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PREPARATION AND CHARACTERISATION OF BIOPOLYMER NANOPARTICLES FOR BLOOD CLOTTING MATERIAL

PROJECT REPORT

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BONAFIDE CERTIFICATE

This is the bonafide record of the project titled **"PREPARATION AND CHARACTERISATION OF BIOPOLYMER NANOPARTICLES FOR BLOOD CLOTTING MATERIAL,"** carried out by the following students of the requirements for the award of the degree of Bachelor of Technology in Textile Technology during the year 2010-2011 under the guidance of Dr.Bhaarathi Dhurai

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ABSTRACT

Blood loss is a major problem during accidents, surgery and wars. The death rate of patients is high because of heavy blood loss. In order to reduce the blood loss we are preparing and characterizing bio polymer nano particles for blood clotting, which can clot quickly.

Bio polymers like Chitosan and Sodium alginate has received much attention as a functional biopolymers for diverse application in pharmaceutics and medicine .Our recent efforts focused on the conversion of these biopolymers to nano form and thus increases the surface contact area .There is no risk in using these nano particles since they are derived from shrimp and sea weeds.

Ball mill technique is used to synthesis the biopolymers . These nano particles are characterized by XRD , FTIR techniques, for their particle size ,mechanical properties and the chemical linkages are also identified.

.The blood clotting properties of these biopolymers are tested by analyzing blood clotting time and the efficiency of these polymers can be determined

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SYMBOLS

? - Bragg's diffraction angle

? - wave length

? W - full width of the x-ray pattern line at half peak height

D - crystallite diameter

ABBREVIATION

RBC - Red Blood Cells

WBC - White Blood Cells

TFPI - Tissue Factor Pathway Inhibitor

TF - Tissue Factor

CP - Chitosan Pure

CM - Chitosan Milled

SAP - Sodium Alginate Pure

SAM - Sodium Alginate Milled

XRD - X-ray diffraction

FTIR - Fourier Transform Infrared Spectroscopy

SAXS - Small angle X-ray scattering

WAXS - Wide angle X-ray scattering

1.INTRODUCTION:

Biopolymers have a long history of use as biomaterials for haemorrhage control.Typical haemostatic biopolymers include proteins and polysaccharides(chitosan and chitin).They have been used in the forms of solid sheets sponges,powders and liquids.Biopolymers are organic polymers and it can be produced without toxic byproducts and biodegrade quickly.Many of the biopolymers are used in the medical field because of its non-toxic nature.So we use some of the biopolymers such as chitosan and sodium alginate in medical applications because they are having anti-bacterial and blood clotting activity.Chitosan a natural cationic polysaccharide has received considerable attentions as a functional , renewable , non-toxic and biodegradable biopolymer for diverse applications especially in pharmaceutics.Chitosan is extracted from shrimp shells and sodium alginate is extracted from seaweeds.These biopolymers are converted into nanoparticles using ball mill technique.A Planetary High-Energy Ball mill is used for producing nanosized powders.The resulting nanoparticles have more surface contact area which in turn increases the blood clotting property.These nanoparticles are characterised by FTIR,XRD and particle analyser methods. Fourier Transform Infrared Spectroscopy (FTIR) provides specific information about chemical bonding and molecular structures, making it useful for analyzing organic materials and certain inorganic materials. Chemical bonds vibrate at characteristic frequencies, and when exposed to infrared radiation, they absorb the radiation at frequencies that match their vibration modes.X-ray diffraction is presently the only portable non destructive method that can quantitatively measure residual stress in crystalline and semi-crystalline materials.

2 LITERATURE SURVEY

2.1 INTRODUCTION ABOUT BLOOD :

BLOOD:

Blood is a liquid tissue that has three major functions; as a transportation, for regulation, and protection. Blood transports materials to and from all the cells of the body Wastes produced by the cells carried away in the blood to organs which remove the wastes. Blood acts as a regulator. Blood can absorb heat from warm areas of the body and releases the heat in cooler areas. The blood usually maintains a constant pH and water balance. Blood also protects the body. It holds specialized cells and chemicals that defend the body against diseases. Blood has the ability to clot, preventing the body from loosing large amounts of blood due to an injury The Components of the Blood - Because blood has many functions you might be able to conclude that the blood is composed of many different parts. The liquid part of the blood is called plasma. Plasma takes up about 55% of the total volume of the blood. The remaining 45% of the blood is made up of red blood cells, white blood cells, and platelets. An adult human has between four and six liters of blood in the body.

Plasma - Plasma is the clear liquid portion of the blood. 90% of plasma is water. The other 10% contains many types of molecules, including nutrients, glucose, vitamins, cellularwastes, salts, and proteins. There are three major types of proteins which exist in plasma. These are albumin, fibrinogen, and globulins. Each proteins has a specific function to perform. The albumin keeps water from leaving the blood and entering the surrounding cells by osmosis. It does this by helping to keep the concentration of the water within the blood the same as the concentration in the body tissues. The fibrinogen aids in the clotting of the blood. Some globulins transport proteins and other substances from one part of the body to the next. Other globulins are known as antibodies, which help to fight of infection. Antibodies are proteins thatattach to and help destroy foreign substances in the body.

Red Blood Cells - These cells are red and carry oxygen and carbon dioxide. They are present in huge numbers within the blood. The human body conatins 30 trillion red blood cells, or approximately 5 million cells per cubic millimeter of blood. The red blood cells transport oxygen from the lungs to the tissues in the body.

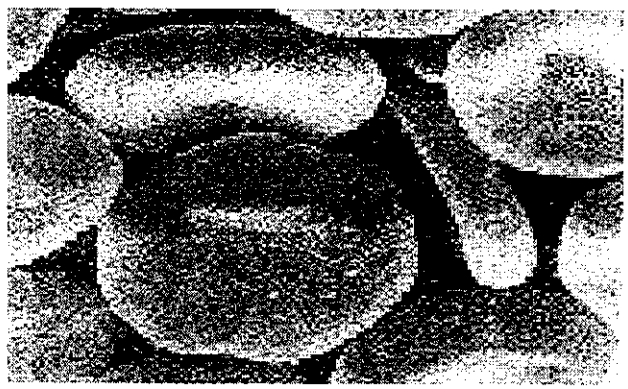


FIG 1 IMAGE OF RED BLOOD CELLS

They also carry carbon dioxide from the body tissues to the lungs. In humans, the matured red blood cells do not contain a nuclei. Their cytoplasm is filled with an iron-containing protein called hemoglobin. Hemoglobin is the substance that gives the blood its red colour. Human red blood cells are constructed by bone marrow and have and a average life span of up to 120 days. New cells are produced at the same rate red blood cells are destroyed . This occurs at pace of about 2 million per second. The old red cells are removed from the body by the spleen and liver and are then broken down. The iron from the hemoglobin is then collected and reused.

When a person has an insufficient amount of hemoglobin or too few red blood cells, this is referred to as anemia. Both of these conditions lowers the amount of oxygen that can be carried throughout the blood. Anemia causes the cells not to recieve the proper amount of oxygen. This is a heriditary disorder, and is caused by an abnormal form of hemoglobin.

White Blood Cells :

There is a variety of colorless blood cells which make the white blood cells, or known as leukocytes. These white blood cells are defenders for the body. They proect the body from

bacteria and viruses, which are disease-causing organisms. Unlike red blood cells, the white blood cells contain a nucleus and are larger than the red blood cells. There are fewer white blood cells than white, but there are still about 60 billion in an adult human body. The bone marrow and lymphatic tissue produce approximately 1 million white blood cells every second. The white bloods cells are distribute themselves throughout the body by moving through the circulatory

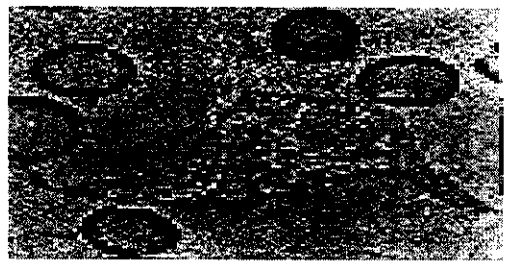


FIG 2 IMAGE OF WHITE BLOOD CELLS

system. When there is an infection within the body, the white blood cells collect in the infected area and attack the foreign organisms.

There are five different types of white blood cells. The majority of them function to protect the body in some form. A portion of the white blood cells are what are called phagocytic(monocytes & neutrophils). They protect the body by fighting the bacterial invaders, and anything which does not belong in the body. The lymphocytes take care of the production of antibodies and the cells that destroy certain substances and uncommon cells. Usually, there are 7000 to 10 000 white blood cells present per cubic millimeter of blood. When an infection of the blood occurs, the numbers of white blood cells may increase to 30,000 or more per cubic millimeter. The phagocytic white blood cells eat the bacteria which they encounter. After these phagocytic cells eat the bacteria some of them die. This is what pus is when it forms around an infected area.

Platelets - The part of the blood which is involved in the clotting of blood. Platelets are formed when bits of cytoplasm are pinched. Even though these bits of cytoplasm contain no nuclei, they surrounded by a membrane. There about a total of 1.5 trillion platelets in the blood of an adult human. There are about 300,000 platelets existing in a cubic millimeter of blood. Their life lasts for about seven days and are produced at about 200 billion per day.

2.2 BLOOD CLOTTING PROCESS:

Unless blood is a free-flowing liquid it will not be able to circulate easily throughout the body's blood vessels. However, in being a liquid it could cause a large variety of problems. If there was an injury that broke a large blood vessel it could lead to a large loss of blood. This problem is resolved by the complex mechanism of clotting. The clots form a temporary barrier to prevent blood loss until the vessel walls have healed.

When a blood vessel is injured, platelets begin to collect near the injury, which forms a barrier known as the platelet plug. When the platelets come in contact with an injured area, they swell up, become sticky, and release certain chemicals.

Prothrombin and fribrinogen are two proteins that are produced by the liver that are always present in the plasma of the blood. The injured tissues and platelets release prothrombin activator and calcium ions (Ca^{2+}) to change prothrombin into the enzyme thrombin. Then the thrombin splits two short amino acid chains from each fibrinogen molecule. The ends of the fibrinogen then join together, forming threads of fibrin. Thesefibrin surround the platelet plug in the damaged area of the blood vessel and provide the shape for the clot. Red blood cells are present within the fibrin which makes the clot appear red. After this the clot stops the bleeding, gets smaller, and hardens. Over time the injury is repaired by the growth of new cells which will replace the cells lost because of the injury. When all the healing has finished an enzyme called plasmin in activated and dissolves the fibrin clot.

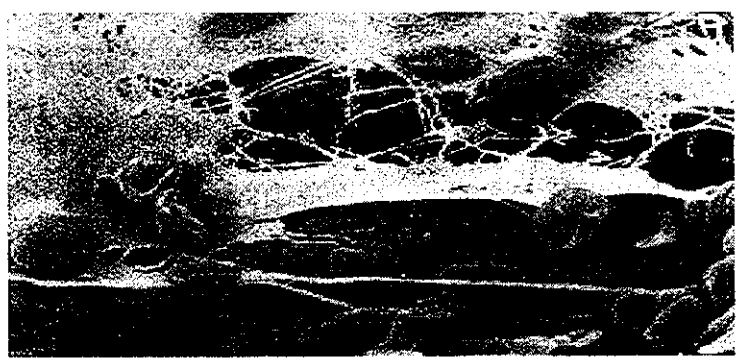


FIG 3 IMAGE OF CLOT

Coagulation is a complex process by which blood forms clots. It is an important part of hemostasis (the cessation of blood loss from a damaged vessel), wherein a damaged blood vessel wall is covered by a platelet and fibrin-containing clot to stop bleeding and begin repair of the damaged vessel. Disorders of coagulation can lead to an increased risk of bleeding (hemorrhage) or obstructive clotting (thrombosis).

Coagulation is highly conserved throughout biology; in all mammals, coagulation involves both a cellular (platelet) and a protein (coagulation factor) component. The system in humans has been the most extensively researched and is therefore the best understood.

Coagulation begins almost instantly after an injury to the blood vessel has damaged the endothelium (lining of the vessel). Exposure of the blood to proteins such as tissue factor initiates changes to blood platelets and the plasma protein fibrinogen, a clotting factor. Platelets immediately form a plug at the site of injury; this is called *primary hemostasis*. *Secondary hemostasis* occurs simultaneously: Proteins in the blood plasma, called *coagulation factors* or *clotting factors*, respond in a complex cascade to form fibrin strands, which strengthen the platele Platelet activation

Damage to blood vessel walls exposes subendothelium proteins, most notably von Willebrand factor (vWF), present under the endothelium. vWF is a protein secreted by healthy endothelium. forming a layer between the endothelium and underlying basement membrane. When the endothelium is damaged, the normally-isolated, underlying vWF is exposed to blood and recruits Factor VIII, collagen, and other clotting factors. Circulating platelets bind to collagen with surface collagen-specific glycoprotein Ia/IIa receptors. This adhesion is strengthened further by additional circulating proteins vWF, which forms additional links between the platelets glycoprotein Ib/IX/V and the collagen fibrils. These adhesions activate the platelets.

