer 2(p-iodobenzoyl) ethyl methacrylate and studied its copolymerization with MMA and HEMA. The polymers obtained were found to have excellent radiopacity with good blood compatibility.

They also synthesised monomers containing three iodine atom such as 2-[2, 3, 5-triiodobenzoyl]-ethyl methacrylate.[11] Davy[12] *et al* synthesized and characterized two to three iodine containing monomers 2-[2, 3, 5-triiodobenzoyl]-ethyl methacrylate and 2-hydroxy-3-methacryloxypropyl (2, 3, 5 triiodo benzoate) for radiopaque denture base acrylics Jayakrishnan et al also reported a new iodine monomer based on 3, 4, 5-triiodobenzoic acid and its copolymerization with monomers such as MMA and HEMA.[13] Biomaterials for cardiovascular applications must meet several design criteria including hemocompatibility, good mechanical strength and flexibility, as well as show X-ray visibility. Poly (DTE carbonate) has been demonstrated as a potential biodegradable polymeric material for cardiovascular applications. In this system, X-ray visibility can be achieved via iodination of the tyrosine ring.

Antibacterial properties can be imparted to Nylon-6 by adsorption of iodine.[14] Natural Rubber (NR) in both its latex and dried form is proposed to be treated with iodine to make it antibacterial in nature[15] was reported by Usala *et al.*

In this part, the synthesis, characterization and radiopacity studies of iodinated Natural rubber is reported.

4.2.2 EXPERIMENTAL

- ***Materials Used***

1. Natural Rubber
2. Compounding ingredients
3. Iodine
4. Potassium Iodide

- ***Preparation of Iodinated natural rubber (INR)***

Centrifuged Natural Rubber (NR) latex was stabilized by stirring with a mixture of 10 % KOH, 10 % potassium oleate and vulcastab VL for 3-4 hours. It was then acidified with 1N HCl. The acidified latex was iodinated with KI/I_2 and the coagulam was washed several times with water and then dried at room temperature.

- ***Preparation of Zinc iso propyl xanthate***

Zinc iso propyl xanthate was prepared in the laboratory by mixing potassium hydroxide, isopropyl alcohol and carbon disulfide in equimolar concentration. It was converted to the zinc salts by the addition of zinc sulphate.[16] The zinc isopropyl xanthat formed was filtered, washed and dried in an oven.

- ***Preparation of INR Vulcanizate***

The INR compound was prepared on a laboratory mixing mill (6x12 inch) as per the formulation given in table 4.1. The optimum cure times of the compound was determined using a Goettfert elastograph model 67085 as per ASTM D 1646(1981) at a temperature of 150 ^{0}C. The compound was compression moulded at 150 ^{0}C, in an electrically heated hydraulic press, into 1mm thick sheets. For this, the mould was pre-heated to 150 ^{0}C, a piece of preformed material was placed directly in the mould cavity and compressed under a hydraulic clamp pressure of 200 kg/cm^2. Upon completion of the required cure cycle, the pressure was released and the sheet were stripped from the mould and suddenly cooled by plunging into cold water. After a few seconds, the sample was taken from water and was dried. Dumbell shaped tensile test specimens were punched out of the compression moulded sheets along the mill direction. The tensile properties of the INR vulcanizate was evaluated on a Zwick universal testing machine using a cross

head speed of 500 nm/minute according to ASTM D 624. The compression moulded sheet was cut in to round shape to measure the radiopacity.

Table 4.1: Formulations for INR Vulcanizate

INGREDIENTS	MIX E (phr)
INR	100
ZnO	5
Stearic acid	2
CBS	0.6
TMTD	0.1
Sulphur	2.5

Iodinated natural rubber is also compounded at room temperature using zinc iso propyl xanthate using the compounding formulation given in table 4.2.

Table 4.2 Formulations for INR (low temperature curing)

INGREDIENTS	MIX G (phr)
Iodinated NR	100
ZnO	5
Stearic acid	2
ZDC	2.5
Sulphur	2.5
Zinc isopropyl xanthate	1.75

- ***Determination of effective atomic number***

Effective atomic number was calculated using the equation,

$\overline{Z} = (a_1 z_1^{2.94} + a_2 z_2^{2.94} + \ldots\ldots\ldots\ldots + a_n z_n^{2.94})^{1/2.94}$ Where, a_1, a_2..... are fractional contents of each element to the total number of electrons in the mixture; Z_1, Z_2, ... refers to the atomic number of the corresponding element.

- ***Determination of Optical Density***

The term optical density refers to the degree of blackening of the film. The degree of blackness is directly related to the intensity of radiation. The measurement of blackness is called photographic density or optical density (OD).

$$OD = \log_{10} (I_0/I_t),$$

Where I_0 is the intensity of light incident on the film and I_t is the intensity of the light transmitted through the film.

- ***Determination of atteunation coeffecient***

The atteunation coeffecient sample was calculated using the following equation

$$I/Io=e^{-\mu x}$$

For the calculation the value of optical density of background of the film was taken as I_0 and optical density of the sample was taken as I. 'x' the thickness of the sample and 'μ' the attenuation coefficient.

4.2.3 RESULTS AND DISCUSSION

4.2.3.1 Studies on Iodinated Natural Rubber

- **Characterization**

Figure 4.1 shows the UV spectrum of iodinated natural rubber in toluene scanned in the 200-700 nm region. Methyl iodide[17] have an absorption maxima of about 254 nm and the spectrum shows a sharp peak at 284.5nm corresponds to the iodinated methyl group.

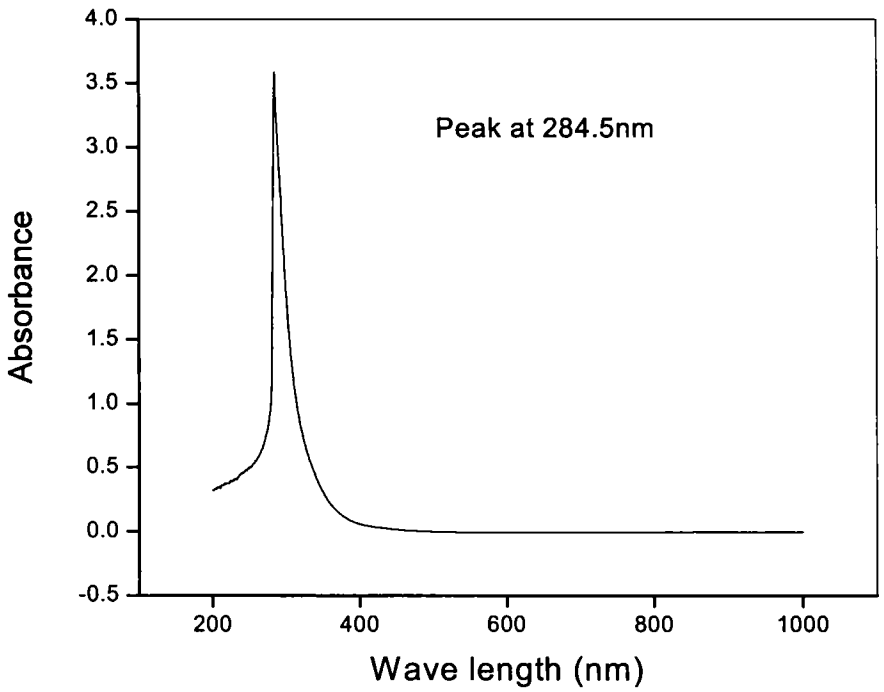

Figure 4.1: UV spectrum of Iodinated Natural Rubber

- **TGA studies**

Thermal stability of iodinated natural rubber was studied using thermogravimetric analysis and is shown in figure 4.2. A peak is observed with a peak max 171.57° C, due to the elimination of iodine. The onset of this decomposition is found to be at 138.37° C and it is noticed that at 150° C around 1.5 % of degradation takes place. Followed by this, a second peak with a peak max at 444.19° C is observed. The onset of this decomposition is observed at 331.33° C which corresponds to a weight loss of 15.9 % and it is due to the decomposition of NR.

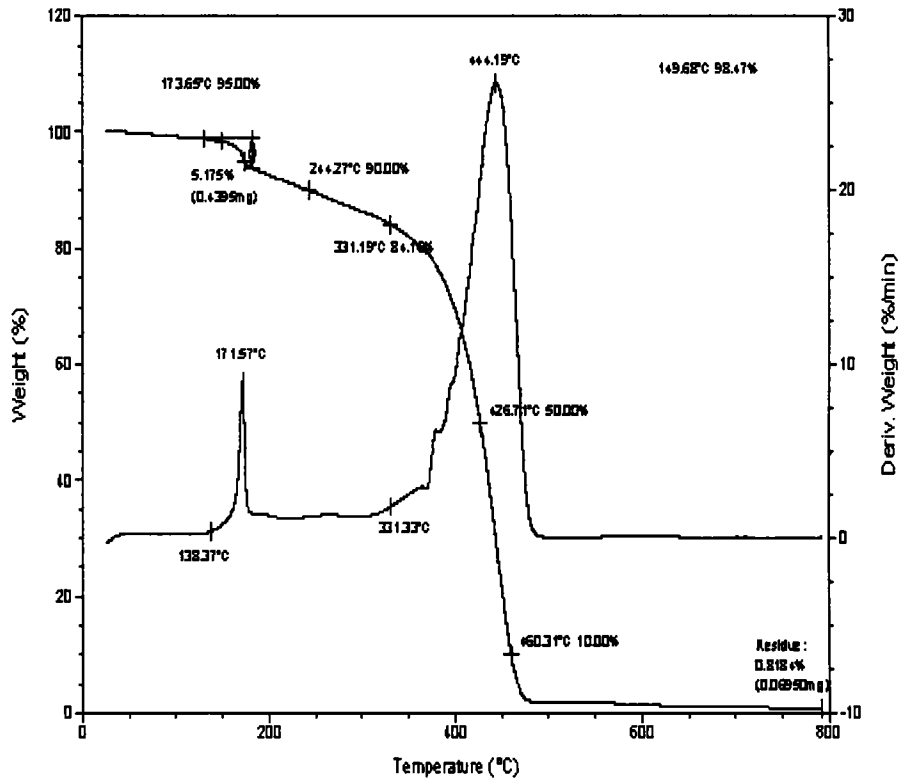

Figure 4.2 : TGA thermogram of iodinated natural rubber

- **X-ray Analysis**

Figure 4.3 shows the positive print of X-ray photograph of INR. It is clear from the figure that the iodinated NR shows good radiopacity. Natural rubber contains only carbon and hydrogen. Hence it is radiolucent in nature. When the NR is iodinated, its electron density increases and hence it shows good radiopacity.

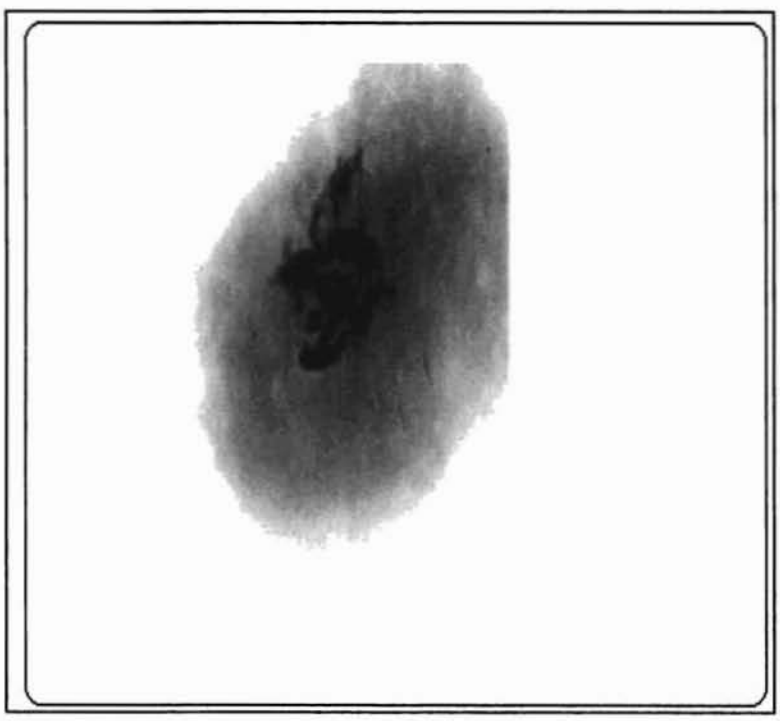

Figure 4.3 : Positive print of X-ray photographs of iodinated NR

- **Anti bacterial studies**

The effectiveness of antibacterial activity depends on the availability of free iodine that subsequently binds to the bacteria. A clear zone of inhibition is visible in the case of iodinated natural rubber as shown in figure 4.4. A wide disparity in colony counts between iodinated natural rubber and natural rubber is observed and it confirm the antibacterial nature of iodinated natural rubber.

