POLYELECTROLYTE COMPLEX COMPOSED OF CHITOSAN AND SODIUM ALGINATE FOR WOUND HEALING

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT OF THE DEGREE OF

Master of Technology

in

Biomedical engineering

by

Aasis Moharana

Under the guidance of

Prof. Devendra Verma

Department of Biotechnology and Medical Engineering

National Institute of Technology,
Rourkela

NATIONAL INSTITUTE OF TECHNOLOGY
ROURKELA

CERTIFICATE

This is to certify that the project entitled "Polyelectrolyte complex composed of chitosan and sodium alginate" submitted by Aasis Moharana (Roll No. 213bm1011) is a genuine work performed by him under my guidance required for the Master of Technology degree in Biomedical Engineering at National Institute of Technology, Rourkela. To the best of my knowledge, this thesis is very authentic and none of its matter has been submitted anywhere else for the award of degree or diploma.

Date:-

Prof. Devendra Verma
Department of Biotechnology and Medical Engineering
NIT Rourkela

ACKNOWLEDGEMENT

With deep regards and profound respect, I avail this opportunity to express my deep sense of gratitude and indebtedness to Prof Devendra Verma, Department of biotechnology and medical Engineering, NIT Rourkela, for introducing the present research topic and for her inspiring guidance, constructive criticism and valuable suggestion throughout this research work. It would have not been possible for me to bring out this report without his help and constant encouragement. I express my sincere gratefulness to Prof. K.pramanik, Head of the Department, biomedical Engineering, NIT Rourkela for giving me an opportunity to work on this project and allowing me the access to valuable facilities in the department. I am really thankful to my dear class mate Mr Prashant Kumar and I am thankful to Mr. Bhisma sir, Ph.D. scholar of biotechnology Engineering Department.

Aasis Moharana

213BM1011

List of figures

Abstract

Wound healing occurs naturally by the body mechanism action. The blood initially clots and the healing procedure starts. A kind of gel is developed which would speed up the process. The gel is made up of chitosan and sodium alginate. This biocompatible gel would provide wet environment and a forms a coating on the wounded area due to which it will refrain from bacterial action and protects the wound from infection. Here we analysed different concentration of sodium alginate and chitosan followed by its characterisation techniques using scanning electron microscope and Fourier transform infrared spectroscopy, viscosity and tensile measurement of the gel. Which shows the comparable results than other Haemostatic gel reported so far. Finally a dynamic syringe is devoloped which is used to produce a homogeneous mixture of chitosan and alginate solution which would successfully help in wound healing process. The basic aim of this study is to optimize the time of formation of the gel.

CHAPTER 1

INTRODUCTION

Introduction

About 70% of the deaths in war fields are due to excessive loss of blood. The blood when drained from a major artery or vein makes severe haemorrhages for which death occurs. Therefore for the control of loss of blood from the victims, different approaches have been developed. Hemcon and Quickclot are some of the widespread products for the dressing of the wound. Other methods such as use of cellulose and carbon nanotubes have also been proposed. Here in this project we have developed a gel capable of accelerating the clotting mechanism. The products to be developed should be bio-degradable, bio-compatible and non toxic. Natural blood clotting produces fibres which form a net like structure to reduce the flow of blood. To replicate the process approaches has to be made so the materials would crosslink with eachother so that it will enhance the mechanical support to the wound.

Some of the objectives are:-

1) The bandage should have fast clotting.

2) Desired environment should be provided for the wound so that it will rectify itself properly.

3) Biocompatible materials should be used.

The fundamental targets for the material needed to be dressed are-

1) To recuperate the harmed territory.

2) stabilise the wound completely.

3) enhance clammy injury recuperating.

Hydrogels

Hydrogels means the combination of gel with water..The content of water should be more than 90 %.It comprises a chain of polymer network which are hydrophilic in nature.The hydrogels has many application because of its resemblance to natural tissue.

Some of the applications of hydrogels are: -

- Drug delivery systems
- Scaffold engineering
- Electrodes for EEG and

There are two major factors of the clotting mechanism of the blood. These are platelets and thrombin. The platelets are tiny cellular elements made in the bone marrow, which travel in the bloodstream waiting for the clotting problem to occur. When bleeding occurs, chemical reaction changes the surface of the platelets to make it sticky. Sticky platelets then said to have activated. These activated platelets begin to adhere to the wall of the blood vessels at the site of bleeding and within a few minutes they form what is called white clot.

The thrombin system consists of the several blood protein that when bleeding occurs, become activated. The activated clotting proteins engage in a cascade of chemical reaction that finally produce a substance called thrombin. It is a long sticky string like substance which sticks to the exposed vessels walls clamping together and forming a web-like strand.

Injured tissue and platelets release the clotting factor prothrombin activator and calcium ion, then prothrombin activator converts the blood protein prothrombin to thrombin, then thrombin splits fibrinogen to form fibrin, fibrin fibres form a mesh over wound trapping RBC and platelets. After the bleeding gets stopped the clot hardens and becomes smaller and new cells grow to repair the wound site and later on enzyme plasmin is released to dissolve the clot.

Hemostatic Bandages

Currently, there are several new blood clotting hemostatic bandages on the market or being tested. Each takes a different approach to stopping bleeding, and each has advantages and disadvantages. Anyone who can apply a bandage can use these products. Within reason, this covers just about everyone in

medicine, the military, and law enforcement. The benefits are lives saved, at least in theory. Therefore, the topic is a must for unbiased discussion.Blood clotting is the body's way of closing a wound to prevent blood loss.For small wounds, a clot begins to form a mesh of platelets and blood cells within one to two minutes.If, however, the wound is large and the blood flow is under pressure, such as in a wound to the femoral artery in the thigh, the normal clotting mechanism fails to stop the bleeding, and the victim can bleed to death within a few minutes. Hemostatic bandages are made to supplement the body's clotting process to stop bleeding. Chitosan and alginate are biocompatible. Therefore, these have been chosen for the study. we can use crosslinkers to mix that solution but as crosslinkers are toxic so we could not use it.so we can use polymer which can crosslink with each other using electrostatic interaction.chitosan is positively charged and alginate is negatively charged, so they can form crosslink but they didn't form a homogeneous gel.Therefore again calcium chloride is added to chitosan which will stabilise the polymer and the calcium chloride is substituted by alginate.still directly mixing does not provide homogeneous mixing so calcium chloride was formerly mixed with alginate so that chitosan would replace calcium chloride and will form link with alginate .

CHAPTER 2

LITERATURE REVIEW

LITERATURE REVIEW

Invention background

A propelled drain administration gauze and methodologies of its application would well increase offered styptic methods. To date, the applying of consistent weight with cotton material remains the famous essential mediation strategy acclimated stem blood stream especially be expected seriously mischief wounds. On the other hand, this methodology neither successfully nor securely stanches serious blood stream. This has been, and keeps on being, a genuine survival drawback inside of the instance of extreme genuine mischief from an injury.

HemCon

HemCon Bandage is manufactured by Hem-Con Inc. of Tigard, Ore. It uses a material called Chitosan, a biodegradable, nontoxic, complex carbohydrate of chitin, which is found in the exoskeletons of shellfish. It will not cause an allergic reaction, according to product literature. A recent study by the U.S. Army Institute of Surgical Research looked at the effectiveness of a chitosan-based hemostatic dressing to prevent blood loss in swine. Based on the results, the team concluded that a chitosan dressing reduced hemorrhage and improved survival after severe liver injury in swine and that further studies are

warranted.The bandage is designed for immediate hemorrhage control and is deployable by an injured soldier, combat medic, or an untrained first responder. The bandage has been tested in animal models of severe bleeding by the U.S. Army and other laboratories.Company literature claims HemCon is superior to all known hemorrhage dressings. But John Hagman, M.D.,medical director for the FBI Hostage rescue team, reported at the December 2003 Special Operations Medical Association (SOMA) conference in Orlando,Fla., that a recent study completed for the U.S. Air Force showed HemCon had
a significant failure rate.