Activated platelets release the contents of stored granules into the blood plasma. The granules include ADP, serotonin, platelet-activating factor (PAF), vWF, platelet factor 4, and thromboxane A_2 (TXA_2), which, in turn, activate additional platelets. The granules' contents activate a G_q-linked protein receptor cascade, resulting in increased calcium concentration in the platelets' cytosol. The calcium activates protein kinase C, which, in turn, activates phospholipase

A_2 (PLA_2). PLA_2 then modifies the integrin membrane glycoprotein IIb/IIIa, increasing its affinity to bind fibrinogen. The activated platelets change shape from spherical to stellate, and the fibrinogen cross-links with glycoprotein IIb/IIIa aid in aggregation of adjacent platelets.

The coagulation cascade:

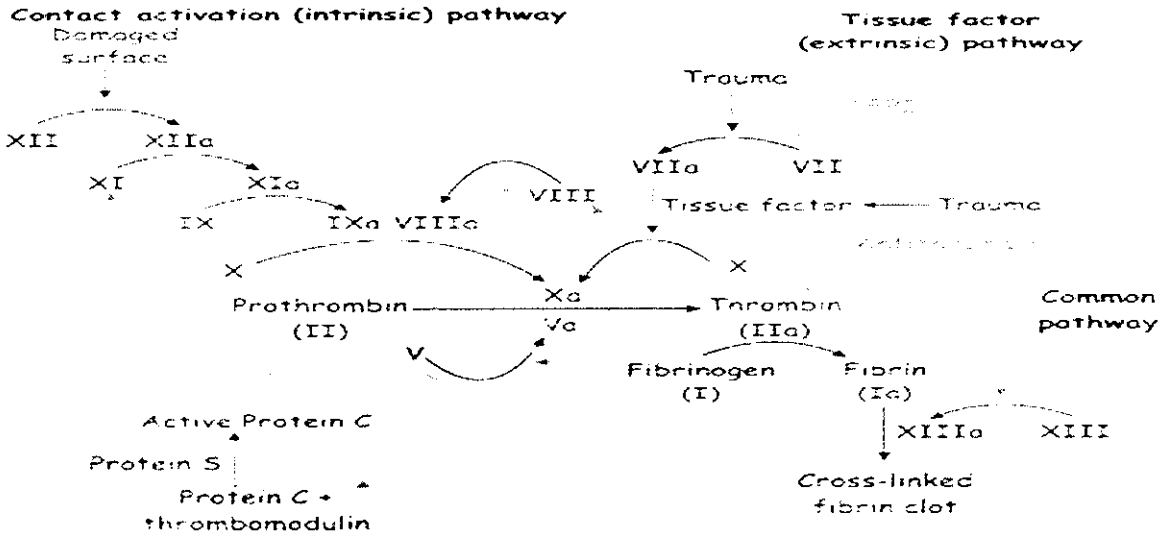


FIG 4 COAGULATION CASCADE

The coagulation cascade of secondary hemostasis has two pathways which lead to *fibrin* formation. These are the *contact activation pathway* (formerly known as the intrinsic pathway), and the *tissue factor pathway* (formerly known as the extrinsic pathway). It was previously thought that the coagulation cascade consisted of two pathways of equal importance joined to a common pathway. It is now known that the primary pathway for the initiation of blood coagulation is the *tissue factor* pathway. The pathways are a series of reactions, in which a zymogen (inactive enzyme precursor) of a serine protease and its glycoprotein co-factor are activated to become active components that then catalyze the next reaction in the cascade, ultimately resulting in cross-linked fibrin. Coagulation factors are generally indicated by Roman numerals, with a lowercase *a* appended to indicate an active form.

The coagulation factors are generally serine proteases (enzymes). There are some exceptions. For example, FVIII and FV are glycoproteins, and Factor XIII is a transglutaminase. Serine proteases

act by cleaving other proteins at specific sites. The coagulation factors circulate as inactive zymogens. The coagulation cascade is classically divided into three pathways. The *tissue factor* and *contact activation* pathways both activate the "final common pathway" of factor X, thrombin and fibrin.

Tissue factor pathway (extrinsic):

The main role of the tissue factor pathway is to generate a "thrombin burst," a process by which thrombin, the most important constituent of the coagulation cascade in terms of its feedback activation roles, is released instantaneously. FVIIa circulates in a higher amount than any other activated coagulation factor.

- Following damage to the blood vessel, FVII leaves the circulation and comes into contact with tissue factor (TF) expressed on tissue-factor-bearing cells (stromal fibroblasts and leukocytes), forming an activated complex (TF-FVIIa).
- TF-FVIIa activates FIX and FX.
- FVII is itself activated by thrombin, FXIa, FXII and FXa.
- The activation of FXa by TF-FVIIa is almost immediately inhibited by tissue factor pathway inhibitor (TFPI).
- FXa and its co-factor FVa form the prothrombinase complex, which activates prothrombin to thrombin.
- Thrombin then activates other components of the coagulation cascade, including FV and FVIII (which activates FXI, which, in turn, activates FIX), and activates and releases FVIII from being bound to vWF.
- FVIIIa is the co-factor of FIXa, and together they form the "tenase" complex, which activates FX; and so the cycle continues. ("Tenase" is a contraction of "ten" and the suffix "-ase" used for enzymes.)

Contact activation pathway (intrinsic):

The contact activation pathway begins with formation of the primary complex on collagen by high-molecular-weight kininogen (HMWK), prekallikrein, and FXII (Hageman factor).

Prekallikrein is converted to kallikrein and FXII becomes FXIIa. FXIIa converts FXI into FXIa. Factor XIa activates FIX, which with its co-factor FVIIIa form the tenase complex, which activates FX to FXa. The minor role that the contact activation pathway has in initiating clot formation can be illustrated by the fact that patients with severe deficiencies of FXII, HMWK, and prekallikrein do not have a bleeding disorder. Instead, contact activation system seems to be more involved in inflammation. Patients without FXII (Hageman factor) suffer from constant infections.[*citation needed*]

Final common pathway:

Thrombin has a large array of functions. Its primary role is the conversion of fibrinogen to fibrin, the building block of a hemostatic plug. In addition, it activates Factors VIII and V and their inhibitor protein C (in the presence of thrombomodulin), and it activates Factor XIII, which forms covalent bonds that crosslink the fibrin polymers that form from activated monomers.

Following activation by the contact factor or tissue factor pathways, the coagulation cascade is maintained in a prothrombotic state by the continued activation of FVIII and FIX to form the tenase complex, until it is down-regulated by the anticoagulant pathways.

Cofactors:

Various substances are required for the proper functioning of the coagulation cascade:

- Calcium and phospholipid (a platelet membrane constituent) are required for the tenase and prothrombinase complexes to function. Calcium mediates the binding of the complexes via the terminal gamma-carboxy residues on FXa and FIXa to the phospholipid surfaces expressed by platelets, as well as procoagulant microparticles or microvesicles shed from them. Calcium is also required at other points in the coagulation cascade.
- Vitamin K is an essential factor to a hepatic gamma-glutamyl carboxylase that adds a carboxyl group to glutamic acid residues on factors II, VII, IX and X, as well as Protein S, Protein C and Protein Z. In adding the gamma-carboxyl group to glutamate residues on

the immature clotting factors Vitamin K is itself oxidized. Another enzyme, *Vitamin K epoxide reductase*, (VKORC) reduces vitamin K back to its active form. Vitamin K epoxide reductase is pharmacologically important as a target for anticoagulant drugs warfarin and related coumarins such as acenocoumarol, phenprocoumon, and dicumarol. These drugs create a deficiency of reduced vitamin K by blocking VKORC, thereby inhibiting maturation of clotting factors. Other deficiencies of vitamin K (e.g., in malabsorption), or disease (hepatocellular carcinoma) impairs the function of the enzyme and leads to the formation of PIVKAs (proteins formed in vitamin K absence); this causes partial or non-gamma carboxylation, and affects the coagulation factors' ability to bind to expressed phospholipid.

Regulators:

Five mechanisms keep platelet activation and the coagulation cascade in check. Abnormalities can lead to an increased tendency toward thrombosis:

- Protein C is a major physiological anticoagulant. It is a vitamin K-dependent serine protease enzyme that is activated by thrombin into activated protein C (APC). Protein C is activated in a sequence that starts with Protein C and thrombin binding to a cell surface protein thrombomodulin. Thrombomodulin binds these proteins in such a way that it activates Protein C. The activated form, along with protein S and a phospholipid as cofactors, degrades FVa and FVIIIa. Quantitative or qualitative deficiency of either may lead to thrombophilia (a tendency to develop thrombosis). Impaired action of Protein C (activated Protein C resistance), for example by having the "Leiden" variant of Factor V or high levels of FVIII also may lead to a thrombotic tendency.
- Antithrombin is a serine protease inhibitor (serpin) that degrades the serine proteases: thrombin, FIXa, FXa, FXIa, and FXIIa. It is constantly active, but its adhesion to these factors is increased by the presence of heparan sulfate (a glycosaminoglycan) or the administration of heparins (different heparinoids increase affinity to FXa, thrombin, or both). Quantitative or qualitative deficiency of antithrombin (inborn or acquired, e.g., in proteinuria) leads to thrombophilia.

- Tissue factor pathway inhibitor (TFPI) limits the action of tissue factor (TF). It also inhibits excessive TF-mediated activation of FIX and FX.
- Plasmin is generated by proteolytic cleavage of plasminogen, a plasma protein synthesized in the liver. This cleavage is catalyzed by tissue plasminogen activator (t-PA), which is synthesized and secreted by endothelium. Plasmin proteolytically cleaves fibrin into fibrin degradation products that inhibit excessive fibrin formation.
- Prostacyclin (PGI_2) is released by endothelium and activates platelet G_s protein-linked receptors. This, in turn, activates adenylyl cyclase, which synthesizes cAMP. cAMP inhibits platelet activation by decreasing cytosolic levels of calcium and, by doing so, inhibits the release of granules that would lead to activation of additional platelets and the coagulation cascade.

2.3 INTRODUCTION ON BIO POLYMERS:-

Biopolymers are polymers that are generated from renewable natural sources, are often biodegradable and non toxic to produce. They can be produced by biological systems (i.e. micro-organisms, plants and animals), or chemically synthesized from biological starting materials (e.g. sugars, starch, natural fats or oils, etc.).Biopolymers are an alternative to petroleum-based polymers (traditional plastics). Some polymers degrade in only a few weeks, while others take several months. Biodegradability and other plastic properties strongly depend on the polymer structure. By changing the structure, these properties can be altered.

There are four main types of biopolymer based respectively on:

1.Starch

2.Sugar

3.Cellulose

4.Synthetic materials

Cellulose, starch and chitin, proteins and peptides, and DNA and RNA are all examples of biopolymers, in which the monomeric units, respectively, are sugars, amino acids, and nucleotides. Some biopolymers are biodegradable. That is, they are broken down into CO_2 and water by microorganisms. In addition, some of these biodegradable biopolymers are compostable.

A major but defining difference between polymers and biopolymers can be found in their structures. Polymers, including biopolymers, are made of repetitive units called monomers. Biopolymers often have a well defined structure, though this is not a defining characteristic (example :ligno-cellulose): The exact chemical composition and the sequence in which these units are arranged is called the primary structure, in the case of proteins. Many biopolymers spontaneously fold into characteristic compact shapes (see also "protein folding" as well as secondary structure and tertiary structure), which determine their biological functions and depend in a complicated way on their primary structures. Structural biology is the study of the structural properties of the biopolymers. In contrast most synthetic polymers have much simpler and more random (or stochastic) structures. This fact leads to a molecular mass distribution that is missing in biopolymers. In fact, as their synthesis is controlled by a template directed process in most in vivo systems all biopolymers of a type (say one specific protein) are all alike: they all contain the similar sequences and numbers of monomers and thus all have the same mass. This phenomenon is called monodispersity in contrast to the polydispersity encountered in synthetic polymers. As a result biopolymers have a polydispersity index Many of these applications can be found in the medical field and can be roughly divided into three categories:

- drug delivery systems,
- wound closure
- healing products,and surgical implant devices

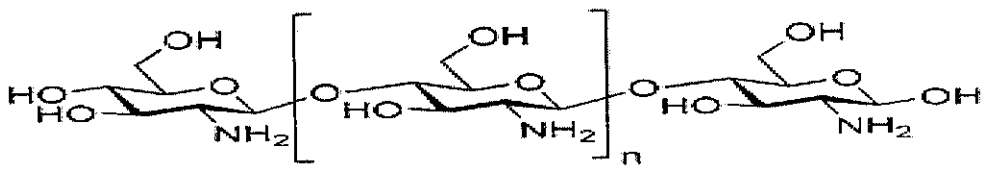
Drug delivery inside the human body can be quite easily controlled with the use of biodegradable capsules . In Wound healing, resorbable non-wovens for the replacement of human tissue , as well as simple sutures, staples, clips or meshes are available . Related to these applications, also the use as bioresorbable scaffolds for tissue engineering is worth mentioning.