Figure 4.4: A clear zone of inhibition in the INR sample

4.2.3.2 Studies on INR vulcanizates

The high temperature cured samples does not show radiopacity due to the elimination of iodine at higher temperature. Hence it does not find a mention in this section.

- **X-ray Analysis**

Radiopacity of the sample depends on the electron density of the material. Figure 4.5 shows the positive print of x-ray photographs of low temperature cured samples with varying thickness from 0.44 to 3.35 mm. The figure shows that the

radiopacity varies with thickness of the samples. It is clear from the figure that the sample with higher thickness give better radiopacity. At higher thickness, the concentration of the iodine is high and hence the electron density also high.

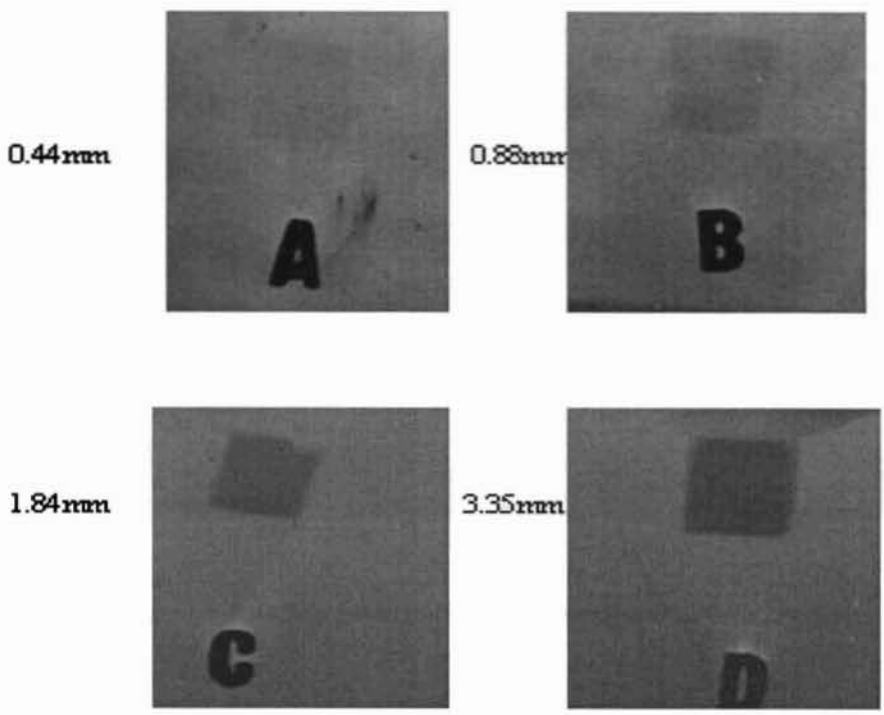

Figure 4.5: Positive print of X-ray photographs of room temperature cured INR samples of various thickness

- **Optical Density Measurements**

The variation of optical density with thickness of the sample is shown in figure 4.6. When the thickness of the sample increases, the optical density decreases since it is inversely related to the radiopacity.

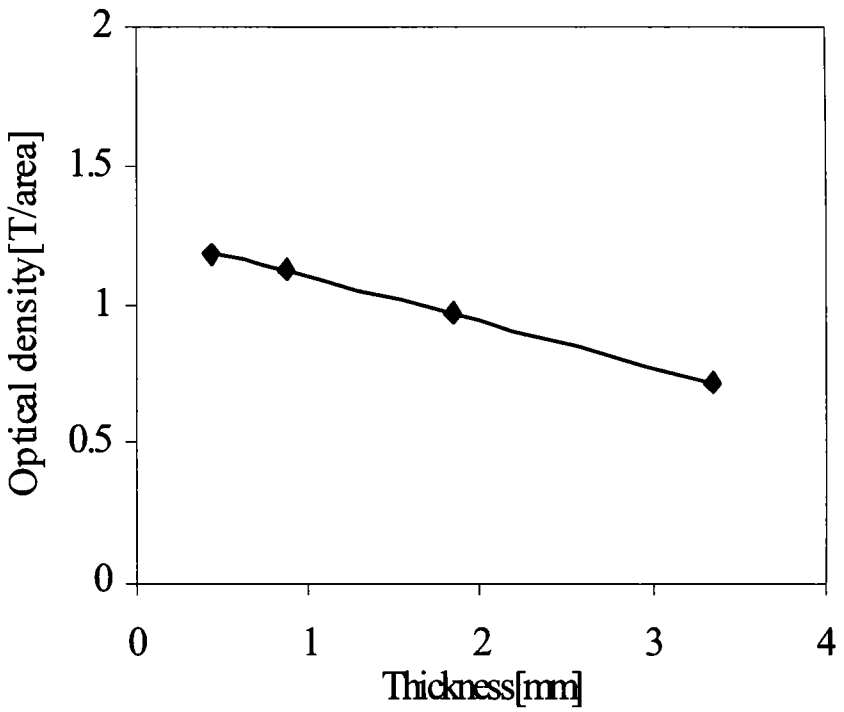

Figure 4.6 : Variation of optical density with thickness

- **Conductivity studies**

An I-V plot of low temperature cured sample is shown in the figure 4.7. Low temperature cured sample shows D.C. conductivity of the order of 7.32 x 10^{-7} S/cm. Natural rubber is commonly used for insulating applications. Natural rubber is an insulator (10^{-12} S/cm) in the normal state and its conductivity is increased to a semi- conductor level by the iodine doping.

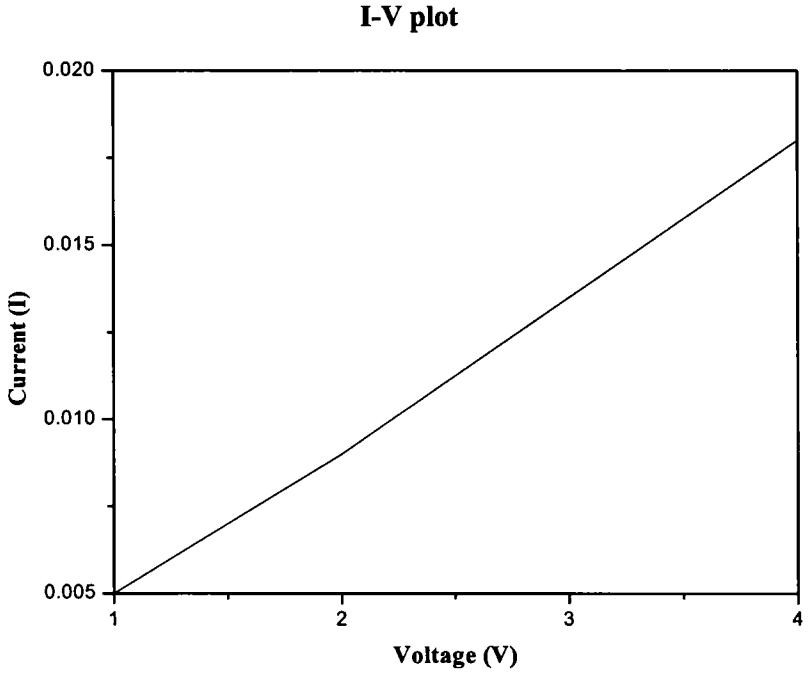

Figure 4.7: I-V plot of low temperature cured INR

4.2.3.3 Comparison of Optical density of NR, INR and Lead sheet

Table 4.3 shows the optical density values of NR, INR and Lead samples. It is evident from the table that after iodination, optical density of natural rubber decreases after iodination and the 3.35 mm thick INR sample have an optical density of 0.76. When the concentration of Iodine in natural rubber increases, the optical density decreases, which in turn increases the radiopacity of the sample. As such, radiopaue iodinated natural rubber with higher thickness could be used for shielding applications.

Table 4.3: Optical density of various samples

	NR	INR	LEAD SHEET
Optical density	1.26	0.76	0.16

4.2.3.4 Physical properties

Table 4.4 shows the physical properties of INR vulcanizates cured at high temperature and low temperature respectively. The tensile strength and tear strength are found to be high for low temperature cured sample compared to high temperature cured sample.

Table 4.4: Physical properties of INR samples

PROPERTY	LOW TEMP	HIGH TEMP
Tensile strength (MPa)	18.76	15.2
Tear strength (N/mm)	37.62	30.6
EB (%)	1840	1930.1

PART II

4.3 STUDIES ON FILLED RADIOPAQUE NATURAL RUBBER

4.3.1 INTRODUCTION

Polymers can be rendered radiopaque by blending them with radiopacifying agents like barium sulfate or metal powders (e.g. tantalum). Heavy metal salts (e.g. barium bromide, bismuth halides, uranyl nitrate) incorporated in to an appropriate polymer ligand via chelation can also impart radiopacity to the matrix polymer. However, the physical and mechanical properties of the base polymer are often adversely affected by the incorporation of these additives Another approach to make the polymer radiopaque by covalently link a radio contrast dye to the polymer. This approach is possible only if the polymer possesses a reactive functional group to which the dye could be attached.

In this part, the preparation, characterization and radiopaque studies of filled radiopaque natural rubber are reported.

4.3.2 EXPERIMENTAL

- ***Materials Used***

1. Natural Rubber
2. Compounding ingredients
3. Barium sulphate (BS) and commercial zinc oxide (ZnO) are added as radiopacifying fillers in natural rubber

- ***Preparation of NR-radiopaque filler vulcanizates***

The compounds as per the formulations given in tables 4.5 and 4.6 were mixed on a two roll mill size (15x33 cm) and the compounds were kept for 24 h for maturation. The compounds were moulded in an electrically heated hydraulic press at 150^0 C at a pressure of 200 kg/cm^2. When the amount of radiopacifier is increased to above 100 phr, the incorporation was difficult. Hence the amount of radiopacifier was limited to 150 phr. The sheets were cut into dumb bell shape to measure the tensile properties and round shape to measure radiopacity.

Table 4.5 : Formulation for NR vulcanizates with Barium sulphate filler

INGREDIENTS	MIX P (phr)	MIX Q (phr)	MIX R (phr)
Natural Rubber	100	100	100
Radiopacifier ($BaSO_4$)	50	100	150
Paraffinic oil	5	7.5	10
Stearic acid	2	2	2
Antioxidant	1.0	1.0	1.0
Zinc oxide	5	5	5
CBS	0.8	0.8	0.8
TMTD	0.15	0.2	0.25
Sulphur	2.5	2.5	2.5

Table 4.6: Formulation for NR vulcanizates with Zinc oxide filler

INGREDIENTS	MIX P (phr)	MIX Q (phr)	MIX R (phr)
Natural Rubber	100	100	100
Radiopacifier (ZnO)	50	100	150
Paraffinic oil	5	7.5	10
Stearic acid	2	2	2
Antioxidant	1.0	1.0	1.0
CBS	0.8	0.8	0.8
TMTD	0.15	0.2	0.25
Sulphur	2.5	2.5	2.5

4.3.3 RESULTS AND DISCUSSIONS

4.3.3.1 Natural rubber-Barium sulphate system

- **X-ray Analysis**

The X-ray film of Natural rubber-Barium Sulphate (NR-BS) samples with different concentrations of $BaSO_4$ is shown in figure 4.9. It is clear from the figure that the radiopacity of NR vulcanizate increases with increase in radiopacifier loading. The figure also shows that the radiopacity is higher for NR vucanizate with 150 phr of Barium Sulphate. In the low energy level, radiopacity of a material is mainly reliant on photoelectric effect and the photoelectric effect depends upon atomic number of the material.

