SYNTHESIS AND CHARACTERIZATION OF CHITOSAN BASED CONTROLLED RELEASE SYSTEMS

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Abstract: Chitosan (Ch) as a drug carrier matrix attract many researchers interest thanks to its natural and biocompatible structure. There are plenty of chitosan modifications via multiple vinyl monomers to form copolymer structures. In this study chitosan polyacrylamide graft copolymer (Ch-g-p(AAm)) is synthesized and characterized. After identifying the reaction conditions the graft copolymer further crosslinked with methylene bisacrylamide (MBA) at different AAm:MBA ratios. The applied AAm:MBA ratios are 15:1 and 20:1 and 30:1 respectively. After synthesizing of one graft copolymer and three crosslinked copolymers overall four copolymers is loaded with active drug molecule acetyl salycilic acide (ASA). The loading capacity of copolymers is determined with IR spectra. After that controlled release of four copolymers at three different pH mediums (pH-2 : 6 : 8,5) is observed at a certain time. The step by step release of copolymers is watched with UV spectra. The slowest release of ASA is estimated at 15:1 ratio copolymer, and fastest one is graft copolymer which shows increase in the crosslinking agents MBA, increase the copolymer controll over slow release of ASA. Spectral characterization are made using TGA, NMR and IR spectras.

Keywords: polymer synthesis, molecular engineering, smart polymers, pH responsive polymers, natural cationic polymers, chitosan, biocompatible polymers, cross-linked copolymers, controlled release.

1. Introduction

Controlled release systems one of the most popular fields that take interest for many researchers. Among the controlled release systems, pH-sensitive systems are the most widely used controlled release systems. Thanks to chitosan being cationic (positively charged) nature in acidic environment, behaviors of swelling in acidic environment and shrinking in basic environment could be manipulated for controlled release systems where neutral and negatively charged polymers in acidic environment can't act that likely pH responsive behaviour. Although having large polymer structure, some kind of chitosans could be water soluble and biocompatible and could pass through the cell membrane that do not resist in the body which gave it the top position among other controlled drug release systems and cause to active use of chitosan as drug carrier matrix. One of the general requisite for binding to the proteins in the body is being cationic structure and additionally being natural polysaccharide as chitosan properties offer. Also offer an irreplaceable carrier in the consider of researchers that present the adjustable chemical medium for many derivative chemical synthesis.

Chitosan (Ch) is polyshaccarides that one of the most found natural polymer in the nature after cellulose. Chitosan hold the beneficial properties for drug industry like biodegradability, biocompatibility, available for chemical modification [1], since the chitosan is positively charged has antimicrobial properties [2] and being partially non-toxic structure. Chitosan could be broken down the amino sugars in the body that does not resist, also some kind of chitosans could pass through the cell membranes. The most human friendly kind of chitosan is 50% Degree of Deacetylation (DDA) chitosans [3], where the Ch—(-NH-CO-CH_3) acetyl group is removed at the half polymer chain.

Figure 1

Actually general perspective for description of chitosan is to have at least DDA of 50% chitin that could be named as chitosan. Deacetylation is realized through the boiling the chitin in NaOH solution.

Figure 2

Amine functionality of chitosan is wide open for modification within vinyl copolymers to form copolymer structures [4–7].

In this study radical generation on the chitosan amine functionality is realized by using ceric ammonium nitrate as oxidizer, considering redox method, the reducing agent is chitosan itself. The radical formation and radical transferring to monomer is shown in figure below.

Figure 3

While the cerium is reduced from (+4) to (+3), on the other side chitosan radical is formed on the consequently reactive carbons 2, 6 and 3 positions [8]. The most reactive one is number 2 carbon related with the amine group, thus the radical could be formed on this amine edge like shown in figure below.

Figure 4

2. Materials & Methods

2.1. Materials

Chitosan 99% DDA (Sigma Aldrich), Nitric acide (Merck), Acrylamide (Merck), Cerium Ammonium Nitrate (Sigma Aldrich), Methylene Bisacrylamide (Fluka), Sodium Hydroxide (Merck), Acetyl Salycilic Acide (Sigma Aldrich), Acetic Acide (Merck), Isopropyl Alcohol, Acetone, KCl and HCl for pH 2 buffer solution, Na_2HPO_4 and NaH_2PO_4 for pH 6 buffer solution, H_3BO_3 and NaOH for pH 8.5 buffer solution.

2.2. Methods & Equipments

FTIR analysis is taken with Thermo Scientific, GladiATR Nicolet 380 FT-IR device. ATR module and diamond crystal edge is used. H NMR analysis is taken with Agilent, VNMRS model device (500 MHz). The measurements are taken in between -10 and 18 ppm via deuterium acetic acid and deuterium water as solvents.

UV analysis is done with Perkin Elmer, Lambda 25 model device at 200-400 cm^{-1} wavelengths.

The handled copolymers are precipitated with Centurion cenrifuge device operated at 3400 rpm (200 G force) for 4.5 minutes along.

2.3. Synthesis of Chitosan-Graft-Polyacrylamide (Ch-g-p(AAm)) Copolymer

In a two or three necked bottom rounded 50 mL flask, 0.1 g. chitosan is solved in 10 mL of 1% (w/v) AcOH for 15 minutes in 40°C temperature. 0.5 g. Acrylamide is added in 10 mL AcOH that AAm:Ch ratio is 5:1. After 15 minutes circulation 6 mL of 0,05 M CAN in

0.1 M HNO_3 solvent is added at three portions step by step each has 5 minutes duration. After the last portion is added the reaction is thoroughly circulated for 3 hours. After the time is ended the reactant volume is mixed with 5 fold volume of IPA. pH is adjusted to 9 basic environment with using 1 M NaOH. For precipitation of polymer chains the reactant is freezed in refrigerator overnight. The day after the mixture is centrifuged and the precipitated copolymer is dryed at vacuum dryer at ambient temperature for 4-5 hours. The copolymer structure is presented in the figure below.

Figure 5

2.4. Synthesis of MBA Crosslinked Copolymer

After the last portion of CAN is added to the reaction medium according to the upper description, the reactants is mixed along 15 minutes and than MBA is added at different ratios of AAm:MBA 15:1, 20:1, 30:1. The reaction time is again 3 hours and handling procedures are the same as described in one step before. The crosslinkled copolymer structure is presented in the figure below.

Figure 6

2.5. ASA Loading of Copolymers

The positively charged copolymers of 108 mg. for each are dissolved in acidic environment using AcOH. And the negatively charged active drug molecule at a w:w ratio of Copolymer:ASA 3:1 meaning that 36 mg ASA is dissolved in distilled water and the pH of ASA solution is adjusted to strong basic medium almost 12-13 using 1 M NaOH solution. The total volume of ASA solution should be equal to the copolymer solution. This technic is also called as PEC method in which copolymers encapsulated the active drug molecules due to ionic interactions and pH responsive behaviour of the chitosan.