2.4 BIOPOLYMERS FOR BLOOD CLOTTING:

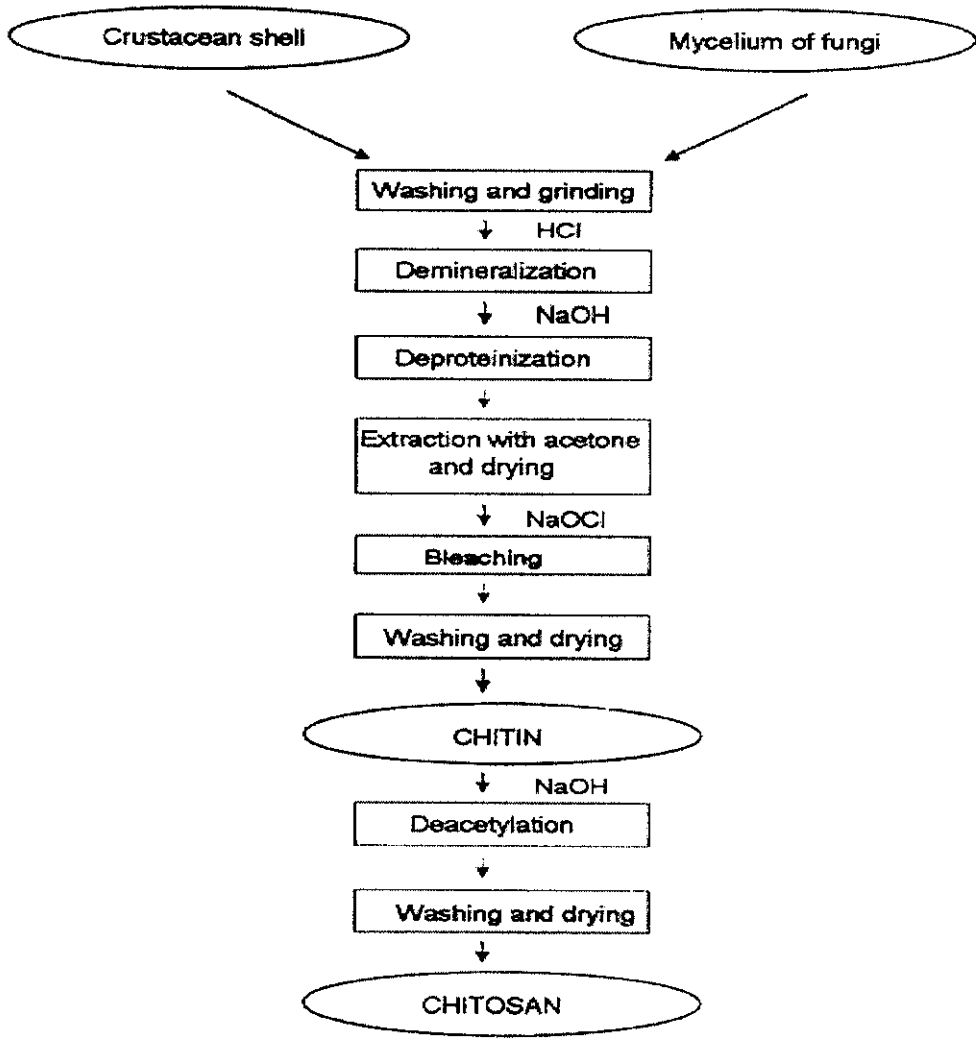
2.4.1 CHITOSAN:

Chitosan (pronounced /' ka? t? sæn/) is a linear polysaccharide composed of randomly distributed ß-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). It has a number of commercial and possible biomedical uses. Chitosan is found in the outer shell of crustaceans .chitosan is the name given to the partially deacetylated form of chitin .chitosan is bio compatible and can be produced in powder,film,bead,fibre,fabric.many of the chitosan properties rely on,its cationic nature,chitosan is used in broad range of orthopaedic,tissue engineering ,wound healing. Chitin and chitosan are described as a family of linear polysaccharides consisting of varying amounts of ß (1->4) linked residues of N-acetyl-2 amino-2-deoxy-D-glucose and 2-amino-2-deoxy-Dglucose residues

Chemical structure of chitosan:



METHODS OF PREPARATION OF CHITOSAN :



BIOLOGICAL PROPERTIES OF CHITOSAN

Biodegradability:

Chitin and chitosan are absent from mammals but they can be degraded in vivo by several proteases (lysozyme, papain, pepsin...). Their biodegradation leads to the release of non-toxic oligosaccharides of variable length which can be subsequently incorporated to glycosaminoglycans and glycoproteins,to metabolic pathways or be excreted Lysozyme, a non-specific protease present in all mammalian tissues, seems to play a degradation role on chitin and

chitosan. The degradation kinetics seem to be inversely related to the degree of crystallinity which is controlled mainly by the degree of deacetylation. Moreover, the distribution of acetyl groups also affects biodegradability since the absence of acetyl groups or their homogeneous distribution (random rather thanblock) results in very low rates of enzymatic degradation.

Biocompatibility:

Chitosan show very good compatibility but this property depends on the characteristics of the sample(natural source, method of preparation. Due to its higher versatility and biological properties the majority of the assays have been carried out on chitosan samples. Although the gastrointestinal enzymes can partially degrade both chitin and chitosan, when the polymers is orally administered they are not absorbed. For this reason, they are considered as not bioavailable. Chitosan presents higher cytocompatibility in vitro than chitin. The cytocompatibility of chitosan has been proved in vitro with myocardial, endothelial and epithellial cells, fibroblast, hepatocytes, condrocytes and keratinocytes. When the positive charge of the polymer increases, the interactions between chitosan and the cells increase too, due to the presence of free amino groups. The adhesion and proliferation of keratinocytes and fibroblasts on several chitosanfilms. The type of cell was a factor that also affected the adhesion, being more favourable for fibroblasts which exhibit a more negative charge surface than for keratinocytes

Haemostatic:

Chitosan may induce the adhesion of erythrocytes with its amino groups or forming a threedimensional network structure in blood that captures the erythrocytes and then make them aggregated. Dynamic blood clotting test showed that chitosan microspheres can shorten the clotting time. It may be related to not only platelet aggregation, but also erythrocyte agglutination, and also it may be due to the large amount of plasma proteins absorbed on the chitosan microsphere surfaces. Chitosan microspheres can induce the adhesion and activation of platelets.It also can directly promote the aggregation of erythrocytes without forming any dimensional network structure or adsorbing any plasma protein at first, but they did not greatly damage the cell membranes of erythrocytes. Chitosan microspheres may be
potential use as thrombospheres.

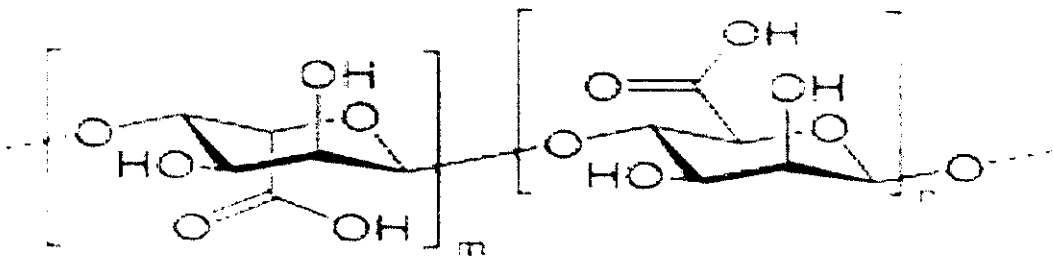
2.4.2 ALGINATE:

"Alginate" is the term usually used for the salts of alginic acid, but it can also refer to all the derivatives of alginic acid and alginic acid itself. ALGINIC acid was discovered by Stanford in in the course of experiments on the extraction of iodine from Scottish kelp 1883].It is now prepared commercially in the British Isles from the sea-weed Laminaria hyperborea. It is coming into general use as an ingredient of foods and to replace agar in many of its uses.

The structure of alginate is a linear copolymer with homopolymeric blocks of (1-4)-linked ß-D-mannuronate (M) and its C-5 epimer a-L-guluronate (G) residues, respectively, covalently linked together in different sequences or blocks.

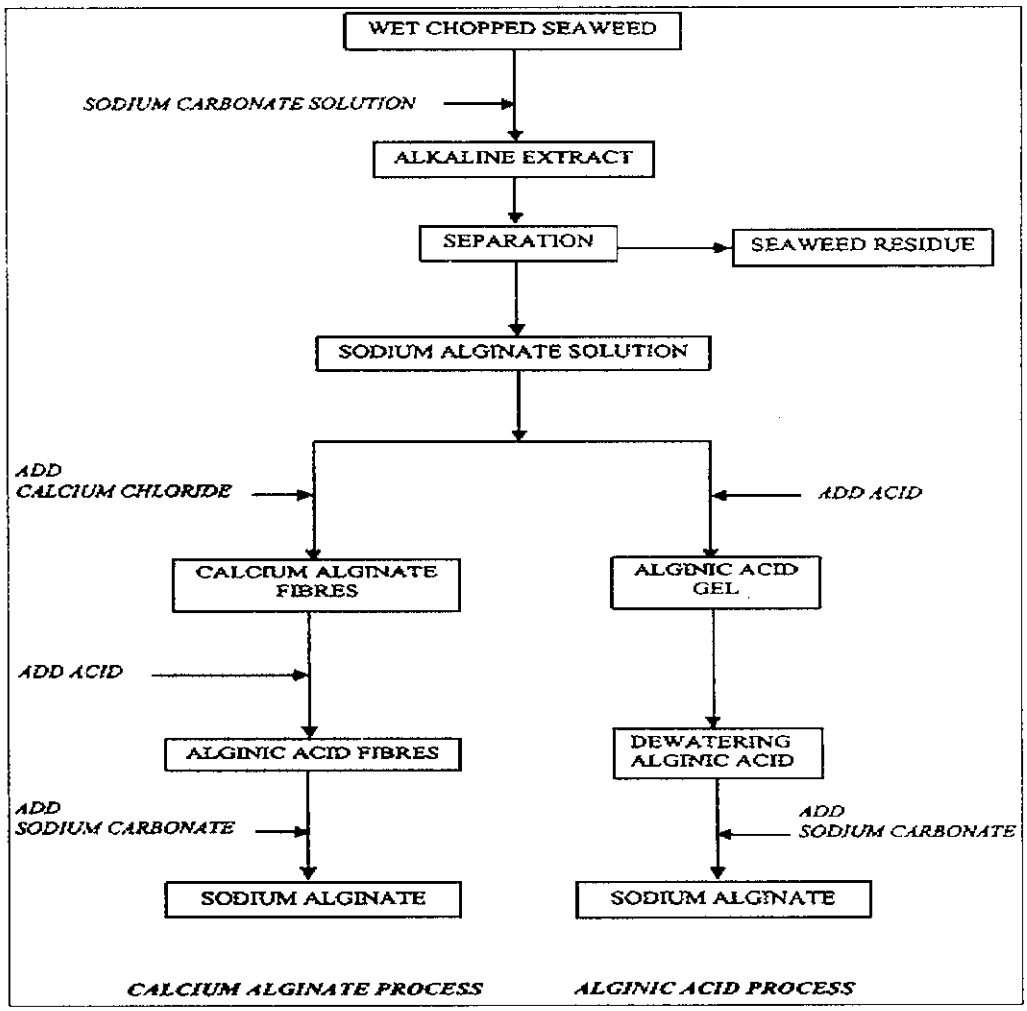
The monomers can appear in homopolymeric blocks of consecutive G-residues (G-blocks), consecutive M-residues (M-blocks), alternating M and G-residues (MG-blocks), or randomly organized blocks.

Chemical structure of sodium alginate:



Sodium alginate is available under the trade name of Manucol in a variety of grades which differ in their average molecular weights. The range of molecular weights which is available includes the molecular weights of the plasma proteins. This suggested that sodium alginate might be a suitable substance for use in the treatment of shock when blood or plasma was not available for transfusion.

PREPARATION OF SODIUM ALGINATE:



Properties:

Direct observation showed that the increased sedimentation rate in the presence of sodium alginate is due to the clumping together of the red cells into large aggregates. This clumping resembles true agglutination both macroscopically and microscopically but is readily distinguished by the ease with which the clumps can be shaken apart.

