Preparation and Characterization of Poly Vinyl Alcohol - Gelatin- Carboxy Methyl Chitosan Polymer Films

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Certificate

This is to certify that the thesis entitled "**Preparation and Characterization of Polymer Films Based on Poly Vinyl Alcohol - Gelatin- Carboxy Methyl Chitosan**" by **Amit Bothra (110BT0542),** in partial fulfilment of the requirements for the award of the degree of Bachelor of Technology in Biotechnology during session 2010-2014 in the Department of Biotechnology and Medical Engineering, National Institute of Technology Rourkela, is an authentic work carried out by him under my supervision and guidance. To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University/Institute for the award of any degree or diploma.

Place: NIT Rourkela

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Abstract

Present work delineate the preparation and characterization of polymer films of polyvinyl alcohol (PVA), gelatin and carboxy methyl chitosan. The films were prepared by esterification followed by glutaraldehyde crosslinking. Swelling behaviour and biodegradability of the polymer films were studied at physiological pH. Further, hemocompatibility and biocompatibility of the polymer film was also evaluated. Result showed that films of different composition have water-swelling capacity around 80%. Biodegradation study revealed that 35% degradation could be achieved by varying the composition. All the polymeric films were found hemocompatible and biocompatible. From this preliminary study it can be said that such films could be used for biomedical application.

KEYWORDS: Polymer films, hemocompatibility, swelling, cytotoxicity, biocompatibility.

Chapter 1

Introduction

1.1 Introduction:

Now a days, biopolymeric devices are utilized to evaluate, treat, augment or substitute any tissue, organ or function of the body. In this regard polymeric films shows great versatility. Polymer films are cross-linked polymeric network. At physiological temperature and pH, these polymeric materials don't dissolve in water but swells extensively.

Polymer films are used in artificial corneas, contact lenses, wound dressing, catheters, sutures and electrode sensors [1]. Cross-linked polymeric films could be obtained by chemical cross-linking (using epichlorohydrin, glutaraldehyde), radical polymerization or by radiation (e.g., gamma radiation, UV radiation). The polymer films characteristics, including the swelling properties, might be regulated by the amount of reagent used in cross-linking. Naturally sensitive films can be obtained by the addition of some special monomers. Solubility properties of water soluble polymers depends upon the presence of functional groups (especially NH_2, OH and COOH) that might be utilized for the preparation of environment sensitive polymer films [2].

1.1.1 PVA (Polyvinyl Alcohol): PVA was initially manufactured by Hermann and Haehnel in 1924 by hydrolysing polyvinyl acetate in ethanol using potassium hydroxide. Commercial manufacturing of PVA is done using polyvinyl acetate, normally by sustained process. In the presence of aqueous sodium hydroxide or anhydrous sodium methylate, the acetate groups are hydrolysed by ester internally change with methanol. The physical characteristics and its particular functional utilizations depend on the degree of polymerization and the degree of hydrolysis [3]. PVA can be divided into two different classes based on hydrolysis: partially hydrolysed and fully hydrolysed. Generally in the foods, partially hydrolysed PVA is used. Vinyl acetate monomer is the essential raw material utilized in the preparation of polyvinyl alcohol. PVA is synthesized using polymerization of vinyl acetate followed by partial hydrolysis [4]. Whole process of hydrolysis is depend on partial replacement of ester group in

vinyl acetate with the hydroxyl group and is finished in the presence of aqueous sodium hydroxide then slow addition of aqueous saponification reagent. After that precipitated PVA is washed and dried. At the time when the saponification reaction is stopped properly, the degree of hydrolysis is determined [5].

PVA is a translucent, tasteless and odourless, cream or white colour granular powder which is utilized as a moisture barrier film for dry foods with inclusions or foods contain inclusions that need to be protected from moisture uptake and for food supplement tablets. PVA is slightly dissolvable in ethanol, soluble in water, but insoluble in other organic solvents. PVA has a melting point of 179°C to 191°C. It has a molecular weight of between 26,250 and 30,250, and a degree of hydrolysis of 85.5% to 90%. Ordinarily a 5% solution of PVA exhibits a pH in the range of 4.5 to 7.0. PVA is unknown to become as a natural product.

Mixtures of PVA with other natural polymers have long been utilized because of film constructing ability of PVA. Molecular weight and the degree of hydrolysis can affect the performance properties of PVA. Atomic weight of PVA is ~161 kDa. As polyethylene, PVA has a planar zigzag structure. PVA grades are promptly dissolvable in water and depend on components like atomic mass, molecule size distribution, and molecule crystallinity. PVA exhibits excellent water retention properties because it's a hydrophilic polymer. For aggregate disintegration, however, PVA needs water temperatures of about 101˚C with a 30 minutes hold time [6].