Quick clot

QuikClot is manufactured by Z-Medica of Newington, Conn. According to product literature, newspaper articles, and press releases from Z-Medica, Quik-Clot was used extensively during Operation Iraqi Freedom. The active ingredient in the product is called granular zeolite, a substance derived from lava rocks. When his material is placed into a bleeding wound, it absorbs the water molecules in the blood and creates a high platelet concentration to promote clotting. This causes an exothermic reaction. In otherwords, it gives off heat. Several U.S. Navy physicians who served in Iraq report that QuikClot produces sufficient heat to cause burns to the skin if measures are not taken to

wipe off water, sweat, and excess blood from the wound and skin before use. In fact, Navy Corpsmen that served with Marine combat units in Iraq reported they observed "second-degree burns" in Iraqi soldiers treated with QuikClot. QuikClot concentrates clotting factors in the blood by promoting extremely rapid adsorption of fluids in and around the wound, creating a matrix for clot formation. As per product literature instructions, QuikClot is to be poured directly onto an open bleeding wound where it promotes formation of a stable powerful clot. The clot, according to Z-Medica, is then easily removed through suction or irrigation of the wound when the patient arrives at a care facility.

The exact formula of QuikClot is proprietary, but it contains no biological or botanical material, thus decreasing the chance of an allergic reaction. Although some organizations are not completely sold on QuikClot, the Navy and Marines performed a limited study using a swine model and were impressed with the results enough to issue the product to combat medics serving in Afghanistan and Iraq. In a press release packet sent to us from Z-Medica dated April 9, 2003, company Vice President Bart Gullong was quoted saying, "Based on QuikClot's performance in Operation Iraqi Freedom, we are now gearing up production to accommodate even faster worldwide adoption of it by all first responders: police, firefighters, EMTs, and anyone who is first on the scene of a severe bleeding injury."

RDH

Fast Deployment Hemostat Bandage, or RDH, is made by Marine Polymer Technologies in Danvers, Mass. The material used to advance blood coagulating, poly-N-acetylglucosamine (p-GlcNAc), is gotten from single-cell green growth found in the sea. The RDH Bandage empowers field faculty with least preparing to quickly and effectively stop blood vessel and other discharge coming about because of limit injury. The improvement of the RDH war zone dressing is the outcome of an effective cooperation between Marine Polymer Technologies and the Office of Naval Research. When the RDH Bandage comes incontact with blood, it animates platelet initiation, which prompts the emission of a substance known as Thromboxane. The Thromboxane fortifies the narrowing of veins close to the injury, which helps moderate blood stream there. As indicated by writing from Marine Polymer Technologies, the system of poly-N-acetylglu-cosamine quickens the ordinary coagulating procedure bringing about the quick control of dying. The dressing is anything but difficult to handle and coats the injury surface. An unmistakable point of preference of the RDH gauze is that it is completely biodegradable and can be left set up on a draining surface to give proceeded with hemostasis after twisted closure. Studies taking a gander at the adequacy of the RDH Bandage have been directed at the Department of Surgery, Ryder Trauma Center, University of Miami School of Medicine in Miami, Fla., and the Department of Surgery, New England Medical

Center in Boston, Mass. The RDH wrap is bundled as a delicate, white 4x4-inch sterile non-woven cushion of a cellulose polymer put on bandage backing in a sterile foil pocket. The expense of the RDH Bandage is dictated by arrangement with the organization on a case-by-case premise, as per John Vournakis, VP of innovative work at Marine Polymer Technologies.

TraumaDEX

TraumaDEX is manufactured by Medafor Inc. of Minneapolis, Minn., and the following information was provided by its executive vice president, Bob Cerza. TraumaDEX is a wound-dressing agent utilizing Microporous Polysaccharide Hemosphere (MPH) technology that has been naturally synthesized from potato starch. When applied directly with pressure to an actively bleeding wound, the particles accelerate natural blood clotting by concentrating blood solids such as platelets and red blood cells, and other blood proteins such as albumin, thrombin, and fibrinogen to form a gel around the particles.

CHAPTER 3

MATERIAL AND METHODS

MATERIALS AND METHODS

Preparation of sodium alginate and calcium chloride solution

To prepare solution of sodium alginate and calcium chloride, we have taken 1mg of sodium alginate in 100 ml of water. Another solution was prepared in which we have taken 3 mg of sodium alginate in 100 ml of water. Then we prepared the solution calcium chloride in which 0.2 mg of calcium chloride was mixed with 100 ml of water and again another solution was prepared with 0.4 mg of calcium chloride in 100 ml of water. 1 ml of sodium alginate and 1 ml of calcium chloride is mixed, in which the concentration of sodium alginate was 1 mg/ml and concentration of calcium chloride was 0.2 mg/ml and the prepared solution is T1.

The other three solutions was prepared with different concentrations, in first solution (T2) concentration of sodium alginate was 1gm and the concentration of calcium chloride was 0.4gm, in second solution (T3) concentration of sodium alginate was 3gm and the concentration of calcium chloride was 0.4gm. And for the last solution the concentration of sodium alginate was 3gm and the concentration of calcium chloride was 0.4gm.

The stock solution of sodium alginate and calcium chloride was prepared of 1% both. Similarly four solution of were prepared as T1,T2,T3,T4. T1 was made whwn 2ml of 1% of SA was added with 0.4ml of calcium chloride.T2 was prepared when 2ml of 1% SA was added with 0.8ml of calcium chloride.T3 was

prepared when 6ml of SA was added with 0.4 ml of calcium chloride. Similarly T4 was prepared when 6ml of 1% SA was added with 0.8ml of calcium chloride.

Concentration of SA= (S1)1mg/ml

(S2)3mg/ml

Concentration of $cacl_2$= (C1)0.2mg/ml

(C2)0.4mg/ml

Final volume= 20ml of solution

Concentration required

T1= 1mg/ml SA + 0.2mg/ml $cacl_2$

T2=1mg/ml SA + 0.4mg/ml $cacl_2$

T3= 3mg/ml SA + 0.2mg/ml $cacl_2$

T4=3mg/ml SA + 0.4mg/ml $cacl_2$

Stock solution

SA =1%

$Cacl_2$=1%

T1=2ml of 1% SA + 0.4ml of $cacl_2$

T2= 2ml of 1% SA + 0.8ml of $cacl_2$

T3= 6ml of 1% SA + 0.4ml of $cacl_2$

T4=6ml of 1% SA + 0.8ml of $cacl_2$

\

- All the solutions were made with adding distilled water upto the volume of 20ml
- All the solution were mixed with magnetic stirrer to mix properly for half hour
- Then the solution were centrifuged with 10000 rpm for 20 minute and then stored in fridge and later viewed under scanning electron microscope.

PREPARATION OF 100 ML SOLUTION OF CHITOSAN

Chitosan are extracted from shrimp shells which is a natural polymer. For 1% chitosan solution 1gram of chitosan is mixed in 100ml of water with 1 molar acetic acid. Therefore for 100ml of chitosan solution, 6ml of acetic acid were poured into 94ml of water and the mixture stirred at a constant speed.

At this moment 1 gm of chitosan is taken and poured into the solution of Acetic acid and water. The solution will form a lump of chitosan. The magnetic bead is rotated at lower rpm. If it rotates at higher rpm the chitosan solution will form small lumps and will not dissolve easily. After that the solution has to be kept overnight to form a viscous solution. Acetic acid is a week acid and it help for dissolving chitosan.

Now the prepared chitosan solution is added to freeze dried T4 solution. The solution was stirred for overnight and we observed the reading that we have taken during analysis of prepared solution.

