International Journal of Drug Delivery 5 (2013) 177-187
http://www.arjournals.org/index.php/ijdd/index

Original Research Article

ISSN: 0975-0215

Biodegradable graft hydrogel membranes for *in-vitro* release studies of Levofloxacin Hemihydrate drug

M.N.Prabhakar[1], A. Chadra Babu[2], M.C.S. Subha[2], K. Chowdoji Rao[1]*

*Corresponding author:

K. Chowdoji Rao

[1]Department of Polymer Science & Technology, Sri Krishnadevaraya University, Anantapur, INDIA
[2]Department of Chemistry, Sri Krishnadevaraya University, Anantapur, INDIA.

Abstract

Controlled release of Levofloxacin Hemihydrate drug through Methyl Methacrylate grafted Poly (vinyl alcohol) (PVA-g-MMA) hydrogel membranes have been investigated. These graft co-polymer hydrogel membranes of various formulations were prepared using conventional solution casting method by varying, monomer, cross-linker and drug content. An attempt has been made to characterize these hydrogel membranes by various instrumental techniques like, Fourier Transform Infrared Spectroscopy (FT-IR), X-Ray Diffraction (XRD), Differential Scanning Calorimetry (DSC), and Scanning Electron Microscopy (SEM). The release patterns of the drug from the hydrogel membrane were carried out in pH 7.4 media and the samples were analysed spectrophotometrically at 294 nm wavelength on a UV Vis spectrophotometer. The mechanical properties of the hydrogel membranes were characterized by UTM. FTIR spectra of the membranes indicated complete esterification of the free carboxyl groups of Methyl Methacrylate. XRD studies indicated that the crystallinity of the membranes was mainly due to Methyl Methacrylate. The experimental results indicated that the hydrogel membrane could be tried for various biomedical applications.

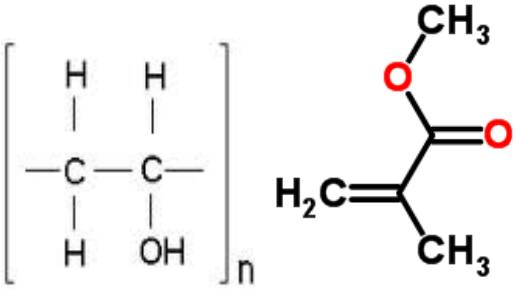
Keywords: Biocompatibility; Poly (Vinyl Alcohol); Methyl Methacrylate, Levofloxine Hemihydrate.

Introduction

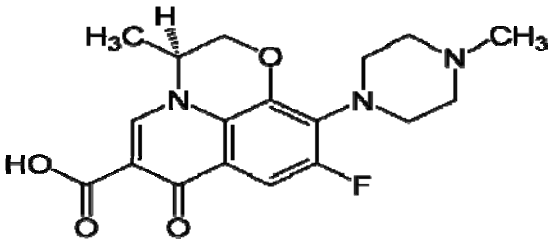
Polymeric membrane materials with hydrophilic/functionalizable groups have gained increasingly more attention in recent years, especially in biological and biomedical applications. Polymeric membranes/matrices are useful in developing the controlled release devices for the effective delivery of drugs in order to improve the patient compliance by maintaining the desired drug concentration in plasma, which helps to achieve a better therapeutic effect. In case of conventional drug therapy, drug is rapidly released from its dosage form, reaching a maximum level, which may be a toxic level, and then decays exponentially to a minimum level, below which the drug is no longer effective until the next administration. In order to maintain the therapeutic level of the drug for longer periods and to decrease its toxic levels, many efforts have been made to use polymers as membrane devices [1-4]. Polymers have been used as coated membranes or as matrices to extend the release rates of the drug. In these systems, drug can be released from a device to the outer medium by diffusion or dissolution mechanisms.