ASA solution is dropwisely added to the copolymer solutions almost 10 minutes. After addition the new loaded copolymer is dropped to acetone in ice bath to precipitate the copolymer. Then centrifuge is applied to form the loaded copolymers. Following that the drying in vacuum dryer. After dryed copolymer is handled the pellet is made under high pressure using pellet apparatus.

2.6. Controlled Release of ASA Loaded Copolymers

ASA release of four copolymers are observed at three different pH buffers (pH 2:6:8.5). Preparation of buffer solutions is as follows. pH 2 buffer solution is made from 7,45 g. KCl and 1,75 mL 37% HCl (770 mg) in 1 liter aqueous solution. pH 6 buffer solution is made from 1,307 g. $Na_2HPO_4 \cdot 7H2O$ and 13,127 g. $NaH_2PO_4 \cdot H_2O$ in 1 liter aqueous solution. And finally pH 8.5 buffer solution is made from 61,83 g. H_3BO_3 and 10 g. $NaOH.H_2O$ in 1 liter aqueous solution.

Copolymer pellets are submerged in 10 mL of buffer solution each at different times. And consequently at a certain times the release of ASA to the medium is estimated with UV spectra. And the values are reported as a table below

Table 1

3. Results & Discussions

3.1. Controlled Release of Copolymers

UV spectrum of controlled release of copolymers datas are reported as a spectrum graph. Also converted to concentration/time graph using calibration curve of ASA.

The ASA release of each copolymer is evaluated separately first.

The ASA release of graft copolymer in acidic, neutral and basic medium is shown figure below

Figure 7

The crosslinked copolymer that has AAm:MBA ratio 15:1 release in acidic, neutral and basic medium is shown figure below

Figure 8

The crosslinked copolymer that has AAm:MBA ratio 20:1 release in acidic, neutral and basic medium is shown figure below

Figure 9

The crosslinked copolymer that has AAm:MBA ratio 30:1 release in acidic, neutral and basic medium is shown figure below

Figure 10

According to these datas all copolymers show increasing release by going from basic medium to asidic medium. In asidic medium copolymers show the fastest release because of chitosan's swelling behaviour and when it swells the loaded drug molecules much available to release in the medium. In basic medium all copolymers show the slowest release because of the chitosan is shrinking and holding the drug molecules and tend not to release or very slow release is observed. And copolymer release behaviour in neutral medium in between those two (asidic&basic).

Secondly all copolymers are collected in same graph classified in pH medium. Copolymer behaviour in asidic medium are reported in figure below.

Figure 11

Copolymer behaviour in neutral medium are reported in figure below:

Figure 12

Copolymer behaviour in basic medium are reported in figure below:

Figure 13

According to these datas the slowest releasing copolymer is the one that highest crosslinked copolymer which is 15:1 ratio copolymer. And the fastest releasing copolymer is graft copolymer which indicate the higher the crosslinking agent used in copolymer the slower release of active drug is obtained.

3.2. Spectral Characterization

Main characteristic signals of chitosan is given in the infrared spectrum of chitosan. Those are –OH and –NH stretching vibrations at 3348 cm^{-1} and 3286 cm^{-1}. Amide I band is at 1643 cm^{-1} and Amide II band is at 1561 cm^{-1}. Chitosans number 1 carbon signal is ocur at 893 cm^{-1} indicating the β conformation of the polyshaccaride. Polyshaccarides C-O-C stretching vibrations occur between 1200-900 cm^{-1}.

Figure 14

Polyacrylamide has amide I and amide II peaks at 1664 and 1537 cm^{-1} respectively.

The amide I peak for only graft copolymer is at 1630 cm^{-1} meaning the 13 cm^{-1} shifting to the low frequency area. And number 1 carbon of chitosan in graft copolymer occur at 899 cm^{-1} instead of 893 in original chitosan.

Crosslinking effect on amide I and amide II bands are given in the figure below.

Figure 15

According to the figure there is a new amide I band because of crosslinking agent MBA. So the amide I bands are at 1652 cm^{-1} and almost 1600 cm^{-1}. The more the crosslinking agents higher the frequency of this new amide I band. The new amide I band for 15:1 crosslinked copolymer clear at 1625 cm^{-1}, for 20:1 copolymer the amide I band is at 1606 cm^{-1}, and 30:1 copolymer amide I band is at 1586 cm^{-1}.

IR spectra of ASA loaded copolymers indicate that as the crosslinking agent is higher, the 1053 cm^{-1} peak gets stronger. So it could be said that the crosslinked copolymer has entrapped/encapsulate more ASA molecules. And graft copolymer has encapsulate the fewest ASA molecule than the other copolymers.

Figure 16

NMR spectrum of copolymers are as given below. The main peak for identifying the crosslinking is at 2,9 ppm because of the number 2 carbon of chitosan. For 30:1 crosslinking ratio copolymer shifting of this peak is 3,3 ppm, for 20:1 ratio copolymer the shifting space come closer to 3,1 ppm and finally this difference is closing much and at 15:1 ratio copolymer this peak occur at 3 ppm. Also it shows that binding of MBA and AAm is on number 2 carbon of chitosan, the most reactive one.

Figure 17

3.3. Thermal Characterization

Thermal Gravimetric Analysis (TGA) is made for thermal characterization of copolymers. Chitosan, graft copolymer and 30:1 copolymer show similar structural stability behaviour in thermal analysis in case of fragmentation at around 100 °C and 300 °C. The most stable copolymer is 15:1 copolymer in which the highest ratio of MBA crosslinking agent is used.

Figure 18

The decomposition starting from 260 °C up to 380 °C is very similar for 20:1, 30:1 crosslinked copolymers and the graft copolymer. Where the 15:1 copolymer shows uninterrupted decreasing curve.

The peak maximums of derivative spectrums for Chitosan, Graft, 30:1, 20:1 and 15:1 consequently are 333°C, 300°C, 310°C, 322°C and 340°C respectively.

4. Conclusion

Synthesis of graft copolymer and crosslinked copolymers 30:1, 20:1 and 15:1 are realized with the effieciencies of 73%, 23%, %25 and %38 consequently. Optimum synthesis conditions are found as 0,05 M CAN concentration in 0,1 M HNO_3 solution, Ch:AAm ratio as 1:5, and AAm:MBA ratio as 15:1. The optimum reaction time is 3 hour and the temperature is 40°C.

Controlled release of 15:1 ratio copolymer is most promising in case of slow release compared to others. Graft copolymer shows the fastest release.

All copolymers shows slow release in basic environment because of the chitosan shrinking behaviour, and as the medium gets more asidic the release become more faster because of the chitosan is swelling and let the ASA molecules go out from copolymer structure to the release medium.