- Good surface detail
- Reaction is faster at higher temperatures

- Elastic enough to be drawn over the undercuts, but tears over the deep undercuts
- Not dimensionally stable on storing due to evaporation
- Non toxic and non irritant

Application :

The uses of alginates are based on three main properties. The first is their ability, when dissolved in water, to thicken the resulting solution (more technically described as their ability to increase the viscosity of aqueous solutions). The second is their ability to form gels; gels form when a calcium salt is added to a solution of sodium alginate in water. The gel forms by chemical reaction, the calcium displaces the sodium from the alginate, holds the long alginate molecules together and a gel is the result. No heat is required and the gels do not melt when heated. This is in contrast to the agar gels where the water must be heated to about 80°C to dissolve the agar and the gel forms when cooled below about 40°C. The third property of alginates is the ability to form films of sodium or calcium alginate and fibres of calcium alginates.

Alginate molecules are long chains that contain two different acidic components, abbreviated here for simplicity to M and G. The way in which these M and G units are arranged in the chain and the overall ratio, M/G, of the two units in a chain can vary from one species of seaweed to another. In other words all "alginates" are not necessarily the same. So some seaweeds may produce an alginate that gives a high viscosity when dissolved in water, others may yield a low viscosity alginate. The conditions of the extraction procedure can also affect viscosity, lowering it if conditions are too severe. All of this results in sellers normally offering a range of alginates with differing viscosities.

Similarly, the strength of the gel formed by the addition of calcium salts can vary from one alginate to another. Generally alginates with a higher content of G will give a stronger gel; such alginates are said to have a low M/G ratio. Good quality stable fibres have been produced from mixed salts of sodium and calcium alginate, and processed into non-woven fabric that is used in wound dressings. They have very good wound healing and haemostatic properties and can be absorbed by body fluids because the calcium in the fibre is exchanged for sodium from the body fluid to give a soluble sodium alginate. This also makes it easy to remove these dressings from

large open wounds or burns since they do not adhere to the wound. Removal can be assisted by applying saline solutions to the dressing to ensure its conversion to soluble sodium alginate. Recently, the consumer division of a multinational pharmaceutical company launched a new line of adhesive bandages and gauze pads based on calcium alginate fibres. They are being promoted as helping blood to clot faster - twice as fast as their older, well established product.

2.5 INTRODUCTION TO NANO TECHNOLOGY:

Nanotechnology is the engineering of functional systems at the molecular scale. This covers both current work and concepts that are more advanced.In its original sense, 'nanotechnology' refers to the projected ability to construct items from the bottom up, using techniques and tools being developed today to make complete, high performance products. Nanotechnology involves manipulating properties and structures at the nanoscale, often involving dimensions that are just tiny fractions of the width of a human hair. Nanotechnology is already being used in products in its passive form, such as cosmetics and sunscreens, and it is expected that in the coming decades, new phases of products, such as better batteries and improved electronics equipment, will be developed and have far-reaching implications. One area of nanotechnology application that holds the promise of providing great benefits for society in the future is in the realm of medicine. Nanotechnology is already being used as the basis for new, more effective drug delivery systems and is in early stage development as scaffolding in nerve regeneration research. Moreover, the National Cancer Institute has created the Alliance for Nanotechnology in Cancer in the hope that investments in this branch of nanomedicine could lead to breakthroughs in terms of detecting, diagnosing, and treating various forms of cancer.Nanotechnology medical developments over the coming years will have a wide variety of uses and could potentially save a great number of lives. Nanotechnology is already moving from being used in passive structures to active structures, through more targeted drug therapies or "smart drugs." These new drug therapies have already been shown to cause fewer side effects and be more effective than traditional therapies. In the future, nanotechnology will also aid in the formation of molecular systems that may be strikingly similar to living systems. These molecular structures could be the basis for the regeneration or replacement of body parts that are currently lost to infection, accident, or disease.

Size matters:

Size effect constitute a fascinating aspect of nano materials

- It deals with structure whose area is smaller than 100 nm atleast in one dimension
- It exploits characteristics and phenomena which occur in the transitional zone
- The technology describes deliberate manufacture of individual nano structure

Nano particles may either be natural or incidental or engineered ,they may either be amorphous or crystalline or polymeric or composites

2.5.1 The "top –down" approach:

Starting from micro technology structures , the components are gradually miniaturized (primraliy featured in physics and physical technology). Enlarging the surface and separation of the particle means an increase in free energy making the system less stable . also some work is lost due to firction effects .

2.5.2 The "bottom-up"approach :

Increasingly complex structures are specially assembled from atomic or molecular components. This approach is popular in chemistry and biology , where dealing with objects of the nanometer scale is a familiar practice . colloidal particles can be produced by sol-gel technique . stable colloids guarantee that the particle size will be of nano size and no work is necessary to enlarge surface .

2.6 CHARACTERISATION OF NANO PARTICLES :

2.6.1 X-RAY DIFFRACTION METHOD :

X-ray diffraction yields the atomic structure of materials and is based on the elastic scattering of X-rays from the electron clouds of the individual atoms in the system. The most comprehensive description of scattering from crystals is given by the dynamical theory of diffraction.[1]

- Single-crystal X-ray diffraction is a technique used to solve the complete structure of crystalline materials, ranging from simple inorganic solids to complex macromolecules, such as proteins.
- Powder diffraction (XRD) is a technique used to characterise the crystallographic structure, crystallite size (grain size), and preferred orientation in polycrystalline or powdered solid samples. Powder diffraction is commonly used to identify unknown substances, by comparing diffraction data against a database maintained by the International Centre for Diffraction Data. It may also be used to characterize heterogeneous solid mixtures to determine relative abundance of crystalline compounds and, when coupled with lattice refinement techniques, such as Rietveld refinement, can provide structural information on unknown materials. Powder diffraction is also a common method for determining strains in crystalline materials. An effect of the finite crystallite sizes is seen as a broadening of the peaks in an X-ray diffraction as is explained by the Scherrer Equation.
- Thin film diffraction and grazing incidence X-ray diffraction may be used to characterize the crystallographic structure and preferred orientation of substrate-anchored thin films.
- High-resolution X-ray diffraction is used to characterize thickness, crystallographic structure, and strain in thin epitaxial films. It employs parallel-beam optics.
- X-ray pole figure analysis enables one to analyze and determine the distribution of crystalline orientations within a crystalline thin-film sample.
- X-ray rocking curve analysis is used to quantify grain size and mosaic spread in crystalline materials.

SCATTERING TECHNIQUE:

Elastic scattering:

Materials that do not have long range order may also be studied by scattering methods that rely on elastic scattering of monochromatic X-rays.

- Small angle X-ray scattering (SAXS) probes structure in the nanometer to micrometer range by measuring scattering intensity at scattering angles 2? close to 0°.[2]

- X-ray reflectivity is an analytical technique for determining thickness, roughness, and density of single layer and multilayer thin films.
- Wide angle X-ray scattering (WAXS), a technique concentrating on scattering angles 2? larger than 5°.

Inelastic scattering:

When the energy and angle of the inelastically scattered X-rays are monitored scattering techniques can be used to probe the electronic band structure of materials.

X-ray crystallography is a method of determining the arrangement of atoms within a crystal, in which a beam of X-rays strikes a crystal and diffracts into many specific directions. From the angles and intensities of these diffracted beams, a crystallographer can produce a three-dimensional picture of the density of electrons within the crystal. From this electron density, the mean positions of the atoms in the crystal can be determined, as well as their chemical bonds, their disorder and various other information.

Since many materials can form crystals — such as salts, metals, minerals, semiconductors, as well as various inorganic, organic and biological molecules — X-ray crystallography has been fundamental in the development of many scientific fields. In its first decades of use, this method determined the size of atoms, the lengths and types of chemical bonds, and the atomic-scale differences among various materials, especially minerals and alloys. The method also revealed the structure and functioning of many biological molecules, including vitamins, drugs, proteins and nucleic acids such as DNA. X-ray crystallography is still the chief method for characterizing the atomic structure of new materials and in discerning materials that appear similar by otherexperiments. X-ray crystal structures can also account for Unusual electronic or elastic properties of a material, shed light on chemical interactions and processes, or serve as the basis fordesigning pharmaceuticals against diseases.

In an X-ray diffraction measurement, a crystal is mounted on a goniometer and gradually rotated while being bombarded with X-rays, producing a diffraction pattern of regularly spaced spots known as *reflections*. The two-dimensional images taken at different rotations are converted into a three-dimensional model of the density of electrons within the crystal using the mathematical

method of Fourier transforms, combined with chemical data known for the sample. Poor resolution (fuzziness) or even errors may result if the crystals are too small, or not uniform enough in their internal makeup.

X-ray crystallography is related to several other methods for determining atomic structures. Similar diffraction patterns can be produced by scattering electrons or neutrons, which are likewise interpreted as a Fourier transform .

2.6.2 FOURIER TRANSFORM INFRARED SPECTROSCOPY :

Fourier transform infrared spectroscopy (FTIR) is a technique which is used to obtain an infrared spectrum of absorption, emission,photoconductivity or Raman scattering of a solid, liquid or gas. An FTIR spectrometer simultaneously collects spectral data in a wide spectral range. This confers a significant advantage over a dispersive spectrometer which measures intensity over a narrow range of wavelengths at a time. FTIR technique has made dispersive infrared spectrometers all but obsolete (except sometimes in the near infrared) and opened up new applications of infrared spectroscopy Fourier Transform Infrared Spectroscopy (FTIR) provides specific information about chemical bonding and molecular structures, making it useful for analyzing organic materials and certain inorganic materials. Chemical bonds vibrate at characteristic frequencies, and when exposed to infrared radiation, they absorb the radiation at frequencies that match their vibration modes. Measuring the radiation absorption as a function of frequency produces a spectrum that can be used to identify functional groups and compounds.

- Identifying the molecular structure of organic compounds for contamination analysis
- Identification of organic particles, powders, films, and liquids (material identification)
- Quantification of O and H in Si, and H in SiN wafers (Si-H vs. N-H)

An FTIR spectrometer works by taking a small quantity of sample and introducing it to the infrared cell, where it is subjected to an infrared light source, which is scanned from 4000 cm-1 to around 600 cm-1. The intensity of light transmitted through the sample is measured at each wavenumber allowing the amount of light absorbed by the sample to be determined as the difference between the intensity of light before and after the sample cell. This is known is the

infrared spectrum of the sample. In the infrared region of the spectrum, the resonance frequencies of a molecule are due to the presence of molecular functional groups specific to the molecule. A functional group is simply a group of two or more atoms, bonded together in a specific way. In the water molecule (H2O), it is the O-H functional group that contributes to the resonance frequency around 3450 cm-1.

Common Molecular Species Measured by FTIR

However, some molecules have very similar functional groups. For example, three common molecules often found in used oil samples: water, glycol and the hindered phenol antioxidant additive BHT.

It is clear that all three molecules possess the same O-H functional group as the water molecule, meaning that they will absorb light in the 3600 to 3400 cm-1 region, although the actual wave number will vary slightly due to the effects of the rest of the molecule.

The similarity of functional groups creates a problem with FTIR. For example, if a sample is analyzed and the infrared absorption recorded in the 3600 to 3400 cm-1 region, one may not be able to differentiate between absorption due to water, glycol contamination or antioxidant additives, because their absorptions peaks are usually fairly broad and may overlap. Fortunately, the glycol molecule also absorbs light in other regions of the infrared spectrum, specifically 880, 1040 and 1080 cm-1, which provides confirmation.

Application :

FTIR can be used in all applications where a dispersive spectrometer was used in the past . In addition, the multiplex and throughput advantages have opened up new areas of application. These include:

- GC-IR (gas chromatography-infrared spectrometry). A gas chromatograph can be used to separate the components of a mixture. The fractions containing single components are directed into an FTIR spectrometer, to provide the infrared spectrum of the sample. This technique is complementary to GC-MS (gas chromatography-mass spectrometry). The GC-IR method is particularly useful for identifyingisomers, which by their nature have identical masses. The key to the successful use of GC-IR is that the interferogram can be captured in a very short time, typically less than 1 second. FTIR has also been applied to the analysis of liquid chromatography fractions.
- Micro-samples. Tiny samples, such as in forensic analysis, can be examined with the aid of an infrared microscope in the sample chamber. An image of the surface can be obtained by scanning. Another example is the use of FTIR to characterize artistic materials in old-master paintings.
- Emission spectra. Instead of recording the spectrum of light transmitted through the sample, FTIR spectrometer can be used to acquire spectrum of light emitted by the sample. Such emission could be induced by various processes, and the most common ones are luminescence and Raman scattering. Little modification is required to an absorption FTIR spectrometer to record emission spectra and therefore many commercial FTIR spectrometers combine both absorption and emission/Raman modes.
- Photocurrent spectra. This mode uses a standard, absorption FTIR spectrometer. The studied sample is placed instead of the FTIR detector, and its photocurrent, induced by the spectrometer's broadband source, is used to record the interferrogram, which is then converted into the photoconductivity spectrum of the sample

3 MATERIALS AND METHODS :

3.1 OBJECTIVE:

- developing a material that can clot blood quickly
- Studying the blood clotting mechanism and identifying a suitable bio polymer for blood clotting .
- Synthesis of nano particle from biopolymer
- Characterization of nano particles
- Testing the bio polymer for blood clotting property

3.2 IDENTIFICATION OF BIO POLYMER AND NANO PARTICLE SYNTHESIS:

Bio polymers are the polymers obtained from the natural sources (renewable sources) From literature survey it is found that chitosan , sodium alginate are having excellent . Biological properties of the above said bio polymer are also good. The biological properties such as bio compatibility , hemostatic and bio degradable properties are good .Due to the above properties they can be used for blood clotting .and there is no risk in using these biopolymer because their skin compatibility is good .bio polymer selected for blood clotting purpose is chitosan and sodium alginate . Chitosan's properties allow it to rapidly clot blood, and has recently gained approval in the United States and Europe for use in bandages and other hemostatic agents. Chitosan hemostatic products have been shown in testing by the U.S. Marine Corps to quickly stop bleeding, and result in 100% survival of otherwise lethal arterial wounds in swine and to reduce blood loss.sodium alginate also form clumps when it comes in contact with blood , Good quality stable fibres have been produced from mixed salts of sodium and calcium alginate, and processed into non-woven fabric that is used in wound dressings. They have very good wound healing and haemostatic properties and can be absorbed by body fluids because the calcium in the fibre is exchanged for sodium from the body fluid to give a soluble sodium alginate. This also makes it easy to remove these dressings from large open wounds or burns

since they do not adhere to the wound. Thus both biopolymers are identified and purchased the composition of chitosan is given below

- Ash -0.40%
- Moisture- 6.68%
- Viscosity (1%)- 184CPS
- Deactylation- 85.16

The composition of sodium alginate is given below:

- Slowly soluble in water 25 kg forms viscous solution insoluble in alcohol ether and chloroform.