Poly (vinyl alcohol) (PVA), is a non-toxic, water-soluble synthetic polymer and has good physical and chemical properties and film-forming ability. The use of this polymer is important in many applications such as controlled drug delivery systems [5], membrane preparation [6], recycling of polymers and packaging [7] etc. Studies on the mechanism of dissolution and changes in crystallinity and swelling behaviour of PVA and its physical gel-forming capabilities, have been carried out [8,9]. PVA has bio inert-ness and it has many uses in medical applications such as artificial pancreas, haemodialysis, and nanofilteration, synthetic vitreous and implantable medical device, anti-thrombogenicity, cell compatibility, blood compatibility and biocompatibility of PVA have been studied extensively [10-12].

Methyl methacrylate (MMA) is one of the most widely used monomers. It has wide-spread biomedical applications, due to its biocompatibility and it can be easily copolymerized with other monomers like sulfopropylmethacrylate [13] and alkyl methacrylate with various acrylic acid derivatives including acrylamide, acrylic acid, butyl ester as well as with styrene [14,15].



Poly (vinyl alcohol) Methyl methacrylate



Levofloxine Hemihydrate

Several Natural polymers such as sodium alginate [16-20], chitosan [21-25], guar gum [26-28], xanthan gum [29-32], pectin [33,34], gellan gum [35,36] have been employed alone or in combination with their native form to control the drug release, but these just had a limited degree of success. In recent years, graft copolymers designed primarily for medical applications have entered the arena of controlled drug release.

Levofloxacin Hemihydrate (LH), a synthetic fluorinated quinolone derivative, is effective for bacterial infection treatment, especially for Helico Bacter pylori (bacteria) [37-40]. It is used to treat infections including: respiratory tract infections, cellulitis, urinary tract infections, prostatitis, anthrax, endocarditis, meningitis endocarditis, meningitis, pelvic inflammatory disease, traveler's diarrhea, tuberculosis and plague [41]. Levofloxacin has a half-life of 5-7 hours and 85% oral bioavailability. Because of this, It is extensively used in many biomedical applications.

In the present study, authors prepared LH incorporated PVA-g-MMA hydrogel membrane and studied the effect of various factors viz., PVA, MMA, LH and glutaraldehyde (GA) (crosslinking agent) concentration on swelling properties and drug release profiles for *In-vitro* release studies and the results are presented.

Materials and Methods

Materials

Table I: Materials used in the present study

Component	Chemical Formula	Application	Manufacturer
PVA (M.W. =50,000)	$(C_2H_4O)n$	base ingredient	Sd.Fine, Mumbai, India
MMA	$CH_2=C(CH_3)COOCH_3$	base ingredient	Sd.Fine, Mumbai, India
Glutaraldehyde (25% aqueous)	$CH_2(CH_2CHO)_2$	Crosslinker	Sd.Fine, Mumbai, India
Levofloxine Hemihydrate (99.39% purity) (gift sample)	$C_{18}H_{20}FN_3O_4.1/2H_2O$	base drug	CIPLA pharma, Bangalore, India
APS	$(NH_4)_2S_2O_8$	base initiator	Sd.Fine, Mumbai, India
Double distilled water was used throughout the study			

Preparation of Graft Hydrogel Membrane

The PVA-g-MMA hydrogel membranes were prepared by a conventional solution casting method. A 2% aqueous solution of PVA was prepared by dissolving PVA in water over night under constant stirring conditions. To this, a aqueous solution containing 50mg of APS was added followed by a known amount of MMA drop by drop with continuous stirring for 5h at 70^0C. To this, the required amount of Levofloxine Hemihydrate drug was added and stirred until complete dispersion of drug in the polymer solution is obtained. For crosslinking, a specific amount of glutaraldehyde (0.5ml of GA and 2-3 drops of HCl) was added to the solution and allowed to stand for 15min. The resulting homogeneous solution was further allowed to stand until trapped air bubbles were removed and poured on a Teflon plate. The membranes were dried in an oven at 37°C, until it shows constant weight. The prepared membranes were stored in a desiccator for further evaluation. The schematic diagram of the formation of crosslinked membrane is shown in Scheme 1.