FIGURES

Figure 1

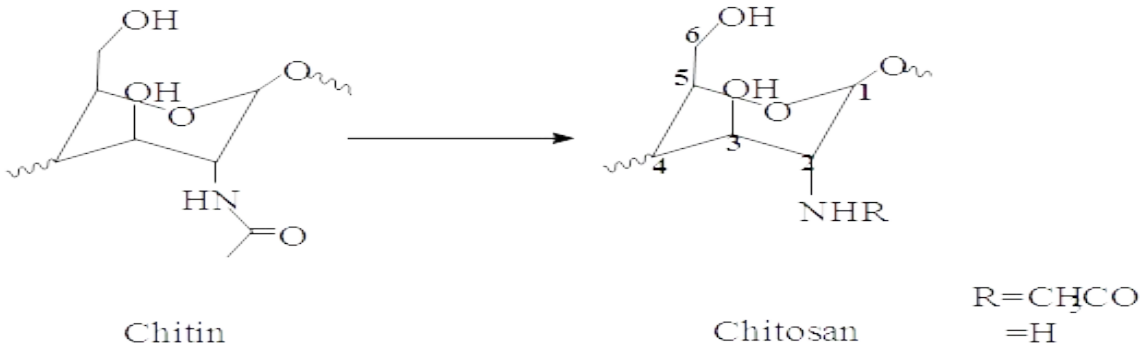

Figure 2

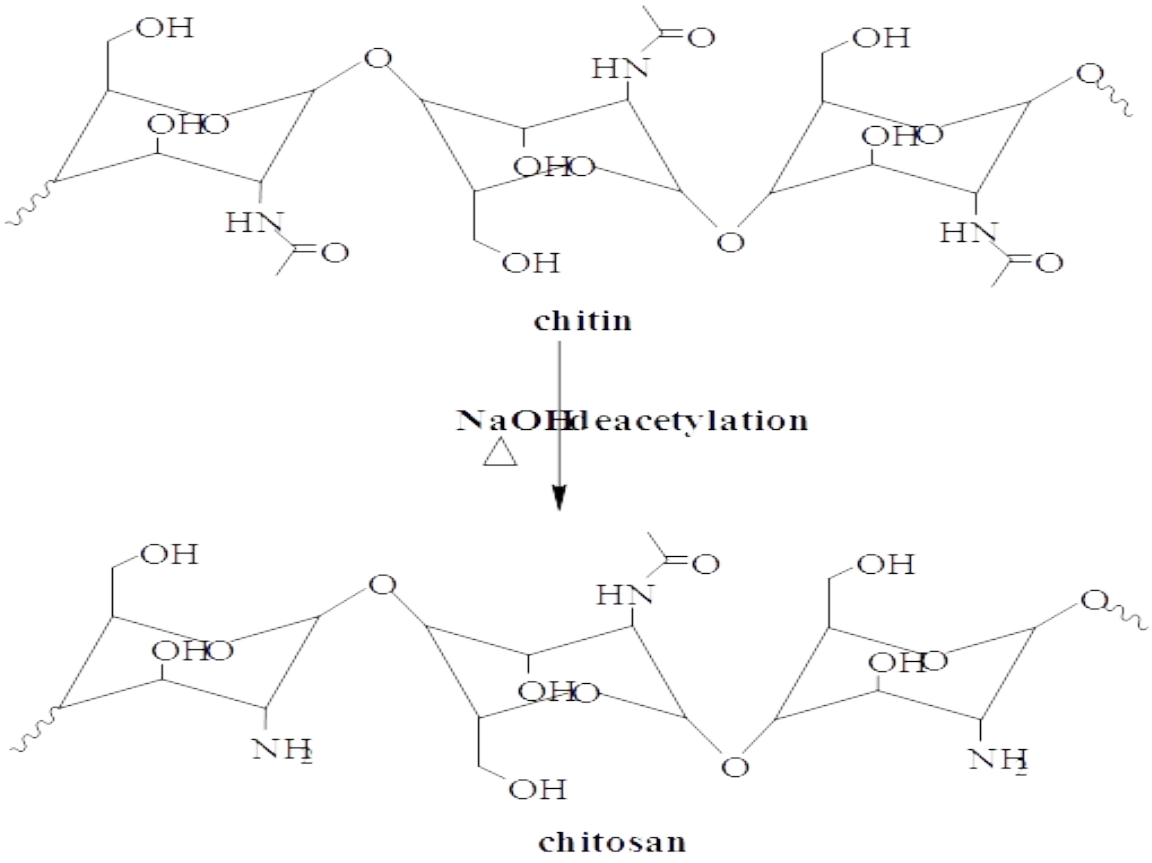

Figure 3

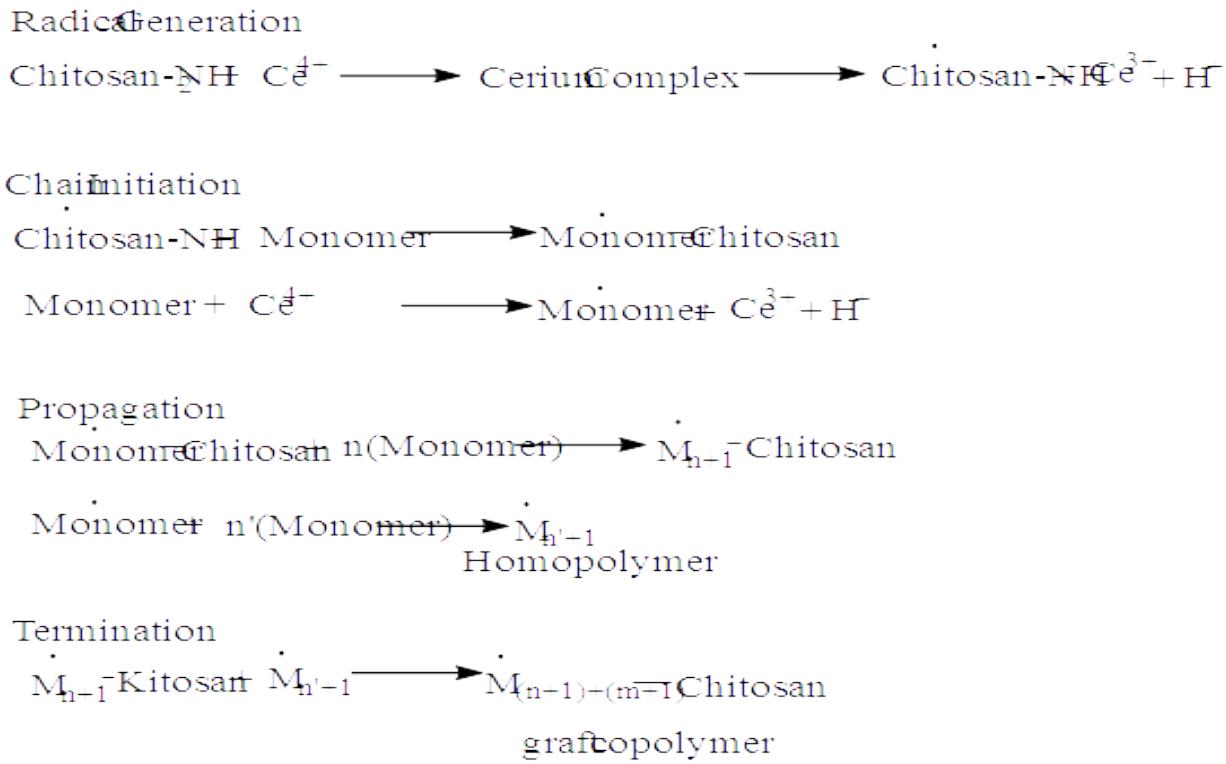
Radical Generation
Chitosan-NH2 + Ce4+ → Cerium Complex → Chitosan-NH• + Ce3+ + H+
Chain Initiation
Chitosan-NH• + Monomer → Monomer-Chitosan
Monomer + Ce4+ → Monomer• + Ce3+ + H+
Propagation
Monomer-Chitosan• + n(Monomer) → Mn-1-Chitosan
Monomer• + n'(Monomer) → Mn'-1
Homopolymer
Termination
Mn-1-Kitosan• + Mn'-1 → M(n-1)-(m-1)-Chitosan
graft copolymer