1.1.2 Gelatin: Gelatin is a natural polymer broadly utilized in pharmaceutical, cosmetic, photographic, and food industries. It is prepared by denaturation and partial hydrolysis of fibrous collagen. Collagen is the most abundant structural protein of animal skin, bones and rarely fish scales. And also the major organic factor of skin and bone of vertebrates is collagen. On the other hand, collagen is consisting a family of proteins with 21 different types of amino acids.

It mainly contains the residues of 3 amino acids—proline, 4-hydroxyproline, and glycine (arranged every third residue)—in its structure. It consists extended left-handed proline helix conformations consolidated with 290 to 4100 amino acids. Presence of higher levels of pyrrolidines in gelatin results in the formation of stronger films or gel. Due to the presence of the triple helixes, gelatin films have good strength. If the triple-helix content increases, the strength of the film increases and decreases the swelling property in aqueous. The gelling properties of the gelatin can be changed by the reaction of chemical cross-links using glutaraldehyde to cross-link lysine to lysine or transglutaminase to cross-link lysine to glutamine residues.

Gelatin swells in cold water and is totally dissolve in hot water. In order to release the needed structure of gelatin in dry state, a temperature of about 60°C is necessary. At the point, when a gelatin solution is spread in a thin layer over a surface and passes from a sol to a gel, it forms a film. Gelatin fulfils numerous other functions, such as: gelling ability, stabilising ability, aerating ability, emulsifying ability, thickening ability, coating, texture improvement, protecting, preventing syneresis, and gluing. The formation of hard and soft capsules and in microencapsulation, these properties can be exploited [7].

1.1.3 Carboxymethyl-chitosan: Chitosan is a polysaccharide similar in structure to cellulose. Chitosan, commonly obtained by partial deacetylation of chitin derived from the exoskeleton of crustaceans, exhibits various useful physico-chemical. It is a polycationic copolymer consisting of glucosamine and N-acetyl glucosamine units.

Carboxymethyl-chitosans are a family of water soluble chitosans which forms semipermeable films and membranes. There are various methods for preparing carboxymethyl-chitosans. The water dissolving nature of the carboxymethyl-chitosan is greatly dependent on manufacturing conditions, especially temperature. It seems that higher preparation temperature leads to more insoluble carboxymethyl-chitosans at neural pH. The nomenclature of carboxymethyl-chitosan

depends on which functional group has been altered during preparation. Modifying the amino or hydroxyl group results in N-carboxymethyl or O-carboxymethyl chitosan, respectively. It is also possible to modify both the groups to obtain N,O-carboxymethyl chitosan. These carboxymethyl-chitosans can be further modified to include different functional groups.

Carboxymethyl-chitosan is a tempting biodegradable and biocompatible polymer which is acquired by the reaction of chitosan with monochloroacetic acid and in basic (alkaline) medium. Film-forming capability of gelatin and ability to interact with various substances and dissolving nature in wide range of pH are because of its antimicrobial activity. It can be used in biomedical and pharmaceutical fields, especially for the controlled drug release. Gelatin is also used in viscosupplementation and tissue engineering. Comparing with generally used chitosan, carboxymethyl-chitosan shows multiple potentials in local drug delivery [8].

General application of drug delivery system can be very helpful, both in terms of, preventing systemic side effects like depression, tachycardia and gastrointestinal complaints and raising drug concentration directly in the action site. Examples of the benefits of site-specific employing carboxymethyl-chitosan vehicles include solubility, pH-sensitivity, absorbability, and bioadhesive ability, controllable biodegradability, nontoxicity of the degradation end products, ease of administration and sustained release potential. The swelling, drug permeation and release properties of Carboxymethyl-chitosan can be controlled by the pH changes due to anionic carboxyl groups and cationic amine groups in matrix [9].