Maximum Limits of Impurities:

- Moisture 15%
- Matter insoluble in water 1%
- Arsenic(As) <3 PPM
- Iron(Fe) 300 PPM
- Lead(as Pb) <10 PPM
- Sulphated ash <30-35%
- Sulphur(S) <0.02%
- Phosphorus(P) <0.02%

NANO PARTICLE SYNTHESIS :

Nano particle can be synthesized by two approach

- The top –down approach
- The bottom-up approach

From the above two approach ,top-down approach is selected ,the reason for selecting this approach is given below

- The materials selected is a bio material so there is chance for char formation in other technique other than this approach
- Bio polymers is used for blood clotting purpose , their property can be changed when synthesized by other technique this is due to the usage of acids or alkalies for the preparation of nano particles

So in order to over come the above issues top down approach is selected ,in the top-down approach ball mill technique is selected .the mechanism and procedure for ball mill technique is given below

Mechanism of ball mill technique :

A Planetary High-Energy Ball mill is for producing nanosized powders and it includes: (a) a main axis which can not only rotate but also up and Down, (b) a roll-bearing to be fixed on nether end of the main axis, a revolvable plate to be fixed on the top of the main axis, and several able-swing shafts installed in the plate. (c) a plurality of planetary motion mill pots are fixed on the support canisters which are supported by able-swing shafts. (d) A stationary ring which is disposed coaxially with the main axis and serves as the orbit for mill pots. (e) impact bars on the bottom of mill pots using magnet technology to disperse doposited powders. The milling method is used for producing a wide variety of nano-scaled ceramic, metal and composite by selecting metal materials from the period table and the ceramic materials from the group of oxide, carbide, nitride, chloride, boride, silicide, sulfite etc.

There are four factors affecting the angle of break of the particles :

1. Speed of Mill
2. Amount of grinding media
3. Amount of material
4. In wet grinding, the consistency or viscosity

Procedure :

- The sample is taken in 3:3 ratio in both the container of ball mill
- The speed of ball mill is 300 rpm
- Time required per sample is 300 min
- No of cycles is 10 per sample
- Pass time is 15 min
- 10 zirconium balls are used
- Weight of each zirconium balls is 4 gms

3.3 CHARACTERISATION OF CHITOSAN NANO PARTICLE :

3.3.1 X-RAY DIFFRACTION METHOD(XRD):

Fig 5 and fig 6 shows the XRD spectra of the chitosan pure and chitosan milled nano particles. The spectra show the well defined peaks of chitosan .this presence of well defined peaks indicates crystallinity of the synthesized solids , the broadening of the peaks in XRD attributed to particle size effects . the mean crystalline size of a powder sample was estimated from the full width at half maximum of the diffraction peak according to the scherrer's equation

$$D = \frac{0.89 \times \lambda}{\Delta W \times \cos\theta}$$

Where λ is the wave length of the incident x-ray beam , θ is the Bragg's diffraction angle

ΔW is the full width of the x- ray pattern line at half peak height in radians

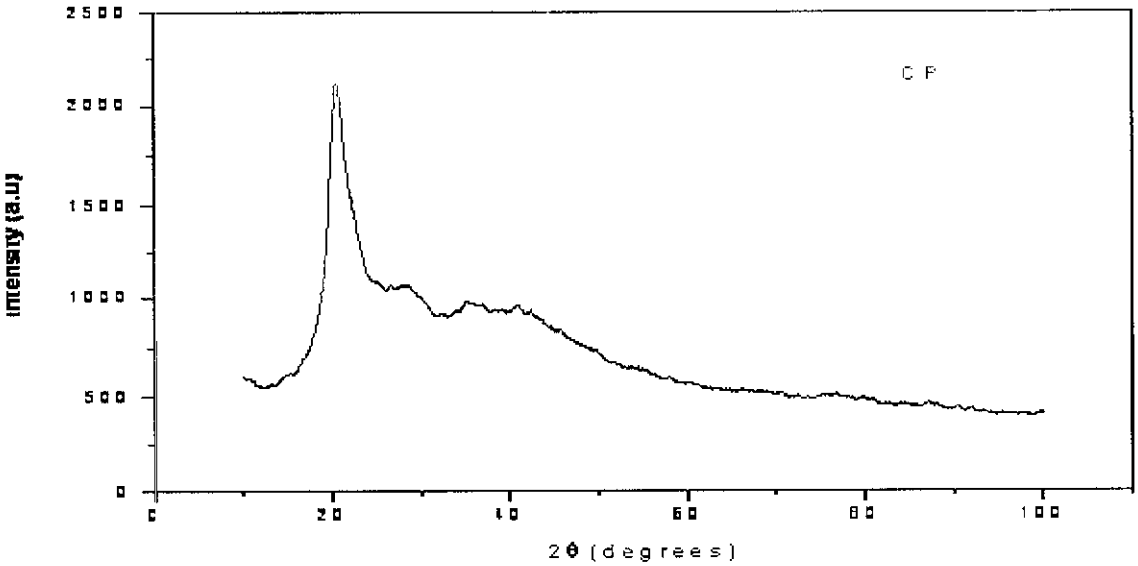


FIG 5 XRD SPECTRA OF CHITOSAN PURE

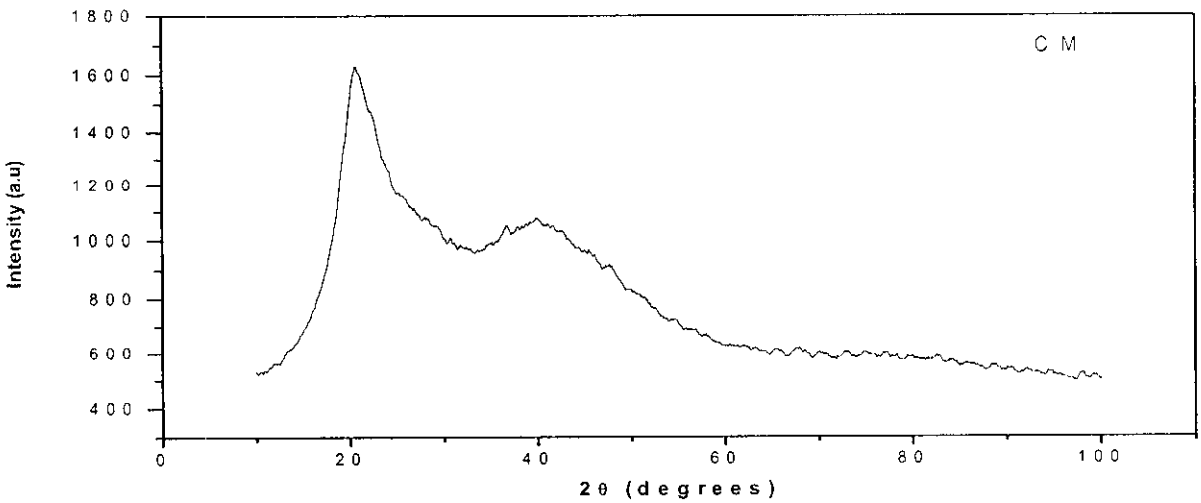


FIG 6 XRD SPECTRA OF CHITOSAN MILLED

3.3.2 FOURIER TRANSFORM SPECTROSCOPY (FTIR):

Fig 7 show the FTIR spectra of chitosan nano particles ,fig shows the absorption band near457.1 cm-1 .the peaks at the peaks at 3416.6 and 1646.1 indicate the presence of –OH and C=O residues