Figure 4

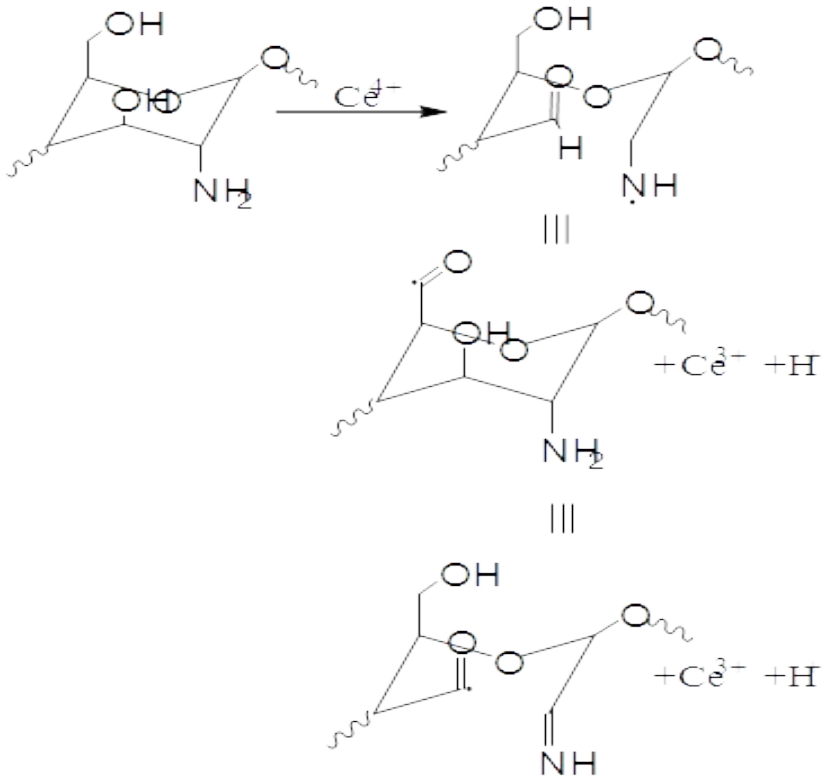

Figure 5

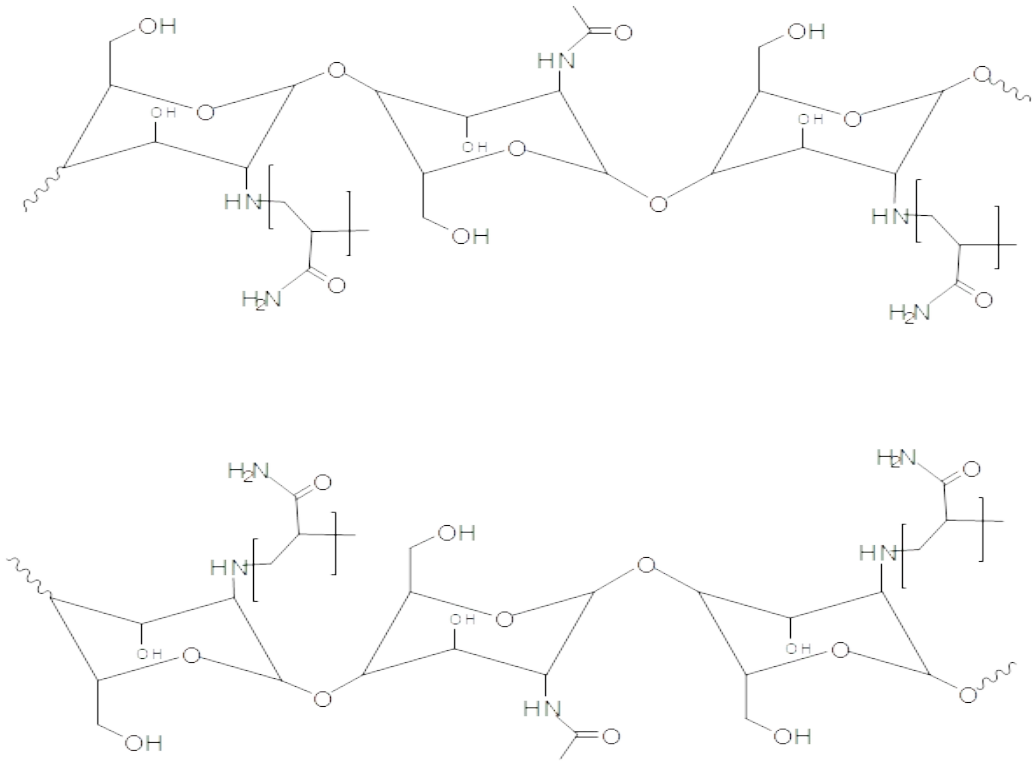

Figure 6

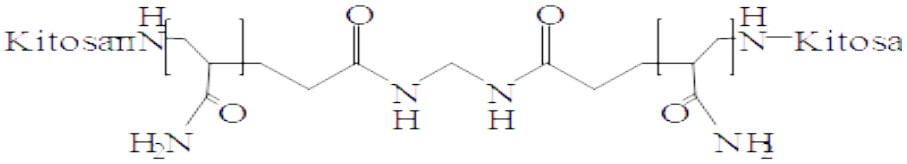

Figure 7

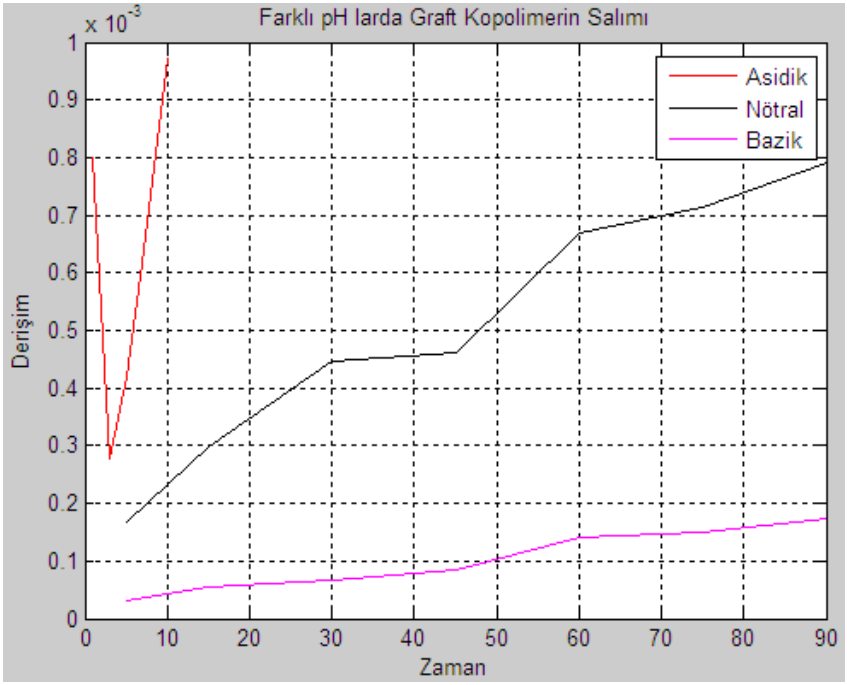

Figure 8

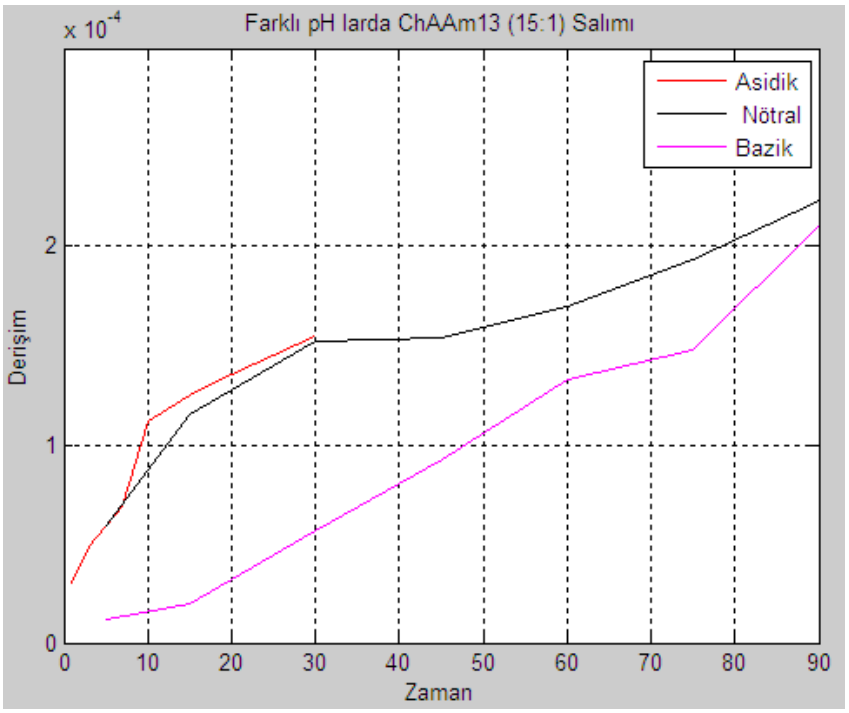

Figure 9

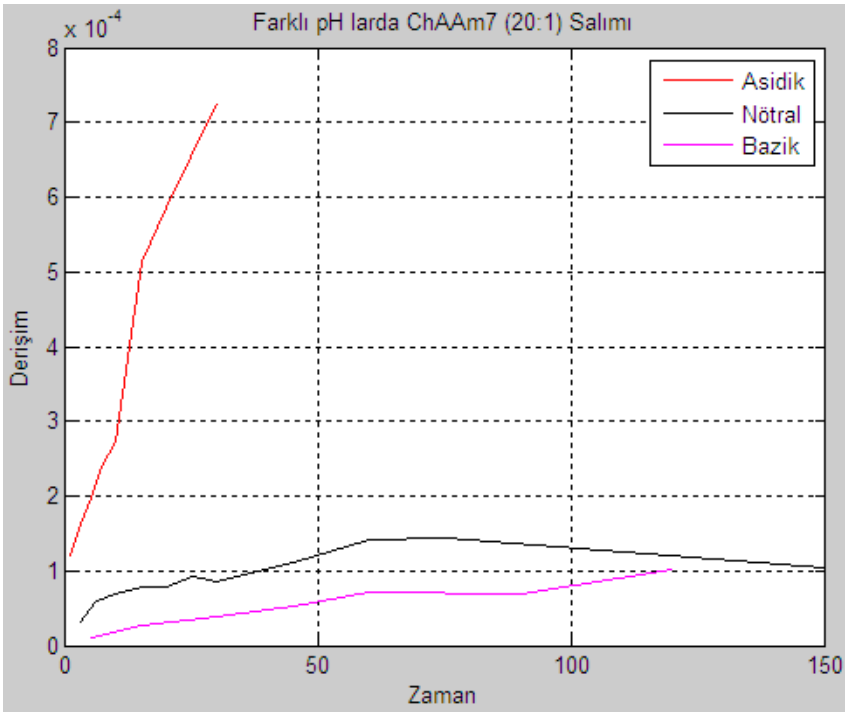

Figure 10

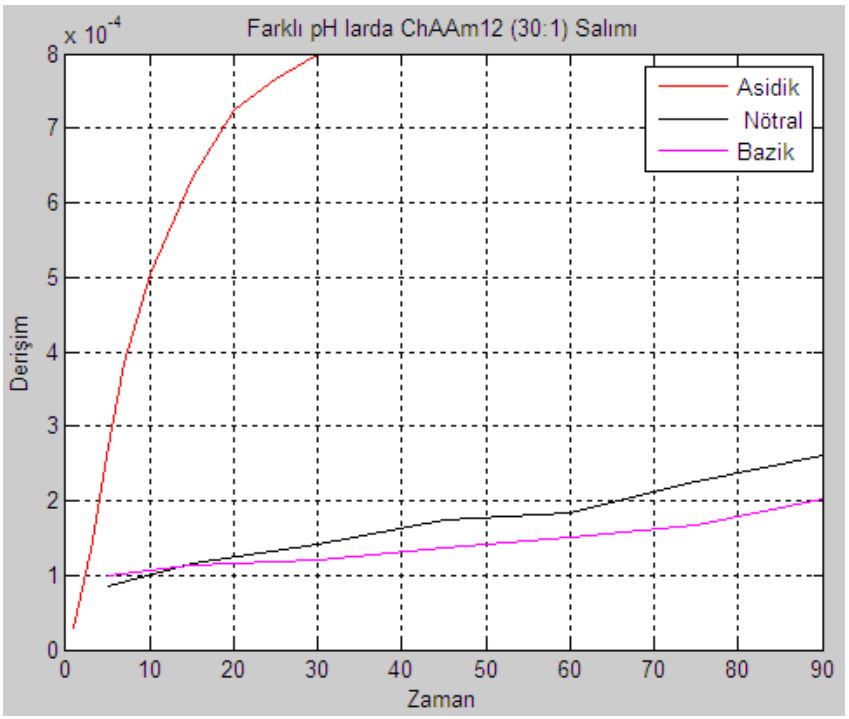

Figure 11

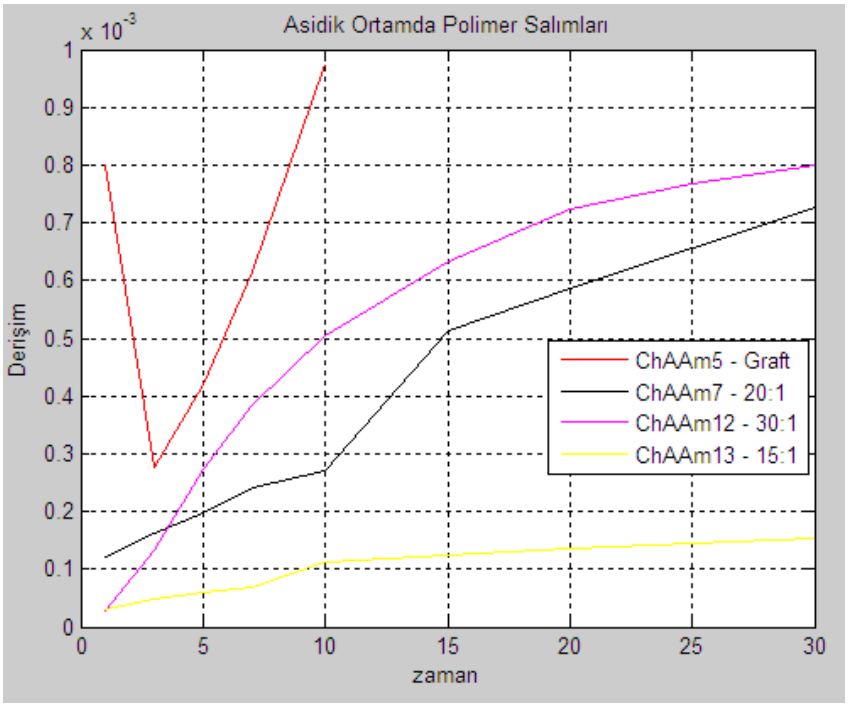

Figure 12

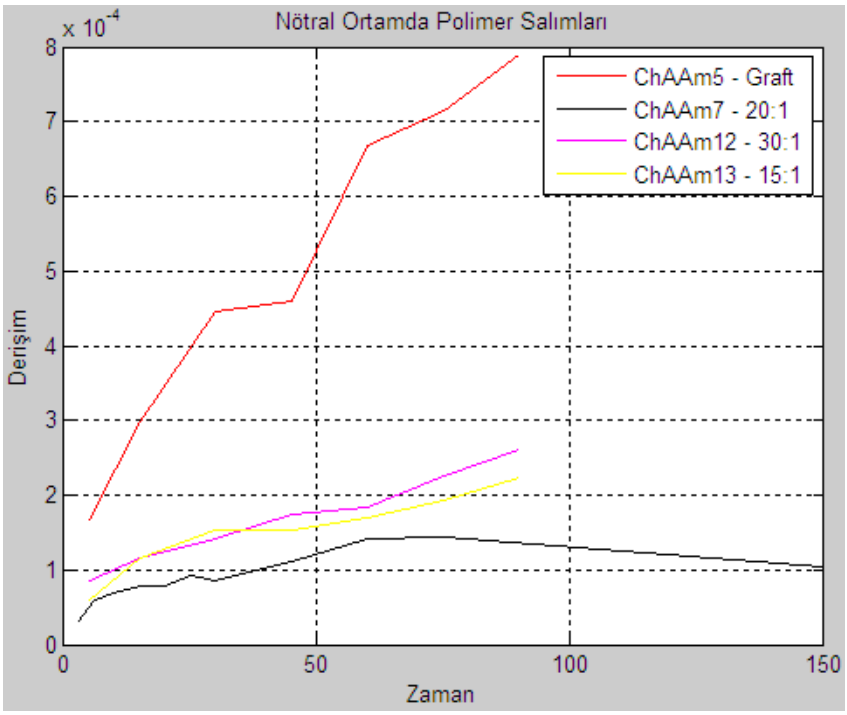

Figure 13