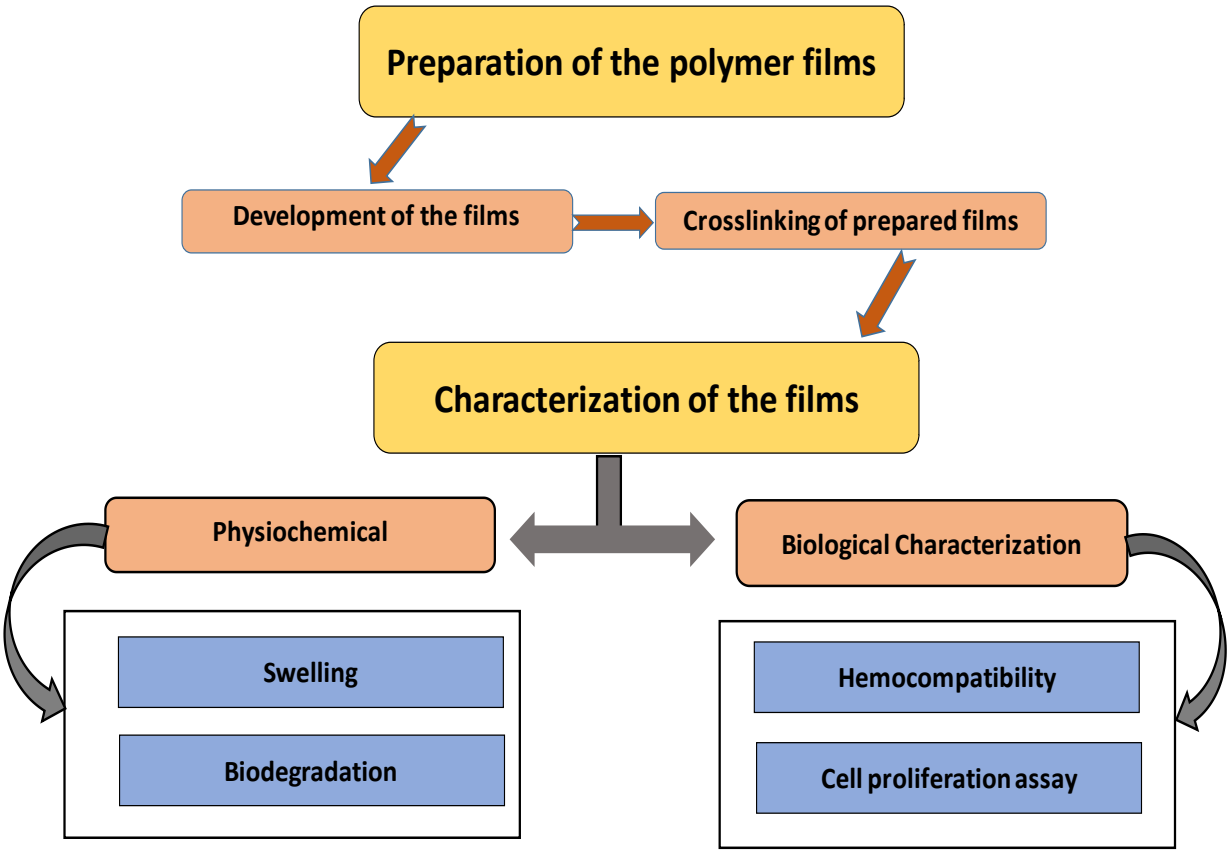
Chapter 2

Objective and Plan of work

2.1 Objective:

1. To prepare the polymer films
    a. Development of the films
    b. Crosslinking of prepared films
2. To characterize the films
    a. Physiochemical characterization
        i. Swelling study
        ii. Biodegradation
    b. Biological characterization
        i. Hemocompatibility
        ii. Cytotoxicity (MTT assay)

2.1 Work plan:



Chapter 3

Material and Methods

3.1 Materials:

a. Polyvinyl alcohol[Aldrich]

b. Gelatin[LOBAL Chemia]

c. Carboxymethyl chitosan[HIMEDIA]

d. Glycine[HIMEDIA]

e. Glutaraldehyde[RANKEM]

f. Distilled water

g. Ethanol[EMSURE]

3.2 Methods:

3.2.1 Preparation of polymer films:

A) Development of polymer films:

Polymer films were developed using PVA, Gelatin and CMC. 50ml of stock solution of PVA and gelatin was prepared in distilled water. Proportion of PVA (10%) and gelatin (2.5%) was kept constant. PVA and gelatin were mixed by stirring at 300rpm, 70˚C for 30 minutes. CMC was weighed for five compositions (Blank, 0.5%, 1.0%, 1.5%, 2.0%). Stock solution was then equally divided into five parts of 10ml each. CMC was weighed for each sample as per the composition of samples mentioned in Table 1. CMC was slowly added into the beakers containing PVA, gelatin solution and stirred at 300rpm, 70˚C. After proper mixing of CMC in the solution, 10 µl of HCl was added for esterification reaction and stirring was continued at 70˚C, 100rpm for 30 minutes. 2.5 ml of thus prepared solution was poured into small petri dishes. Petri dishes were labelled with sample code and kept on levelled surface in laminar air flow for 72

hours for drying the samples. Polymer films were ready that were taken out using scalpel (if needed) [10].

Table 1: Compositions of CMC for polymer films

% of CMC in films (Sample code)	Weight of CMC(in grams)
Blank (0)	0
0.5	0.05
1.0	0.10
1.5	0.15
2.0	0.20

Table1 for 10 ml of solution carboxymethyl chitosan compositions for polymer films.