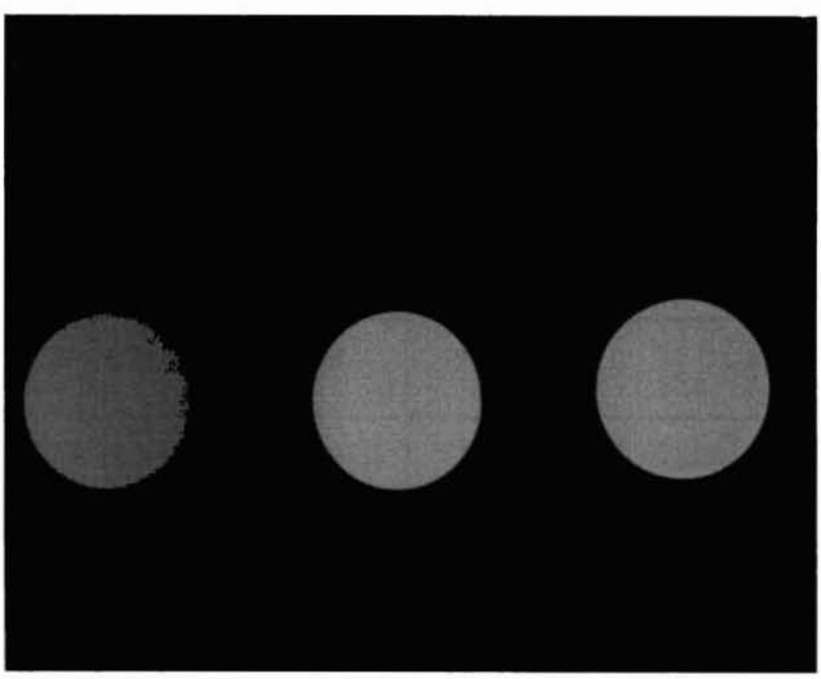

Figure 4.8: X-ray photographs of NR-BS systems at concentrations varying from 50 - 150 phr

- **Optical Density**

The variation of optical density with concentration of barium sulphate is given in table 4.7. When the amount of radiopacifier in the NR matrix increases the optical density decreases, as it has inverse link with radiopacity.

Table 4.7: The variation of optical density with amount of $BaSO_4$

SAMPLE	OPTICAL DENSITY
NR-50BS	0.271
NR-100BS	0.19
NR-150BS	0.17

- **Determination of atteunation coefficient**

Figure 4.10 shows the X-ray photograph of NR-150 BS using cone in order to reduce the scattering. The attenuation coefficient is found to be 1.155. The higher the value of attenuation coefficient, the better will be the radiopacity.

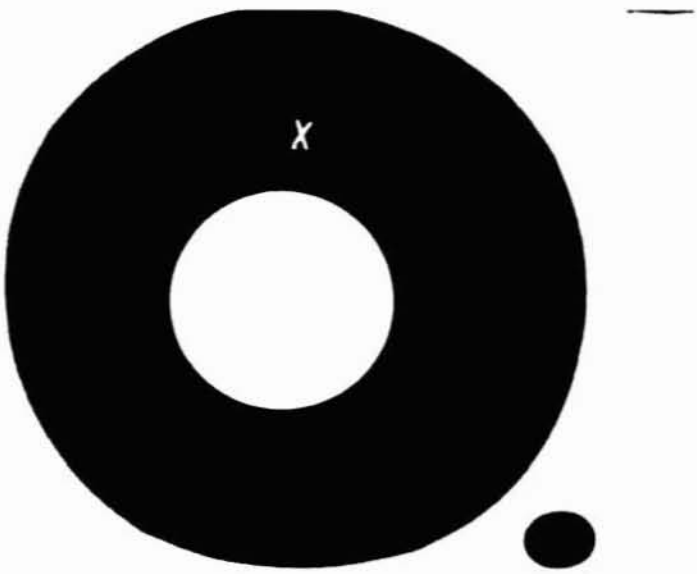

Figure 4.9: X-ray photographs of NR-150BS system (by using cone)

- **Effective atomic number**

The compositional percentage of NR-BS vulcanizates is shown in the table 4.8 and the effective atomic number of Natural Rubber – Barium Sulphate system is shown in table 4.9. It is clear from table that the effective atomic number of NR vulanizate increases with increase in radiopacifier and the effective atomic number of the system containing 150 phr radiopacifier are 36.54. When the effective atomic number increases, the electron density of the vulcanizate also increases, which in turn increases the radiopacity. This may be the reason why NR-BS system with 150 phr Barium sulphate gives higher radiopacity.

Table 4.8: The compositional percentage of elements

Elements	**Compositional %**			**Fractional content of elements**		
	NR-50BS	NR-100BS	NR-150 BS	NR-50 BS	NR-100 BS	NR-150 BS
Carbon	55.464	42.28	34.166	0.5307	0.3689	0.3466
Hydrogen	8.3175	6.341	5.124	0.1579	0.1038	0.1031
Oxygen	9.2763	13.565	16.335	0.0887	0.1183	0.1657
Nitrogen	0.0636	0.0513	0.0345	0.0006	0.0004	0.00035
Sulphur	5.999	6.653	8.900	0.0572	0.0579	0.0901
Zinc	2.5035	1.9097	1.5417	0.0219	0.3015	0.0143
Barium	18.289	27.881	33.789	0.1427	0.1983	0.2796

Table 4.9 : Effective atomic number of NR-BS vulanizate

Effective atomic number	NR-50BS	NR-100BS	NR-150BS
	29.2855	32.5647	36.5452

- **TGA studies**

Figure 4.10 shows the TGA thermogram of NR vulanizate with different amount of barium sulphate.

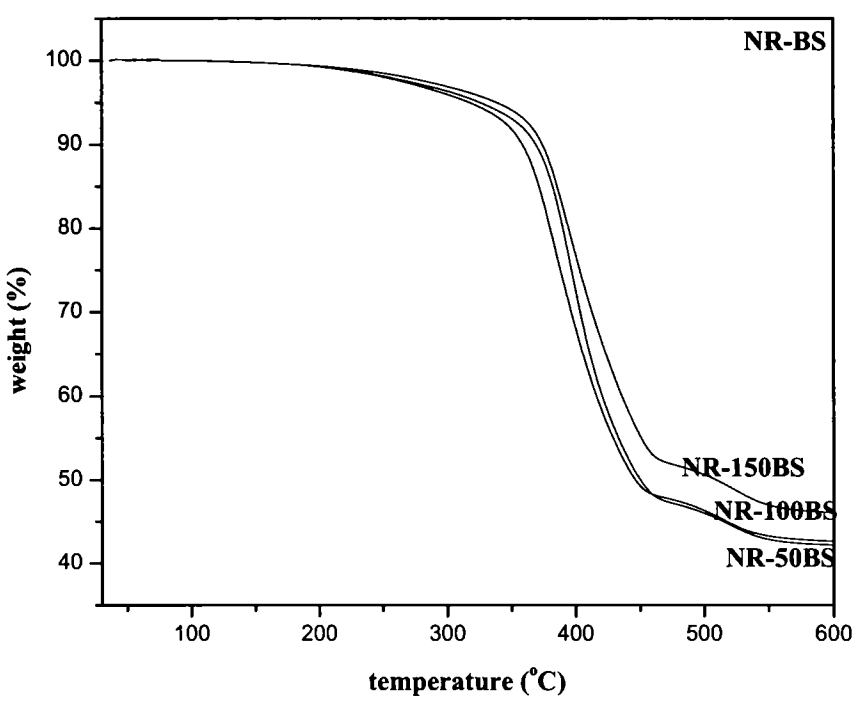

Figure 4.10: The TGA thermogram of NR vulanizate with different amount of barium sulphate

In all cases, the initial weight loss is observed between 100-300^{O} C and it is due to the evaporation of moister content present in the system. In the case of NR-BS system, 5 % degradation of NR chain is observed at 317° C for NR-BS system containing 50 phr barium sulphate, which is increased to 322 ° C for 100 phr barium sulphate system and it is further increased to 328° C for 150 phr barium sulphate system. The thermogram indicates that a degradation of 50 % is observed between 350 – 450° C. This indicates that the NR is more stabilized on compounding with barium sulphate.

- **Physical properties**

The variation of tensile properties with amount of barium sulphate is shown in figure 4.11.

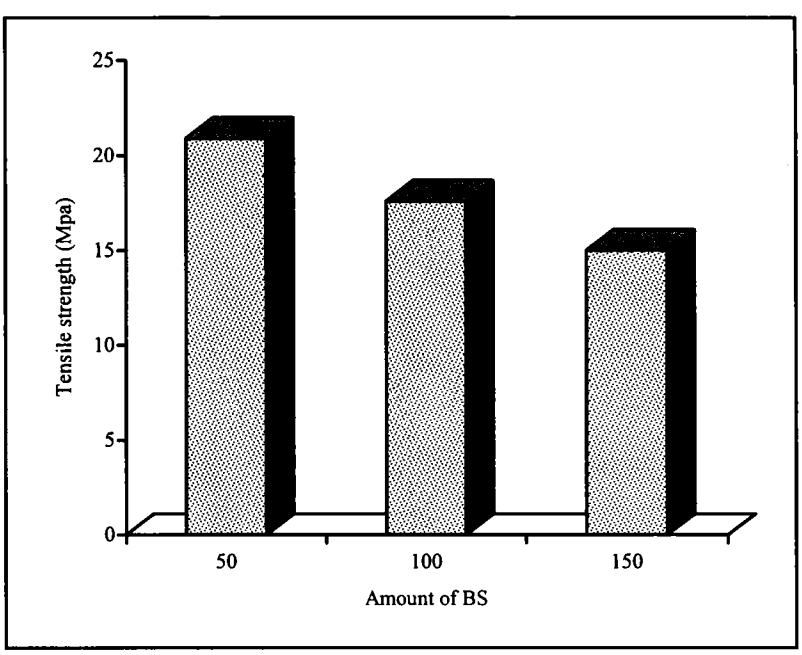

Fig 4.11: Variation of tensile strength with concentration of barium sulphate

It is clear from the figure that the tensile strength of NR vulcanizates with 150 phr of barium sulphate shows lower value. When the amount of inorganic filler increases in the NR matrix, its tensile strength decreases.

4.3.3.2 Natural rubber- Commercial Zinc oxide system

- **X-ray Analysis**

The X-ray photographs of Natural rubber–commercial ZnO (NR-ZnO) having radio pacifiers 50 phr, 100 phr and 150 phr are shown in figure 4.13. It is clear from the figure that the NR vulcanizate with 150 phr Zinc oxide give higher radiopacity as in the case of barium sulphate filler.

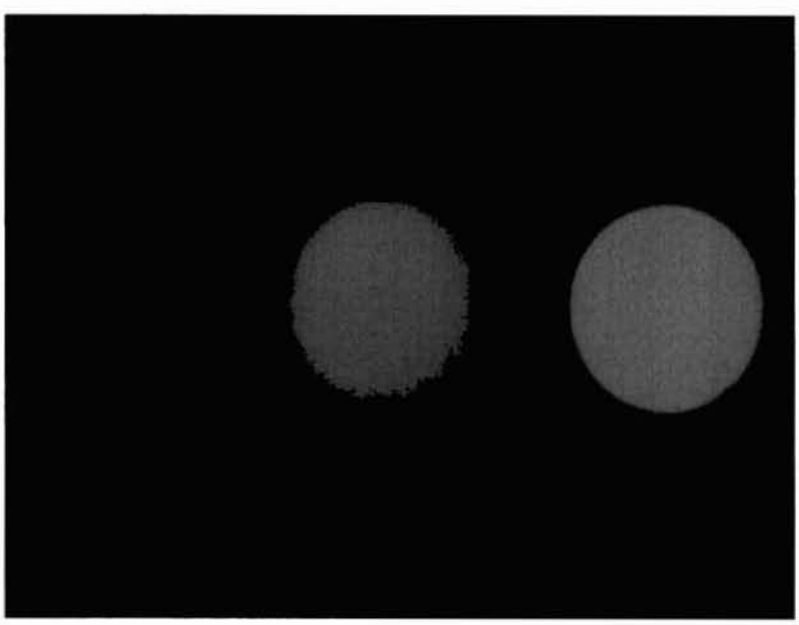

Figure 4.12: X-ray photographs of NR-ZnO systems at concentrations varying from 50 - 150 phr

- **Optical density studies**

The optical density values of X-ray film of NR vulcanizates containing commercial zinc oxide are shown in table 4.10. Natural rubber containing 150 phr radiopacifier have lower optical density (higher radiopacity) than that of 50 phr and 100 phr.