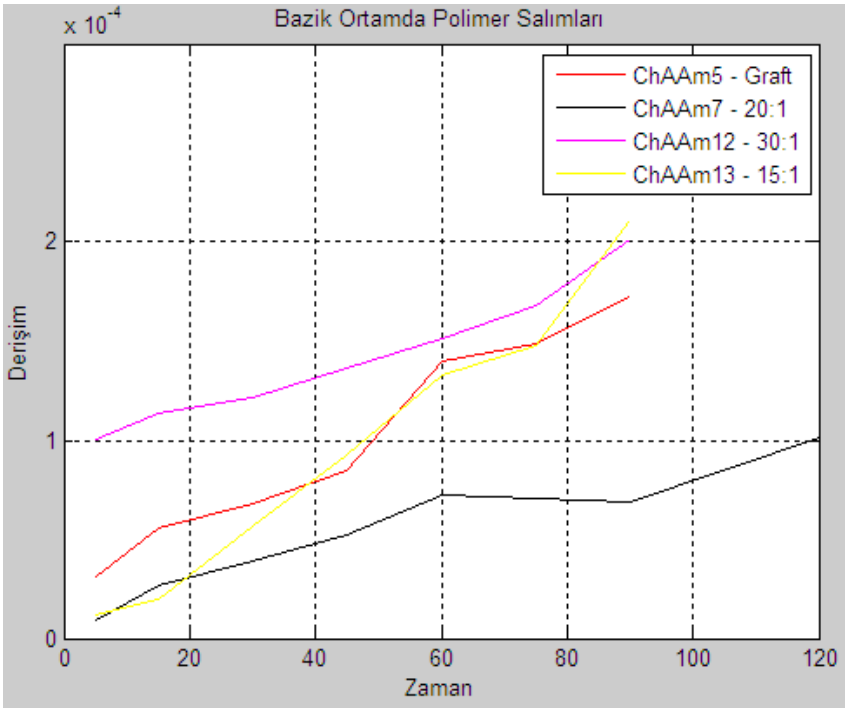

Figure 14

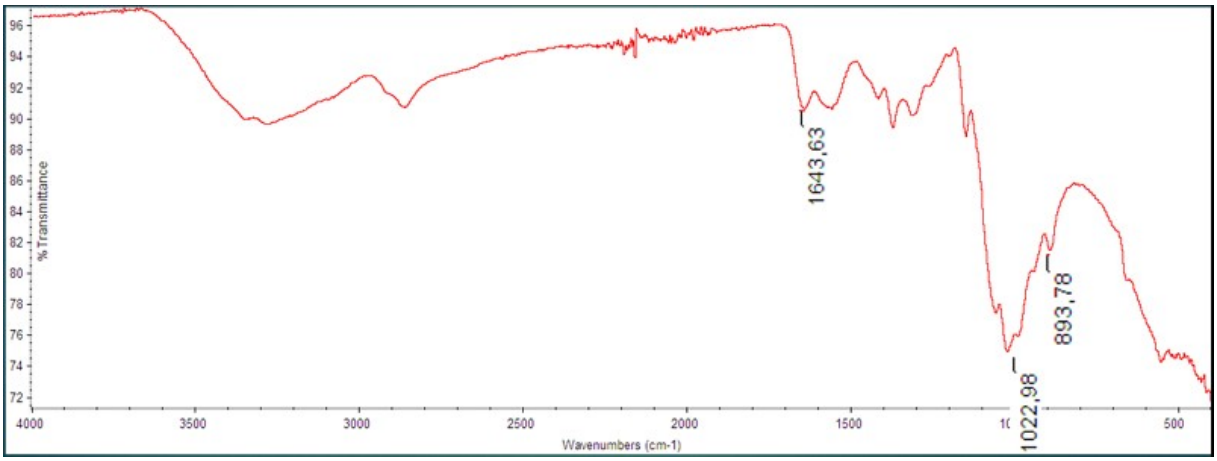

Figure 15

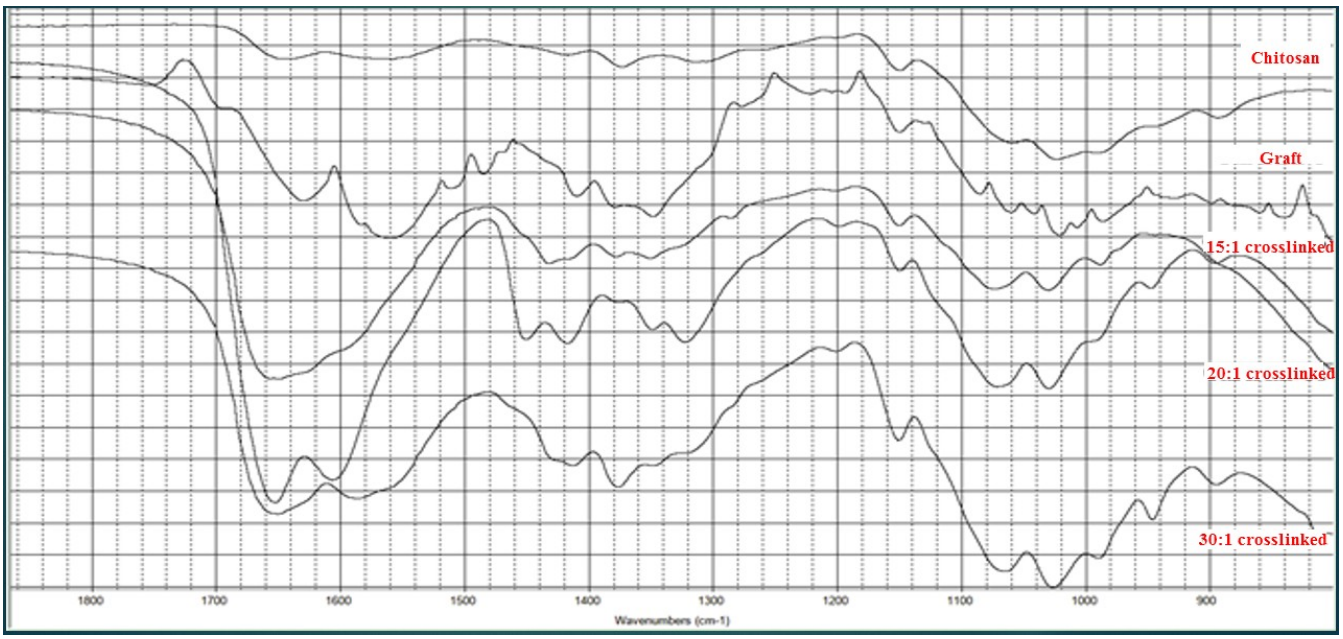

Figure 16

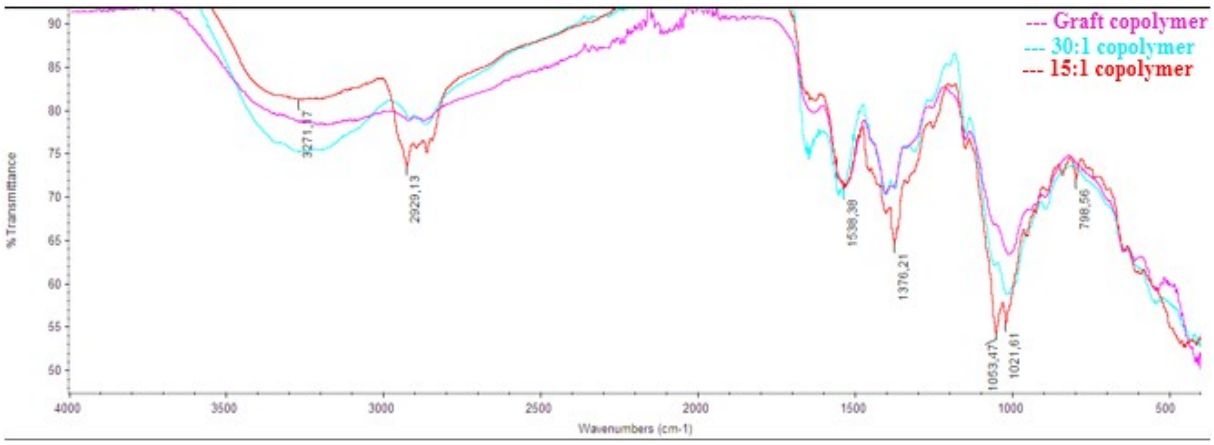

Figure 17

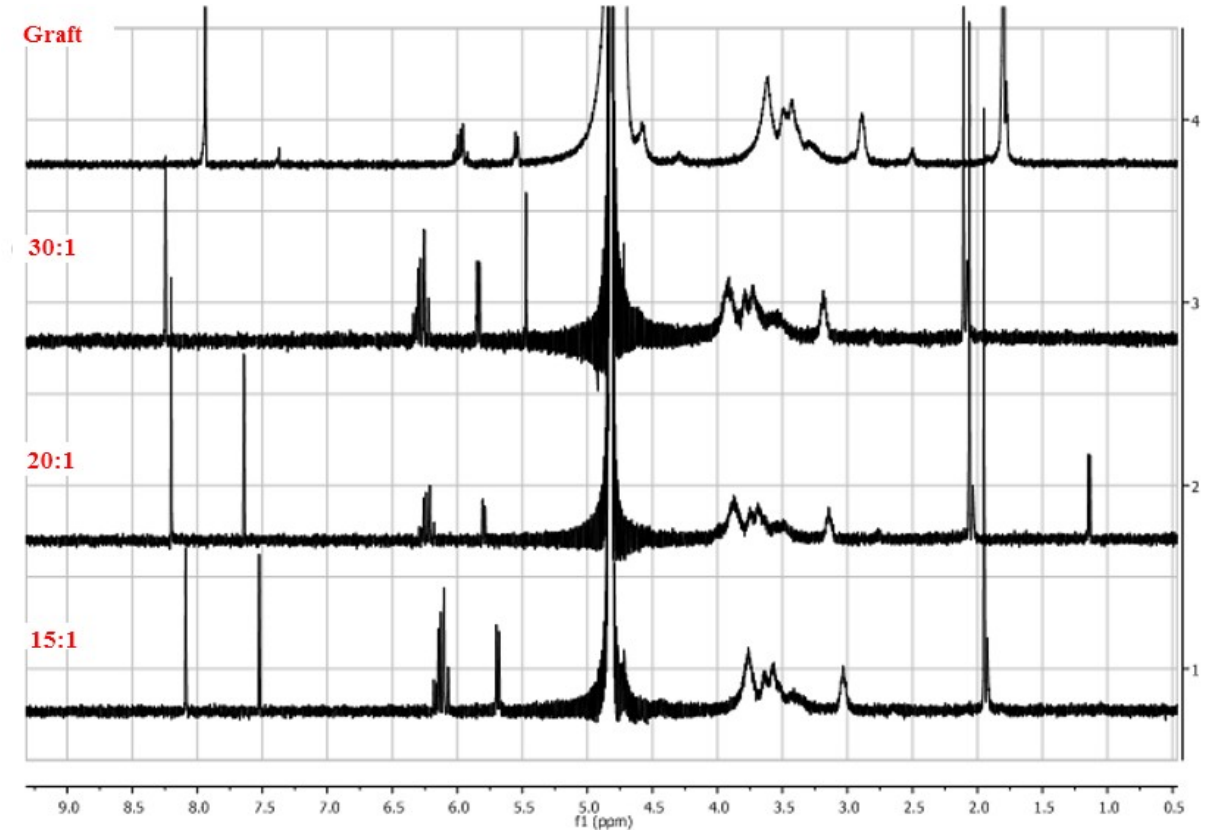

Figure 18

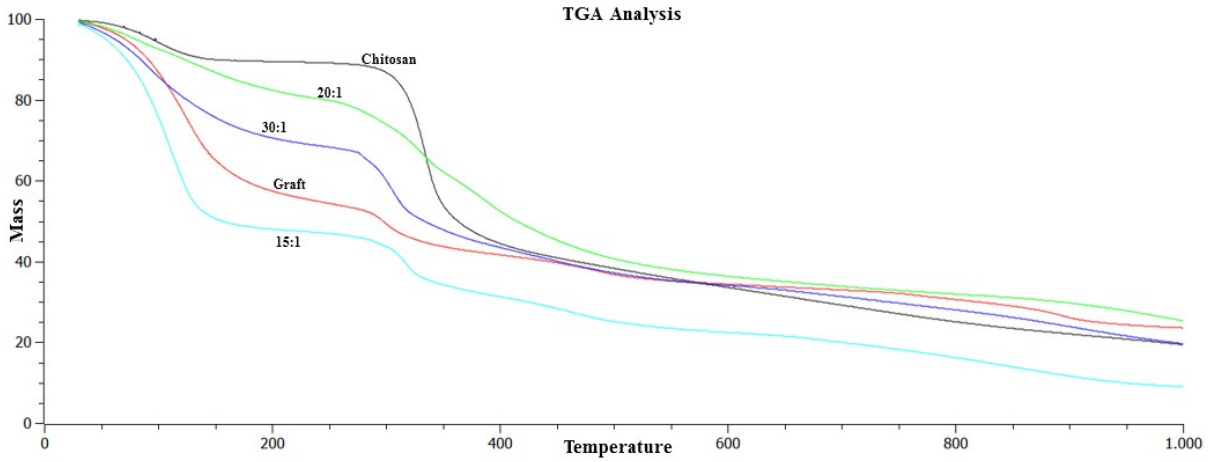
TGA Analysis
Chitosan
20:1
30:1
Graft
15:1
Mass
Temperature
100
80
60
40
20
0
0
200
400
600
800
1.000

Table 1

COPOLYMERS		ACIDIC MEDIUM								