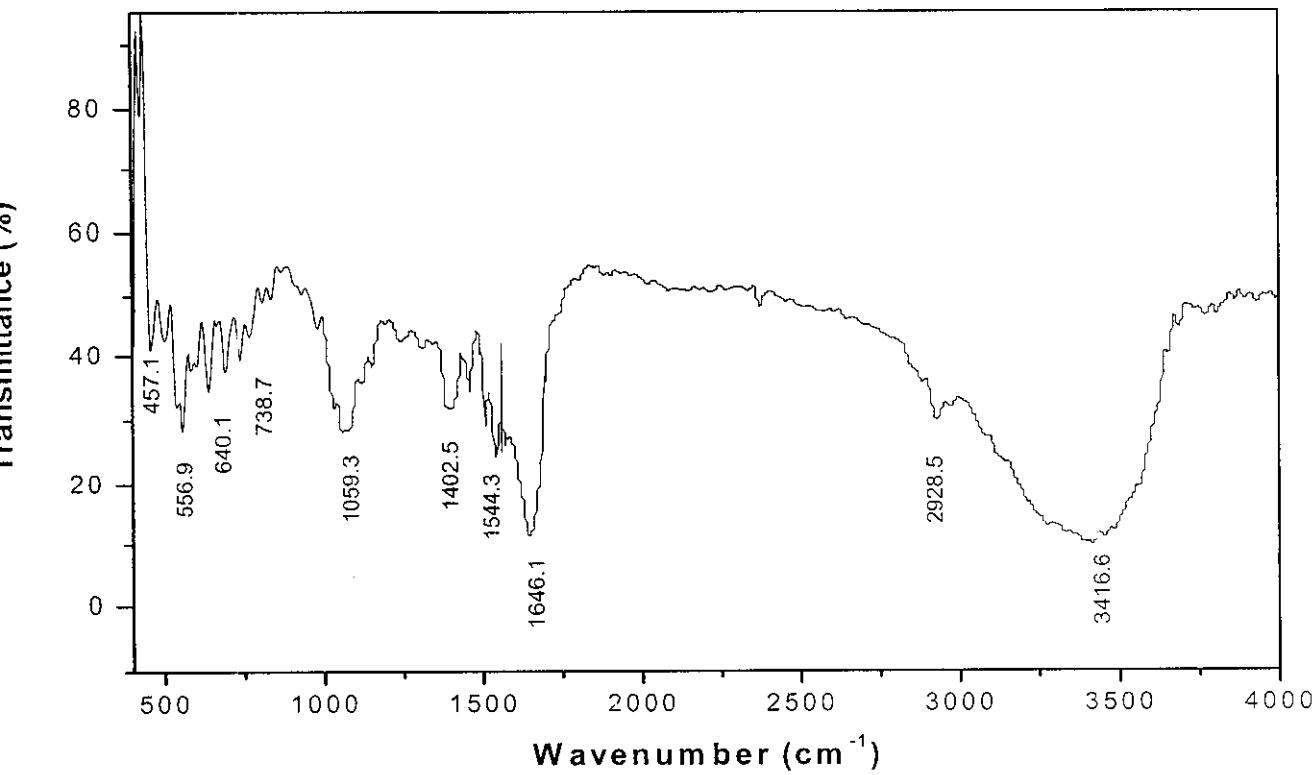


FIG 7 FTIR SPECTRA OF CHITOSAN

3.3.3 PARTICLE SIZE DISTRIBUTION :

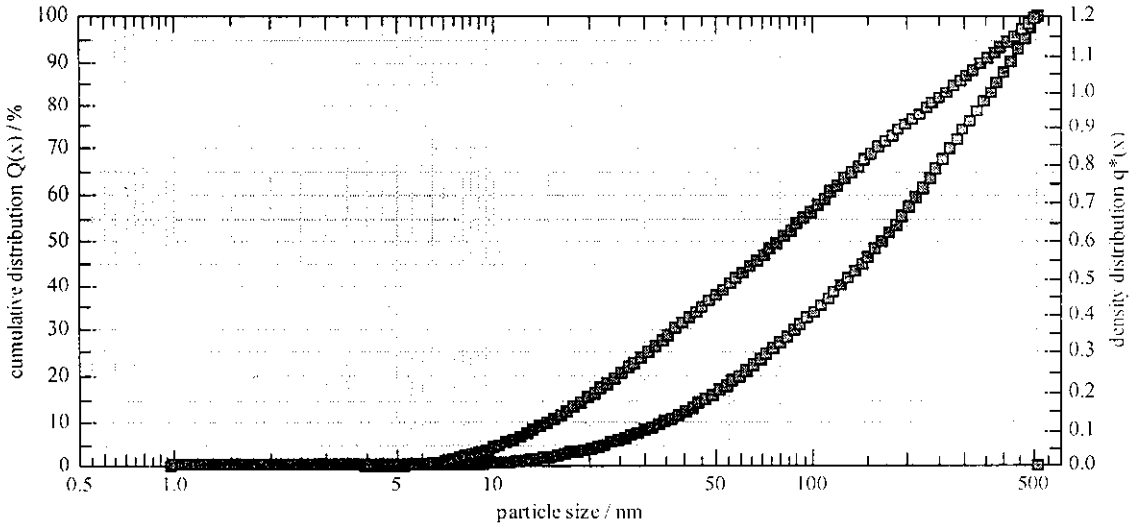


FIG 8 PARTICLE SIZE DISTRIBUTION OF CHITOSAN

cumulative distribution

x_0/nm	Q_3/%	x_0/nm	Q_3/%	x_0/nm	Q_3/%	x_0/nm	Q_3/%
1.02	0.00	4.91	0.00	23.48	5.20	112.41	36.61
1.08	0.00	5.15	0.00	24.66	5.69	118.04	38.15
1.13	0.00	5.41	0.00	25.90	6.21	123.96	39.73
1.19	0.00	5.68	0.00	27.20	6.76	130.18	41.34
1.25	0.00	5.97	0.00	28.56	7.34	136.71	42.98
1.31	0.00	6.27	0.01	29.99	7.94	143.57	44.66
1.37	0.00	6.58	0.02	31.50	8.58	150.77	46.38
1.44	0.00	6.91	0.04	33.07	9.25	158.33	48.12
1.52	0.00	7.26	0.07	34.73	9.94	166.27	49.90
1.59	0.00	7.62	0.10	36.48	10.67	174.61	51.72
1.67	0.00	8.00	0.14	38.30	11.43	183.36	53.57
1.76	0.00	8.40	0.19	40.23	12.22	192.56	55.45
1.84	0.00	8.82	0.25	42.24	13.05	202.21	57.37

1.94	0.00	9.27	0.33	44.36	13.90	212.36	59.32
2.03	0.00	9.73	0.42	46.59	14.79	223.01	61.30
2.14	0.00	10.22	0.52	48.92	15.71	234.19	63.31
2.24	0.00	10.73	0.64	51.38	16.67	245.93	65.36
2.35	0.00	11.27	0.77	53.95	17.66	258.27	67.44
2.47	0.00	11.84	0.92	56.66	18.68	271.22	69.56
2.60	0.00	12.43	1.08	59.50	19.74	284.82	71.71
2.73	0.00	13.05	1.27	62.48	20.83	299.11	73.89
2.86	0.00	13.71	1.47	65.62	21.95	314.11	76.10
3.01	0.00	14.40	1.70	68.91	23.11	329.86	78.34
3.16	0.00	15.12	1.94	72.36	24.31	346.40	80.62
3.32	0.00	15.88	2.21	75.99	25.53	363.78	82.93
3.48	0.00	16.67	2.50	79.80	26.80	382.02	85.27
3.66	0.00	17.51	2.81	83.81	28.10	401.18	87.64
3.84	0.00	18.39	3.15	88.01	29.43	421.30	90.05
4.03	0.00	19.31	3.51	92.42	30.80	442.43	92.49
4.24	0.00	20.28	3.89	97.06	32.20	464.61	94.96
4.45	0.00	21.29	4.30	101.93	33.63	487.91	97.46
4.67	0.00	22.36	4.74	107.04	35.11	512.38	100.00

density distribution (log.)

x_m/nm	$q_3$lg	x_m/nm	$q_3$lg	x_m/nm	$q_3$lg	x_m/nm	$q_3$lg
1.00	0.00	4.79	0.00	22.91	0.22	109.69	0.71
1.05	0.00	5.03	0.00	24.06	0.23	115.19	0.73
1.10	0.00	5.28	0.00	25.27	0.24	120.97	0.74
1.16	0.00	5.54	0.00	26.54	0.26	127.03	0.76
1.22	0.00	5.82	0.00	27.87	0.27	133.41	0.77
1.28	0.00	6.11	0.00	29.27	0.29	140.10	0.79
1.34	0.00	6.42	0.01	30.73	0.30	147.12	0.81
1.41	0.00	6.74	0.01	32.28	0.31	154.50	0.82
1.48	0.00	7.08	0.01	33.89	0.33	162.25	0.84

1.55	0.00	7.44	0.02	35.59	0.34	170.39	0.85
1.63	0.00	7.81	0.02	37.38	0.36	178.93	0.87
1.71	0.00	8.20	0.02	39.25	0.37	187.90	0.89
1.80	0.00	8.61	0.03	41.22	0.39	197.33	0.90
1.89	0.00	9.04	0.04	43.29	0.40	207.22	0.92
1.98	0.00	9.50	0.04	45.46	0.42	217.62	0.93
2.08	0.00	9.97	0.05	47.74	0.43	228.53	0.95
2.19	0.00	10.47	0.05	50.13	0.45	239.99	0.96
2.30	0.00	11.00	0.06	52.65	0.47	252.03	0.98
2.41	0.00	11.55	0.07	55.29	0.48	264.67	0.99
2.53	0.00	12.13	0.08	58.06	0.50	277.94	1.01
2.66	0.00	12.74	0.09	60.97	0.51	291.88	1.03
2.79	0.00	13.38	0.10	64.03	0.53	306.52	1.04
2.93	0.00	14.05	0.11	67.24	0.55	321.89	1.06
3.08	0.00	14.75	0.12	70.62	0.56	338.03	1.07
3.24	0.00	15.49	0.13	74.16	0.58	354.98	1.09
3.40	0.00	16.27	0.14	77.88	0.59	372.79	1.10
3.57	0.00	17.08	0.15	81.78	0.61	391.48	1.12
3.75	0.00	17.94	0.16	85.88	0.63	411.11	1.13
3.94	0.00	18.84	0.17	90.19	0.64	431.73	1.15
4.13	0.00	19.79	0.18	94.71	0.66	453.38	1.16
4.34	0.00	20.78	0.19	99.46	0.68	476.12	1.18
4.56	0.00	21.82	0.21	104.45	0.69	500.00	1.19

product: Chitosen

refraction:1.33 - 0.00

liquid: WATER

refraction:0.79

viscosity:0.89 mPas

measuring cond.: 80/80/5/100/25

duration: 100.77 s

temperature: 24.99 °C

laser power: 82.00 %

evaluation: WINDOX 5.4.0.0

rule: 1..500nm:128(log) 0.001..5ms -1%

measured correlation function:

amplitude: 12.92 %

mean count rate ch.1:151.18 kcps

mean count rate ch.2:109.01 kcps

single scattering ratio:63.91 %

user parameters:

Parameter 1:

Parameter 2:

Parameter 3:

Parameter 4:

3.4 CHARACTERISATION OF SODIUM ALGINATE NANO PARTICLE :

3.4.1X-RAY DIFFRACTION METHOD

Fig9 and fig 10 the XRD spectra of the sodium alginate pure and sodium alginate milled nano particles. The spectra show the well defined peaks of sodium alginate .this presence of well defined peaks indicates crystallinity of the synthesized solids , the broadening of the peaks in XRD attributed to particle size effects . the mean crystalline size of a powder sample was estimated from the full width at half maximum of the diffraction peak according to the scherrer's equation

$$D = \frac{0.89 \times \lambda}{\Delta W \times \cos\theta}$$

Where λ is the wave length of the incident x-ray beam , θ is the Bragg's diffraction angle

ΔW is the full width of the x- ray pattern line at half peak height in radians

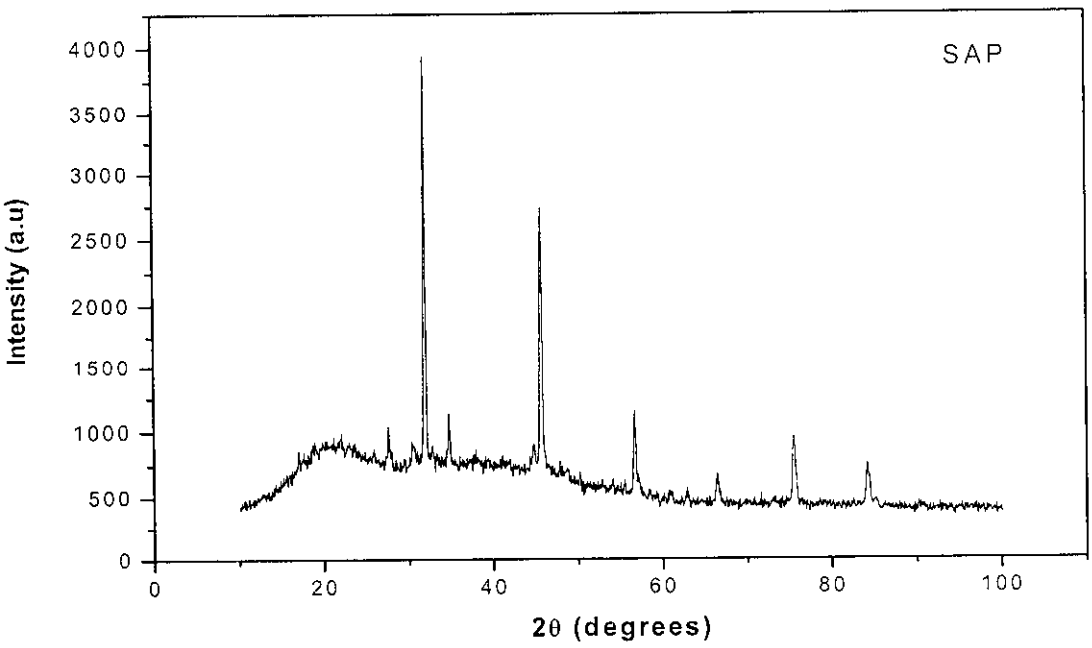


FIG 9 XRD SPECTRA OF SODIUM ALGINATE PURE

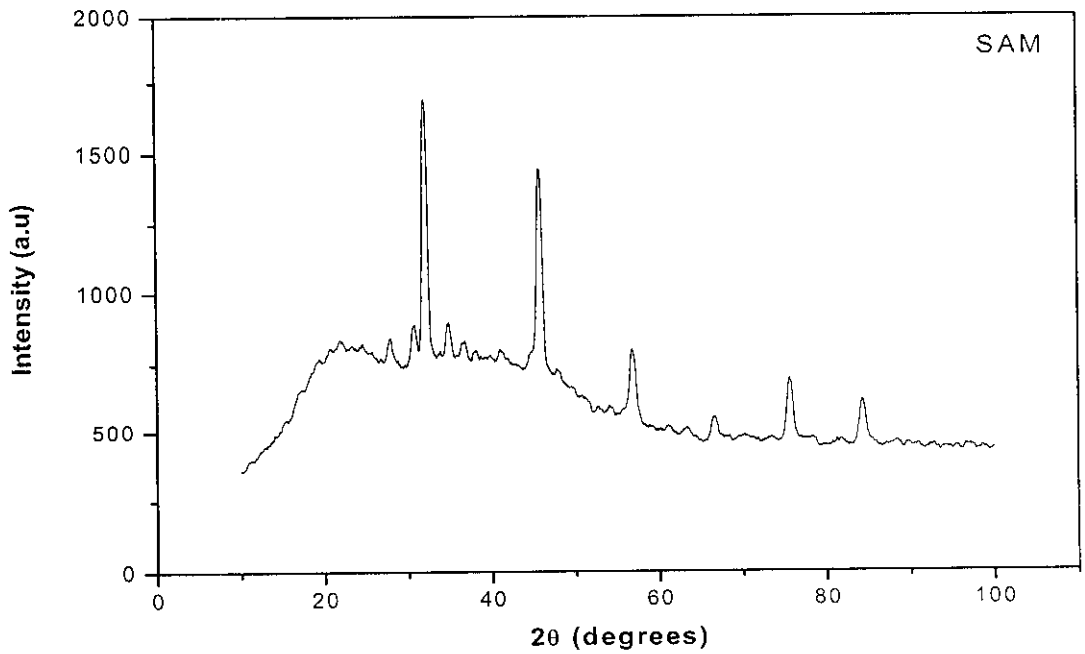


FIG 10 XRD SPECTRA OF SODIUM ALGINATE MILLED

3.4.2 FOURIER TRANSFORM SPECTROSCOPY (FTIR):

Fig 11 show the FTIR spectra of chitosan nano particles ,fig shows the absorption band near 469.9 cm-1 .the peaks at the peaks at 3512.2 and 1630.6 indicate the presence of –OH and C=O residues