B) Crosslinking of polymer films:

Films were washed in distilled water for 5 minutes to remove HCl. 90% ethanol solution was prepared in 50ml falcon tube (45 ml 100% ethanol + 5 ml water). It was divided equally into five parts. To each part 25µl of glutaraldehyde (25%) was added. The films were placed in above mentioned solution for 3 hours for crosslinking. After that films were kept in laminar air flow for 24 hours to dry. Thus prepared polymer films were used for physico-chemical characterization of the polymer films [11].

3.2.2 Swelling and biodegradation study:

Swelling and biodegradation study of prepared polymer films was done using PBS solution. 100ml of PBS solution was prepared [12].

Table 2: Composition of PBS (pH 7.4) solution for 100 ml

Na_2HPO_4	0.238g
KH_2PO_4	0.019g
NaCl	0.80g

Polymer films were taken and cut into four pieces. Each piece of all the five samples were weighed. Each piece of the polymer film was added to the falcon tube containing 5ml PBS. Film pieces were weighed after every 15 minutes for swelling study and the readings recorded. Readings were taken till 96 hours (Readings were taken after 15, 30, 45, 60, 120, 180, 240 minutes and at 24, 48, 72, 96 hours). After 96 hours, film pieces were removed and placed in small petri dishes to dry, then weighed and the weight was recorded. Biodegradation was calculated relative to the dry weight of the samples. The swelling percentage was calculated as per the below mentioned formula [13].

$$\% \text{ Swelling} = [(Wt \neg Ws)/Ws] \times 100$$

Where; Wt = Weight at time during swelling

Ws = Weight before swelling started

Following formula was used to calculated % biodegradation.

$$\% \text{ Biodegradation} = [(Wt \neg Ws)/Wt] \times 100$$

Where; Wt = Dry weight after 96hours

Ws = Weight before swelling started

3.2.3 Hemocompatibility:

Hemocompatibility test of films was done using goat blood. Fresh goat blood was collected in an EDTA containing tube (EDTA was used for less clotting). Goat blood

was diluted with normal saline in 8:10 ratio. (Normal saline = 9% NaCl in distilled water). For hemocompatibility study, polymer film pieces were neutralised using 0.1M glycine for 2 hours at room temperature to neutralize glutaraldehyde. After glycine treatment the polymer film pieces were dipped in 10ml normal saline at 37˚C for an hour. After that polymer film pieces were removed and the leachent was added to tubes containing 0.2ml of diluted goat blood. Samples were then kept at 37˚C for an hour. 0.1 N HCl in diluted goat blood and normal saline were taken as positive and negative controls respectively. Samples were centrifuged at 4000rpm for 15 minutes. OD value was measured at 545nm. Positive control was considered as 100% and negative control as 0% hemolysis. Percentage hemolysis was calculated using following formula [14].

$$\% \text{ Hemolysis} = [\{(OD)_{test} \neg (OD)_{-ve}\} \div \{(OD)_{+ve} \neg (OD)_{-ve}\}] \times 100$$

Where; $(OD)_{test}$ = OD value of a sample

$(OD)_{-ve}$ = OD value of negative control

$(OD)_{+ve}$ = OD value of positive control

3.2.4 Cytotoxicity test of polymer films (MTT assay):

A) Sample preparation from films:-

Films were neutralized using 0.1M glycine for 2 hours and then washed using PBS to neutralize remaining glutaraldehyde. For leachent preparation film pieces of equal weight were dipped into 10ml PBS and kept at 37˚C for an hour. After that films were removed and leachent was stored at room temperature [15].

B) Seeding of HaCat cells in a 96 well plate for MTT assay:-

A 96 well plate was taken. 1X 10^4 cells were seeded in each well. 200µl of DMEM media was added to each well. The cells were maintained in an incubator at 5%

CO_2, 37˚C. 20µl of different concentration of leachents were added to the wells in triplicates (1mg/ml, 100µg/ml, and 10µg/ml). Cells without leachents were taken as control [16].