Preparation of four solutions s1, s2, s3 and s4

S1=30ml of SA + water (100ml)

S2=2g of SA + water (100ml)

S3=2% chitosan + 4 ml of cacl2 +6ml acetic acid

S4=2% chitosan + 30 ml cacl2 +6ml acetic acid

Solutions prepared

Fig.1 Shows the solution

CHAPTER 4

RESULTS AND DISCUSSION

Measurement of Viscosity

Viscometer- It is an instrument used to measure viscosity of the fluid. Rotational viscometer uses the idea that the force required to turn an object in a fluid that indicate the viscosity of the fluid being measured. The viscometer determines the required force for rotating a disk or bob in a fluid at known speed. 'Cup and bob' viscometers work by defining the exact volume of sample which is to be sheared within a test cell, the torque required to achieve a certain rotational speed is measured. There are two classical geometries in "cup and bob" viscometers, known as either the "Couette" or "Searle" systems.

Viscosity of different samples

S1= chitosan: SA =1:1

S2= chitosan: SA =1:2

S3= chitosan: SA =1:3

S4= chitosan: SA =2:1

VISCOSITY CURVE ANALYSIS

S1= chitosan: SA (1:1)

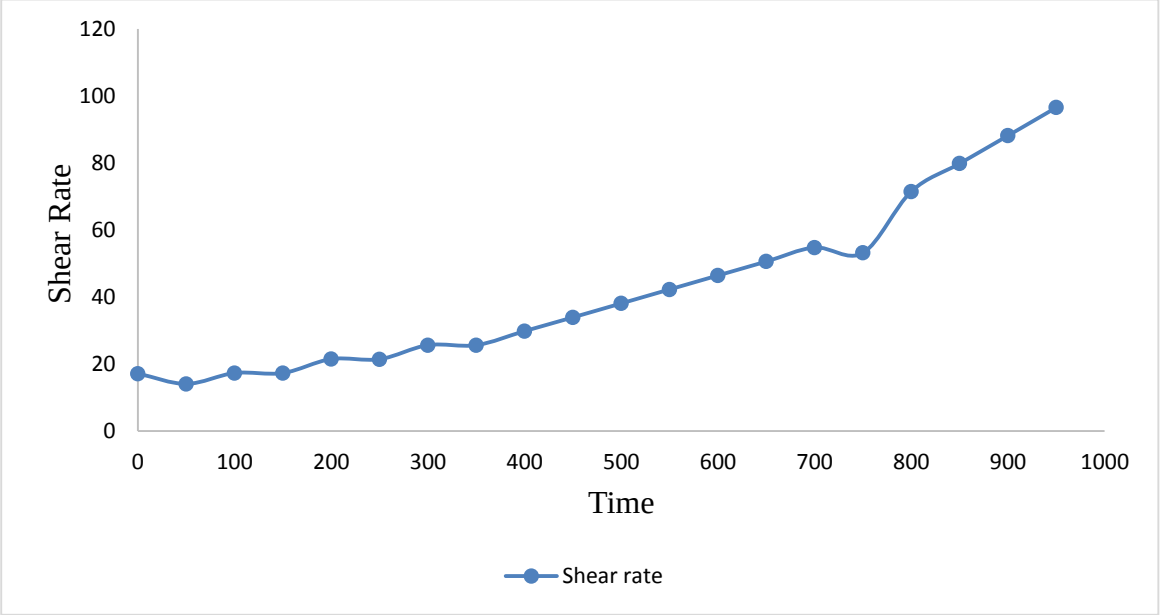

Fig. 4.1: Indicates the shear stress of Chitosan: SA (1:1)

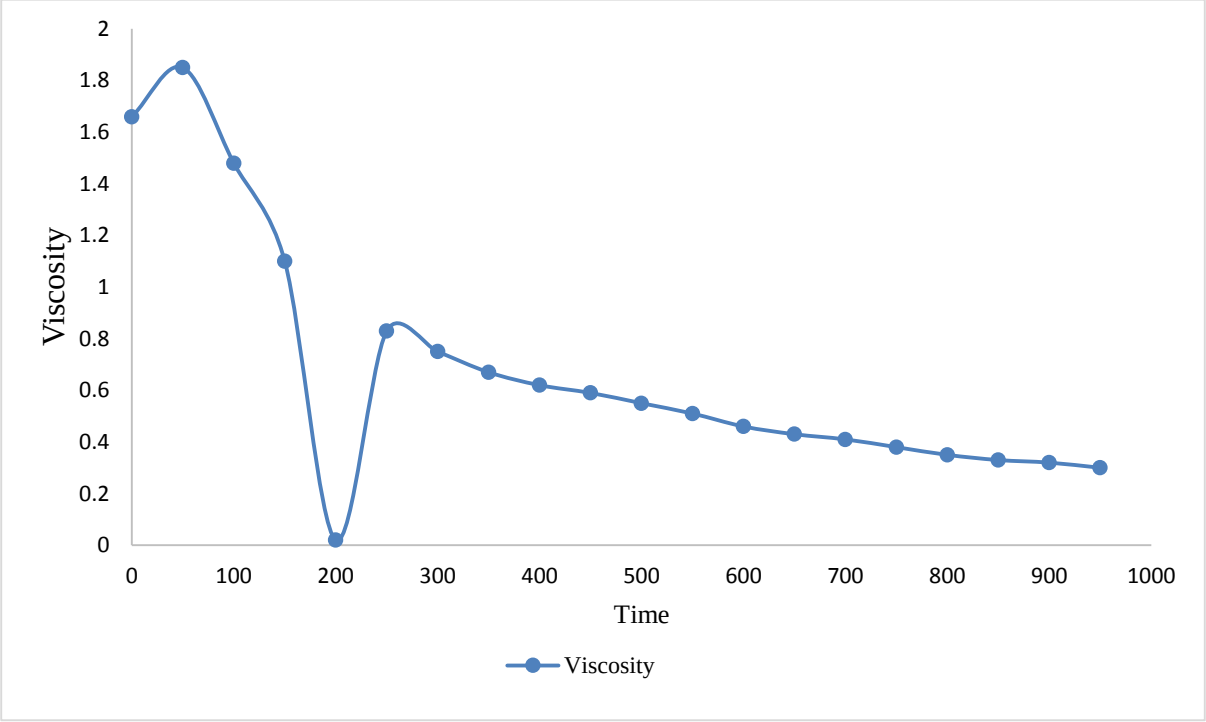

Fig. 4.2: Indicates the viscosity of Chitosan: SA (1:1)

S2= Chitosan: SA (1:2)

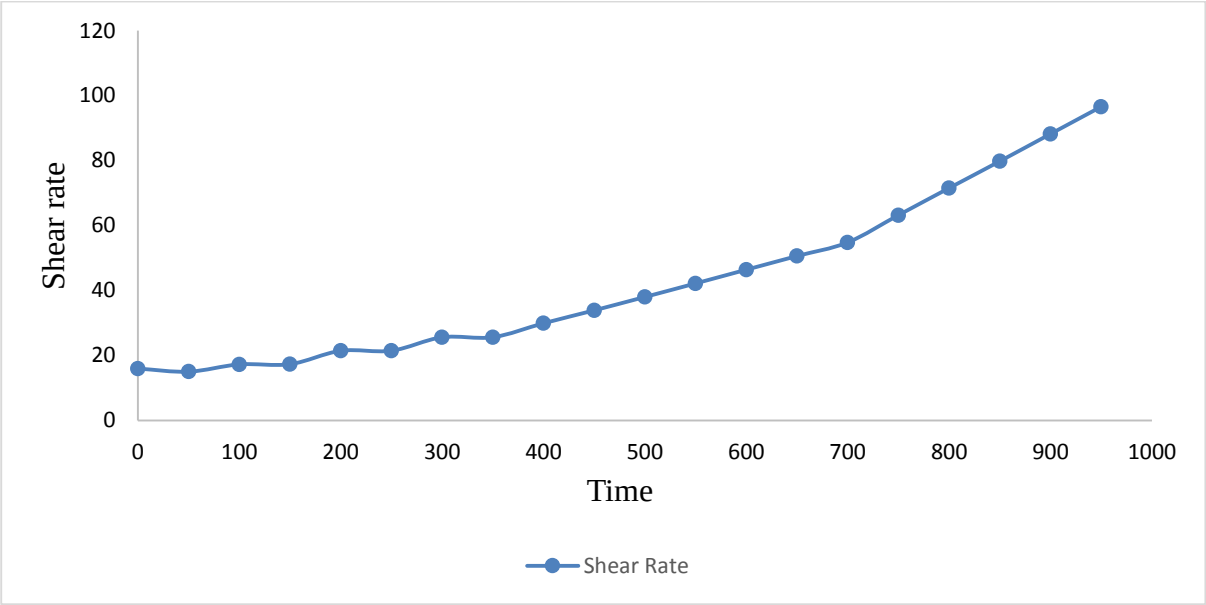

Fig. 4.3: Indicates the shear stress of Chitosan: SA (1:2)