Table 4.12: Variation of optical density with amount of zinc oxide

SAMPLE	OPTICAL DENSITY
NR-50ZnO	0.88
NR-100ZnO	0.313
NR-150ZnO	0.19

- **Determination of atteunation coefficient**

The attenuation coefficient of NR 150 ZnO sample is measured using X-ray (cone) as shown in figure 4.13 and is found to be 1.1333. The higher the value the better will be the material for radiopaque applications.

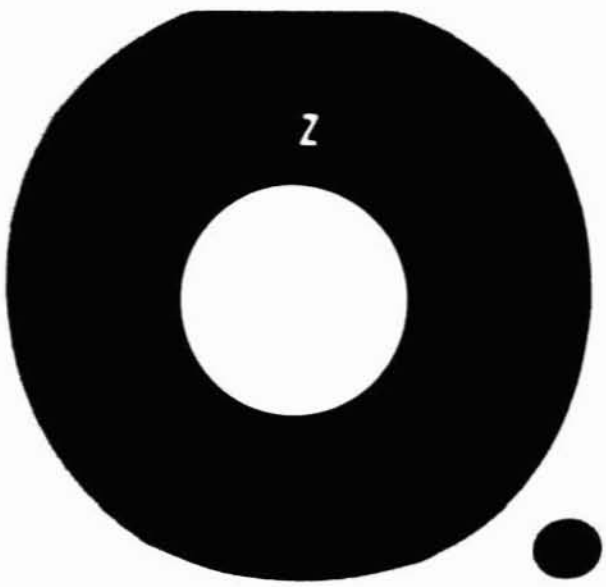

Figure 4.13: X-ray photographs of NR-150ZnO system (using cone)

- **Determination of effective atomic number**

Tables 4.11 and 4.12 show the compositional percentage of each element present in NR-ZnO vulcanizate and the effective atomic number of the NR vulcanizate containing zinc oxide respectively. The effective atomic number of NR-ZnO having 150 phr zinc oxide system is 23.51. The photoelectric absorption of X-ray is directly proportional to the atomic number and when the effective atomic number increases, radiopacity also increases.

Table 4.11: The compositional percentage of elements

Elements	Compositional %			Fractional content element		
	NR-50 ZnO	NR-100 ZnO	NR-150 ZnO	NR-50 ZnO	NR-100 ZnO	NR-150 ZnO
Carbon	57.25	43.106	34.835	0.5385	0.4189	0.3441
Hydrogen	8.58	6.495	5.224	0.1602	0.1252	0.1023
Oxygen	6.45	9.675	11.626	0.0606	0.0940	0.1149
Nitrogen	0.066	0.0015	0.0352	0,0006	0.0005	0.0003
Sulphur	1.784	1.3109	1.106	0.0167	0.0127	0.1090
Zinc	25.86	39.095	47.157	0.2232	0.3486	0.4273

Table 4.12: The effective atomic number of NR-ZnO Vulcanizate

	NR-50ZnO	NR-100ZnO	NR-150ZnO
Effective atomic number	18.2466	21.11	22.58247

- **TGA studies**

The thermograms of NR-ZnO of different zinc oxide loading such as 50phr, 100 phr and 150 phr are shown in figure 4.14. In all cases the initial weight loss is observed between 100 - 300° C due to the evaporation of water content present in the system. It is clear from the thermogram that 5 % degradation of NR chain is observed at 338° C for NR-ZnO system containing 50 phr commercial zinc oxide, which is reduced to 331° C for 100 phr commercial zinc oxide system and it further reduces to 327° C for 150 phr zinc oxide system. The TGA thermograms indicate that the thermal stability of the natural rubber decreases with increase in zinc oxide content.

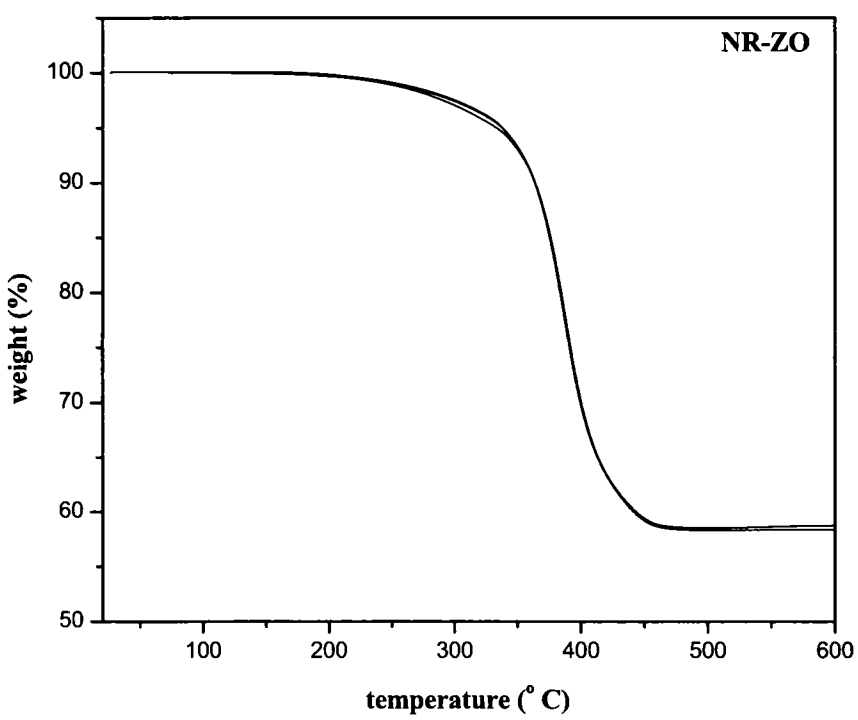

Figure 4.14: TGA thermogram of NR vulcanizates with different phr of zinc oxide.

- **Physical properties**

When the amount of zinc oxide in the NR vulcanizate increases, the tensile strength decreases as shown in figure 4.15. The NR Vulcanizate with 150 phr of zinc oxide shows poor tensile values compared to 100 phr and 50 phr system as in the case of NR-BS system.

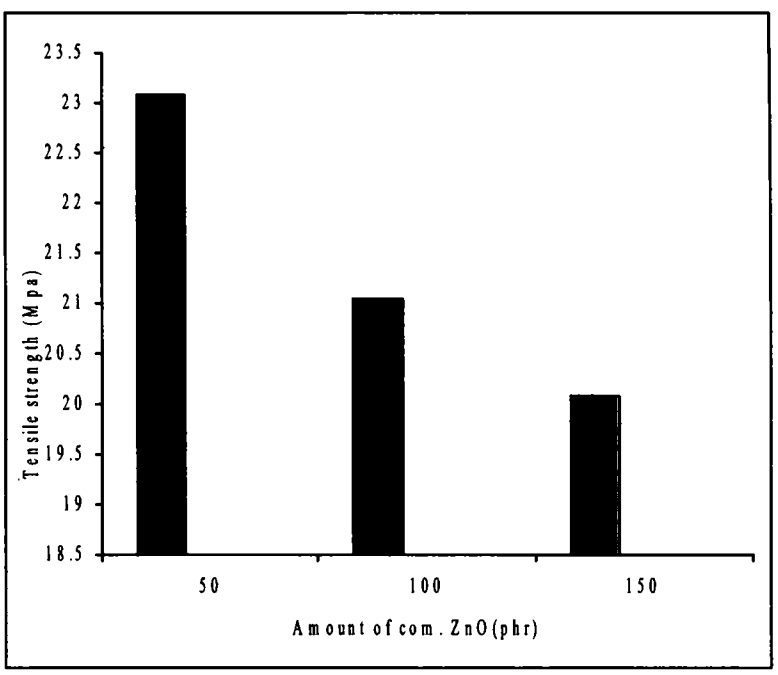

Figure 4.15: Variation of tensile strength with amount of zinc oxide

4.4 CONCLUSIONS

- Radiopaque Natural Rubber can be prepared by the iodination of Natural Rubber
- Low temperature cured Iodinated Natural Rubber shows better radiopacity than high temperature cured sample
- Iodinated natural rubber shows excellent antibacterial property
- The radiopacity of the Iodinated natural rubber is found to be dependent on the thickness of the sample and it increases with increase in thickness
- The optical density of the Iodinated natural rubber is found to be decreases with increase in thickness of the sample
- The D.C. conductivity of the INR sample is found to be in the order of 7.32×10^{-7} S/cm
- Radiopacifier filled radiopaque natural rubber is prepared using barium sulphate and commercial zinc oxide
- The NR vulcanizate with 150 phr of barium sulphate and zinc oxide gives better radiopacity than that of 100 phr and 50 phr
- The NR vulcanizate with barium sulphate system shows better radiopacity than that of zinc oxide
- The tensile properties of the NR vulcanizates decrease with increase in the concentration of radiopacifier

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CHAPTER 5

PREPARATION OF ZINC OXIDE IN CHITOSAN MEDIUM AND ITS EFFECT ON RADIOPACITY OF NATURAL RUBBER

5.1 INTRODUCTION

Radiopacifiers are the substances that are added to polymers to impart radiopacity. For example metal inserts such as fine wire, gold gauze or lead foil have been introduced into dental methacrylic resin to impart radiopacity, but they are of little use as they degrade the denture. Barium sulphate is one of the most widely used compounds for dental resins and bone cements. It is very stable and less expensive. Bismuth compounds are also used, but are more expensive than barium sulphate; it has higher density. Bismuth produces a brighter and sharper X-ray image than barium sulphate does. Another example is tungsten which is compatible with most polymers. Tungsten is more than twice as dense as bismuth and provides a high level of radiopacity; loading levels of up to 95 % by weight is

possible. Host compounds containing tungsten are dark grey in color, which limits coloring option.

5.1.1 ZINC OXIDE: MATERIAL FOR 21ST CENTURY

Zinc oxide is a material, which combines semi-conductivity, piezoelectric and electromechanical properties. It is an important functional oxide, exhibiting near fluorescent emission. Because of its nano-central symmetry, ZnO is piezoelectric, which is the key factor in building sensors and transducers. ZnO nano materials are promising candidates for nanoelectronics and photonics. A great variety of nano structures like nano belts, nano rods, nano rings etc, can be made of ZnO. Due to these factors it was cited as the material of the 21st century in Materials Today. [1]

Recently, nano materials obtained a great deal of attention in both fundamental and industrial research. Fine particle dimensions and narrow size distribution are often important while fabricating ceramic material of desired microstructure and with characteristic performance. Fine powders can exhibit unique electrical/optical characteristics compared to bulk materials, since surface characteristics are no longer negligible to volume characteristics in this regime.[2]

Zinc oxide-Euginol system is commonly used as radiopaque filler in dentistry. In addition to this, zinc oxide is a common additive in natural rubber. Hence a trial has been made to incorporate zinc oxide in natural rubber so as to impart radiopacity to the latter. From previous studies, it is clear that higher amount of zinc oxide is required to deliver better radiopacity. The control of particle size, morphology and crystalline phase of the particles during the synthesis process is essential to achieve key properties for the applications. The search for novel methods to prepare nano materials with controlled size and morphology still

remains a technical challenge. The synthesis of ZnO via aqueous carboxylate gelation route from zinc acetate is reported.[3]

In this part of the study the preparation of nano ZnO powder in chitosan medium is reported.

PART I

5.2 PREPARATION AND CHARACTERIZATION OF ZINC OXIDE

Nano-sized powders with uniform shape and narrow size distribution are shown to possess many interesting properties not shown by their bulk counterparts. They have larger surface area and wider band gap between valence and conduction bands. In many ways, they exhibit atom like behaviors. This can effectively enhance optical, chemical and electromagnetic properties. They sinter to dense bodies at lower temperatures, their microstructures are uniform after sintering, and they show quantum-confinement effects.[4] There are various methods for the synthesis of nanopowders. Main criteria for a good method are reproducibility in size and shape and control over average size. Basically there are two approaches in getting submicron powders; one is top to bottom approach i.e. mechanical break process by attrition etc. and the other, bottom to top i.e., building up process or nucleation process. This involves the phase change from vapor or liquid to solid. These methods are broadly classified into (a) low temperature and (b) high temperature methods. Among the low temperature techniques, chemical precipitation and replication methods have been widely used. Chemical precipitation technique include precipitation of solution from room temperature to 100° C, hydrothermal synthesis (> 100° C >1 atm pressure), inverse micelle method, sol-gel synthesis etc. These methods are ideally suited for precise control

of size and shape of nano particle size. In addition, they are cost effective because of less energy consumption. The main drawback in the precipitation technique is chemical contamination. The replication method has been used to produce nano oxides or metals by carrying reactions in micro pores and mesopores of either crystalline or amorphous materials [5].