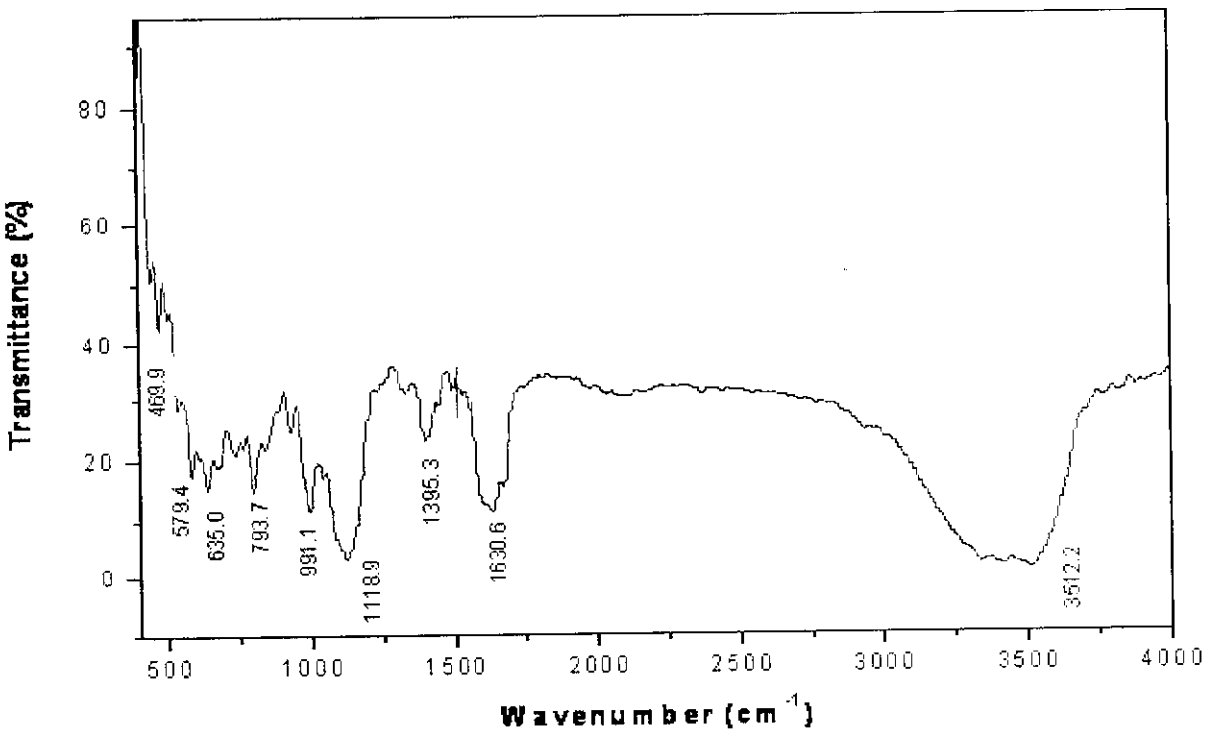


FIG 11 FTIR SPECTRA OF SODIUM ALGINATE

3.4.3.PARTICLE SIZE DISTRIBUTION :

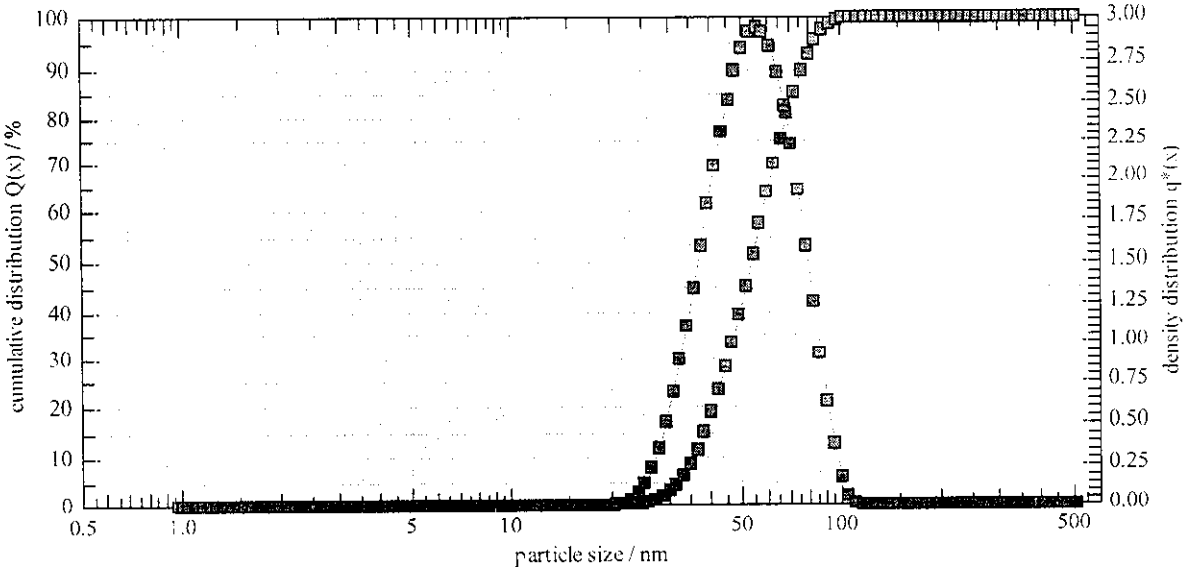


FIG 12 PARTICLE SIZE DISTRIBUTION OF SODIUM ALGINATE

cumulative distribution

x_0/nm	Q_3/%	x_0/nm	Q_3/%	x_0/nm	Q_3/%	x_0/nm	Q_3/%
1.02	0.00	4.91	0.00	23.48	0.09	112.41	100.00
1.08	0.00	5.15	0.00	24.66	0.25	118.04	100.00
1.13	0.00	5.41	0.00	25.90	0.55	123.96	100.00
1.19	0.00	5.68	0.00	27.20	1.05	130.18	100.00
1.25	0.00	5.97	0.00	28.56	1.80	136.71	100.00
1.31	0.00	6.27	0.00	29.99	2.88	143.57	100.00
1.37	0.00	6.58	0.00	31.50	4.33	150.77	100.00
1.44	0.00	6.91	0.00	33.07	6.20	158.33	100.00
1.52	0.00	7.26	0.00	34.73	8.55	166.27	100.00
1.59	0.00	7.62	0.00	36.48	11.40	174.61	100.00
1.67	0.00	8.00	0.00	38.30	14.77	183.36	100.00
1.76	0.00	8.40	0.00	40.23	18.67	192.56	100.00

1.84	0.00	8.82	0.00	42.24	23.08	202.21	100.00
1.94	0.00	9.27	0.00	44.36	27.99	212.36	100.00
2.03	0.00	9.73	0.00	46.59	33.32	223.01	100.00
2.14	0.00	10.22	0.00	48.92	39.03	234.19	100.00
2.24	0.00	10.73	0.00	51.38	45.02	245.93	100.00
2.35	0.00	11.27	0.00	53.95	51.20	258.27	100.00
2.47	0.00	11.84	0.00	56.66	57.44	271.22	100.00
2.60	0.00	12.43	0.00	59.50	63.63	284.82	100.00
2.73	0.00	13.05	0.00	62.48	69.63	299.11	100.00
2.86	0.00	13.71	0.00	65.62	75.32	314.11	100.00
3.01	0.00	14.40	0.00	68.91	80.57	329.86	100.00
3.16	0.00	15.12	0.00	72.36	85.28	346.40	100.00
3.32	0.00	15.88	0.00	75.99	89.36	363.78	100.00
3.48	0.00	16.67	0.00	79.80	92.74	382.02	100.00
3.66	0.00	17.51	0.00	83.81	95.41	401.18	100.00
3.84	0.00	18.39	0.00	88.01	97.38	421.30	100.00
4.03	0.00	19.31	0.00	92.42	98.71	442.43	100.00
4.24	0.00	20.28	0.00	97.06	99.50	464.61	100.00
4.45	0.00	21.29	0.00	101.93	99.88	487.91	100.00
4.67	0.00	22.36	0.02	107.04	99.99	512.38	100.00

density distribution (log.)

x_m/nm	$q_3$lg	x_m/nm	$q_3$lg	x_m/nm	$q_3$lg	x_m/nm	$q_3$lg
1.00	0.00	4.79	0.00	22.91	0.03	109.69	0.00
1.05	0.00	5.03	0.00	24.06	0.07	115.19	0.00
1.10	0.00	5.28	0.00	25.27	0.14	120.97	0.00
1.16	0.00	5.54	0.00	26.54	0.23	127.03	0.00
1.22	0.00	5.82	0.00	27.87	0.36	133.41	0.00
1.28	0.00	6.11	0.00	29.27	0.51	140.10	0.00
1.34	0.00	6.42	0.00	30.73	0.68	147.12	0.00
1.41	0.00	6.74	0.00	32.28	0.88	154.50	0.00

1.48	0.00	7.08	0.00	33.89	1.10	162.25	0.00
1.55	0.00	7.44	0.00	35.59	1.34	170.39	0.00
1.63	0.00	7.81	0.00	37.38	1.59	178.93	0.00
1.71	0.00	8.20	0.00	39.25	1.84	187.90	0.00
1.80	0.00	8.61	0.00	41.22	2.08	197.33	0.00
1.89	0.00	9.04	0.00	43.29	2.31	207.22	0.00
1.98	0.00	9.50	0.00	45.46	2.51	217.62	0.00
2.08	0.00	9.97	0.00	47.74	2.69	228.53	0.00
2.19	0.00	10.47	0.00	50.13	2.82	239.99	0.00
2.30	0.00	11.00	0.00	52.65	2.91	252.03	0.00
2.41	0.00	11.55	0.00	55.29	2.94	264.67	0.00
2.53	0.00	12.13	0.00	58.06	2.91	277.94	0.00
2.66	0.00	12.74	0.00	60.97	2.82	291.88	0.00
2.79	0.00	13.38	0.00	64.03	2.68	306.52	0.00
2.93	0.00	14.05	0.00	67.24	2.47	321.89	0.00
3.08	0.00	14.75	0.00	70.62	2.22	338.03	0.00
3.24	0.00	15.49	0.00	74.16	1.92	354.98	0.00
3.40	0.00	16.27	0.00	77.88	1.59	372.79	0.00
3.57	0.00	17.08	0.00	81.78	1.26	391.48	0.00
3.75	0.00	17.94	0.00	85.88	0.93	411.11	0.00
3.94	0.00	18.84	0.00	90.19	0.63	431.73	0.00
4.13	0.00	19.79	0.00	94.71	0.37	453.38	0.00
4.34	0.00	20.78	0.00	99.46	0.18	476.12	0.00
4.56	0.00	21.82	0.01	104.45	0.06	500.00	0.00

product: Sodium Alg

refraction:1.64 - 0.00 i

liquid: SHMP

refraction:1.48

viscosity:0.89 mPas

measuring cond.: 80/80/5/100/25

duration: 100.77 s

temperature: 24.99 °C

evaluation: WINDOX 5.4.0.0

rule: 1..500nm:128(log) 0.001..5ms -1%

measured correlation function:

amplitude: 9.19 %

mean count rate ch.1:58.35 kcps

mean count rate ch.2:49.54 kcps

single scattering ratio:45.45 %

user parameters:

Parameter 1:

Parameter 2:

Parameter 3:

Parameter 4:

3.5 TESTING:

The blood clotting efficiency of the bio polymer is tested by conducting blood clotting test .