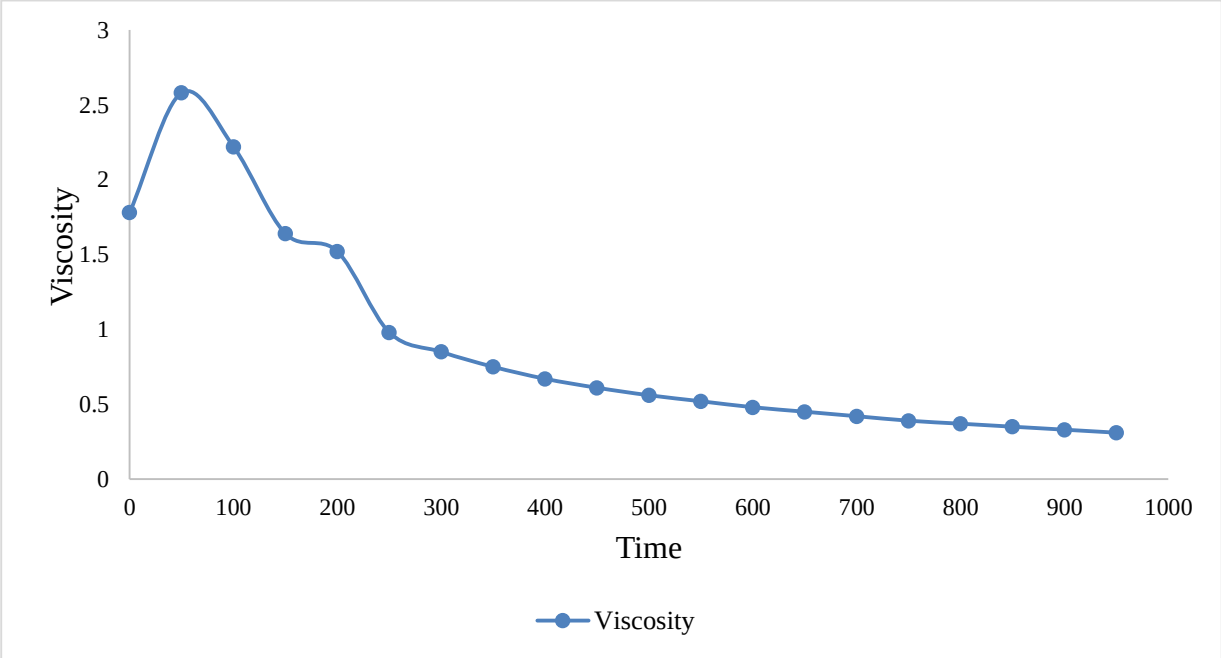

Fig. 4.4: Indicates the viscosity of Chitosan: SA (1:2)

S3=Chitosan: SA (1:3)

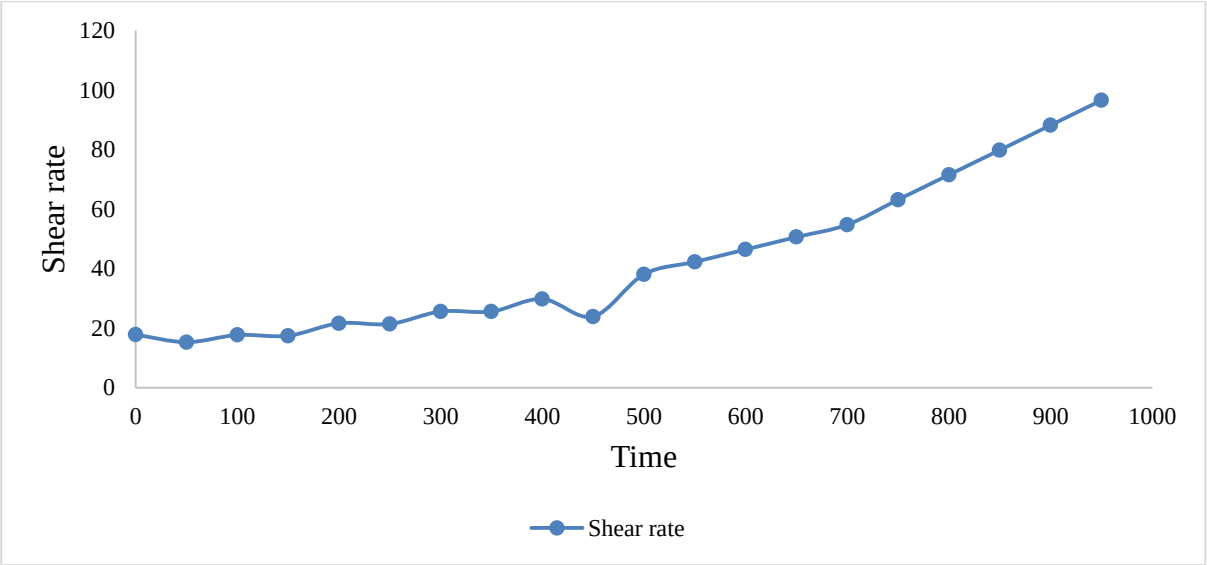

Fig. 4.5: Indicates the shear stress of Chitosan: SA (1:3)

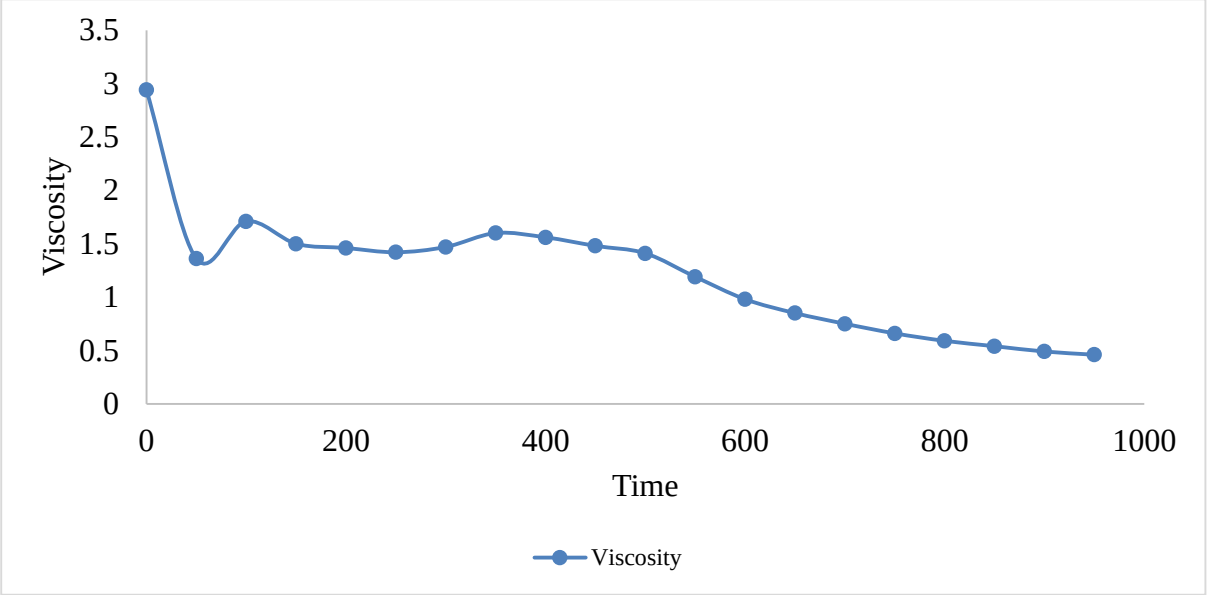

Fig. 4.6: Indicates the viscosity of Chitosan: SA (1:3)