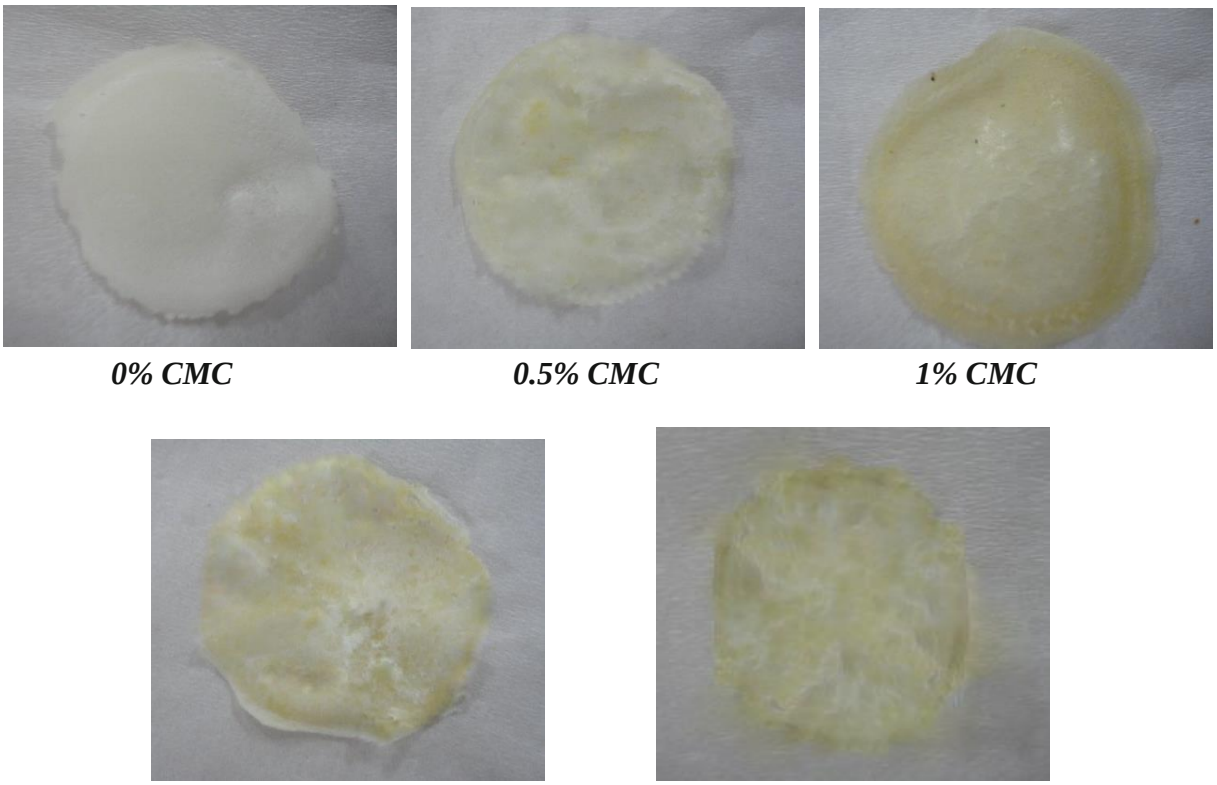
C) MTT assay of seeded HaCat cells:-

After a day of cell seeding, 20µl of MTT reagent was added to each well. Cells were incubated for 3 hours with the reagent in an incubator at 37˚C and 5% CO_2. After that the media was removed. 200µl of DMSO was added to each well to dissolve the formazan crystals. OD value was measured at 595nm [16].

Chapter 4

Results and Discussion

4.1 Preparation of polymer films:



0% CMC *0.5% CMC* *1% CMC*

1.5% CMC *2% CMC*

Figure 1: Polymer films with different proportions of CMC

4.2 Swelling and biodegradation:

4.2.1 Swelling study: Swelling study shows that polymer film sample without CMC showed minimum swelling which was around 65%. While films containing the CMC showed increased swelling and in all the samples containing CMC the swelling was around 80%. This concludes that CMC is increasing the swelling property of the polymer films. Polymer films with high swelling can be tried for several biomedical applications.

Table 3: Swelling of polymer films with time

Time(Minutes)	0% CMC	0.5% CMC	1% CMC	1.5% CMC	2% CMC
15	61.44	77.1	69.77	76.15	78.87
30	63.58	79.15	74.71	78.89	79.31
45	64.24	79.32	76.19	80	79.59
60	64.46	79.41	77.11	80.34	79.73
120	64.67	79.5	78.33	80.47	79.87
180	65.09	79.5	78.47	80.47	80
240	65.29	79.58	78.47	80.55	80