- The blood from two donor is taken , normal clotting time is calculated in microbiology lab in ganga hospital , Coimbatore .
- The results obtained is consider as a reference to calculate the clotting efficiency of tho bio polymers
- In the a test tube , both milled and pure bio polymer is taken separately .
- Now again blood is taken from the donor
- No anti coagulants are added to it
- As soon as the blood is taken from the donor ,immediately it is transferred to the test tube without directly exposing it to the atmosphere .
- Now the clotting time is calculated using stop watch.
- Blood sample will change in to cake or gel form .now the time in the stop watch is noted
- In this test the blood samples are not exposed to atomsphere
- chitosan and sodium alginate both pure and nano particle is tested by the procedure and clotting time is calculated

TEST REPORT

:

GANGA LABORATORY

Patient Name : Mr. VEERA SENAN
Age/Sex : 22/MALE
Patient ID : 11034234
Referred By : Dr. S. Rajasekaran M.S (Ortho.)., D. Ortho., Dip. N.B., F.R.C.S(Ed)., M.Ch. Ortho., (Liverpool)

Lab No : 164712
Request Date : 24-03-2011 11:53 AM
Report Print Date : 24-03-2011 02.39 PM
Page : 1

BIOCHEMISTRY

Test	Patients Results		Reference Value
APTT			
TEST	: 31.6	Seconds	
NORMAL	: 23-35	Seconds	
PROTHROMBIN TIME			
TEST	: 16.1	Seconds	
CONTROL	: 14.0	Seconds	
INR	: 1.2		

CLINICAL PATHOLOGY

BLOOD GROUP			
BLOOD GROUP	: "A1"		
RH TYPING	: Positive		
BLEEDING TIME	: 2 mts 30 sec		(1 - 5)
CLOTTING TIME	: 3 mts 30 sec		(4 - 9)

Dr. M.Shyam Kumar M.B.B.S.,D.C.P
Clinical Pathologist

*** End of Report ***

GANGA MEDICAL CENTRE & HOSPITAL (P) LTD
313, Mettupalayam Road, Coimbatore - 641 043, Tamilnadu, India. Ph: 0422-2485000,
E-mail: srs@gangahospital.com, sr@gangahospital.com Website: www.gangahospital.com

GANGA LABORATORY

Patient Name	: Miss SUNDHA MONISHA	Lab No	: 164710
Age/Sex	: 22/FEMALE	Request Date	: 24-03-2011 11:52 AM
Patient ID	: 11034233	Report Print Date	: 24-03-2011 01:32 PM
Referred By	: Dr. S. Rajasekaran M.S (Ortho.)., D. Ortho., Dip. N.B., F.R.C.S(Ed)., M.Ch. Ortho., (Liverpool)	Page	: 1

BIOCHEMISTRY

Test		Patients Results		Reference Value
APTT				
TEST		: 31.3	Seconds	
NORMAL		: 23-35	Seconds	
PROTHROMBIN TIME				
TEST		: 15.2	Seconds	
CONTROL		: 14.0	Seconds	
INR		: 1.11.		

CLINICAL PATHOLOGY

BLEEDING TIME	*	: 3 mts 15 sec	(1 - 5)
CLOTTING TIME	*	: 5 mts 00 sec	(4 - 9)

Dr. M.Shyam Kumar M.B.B.S.,D.C.P
Clinical Pathologist

*** End of Report ***

particulars	Donor 1	Donor 2
Normal clotting time	5 min	3 min 30 sec
Chitosan-pure	4.12 min	2.45 min
Sodium alginate-pure	4.25 min	2.75 min
Nano chitosan	3.55 min	2.20
Nano sodium alginate	4 min	2.52 min

TABLE 1 CLOTTING TIME OF CHITOSAN AND SODIUM ALGINATE

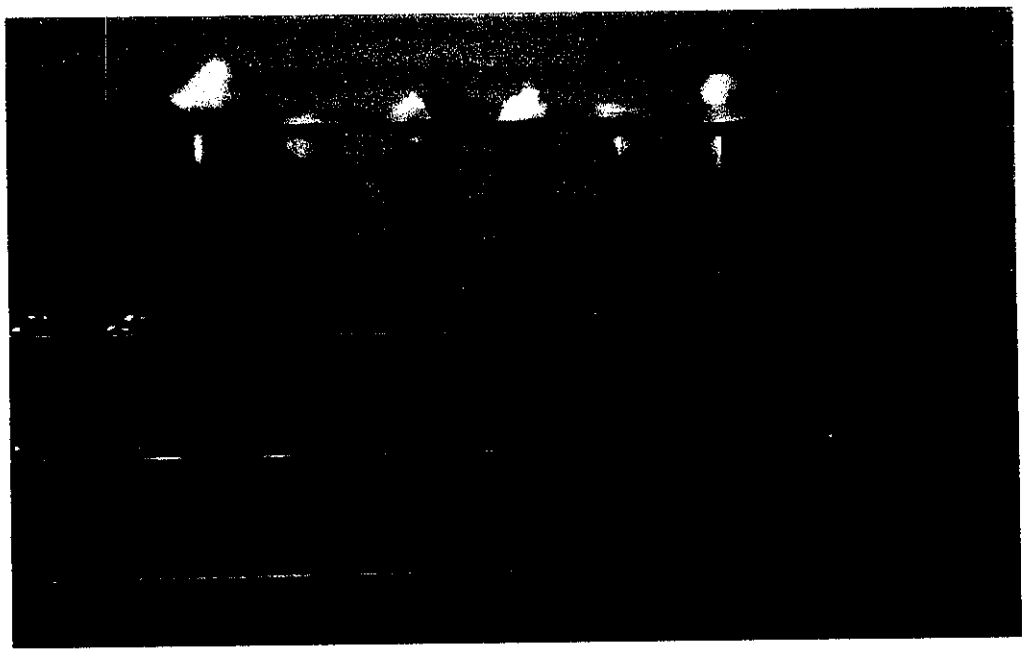


FIG 13 IMAGE OF BLOOD CLOT

FIG 14 IMAGE OF BLOOD CLOOT IN TEST TUBE

4 RESULT AND DISSCUSSION

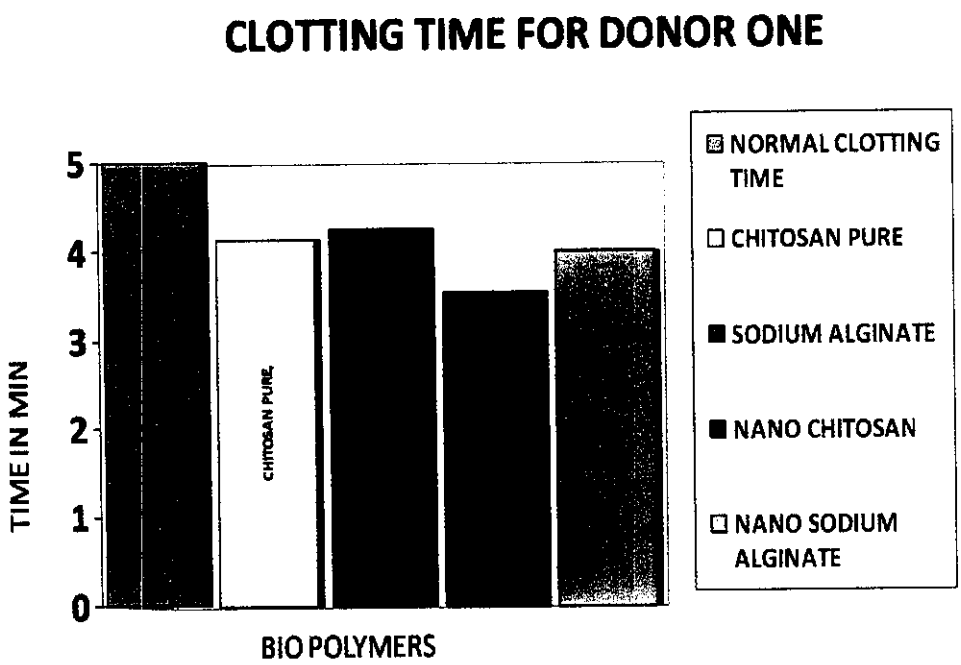


chart 1 clotting time of donor one

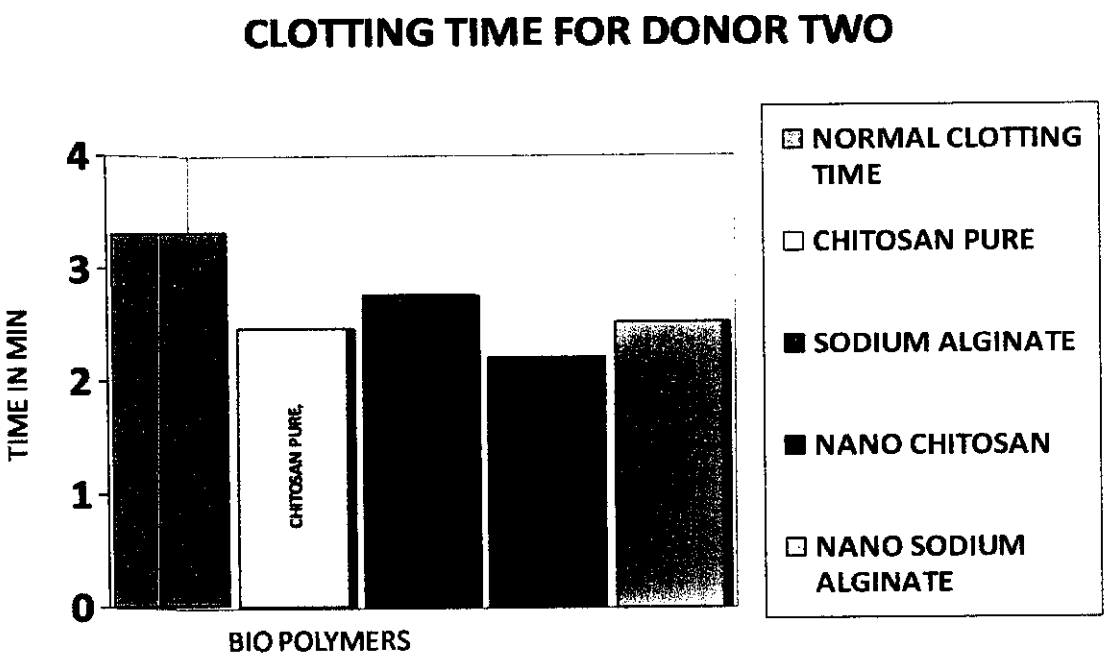


Chart 2- clotting time of donor two

- The normal clotting time of donor 1 and donor 2 is compared with the clotting time of blood samples having chitosan pure, nano chitosan, sodium alginate pure and nano sodium alginate.
- From this comparison it is found that biopolymer nanoparticles reduces the clotting time.
- Among these biopolymer nanoparticles, the performance of chitosan nanoparticle is good.
- And the performance of chitosan is better than sodium alginate both in micro and nano level
- From XRD spectra the size of the crystalline nano particles are determined

- Presence of OH and C=O bond in chitosan is determined from the FTIR spectra from the peaks at 1646.1 and 3416.6 in the spectra
- Presence of OH and C=O bond in sodium alginate is determined from the FTIR spectra from the peaks at 1630.6 and 3512.2 in the spectra
- From the particle size distribution graph , the average nano particle size of chitosan is found to be 100 nm
- From the particle size distribution graph , the average nano particle size of sodium alginate is found to be 50 nm

CONCLUSION :

- Biopolymers are biomaterials which have high blood clotting properties .
- From literature survey it is found that biopolymers like chitosan and sodium alginate have anti-bacterial , biodegradable and blood clotting properties.
- The efficiency of the biopolymers at the nano scale is higher when compared to its macro or micro level because of the surface contact area is more at nano level.
- As a result of the blood clotting test it is found that nano chitosan is more effective than nano sodium alginate.

FUTURE SCOPE:

The present investigation have paved the way for further research on the following aspects

- The studies can be extended by applying nano particle on a textile substrate and its properties can be checked
- The chemicals now used for blood clotting are having side effects such as trauma, irritation and swelling , so these bio polymers can be used as alternative for those chemicals

References:

1. Ravi Kumar,M.N.V.; Muzzarelli, C.; Sashiwa,H.; Domb, A.J. Chitosan chemistry and pharmaceutical perspectives. Chem.. Rev.2004,104,6017-6084.

2. Shahidi, F.; Arachchi,J.K.V.; Jeon, Y.J. Food applications of chitin and chitosan.Trends Food Sci.Technol.1999,10,37-51.

3. Dodane, V.; Vilivalam, V.D. Pharmaceutical applications of chitosan. Pharm. Sci. Technol. Today 1998,1,246-253.

4. Jeon, Y.J.; Shahidi, F.; Kim, S.K. Preparation of chitin and chitosan oligomers and their application in physiological functional foods. Food Rev.Int.2000,16, 159-176.

5. Rinaudo, M. Chitin and Chitosan: Properties and applications. Prog. Polym. Sci. 2006, 31, 603-632.

6. Kurita,K. Chemistry and application of chitin and chitosan. Polym.Degrad. Stab.1998,59(1-3),117-120.

7. Ouakfaoui, S.E.; Asselin, A. Diversity of chitosanase activity in cucumber. Plant Sci.1992, 85, 33-41.

8. Xia, W.S. Physiological activities of chitosan and its application in functional foods.J.Chin. Inst. Food Sci. Technol.2003,3(1),77-81.

9. Abdel-Aziz, S.M.; Mostafa, Y.A.; Moafi,F.E. Partial Purification and some properties of the chitosanases produced by Bacillus Alvei Nrc-14.J.Appl. Sci. Res.2008,4(10),1285-1290.a

10. Chen, X.; Xia, W.S.; Yu, X. Purification and characterisation of two types of chitosanase from Aspergillus sp.CJ22-326. Food Res. Int.2005,38,315-322.