S4= Chitosan: SA (3:1)

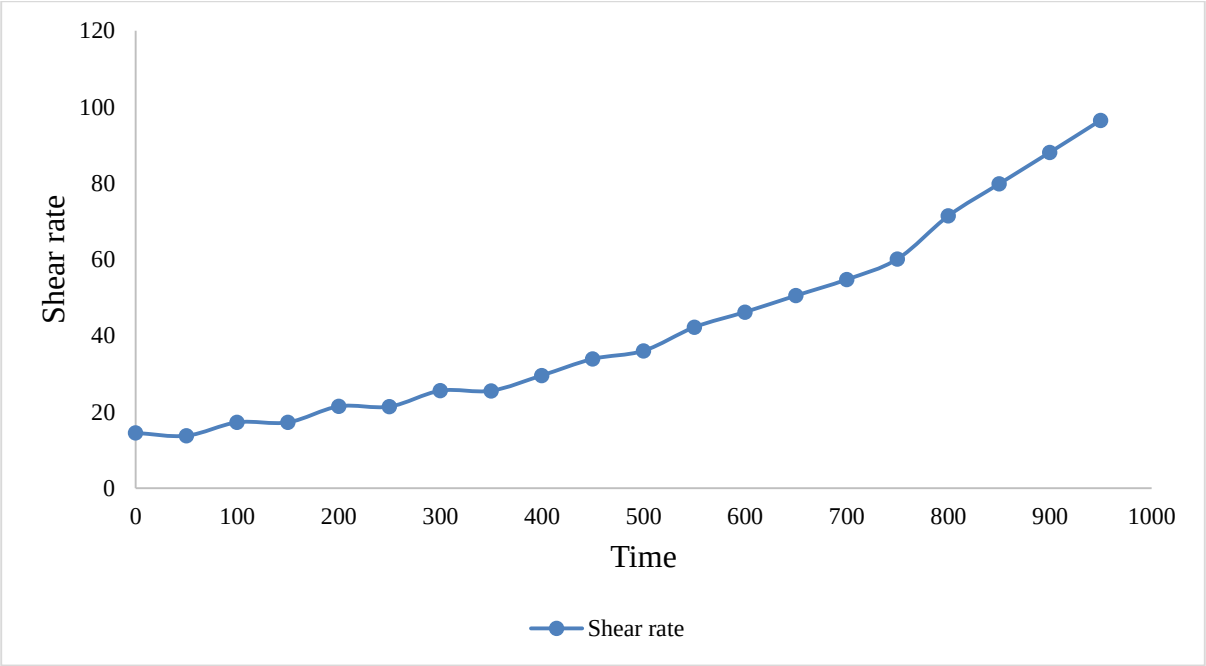

Fig 4.7: Indicates the shear stress of Chitosan: SA (3:1)

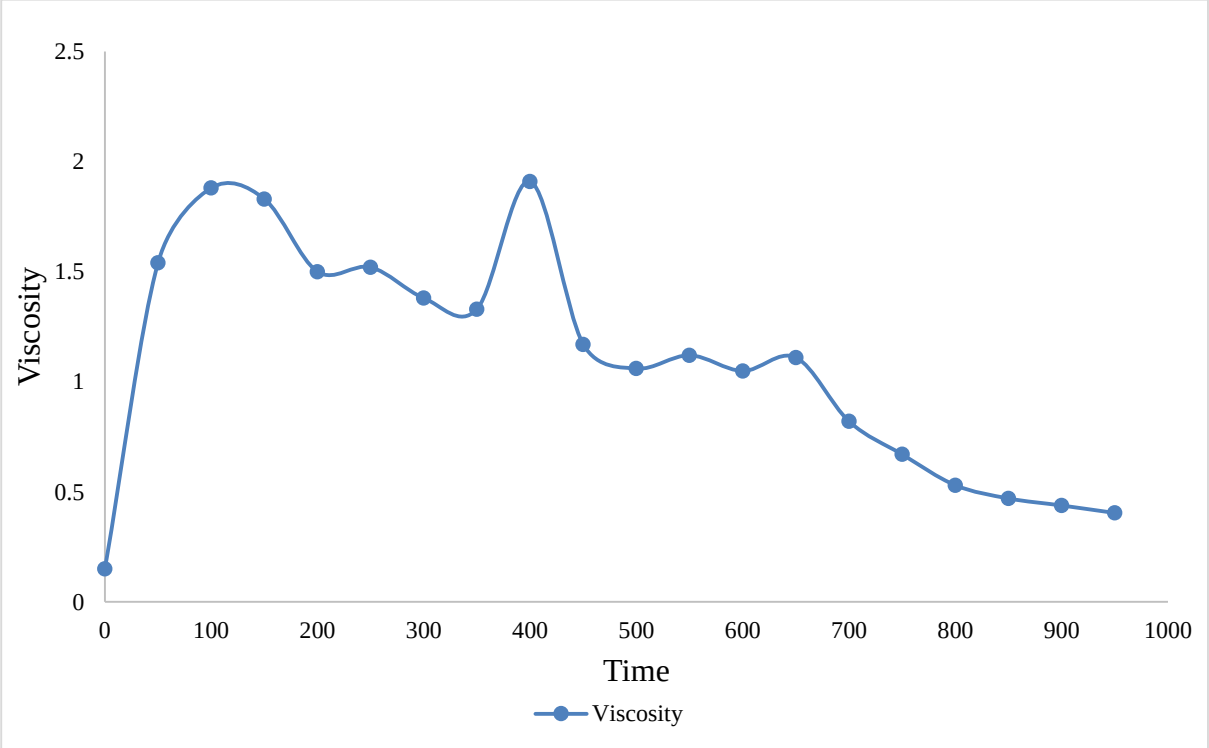

Fig 4.8: Indicates the viscosity of Chitosan: SA (3:1)

FTIR ANALYSIS OF SAMPLES

Principle

FTIR stands for Fourier transform infrared spectroscopy. In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like a fingerprint no two unique molecular structures produce the same infrared spectrum. This makes infrared spectroscopy useful for several types of analysis. So, what information can FT-IR provide?