The high temperature method includes gas condensation, self propagating high temperature synthesis, spray pyrolysis, laser ablation etc. In the gas condensation technique, metal is volatilized in inert atmosphere to produce nano powders. Laser ablation method use pulsed laser to evaporate metal atoms to form hot plasma, which then condenses to form nanoclusters. Chemical methods give the ability to produce powders with an exceptionally small size (nanometer range).[6] Different synthetic routes were proposed for the preparation of fine metal oxides. These are precipitation routes[7], micro emulsion[8], a combustion synthetic route[9], an adapted pechini process[10-12] etc.

5.2.1 EXPERIMENTAL

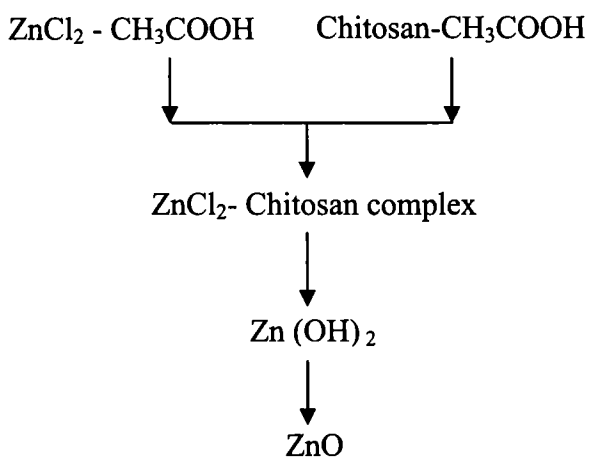
The following are the materials used in this part of the work: Zinc chloride, Zinc acetate, Zinc nitrate, 2 % chitosan solution, Acetic acid and Sodium hydroxide.

- ***Preparation of ZnO***

1. Using zinc chloride

Zinc oxide was prepared using *in situ* precipitation method. The medium for precipitation was prepared using a 2 % chitosan solution of degree of deacetylation > 85 %. Figure 5.1 shows the schematic overview of the preparation of zinc oxide. The zinc chloride salt was added to a solution of chitosan in acetic acid and was kept for 24 h. Then sodium hydroxide solution was added drop wise and kept for 12 h. The precipitate formed was filtered, washed several times with

water and dried at 100° C. Zinc hydroxide obtained was dried and calcined at different temperatures like 350° C, 450° C and 550° C.



5.1 Schematic overview of precipitation of zinc oxide

2. Using zinc acetate and zinc nitrate

Zinc oxide was prepared from zinc acetate and zinc nitrate using the *in situ* precipitation reaction (procedure mentioned in the above section). The zinc oxide formed was calcined at 550 ° C.

3. Characterization

The zinc oxide samples prepared were characterized using SEM, FTIR and XRD analysis (The description of these techniques are summarized in sections 2.2 of this thesis). Radiopacity of NR vulcanizates containing zinc oxide was also measured.

4. Crystallite Size

The crystallite size of the precipitated samples of zinc oxide was calculated using Scherer equation,

$$CS = 0.94/\beta\cos\theta \quad (1)$$

Where, CS is the grain size, β is full width at half-maximum (FWHM $_{hkl}$) of an *hkl* peak at θ value.

5.2.2 RESULTS AND DISCUSSION

5.2.2.1 Studies on zinc oxide prepared from zinc chloride (ZnO/E)

- **IR Spectroscopy**

Figure 5.2 shows the IR spectrum of the intermediate products formed at different temperatures during calcination.

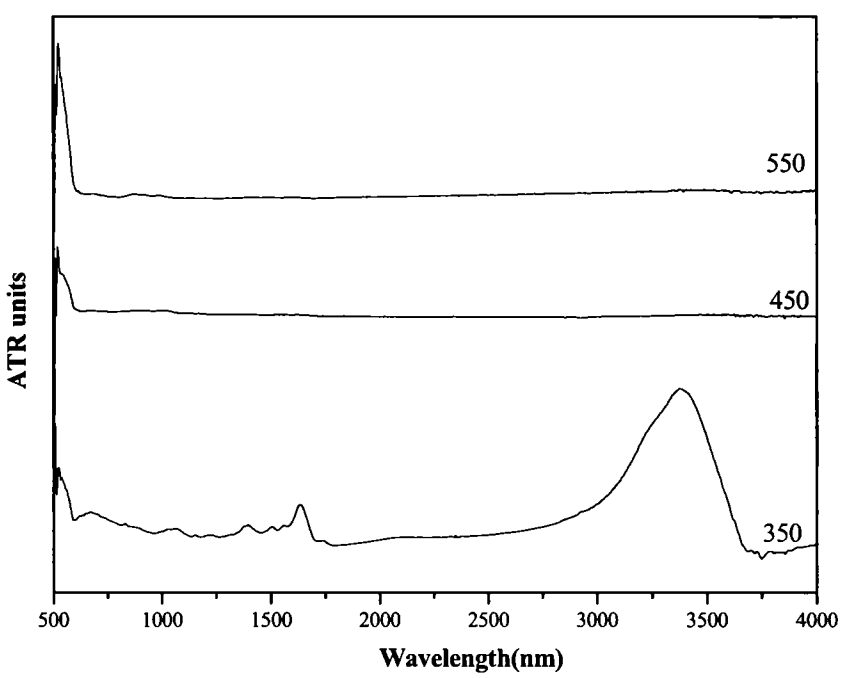


Figure 5.2: FTIR spectra of zinc oxide samples at different calcination temperatures

A broad peak in the range 3250 to 3600 cm^{-1} for the sample heated at 350° C indicates the presence of -OH stretching vibrations, which is due to the presence

of zinc hydroxide. It indicates that at 350° C the zinc hydroxide is not fully converted to zinc oxide. It is seen from the spectra that this peak is disappeared for the samples calcined at higher temperatures.

It is also clear from the spectra that the intensity of the characteristic peaks of zinc oxide in the range of 500-525cm^{-1} is increased when the calcinations temperature is increased.

- **XRD Studies**

Figure 5.3 shows the XRD patterns of zinc oxide samples formed at different calcination temperatures.

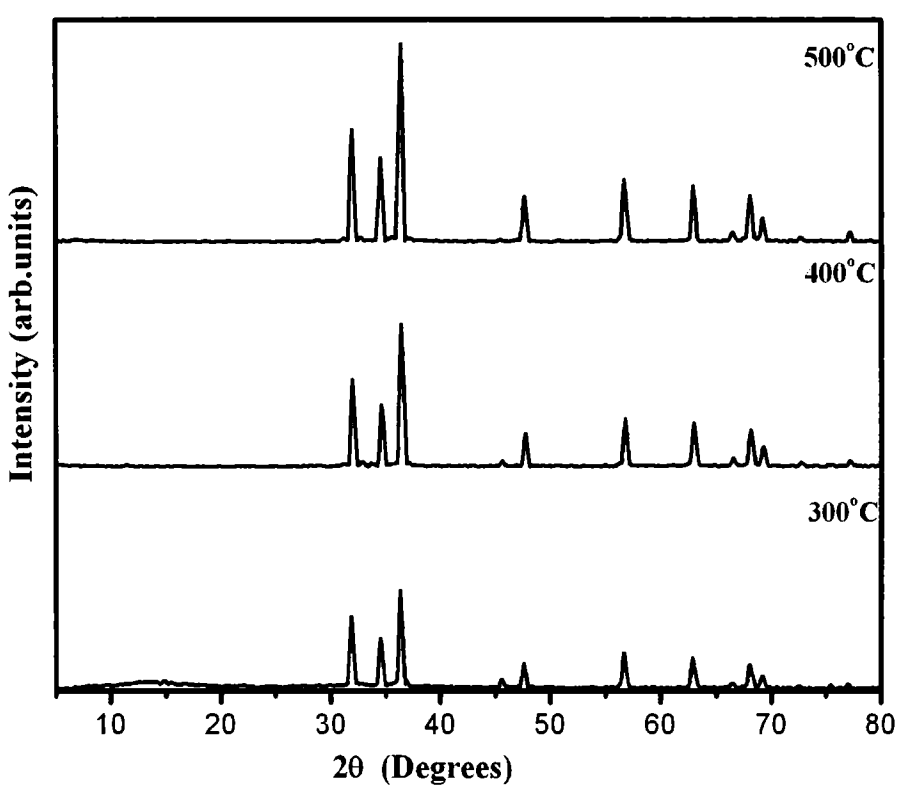


Figure 5.3: XRD patterns of zinc oxide samples calcined at different temperatures

The Bragg reflections of zincite are visible at all temperatures as shown in the figure. But at lower temperature, in the precursor, more amorphous regions are present, along with crystalline zinc oxide particles. When the temperature is increased, this amorphous region disappeared as shown in the XRD pattern of higher temperature samples.

It is also clear from the figure that the increase in calcination temperature, the crystallinity increases as indicated by the intensity of the XRD pattern. The zinc oxide samples calcined at 550° C showed lower particle size as indicated by high peak intensity[13].

Figure 5.4 shows the XRD pattern of zinc oxide prepared from zinc chloride, calcined at 550° C.

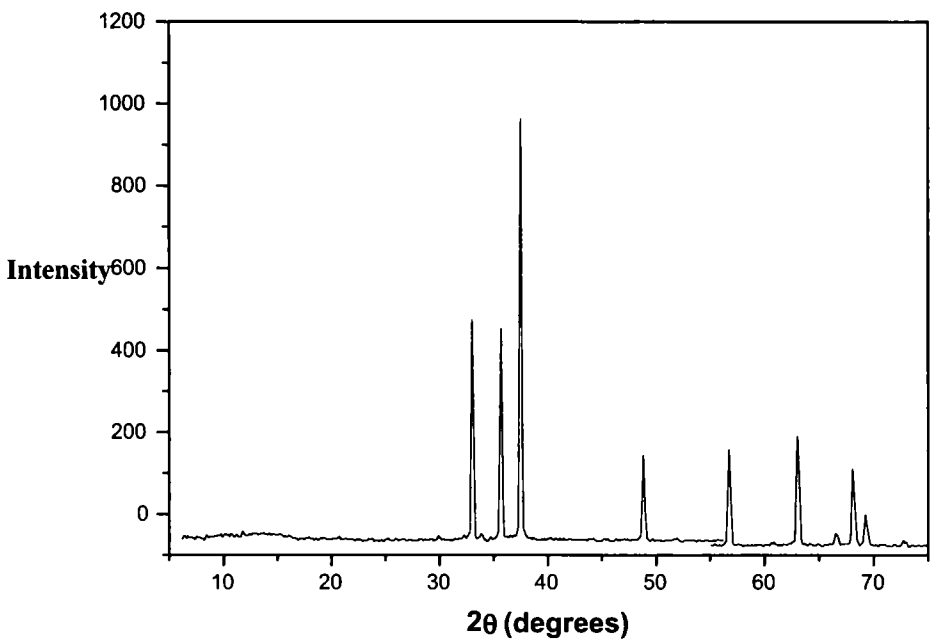


Figure 5.4: XRD patterns of zinc oxide samples calcined at 550 ^{0}C

It is clear from the figure that the major reflections between 30° and 40° (2θ values) indicate higher crystalline regions in the zinc oxide sample. Also the less intense peaks at 47.5°, 56°, 63° (2θ's) indicate the high crystallinity of zinc oxide samples.

- **SEM analysis**

The scanning electron micrographs of the zinc oxide sample prepared from zinc chloride without stirring is shown in figure 5.5. It is clear from the figure that the zinc oxide particles formed have uniform and specific plate like shape. It is also clear from the photograph that the zinc oxide formed is crystalline as its shape is changed from the honey comb structure of amorphous commercial zinc oxide as shown in figure 5.6. Also the particle size of the zinc oxide is reduced from 5 μm in the case of commercial zinc oxide to 200 nm in the case of zinc oxide prepared from zinc chloride salt.