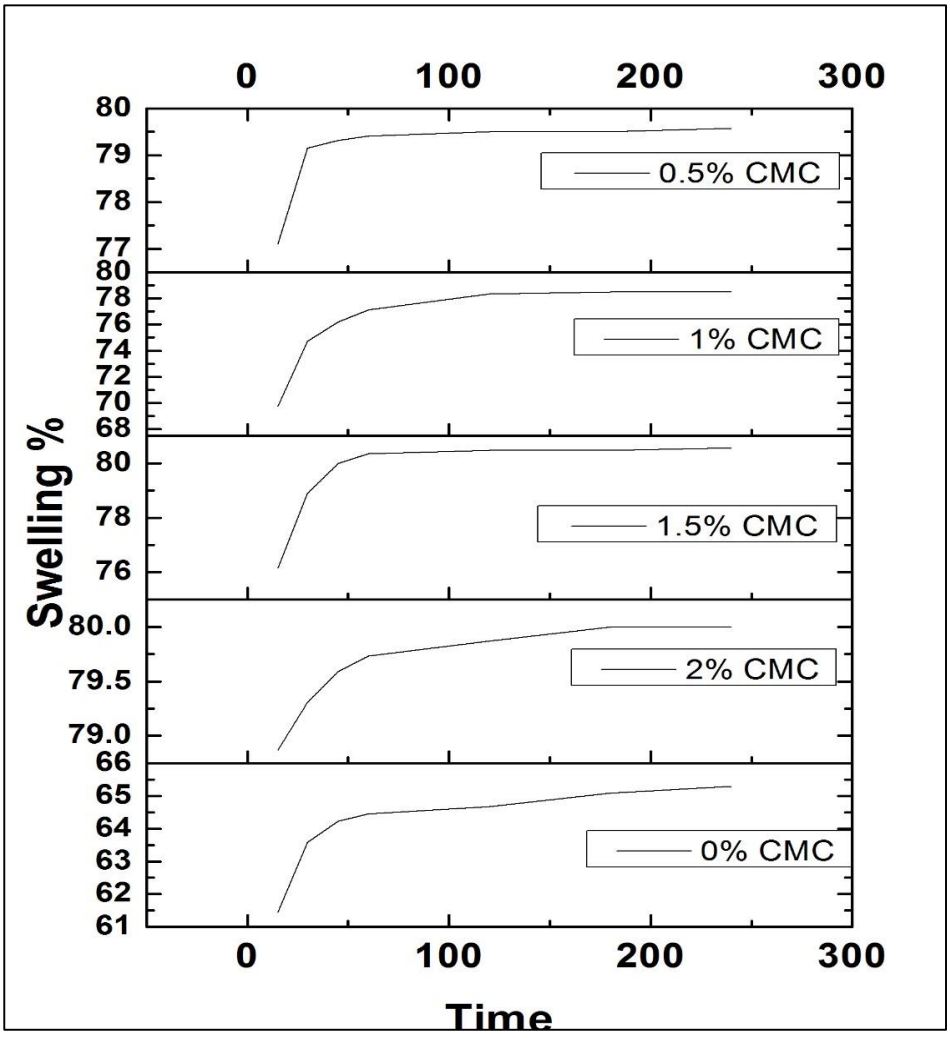


Figure 2: Percentage swelling of polymer films with time

4.2.2 Biodegradation study: Biodegradation study indicated that polymer films were slowly biodegraded. After 96 hours approximately 30% of all the polymer film samples were biodegraded. Film without CMC polymer degraded to higher extent in comparison

of films containing CMC. As the proportion of CMC is increased, degradability also increased to a small extent (around 35%).

Table 4: Biodegradation data for polymer film samples

S.No.	Samples	Dry weight		% Biodegradation
		At 0 hours	At 96 hours	
1	Blank	0.059	0.041	30.5
2	0.50%	0.049	0.033	32.65
3	1.00%	0.065	0.042	33.84
4	1.50%	0.057	0.037	35.09
5	2.00%	0.03	0.019	36.67