• It can identify unknown materials

• It can determine the quality or consistency of a sample

• It can determine the amount of components in a mixture

Observation

S1=Chitosan: SA (1:1)

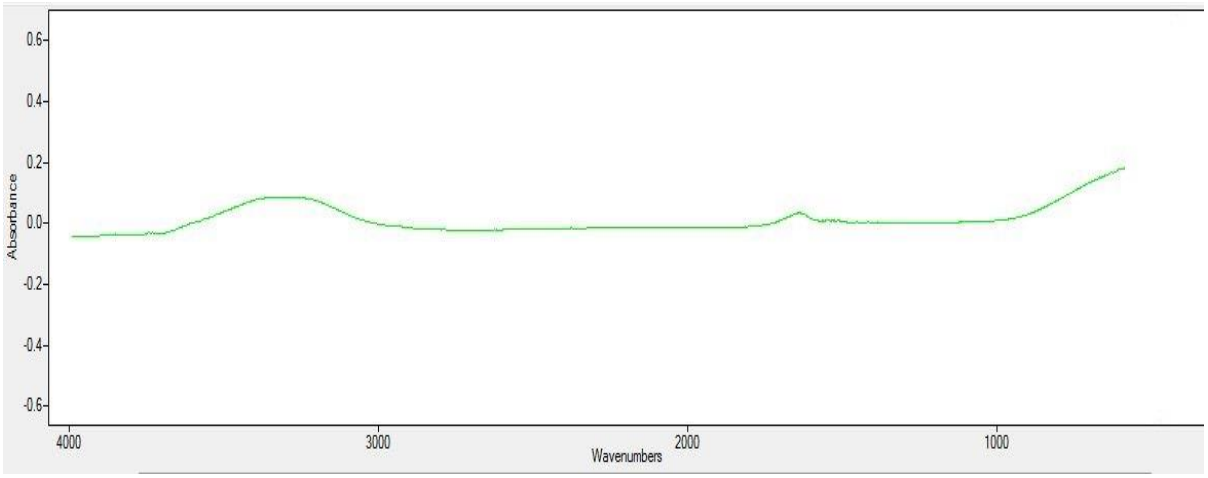

Fig. 4.9: FTIR analysis of Chitosan: SA (1:1).

S2=Chitosan: SA (1:2)

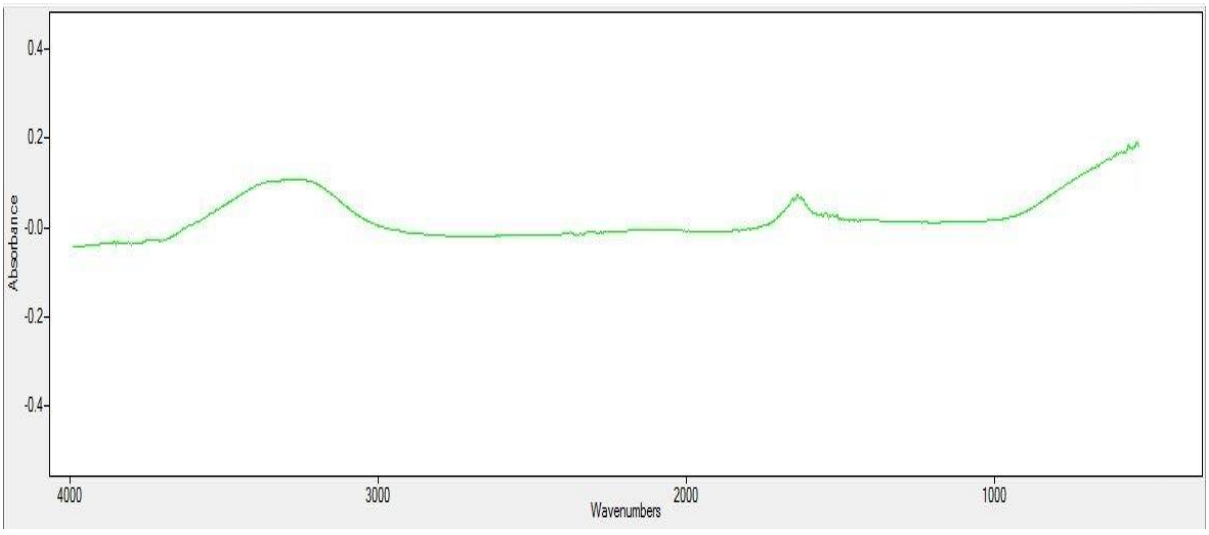

Fig.4.10: FTIR analysis of Chitosan: SA (1:2)

S3= Chitosan: SA (1:3)

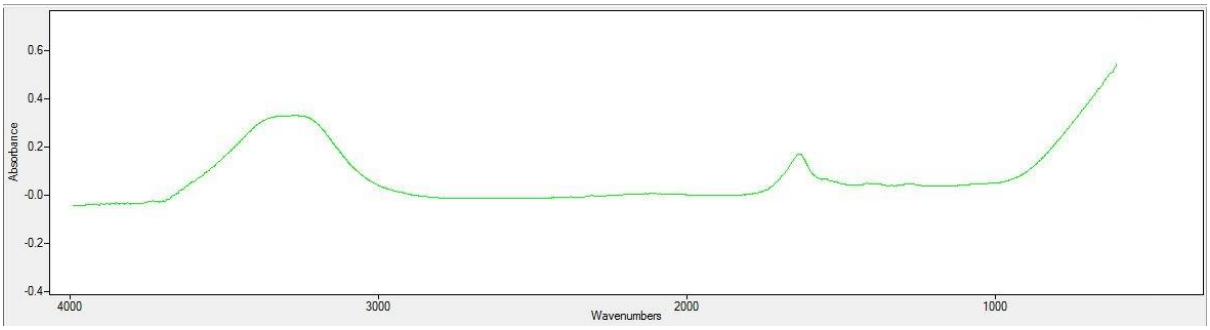

Fig.4.11: : FTIR analysis of Chitosan: SA (1:3).

ANALYSIS OF SAMPLES USING FTIR (with different concentration of calcium chloride)

T1 = Chitosan: SA (1:1) [12% concentration]

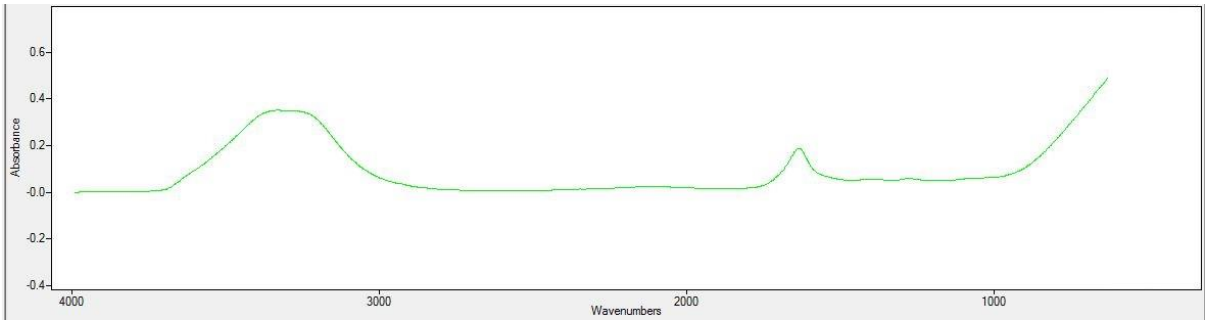

Fig.4.12: FTIR analysis of Chitosan: SA (1:1) (12% concentration).

T2=Chitosan: SA [15% concentration]

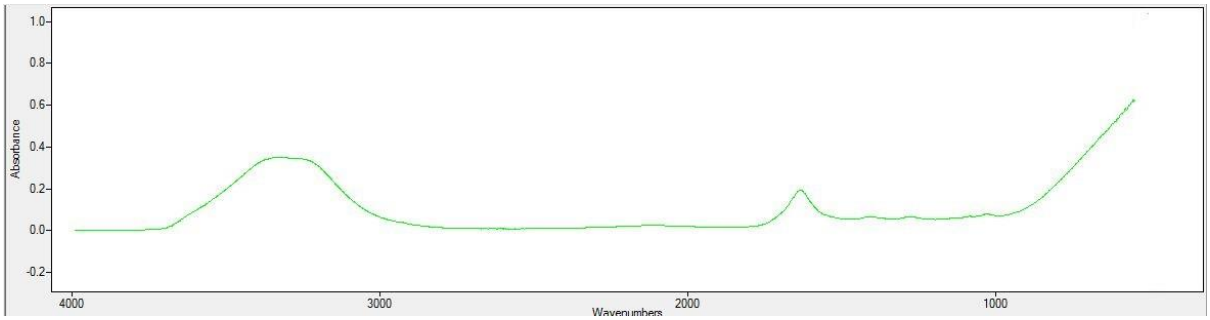

Fig.4.13: FTIR analysis of Chitosan: SA (1:1) (15% concentration).