Figure 5.7 shows the SEM photograph of zinc oxide prepared from zinc chloride with constant stirring. ZnO particles of uniform rode like structure having high aspect ratio, is obtained as shown in the figure. It is also clear from the figure that the particle size of 2 μm with more oriented zinc oxide particles is formed.

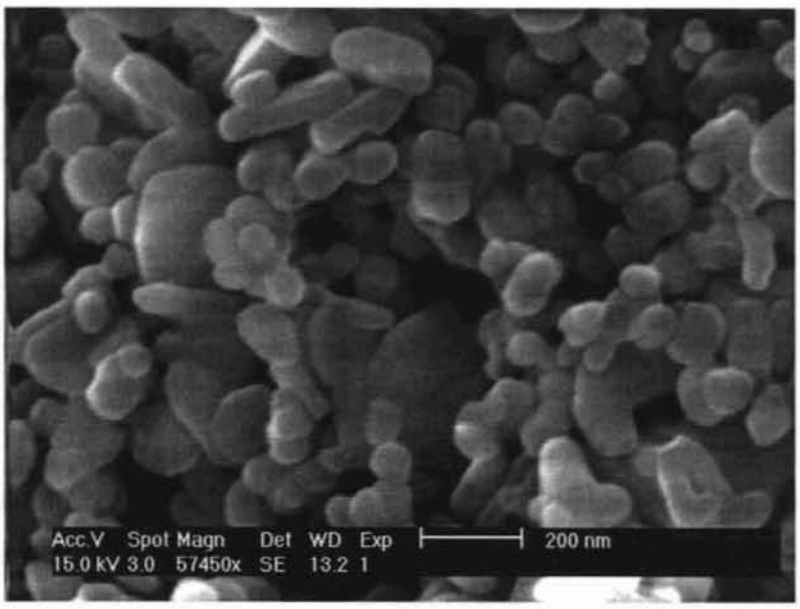


Figure 5.5: SEM photograph of zinc oxide prepared from zinc chloride salts (without stirring)

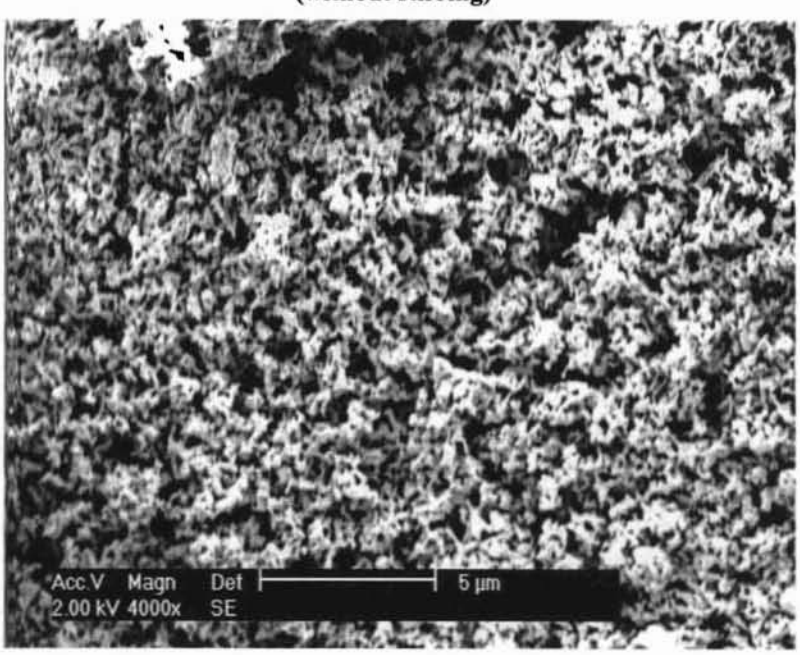


Figure 5.6: SEM photograph of commercial zinc oxide

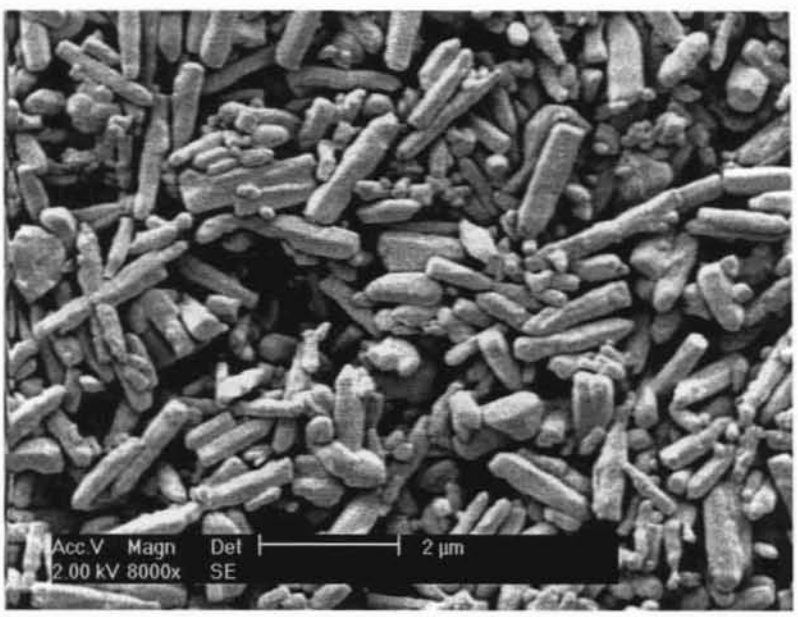


Figure 5.7: SEM photograph of zinc oxide prepared from zinc chloride (with stirring)

5.2.2.2 Studies on zinc oxide prepared from zinc acetate (ZnO/A) and zinc nitrate(ZnO/C)

- **SEM Analysis**

Figures 5.8 and 5.9 show the SEM photographs of zinc oxide prepared from zinc acetate salt and zinc nitrate salt respectively. It is clear from the figure 5.8 that the zinc oxide formed has a cauli flower structure. Zinc oxide prepared from zinc nitrate salt shows a coral structure.

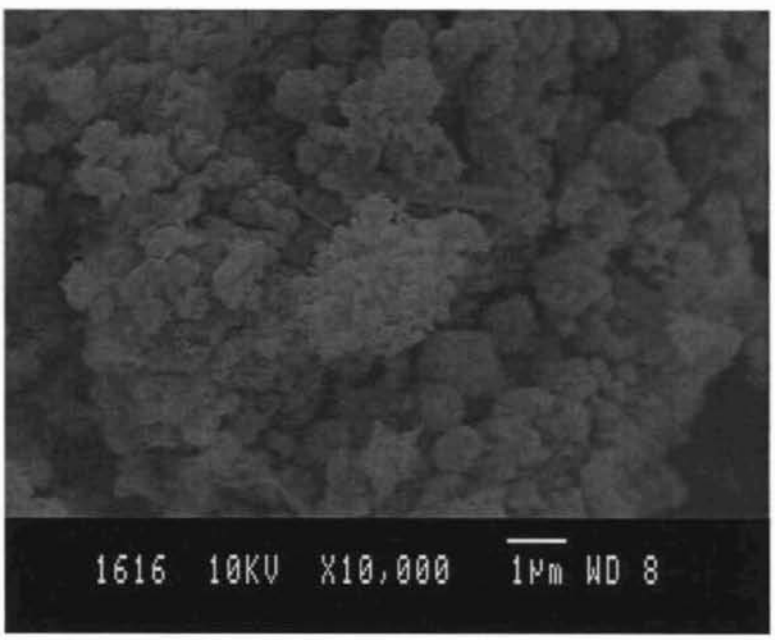


Figure 5.8:SEM photograph of zinc oxide prepared from zinc acetate

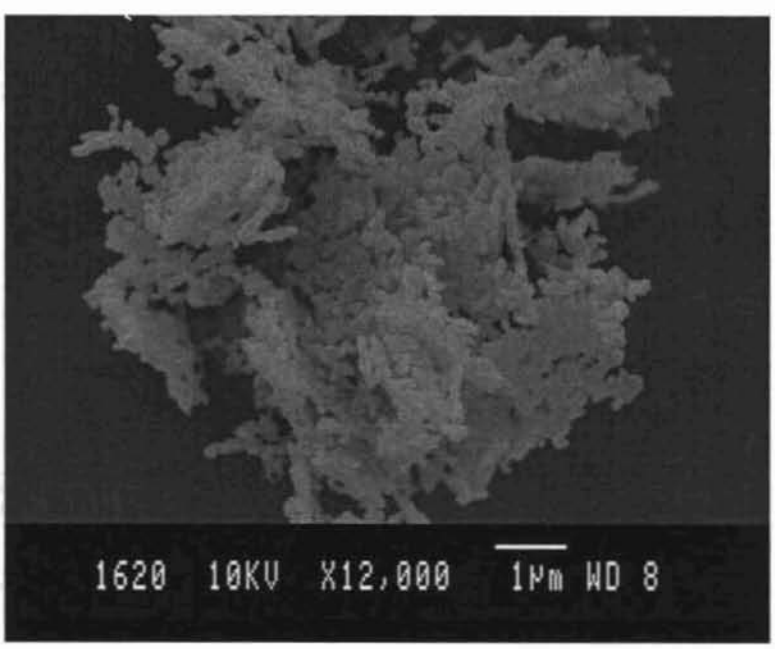


Figure 5.9: SEM photograph of zinc oxide prepared from zinc nitrate

- **X-ray Diffraction studies**

Figure 5.10 and figure 5.11 show the XRD patterns of zinc oxide samples prepared from zinc acetate and zinc nitrate respectively. It is clear from the two figures that the crystalline nature is similar to zinc oxide prepared from zinc chloride.

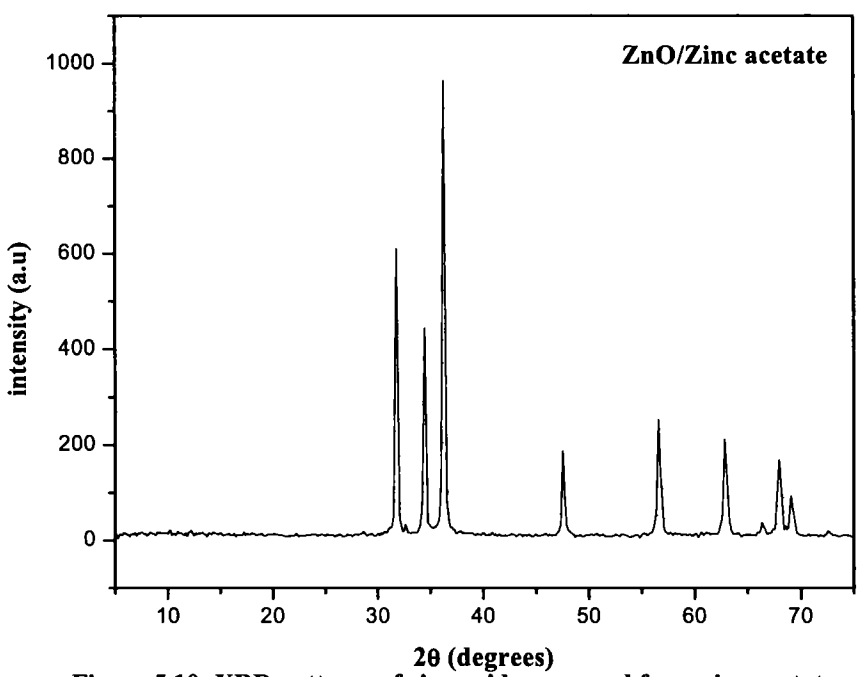


Figure 5.10: XRD patterns of zinc oxide prepared from zinc acetate

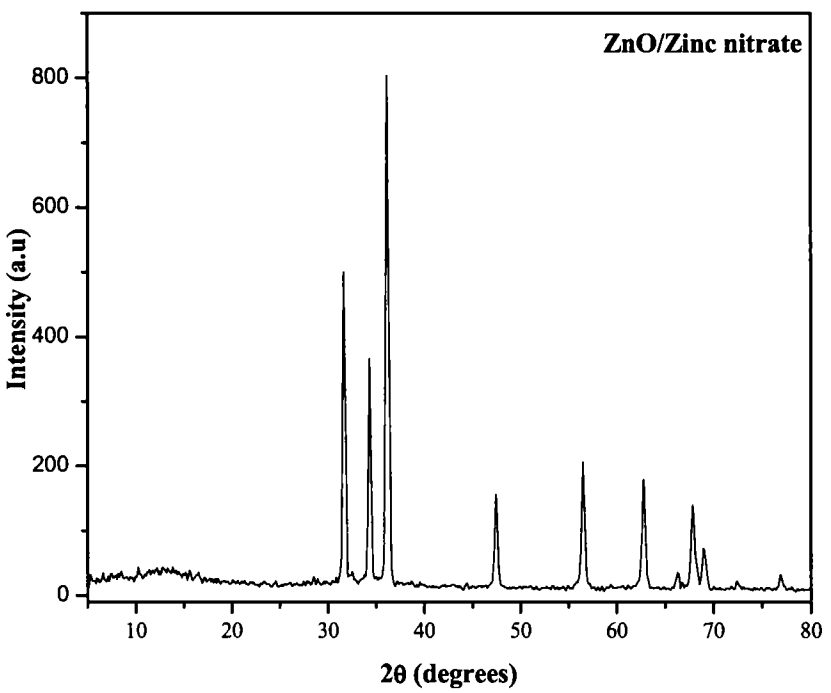


Figure 5.11: XRD patterns of zinc oxide prepared from zinc nitrate.