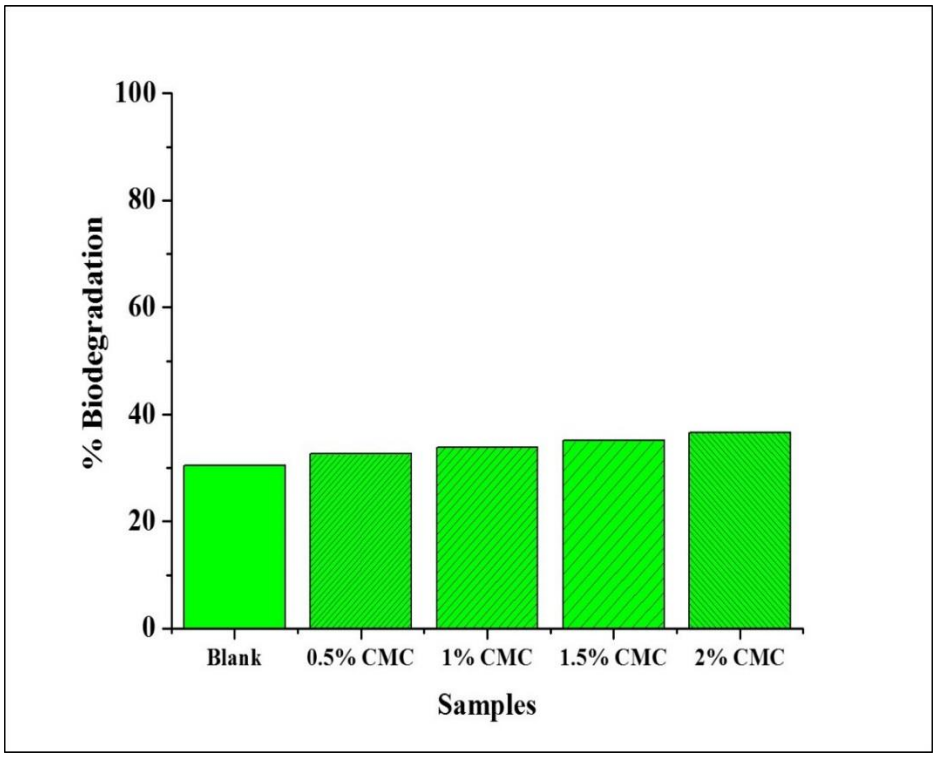


Figure 3: Degradability index of polymer film samples.

4.3 Hemocompatibility:

Hemocompatibility study of the polymer films were performed to check the applicability of the polymer films for various bio medical applications. The results

indicated that films are hemocompatible and can be tried for drug delivery. With an increase in the percentage of CMC the % hemolysis of blood decreased. This is to be noted that these polymer films can be tried for various bio medical applications.

Table 5: Absorbance readings of the hemocompatibility test for various samples.

S.No.	Samples	OD at 545nm	% Hemolysis
1	Positive Control	0.629	100
2	Blank	0.069	4.76
3	0.50%	0.065	4.08
4	1.00%	0.06	3.23
5	1.50%	0.053	2.04
6	2.00%	0.045	0.6
7	Negative Control	0.041	0

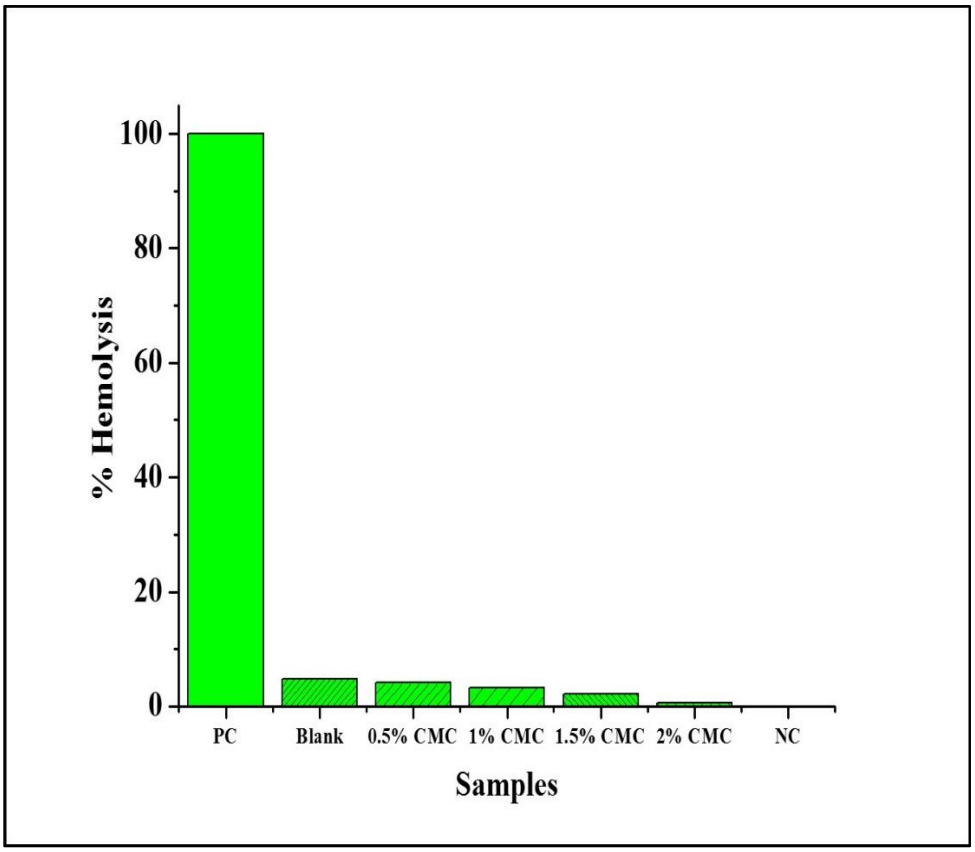


Figure 4: % Hemolysis for PVA-Gelatin-CMC film samples.