T3 = Chitosan: SA [20 % concentration]

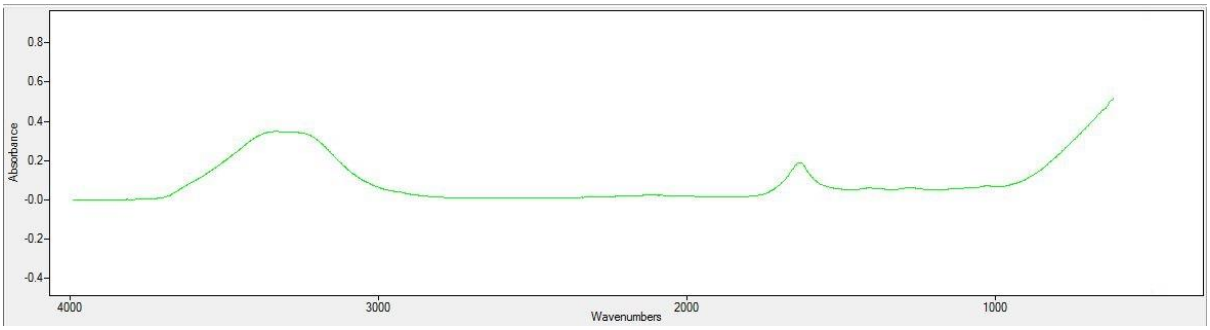

Fig.4.14: FTIR analysis of Chitosan: SA (1:1) (20% concentration).

Biodegradability

Biodegradability is a test performed to know how the material is degraded if it is put under body fluids. Body fluid cannot be accessed easily. For that reason sbf is used. SBF stands for simulated body fluids. The SBF contains different chemicals similar to that of the human body. Two samples were made of ratio (chitosan: SA) 1:1 and 1:2.those samples were then dried and weighed to be 1 gram. Then after different days the dry weight in grams was measured.

Table 4.1: Shows the biodegradability reading associated with Chitosan: SA.

Ratio	Dry-weight (1day)	Dry-weight (3day)	Dry-weight (9day)	Dry-weight (15day)
1:1	0.99	0.931	0.804	0.7414
1:2	0.996	0.852	0.61	0.4412

It was noted that the degradation went 25% and 55% respectively for the two samples. As shown in Figure 4.11 the weights of the Chitosan: SA (1:1) and Chitosan: SA (1:2) are decreasing. Blue and red curves indicate the biodegradability curves associated with Chitosan: SA (1:1) and Chitosan: SA (1:2). Both the curves have decreasing slope which indicates, as the number of days increases the weight of samples also decreases. Initially the decreasing rate for both the samples is almost same but after few days the decreasing rate of Chitosan: SA (1:2) increases which results larger degradation of sample when compared with Chitosan: SA (1:1). The degradation rate of Chitosan: SA (1:1) is almost constant whereas the degradation rate of other sample varies nonlinearly.

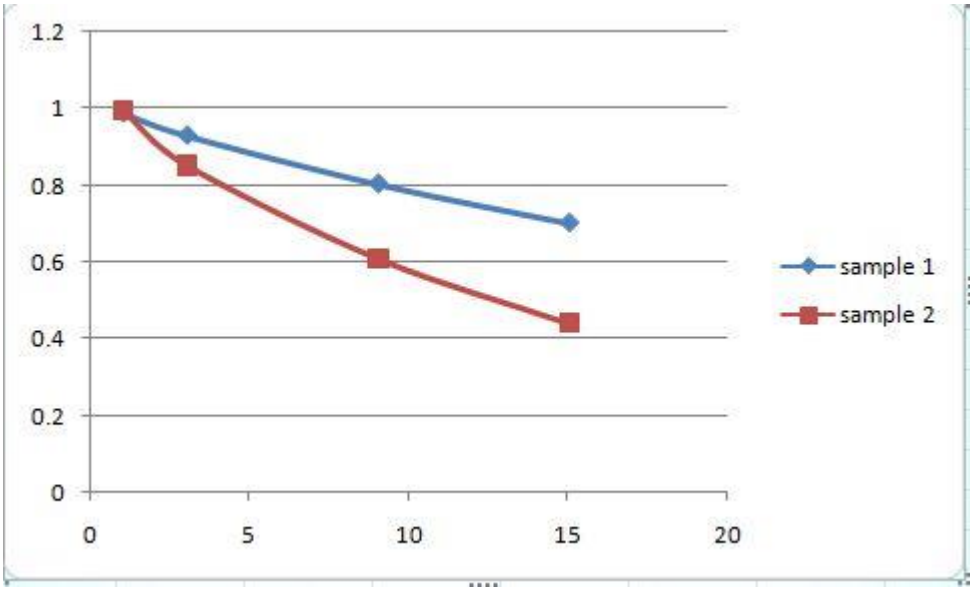

Fig. 4.15: Shows the biodegradability curve of Chitosan: SA (1:1) and Chitosan: SA (1:2).

DYNAMIC SYRINGE MIXER

The dynamic syringe mixer is used to provide a homogeneous mixer of chitosan and sodium alginate. It has several rotating buds inside it which under the pressure of the two solutions mixes properly and can be directly applied to the wound site. The mixer is portable and easy to handle and does not use complex mechanism which makes it easy to use.

Catia v5 r21

The 3D model of the dynamic stirrer mixer is made on the part design orkbench of the Catia. The stirrer consists of three major components: part 1 is the top part which consists of two passages through which solution can be injected with the help of syringe. The second part is the lower part which consists of rotating platforms; the solution injected enters the third part where mixing process starts.

TOP PART OF MIXER

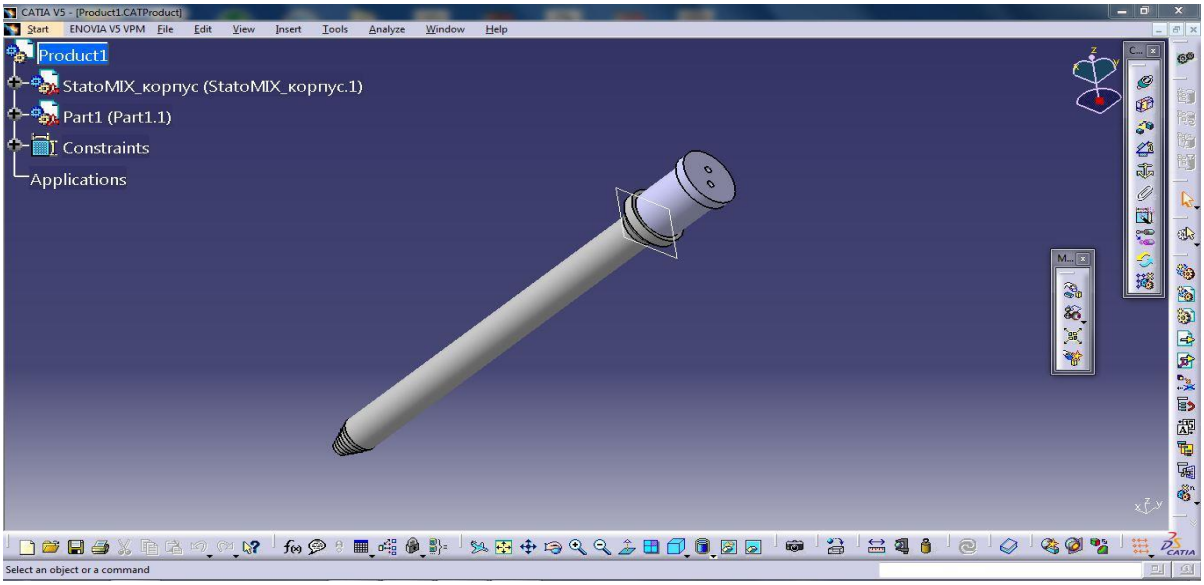

Fig. 4.16 Shows the top part of the mixer.