5.2.2.3 Comparison of particle size of zinc oxides prepared from three salts

- **Crystallite Size**

Table 5.1 shows the crystallite size of zinc oxide prepared from zinc chloride, zinc acetate and zinc nitrate salts. The full width at half-maximum of an *hkl* peak at θ value and crystal size of all the zinc oxide samples shows that the zinc oxides prepared from zinc acetate have smaller crystal size compared to the zinc oxides prepared from other salts. It is also seen that the zinc oxide prepared from zinc chloride shows high particle size but lower FWHM value.

Table 5.1: Crystallite size and FWHM values of zinc oxide

ZnO sample	FWHM	Crystallite size(nm)
ZnO/E	0.17253	50.59
ZnO/A	0.25982	33.59
ZnO/C	0.2398	36.40

- **Density measurements**

The density of different precipitated zinc oxide samples and commercial zinc oxide are shown in table 5.2. It is clear from the table that the density is higher for zinc oxide precipitated from zinc acetate. According to the principle of ultrasound imaging the radiopacity of the substance is directly related to its density and hence it confirms the higher radiopacity of zinc oxide prepared from zinc acetate.

Table 5.2: Comparison of density of zinc oxides

Sample	Density
ZnO/A	2.802
ZnO/E	2.74
ZnO/C	2.63
ZnO(commercial)	2.34

PART II

5.3 STUDIES ON THE EFFECT OF ZINC OXIDE ON THE RADIOPACITY OF NATURAL RUBBER

From previous studies, it is clear that higher amount of zinc oxide is required for good radiopacity. The control of particle size, morphology and crystalline structure of the particles during preparation process is essential to achieve key properties for the applications. In this section a detailed study on the radiopacity of natural rubber compounded with zinc oxide prepared from different zinc salts is reported.

5.3.1 EXPERIMENTAL

- ***Materials Used***

1. Natural Rubber
2. Compounding ingredients like activator,accelerator, curative and antioxident.
3. Zinc oxide as radiopacifier

- ***Preparation of NR-ZnO blends***

The compounds as per the formulations given in table 5.3 were mixed on a two roll mill size (15 x 33 cm) and were kept for 24 hrs for maturation. The compounds were moulded in an electrically heated hydraulic press at 150^0 C at a pressure of 200 kg/cm^2 up to respective optimum cure times. When the amount of radiopacifier is increased, it becomes very difficult to compound. Hence the amount of radiopacifier was limited to 150 phr. The sheets were cut into dumb bell shape to measure the tensile properties and round shape (1cm thickness and 60mm diameter) to measure radiopacity.

Table 5.3 : Formulation for NR Vulcanizates with precipitated zinc oxide

INGREDIENTS	MIX P (phr)	MIX Q (phr)	MIX R (phr)
Natural Rubber	100	100	100
Zinc oxide	50	100	150
Parafinic oil	5	7.5	10
Stearic acid	2	2	2
CBS	0.8	0.8	0.8
TMTD	0.15	0.2	0.25
Sulphur	2.5	2.5	2.5
Antioxidant	1.0	1.0	1.0

- **Optical density measurements**

The optical density is directly proportional to radiation exposure. It was calculated using the equation

$$OD = \log_{10} (I_0/I_t)$$

Where I_0 is the intensity of light incident on the film and I_t is the intensity of the light transmitted through the film. $I_0/I_{t\,is}$ the light stopping effect and opacity.

- **Attenuation coefficient**

The atteunation coeffecient was calculated using the equation

$$I/Io = e^{-\mu x}$$

Where I_0 is the value of optical density of background of the film and I_{is} the optical density of the sample 'x' the thickness of the sample and 'μ' the attenuation coefficient.

5.3.2 RESULTS AND DISCUSSIONS

5.3.2.1 Studies on NR-ZnO/E

- **X-ray studies**

The X-ray film of Natural rubber-Zinc oxide (NR-ZnO/E) samples with different concentrations of Zinc oxide is shown in figure 5.12. It is clear from the figure that the radiopacity of NR vulcanizate increases with increase in radiopacifier loading. The figure also shows that the radiopacity is higher for NR vucanizate with 150 phr of zinc oxide.

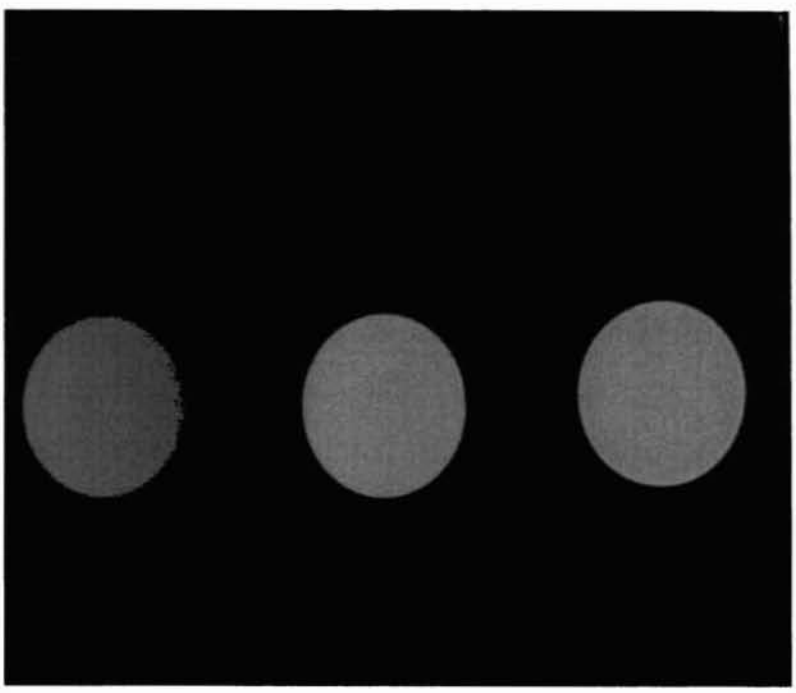


Figure 5.12: X-ray photograph of NR-ZnO/E systems at amount varying from 50 - 150 phr

- **Optical density**

The variation of optical density with amount of precipitated zinc oxide is given in table 5.4. When the amount of radiopacifier in the NR matrix increases the optical density decreases, as it is inversely related to radiopacity.

Table 5.4 The variation of optical density with amount of precipitated zinc oxide prepared from zinc chloride

SAMPLE	OPTICAL DENSITY
NR-50ZnO	0.271
NR-100ZnO	0.17
NR-150ZnO	0.15

The optical density values show that the precipitated zinc oxide samples is superior in radiopaque properties than that of commercial zinc oxide samples (as shown in chapter 4). From the optical density values it is clear that natural rubber containing 100 phr precipitated zinc oxide prepared from zinc chloride possesses an optical density value of 0.17 which is equivalent to that of natural rubber containing 150 phr barium sulphate (chapter 4).

Since it is very difficult for compounding with higher amount of fillers and also the radiopaque properties of 100 phr of zinc oxide samples are comparable with 150 phr barium sulphate, the amount of precipitated zinc oxide filler in NR is optimized to 100 phr.

- **Attenuation coefficient**

Figure 5.13 shows the x-ray photograph (using cone) of NR vulcunizates containing 100 phr zinc oxide prepared from zinc chloride. The attenuation coefficient of the sample is found to be 1.1813.

Figure 5.13 X-ray photograph of NR vulcanizates containing 100 phr precipitated zinc oxide prepared from zinc chloride(NR-ZnO/E)

5.3.2.2 Studies on (NR-ZnO/ A) & (NR-ZnO/ C) systems

- **Radiopacity studies**

The X-ray film of NR vulcanizates containing 100 phr of precipitated zinc oxide prepared from zinc acetate (ZnO/A) and zinc nitrate (ZnO/C) is shown in figure 5.14.

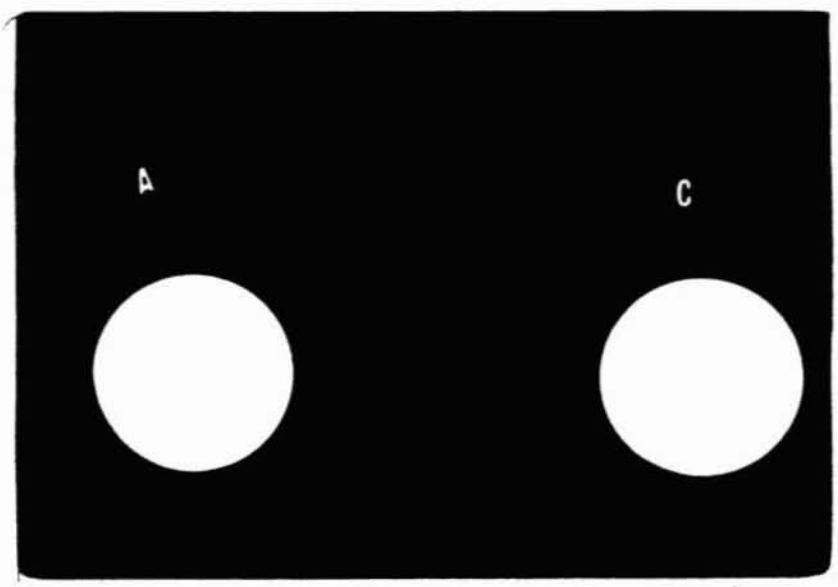


Figure 5.14 X-ray film of NR vulcanizates containing 100phr precipitated zinc oxides

(A-ZnO/A, C-ZnO/C)

It is clear from the figure that 100 phr of zinc oxide (prepared from zinc acetate and zinc nitrate) shows excellent radiopaque property in NR.

- **Optical density studies**

The optical density of NR vulcanizates containing 100 phr precipitated zinc oxide prepared from zinc acetate and zinc nitrate is given in table 5.5. The table indicates that the optical density of ZnO/A is less compared to ZnO/C system. Zinc oxide prepared from zinc acetate offer better radiopacity in NR than that of zinc oxide prepared from zinc nitrate.

Table 5.4: Optical density of NR vulcanizates containing precipitated ZnO

SAMPLE	OPTICAL DENSITY
NR-100ZnO (ZnO/C)	0.17
NR-100ZnO (ZnO/A)	0.15
Background	2.36

- **Attenuation coefficient**

The x-ray photographs of different NR vulcanizates containing 100 phr precipitated zinc oxide prepared from different zinc oxide samples prepared from zinc chloride, zinc acetate and zinc nitrate are shown in figures 5.15 and 5.16 respectively and the attenuation coefficient values are shown in table 5.6.