Graft	Concntr.	0,000799	0,000276	0,000418	0,000615	0,000974				
	Time	1	3	5	7	10				
15:1	Concntr.	0,000031	0,000049	0,000059	0,000069	0,000112	0,000125	0,000135	0,000155	
	Time	1	3	5	7	10	15	20	30	
20:1	Concntr.	0,000122	0,000162	0,000198	0,000240	0,000271	0,000513	0,000585	0,000725	
	Time	1	3	5	7	10	15	20	30	
30:1	Concntr.	0,000029	0,000134	0,000273	0,000384	0,000505	0,000632	0,000723	0,000767	0,000799
	Time	1	3	5	7	10	15	20	25	30

		NEUTRAL MEDIUM										
Graft	Concntr.	0,000167	0,000298	0,000446	0,000460	0,000669	0,000715	0,000789				
	Time	5	15	30	45	60	75	90				
15:1	Concntr.	0,000059	0,000116	0,000153	0,000154	0,000169	0,000194	0,000223				
	Time	5	15	30	45	60	75	90				
20:1	Concntr.	0,000032	0,000059	0,000069	0,000078	0,000079	0,000092	0,000086	0,000111	0,000141	0,000144	0,0004
	Time	3	6	10	15	20	25	30	45	60	75	150
30:1	Concntr.	0,000085	0,000116	0,000140	0,000173	0,000184	0,000226	0,000260				
	Time	5	15	30	45	60	75	90				

		BASIC MEDIUM										
Graft	Concntr.	0,000031	0,000056	0,000068	0,000084	0,000140	0,000149	0,000172				
	Time	5	15	30	45	60	75	90				
15:1	Concntr.	0,000011	0,000019	0,000057	0,000092	0,000133	0,000148	0,000211				
	Time	5	15	30	45	60	75	90				
20:1	Concntr.	0,000009	0,000027	0,000039	0,000052	0,000072	0,000069	0,000101				
	Time	5	15	30	45	60	90	120				
30:1	Concntr.	0,000100	0,000114	0,000122	0,000137	0,000151	0,000168	0,000201				
	Time	5	15	30	45	60	75	90				

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