BOTTOM PART

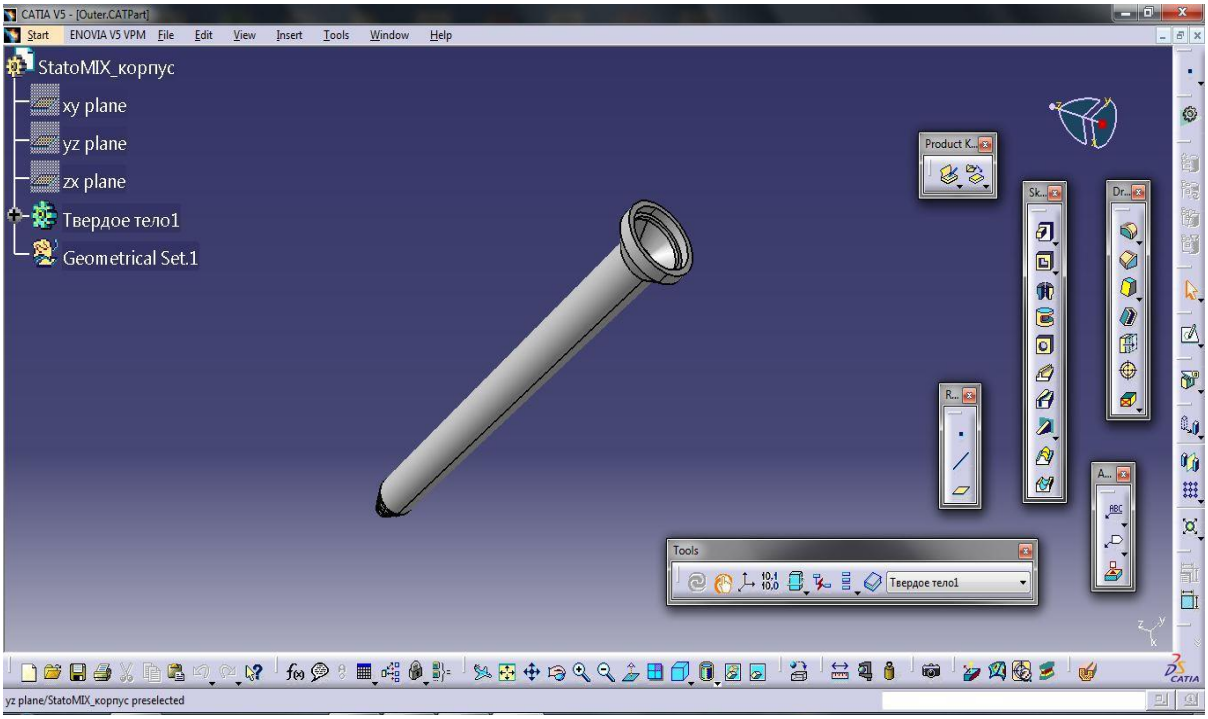

Fig. 4.17: Shows the bottom part of the mixer.

Rotating platform

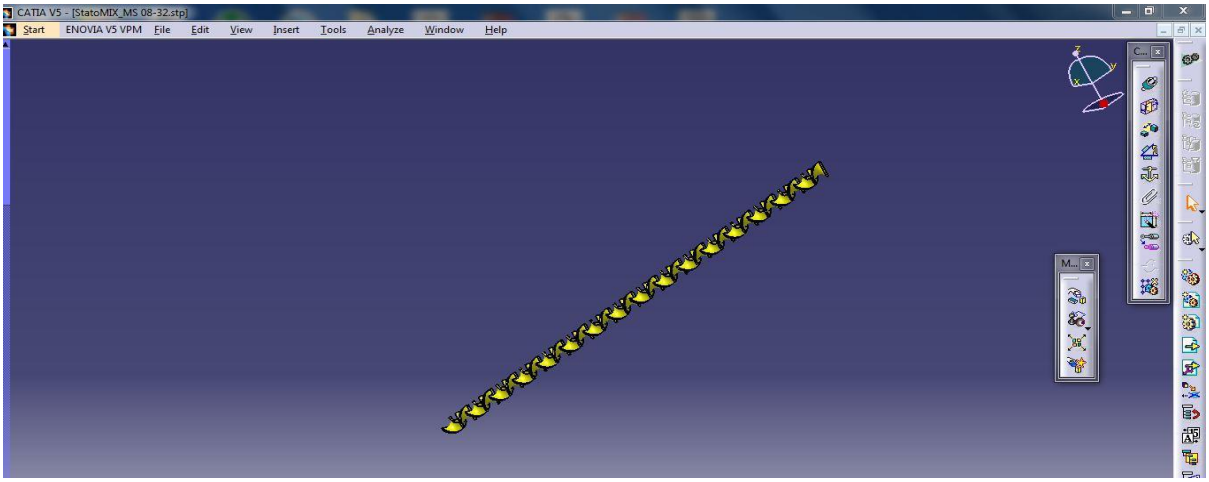

Fig. 4.18: Shows the rotating platform of the mixer.

RAPID PROTOTYPING

The rapid prototyping refers to a class of technologies that are used to produce physical objects layer by layer directly from computer aided design. These techniques allow designers to produce tangible proto types of their designs quickly, rather than just two dimensional pictures.

Fig.4.19: shows the prototype dynamic syringe mixer.

CHAPTER 5

CONCLUSION

Conclusion

The experiments that we have performed during the entire project work conclude that, the prepared gel has the capability of wound healing and comparable bio-degradable property. Dynamic syringe mixer is designed in such a way that it minimises the time of formation of gel when the solutions are mixes inside the rotating platform. In FTIR analysis we have taken various concentration of Chitosan: Sodium alginate. FTIR results indicate the sharp peak which shows the bonds in chitosan and sodium alginate. The viscosity test of the samples were carried out and analysed.

REFERENCE

1) Polyelectrolyte complex composed of chitosan and sodium alginate for wound dressing application by Yun-Jung Kima, Hyun-Chul Leeb, Jong-Suk Ohc, Boo-Ahn Shind, Chang-Seokohe, Ro-Dong Parkf, Kap-Seung Yangg & Chong-Su Choh, pages 543-556.

2) Chitosan and alginate polyelectrolyte complex membranes and their properties for wound dressing application by Xin Meng ,Feng Tian , Jian Yang, Fan Li .pages 1-6 J Mater Sci.

3), Basic Principles of Wound Healing. Wound Care Canada, 2004 Heather L. Orsted and D.K., Louise Forest Lalande, Marie Françoise Mégie. **9**(2): p. 4-12.

4), Wound dressings: principles and practice. Abdelrahman, T. and H. Newton Surgery (Oxford), 2011. **29**(10): p. 491-495.

5 Polymer nanocomposites. Koo, J.H., 2006: McGraw-Hill Professional Pub. New York.

6) Hemostatic wound dressing patent US 5836970 A.

7) Rapid prototyping from www.emeraldgrouppublishing.com

8) Synthetic hydrogels VI. Hydrogel composites as wound dressings and implant materials. Biomaterials, 1989 Corkhill, P.H., C.J. Hamilton, and Tighe, B.J.,. **10**(1): p. 3-10.

9., Chemical and physical properties of a hydrogel wound dressing. Biomaterials, 1986) Kickhöfen, B., Wokalek, H., Scheel, D. and Ruh, H. **7**(1): p. 67-72.