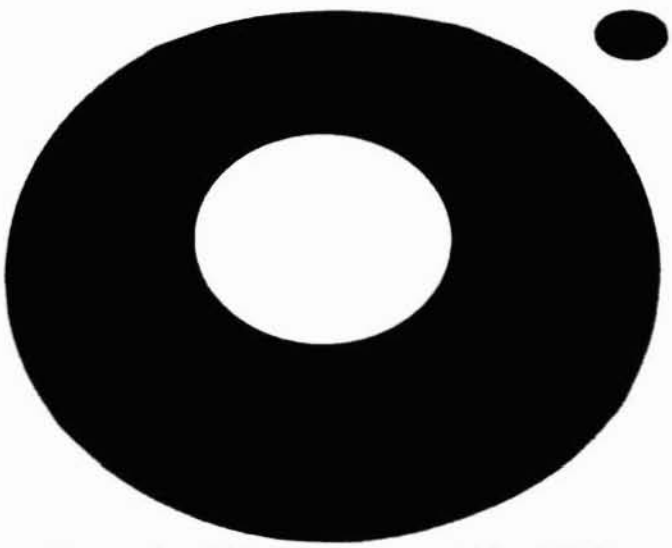


Figure 5.15 X-ray photographs of NR vulcanizates containing 100 phr precipitated zinc oxide prepared from zinc acetate (NR-ZnO/A)

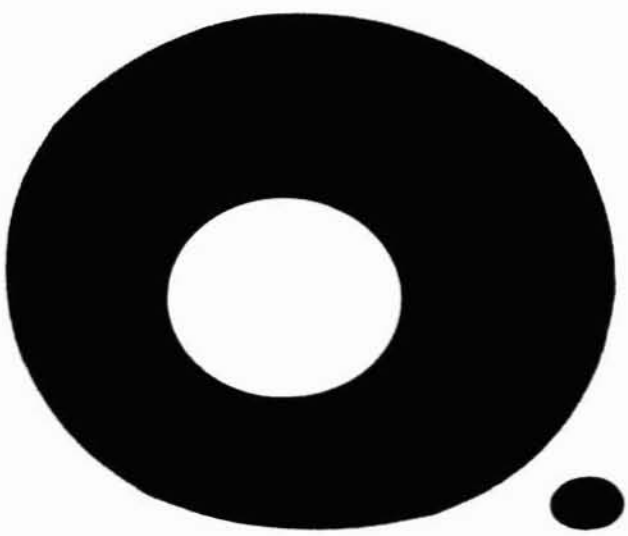


Figure 5.16 X-ray photographs of NR vulcanizates containing 100 phr precipitated zinc oxide prepared from zinc nitrate (NR-ZnO/C)

Table 5.6. Atteunation coefficient of NR vulcanizates containing precipitated zinc oxide prepared from zinc acetate and zinc nitrate

Sample	Io	I	Atteunation coefficient
NR-(ZnO/C)	2.183	0.17	1.0935
NR-ZnO/A	2.193	0.I2	1.2618

It is clear from the table that the attenuation coefficient is higher for zinc oxide prepared from zinc acetate. The higher the value, the better will be the material for radiopaque applications.

- ***Comparison of ZnO/E, ZnO/A and ZnO/C***

The optical density, crystallite size and attenuation coefficient of zinc oxide samples prepared from zinc nitrate, zinc acetate and zinc chloride are given in table 5.7.

Table 5.6. Comparison of properties of ZnO/E, ZnO/A and ZnO/C

Sample	Crystallite size	Optical density	Density	Attenuation coefficient
ZnO/E	50.59	0.166	2.74	1.1813
ZnO/A	33.59	0.12	2.802	1.2618
ZnO/C	36.4	0.17	2.63	1.0932

It is clear from the table that the crystallite size of ZnO/A is less, compared to ZnO/C and ZnO/E. Also the optical density of ZnO/A is less and the attenuation coefficient is higher for ZnO/A. The density is also higher for ZnO/A. Therefore it is confirmed that the zinc oxide prepared from zinc acetate shows better radiopacity and it is a very promising material for applying as radiopaque filler.

5.4 CONCLUSIONS

- Zinc oxide with higher crystallinity can be prepared from zinc chloride, zinc nitrate and zinc acetate salts, using chitosan medium
- Zinc oxide prepared from zinc acetate salt is found to be lesser in size compared to zinc oxide prepared from nitrate and chloride salts
- The optical density is lower and the attenuation coefficient is higher for zinc oxide prepared from zinc acetate salt
- The radiopacity is found to be higher for NR-ZnO/A vulcanizates compared to NR-ZnO/E and NR-ZnO/C
- NR compound with 100 phr zinc oxide prepared from zinc acetate is an excellent material for applications in the area of radiopaque materials

5.5 REFERENCES

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CHAPTER 6

SUMMARY AND CONCLUSIONS

Radiopaque materials open up a new outlook to various technological applications like biomedical, radiation shielding, toy manufacturing, plastic explosives etc. In view of the scope and ample potential of radiopaque polymers, an attempt has been made in the present investigation to develop radiopaque systems from natural polymers, which are promising to be applicable in medical field. Most of the conventional radiopaque systems are based on synthetic polymers. Chitosan and natural rubber, two of the most versatile natural polymers, were selected as the base polymers for the present work, due to their excellent performance characteristics.

A brief literature survey tracing the developments of radiopaque polymers, their advantages, classifications and a short review on chitosan and natural rubber that lead to the scope and objectives of the present investigation are given. A description of the evolution and properties of biomaterials and the new area of applications based on these materials are also given. The essential properties of

radiopaque polymers, methods to impart radiopacity in polymers and important technological applications have been discussed in detail. The recent trends in the field of radiopaque polymers and their applications in the medical field are highlighted.

The promising area of applications of radiopaque polymers leads to an exploitation of radiopaque properties like x-ray visibility, optical density, effective atomic number, attenuation coefficient of biopolymers like chitosan, chitosan formate, chitosan acetate, carboxy methyl chitosan and natural rubber. The radiopaque properties of these materials highly depend upon the size, shape, amount of radiopacifier and crystallinity of the radiopaque material.

Radiopaque chitosan microspheres were prepared by cross linking with glutaraldehyde followed by the encapsulation of barium sulphate. The effect of different emulsion systems on the morphology of chitosan microspheres were studied. Naphthenic oil / ammonium oleate, silicon oil / ammonium oleate and liquid paraffin / sorbitan sesqueoleate systems were used for the preparation of microspheres. Among these emulsion systems, liquid paraffin / sorbitan sesqueoleate gave good quality, free flowing microspheres with narrow particle diameter distribution. Water soluble derivatives of chitosan like chitosan formate, chitosan acetate and carboxy methyl chitosan were successfully prepared and characterized using infrared spectroscopy. Radiopaque microspheres of these derivatives were also prepared using liquid paraffin / sorbitan sesqueoleate system. Chitosan formate resulted in microspheres with perfect spherical morphology where as chitosan acetate does not. In the case of carboxy methyl chitosan, only micro particles were obtained. Microspheres were also prepared from the blend of poly (vinyl alcohol) / carboxy methyl chitosan. These microspheres show better surface morphology with excellent radiopacity.

Another important application of radiopaque polymers is radiation shielding. Conventionally lead is used for shielding purposes but not without health and environmental concerns. In this study, an attempt has been made to prepare radiopaque natural rubber for shielding applications. At first natural rubber was iodinated to make it radiopaque. This iodinated natural rubber shows good radiopacity. When the iodinated natural rubber vulcanizate is cured at high temperature, its radiopacity decreases due to the elimination of iodine at higher temperatures. But the low temperature cured iodinated NR shows good radiopacity. Also the idonated NR possesses anti-bacterial property. The DC conductivity of low temperature cured iodinated natural rubber is found to be in the order of 10^{-7} Scm^{-1}. Radiopaque natural rubber is also prepared by addition of radiopaque fillers such as barium sulphate and commercial zinc oxide. Natural rubber vulcanizates with 150 phr of barium sulphate and commercial zinc oxide gave better radiopacity than that of 100 phr and 50 phr. Among these NR vulcanizates barium sulphate system show better radiopacity than that of zinc oxide. The physical properties of natural rubber decreases with increasing amount of radiopacifier. A drastic decrease in tensile properties was observed in the case of natural rubber containing barium sulphate. A noteworthy finding of this study is that 150 phr of a common additive in rubber compounding like zinc oxide gave fairly good radiopacity and physical property in NR. For the same reason further studies were concentrated on natural rubber vulcanizates containing zinc oxide system.

Zinc oxide is an important functional oxide, available with a great variety of nano structures like nano belts, nano rods, nano rings etc. From the preliminary studies, it has become clear that a higher amount of zinc oxide is required to deliver better radiopacity to polymer matrices like NR. In order to improve the particle size, morphology and crystalline phase of the zinc oxide particles, a

novel method for the preparation of zinc oxide is adopted. It was prepared by using a natural polymer, chitosan, as the medium for precipitation. Different salts of zinc are used as the starting material for its preparation. A detailed comparison of their crystallite size and surface morphology was done. Zinc oxide prepared from different zinc salts shows different surface morphology and crystallite size. By varying the amount of precipitated zinc oxide (prepared from zinc chloride) in NR, radiopacity studies were carried out. A remarkable observation of this study is that 100 phr precipitated zinc oxide is equivalent to 150 phr of barium sulphate in natural rubber to offer same extent of radiopacity. Reducing the content of radiopaque filler in the matrix polymer matrix, substantially reduces the processing difficulty to a great extent.

A detailed radiopacity study was done in natural rubber containing 100 phr precipitated zinc oxide prepared from different zinc salts. Variation in optical density was observed in these systems as wafer thin. In order to determine the attenuation coefficient of these systems, X-ray of these samples was taken using a cone that helps reducing scattering. One of the significant findings of this investigation is that NR vulcanizates containing precipitated zinc oxide (from zinc acetate) shows higher attenuation coefficient. Among all zinc oxide samples, the zinc oxide prepared from zinc acetate salt is superior in properties such as radiopacity, crystallite size, optical density and attenuation coefficient. These interesting findings reveal the applications of these natural radiopaque systems in various fields like surgical tools, medical tubings, catheters, radiation shielding, etc.

LIST OF PUBLICATIONS

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2. Nisha V.S, Rani Joseph, "Preparation and characterization of Radiopaque Natural Rubber" (Journal of *rubber chemistry and technology, Accepted for publication*)

3. Nisha V.S, Rani Joseph, "Preparation and properties of iodine doped radiopaque natural rubber" (communicated to *Journal of applied polymer science*)

4. Nisha V.S, Rani Joseph, "Effect of various emulsion systems on the preparation of radiopaque chitosan microspheres" (communicated to *Asian journal of chitin*)

5. Nisha V.S, Rani Joseph, Santhosh V.S, Babu.B.R.S "A comparative study of filled radiopaque natural rubber containing barium sulphate and zinc oxide" (Communicated to *Journal of applied polymer science*)

International/National Conferences

1. Nisha V.S, Rani Joseph, Preparation and properties of radiopaque chitosan microspheres, 16th AGM, MRSI 2005.

2. Nisha V.S, Rani Joseph, "Studies on radiopaque iodinated natural rubber" ICBC, Kottayam, 2005.

3. Nisha V.S, Sona Stanly, Sinto Jacob, Rani Joseph, "Effects of active zinc oxide on the properties of NR and Neoprene rubber", International seminar on Polymer composites. SB College, Changanacherry.

4. Nisha V.S, Rani Joseph, "Preparation and characterization of radiopaque Natural Rubber", Technical Meeting of The Rubber Division, American Chemical Society, Pittsburg, USA.

5. Nisha V.S, Sona Stanly, Sinto Jacob, Rani Joseph, "Insitu precipitation of zinc oxide in chitosan medium and its effects on NR and Neoprene rubber vulcanizates", IRMRA, Mumbai.

6. Nisha V.S, Rani Joseph, "Insitu precipitation of zinc oxide in chitosan medium and its effect on radiopacity in natural rubber", EMTIC, University of Calicut.

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LIST OF ABBREVIATIONS

%	Percentage
ACT	Acetone
BS	Barium sulphate
CHN	Chitosan
cm	Centimeter
CMC	Carboxy methyl chitosan
CS	Crystallite size
D.C.	Direct current
DEE	Diethyl ether
GA	Glutaraldehyde
gm	Gram
HCl	Hydrochloric acid
I_2	Iodine
INR	Iodinated natural rubber
IR	Infra red
m	Meter
min	Minutes
nm	Nanometre
NO	Naphthenic oil
NR	Natural rubber

°C	Degree Celsius
OD	Optical density
PVA	Poly (vinyl alcohol)
S	Seimen
Sec	Seconds
SEM	Scanning electron microscopy
Sil	Silicone oil
TGA	Thermo gravimetric analysis
x	Thickness
XRD	X-ray diffraction
ZnO	Zinc oxide
μ	Attenuation coefficient