4.4 Cytotoxicity (MTT assay):

The viability of HaCaT cells for different polymer film samples were compared with respect to control (cells without leachent). From the observations of MTT assay, it was clear that all films samples are biocompatible and can be tried for therapeutic applications. These films can also be very helpful for tissue engineering as well as for other biomedical applications.

Table 6: MTT assay absorbance at 595nm (readings of triplicates)

Samples	Control	Blank	0.5% CMC	1% CMC	1.5% CMC	2% CMC
1	0.659	0.597	0.591	0.584	0.593	0.589
2	0.616	0.61	0.59	0.589	0.581	0.584
3	0.635	0.607	0.586	0.609	0.572	0.589
4	0.637	0.586	0.602	0.577	0.586	0.579
Average	0.63675	0.6	0.59225	0.58975	0.583	0.58525
Std. deviation	0.01759498	0.01086278	0.006849574	0.013744696	0.008831761	0.004787136
compared to 1	1	0.957319505	0.944954128	0.940965297	0.930195453	0.933785401
Std. deviation	0.027632478	0.017331919	0.010928718	0.021930109	0.014091362	0.00763803

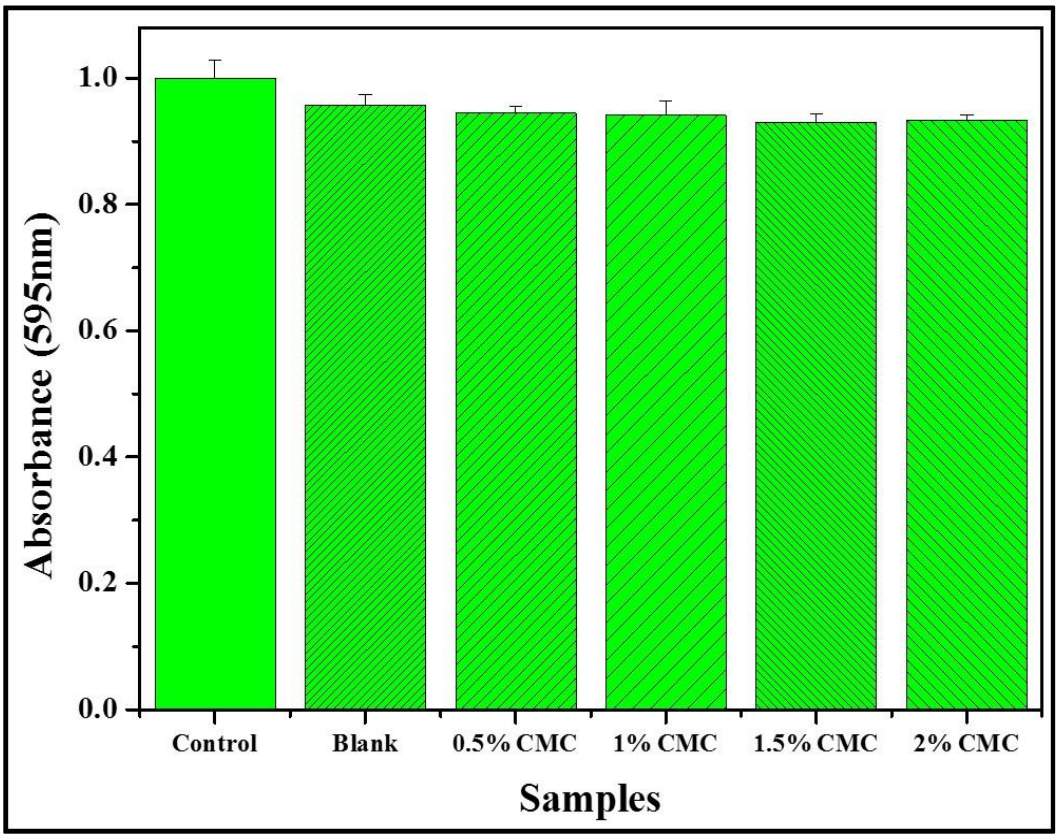


Figure 5: Cell viability index of the polymer film samples

4.5 Discussion:

Our study on the polymer films showed that these films can be formed with less proportion of CMC. Swelling nature of polymer films is constant around 80% and have good swelling strength. In case of biodegradation, it is increased with increase in proportion of CMC and is constant around 35%. Hemocompatibility of polymer films is negligible and can be tried for various tissue engineering applications. Cytotoxicity (MTT assay) results showed that polymer films biocompatible and can be tried for several biomedical applications.

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