FT-IR Studies

The FT-IR spectra of dry hydrogel membranes were obtained using FT-IR Spectrophotometer (Bomem, Model: MB3000, Canada). Percentage transmittance (%T) was recorded in the spectral region of 500–4000cm^{-1} using a resolution of 4 cm^{-1} and 40 scans. The dry membrane powder was thoroughly grounded with KBr (IR grade, Merck-Germany) at a ratio of 1:200 and pressed into a pellet and the spectrum was then recorded.

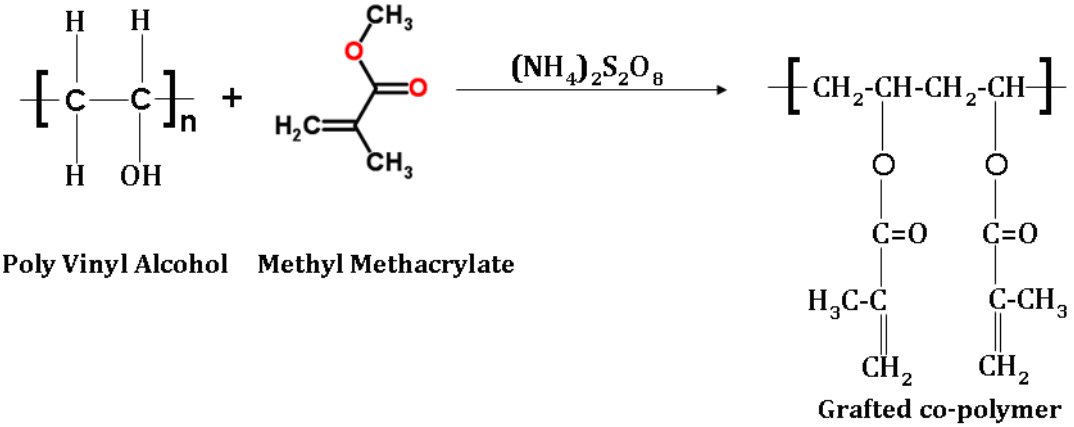
Thermal Studies

Thermal decomposition temperature of grafted hydrogel films were carried out on a Waters apparatus DSC-TGA Q-600 model instrument (UK) in a nitrogen rich atmosphere at a heating range of 35^0C-700^0C at the rate of 10^0C/min. The weight of the samples taken for each record was about 9-12mg. The incept point of the slopes was taken as glass-transition temperature (T_g)

Tensile strength of the membrane

The tensile properties of all grafted hydrogel membranes were determined by using INSTRON 3369 Universal Testing Machine

(Norwood, Massachusetts, USA) running at a crosshead speed of 5 mm/min. The sample membranes were cut into 1x 10 cm. The tensile parameters were



Cross-linked Graft Co-Polymer Hydrogel Membrane

Scheme 1: Schematic representation of the formation of Cross-linked membrane

measured using 10 kg load cell. In each case, 3 samples were tested and the average values are reported.

Swelling Measurements

Equilibrium swelling studies of membranes were performed in water at room temperature. The weight of the dried membranes (W_d) measured directly on an electronic microbalance (Adam PW214, London, with an accuracy 0.0001 g) and then the dried membranes were suspended in glass vessels containing 50 ml of water at 37° C. After 24 h the swollen membranes (W_s) were removed from water and immediately weighed after removal of excess water by using a blotter. The procedure was repeated until the membranes reached constant weight (equilibrium water uptake). The swelling ratio of membrane was calculated from the following Equitation 1.

$$\% \ SR = \left[\frac{W_s - W_d}{W_d} \right] X \ 100 \quad \text{----------} \quad (1)$$

Here W_d and W_s were the weight of dry and swollen membranes, respectively.

Drug content

The membrane of specified area (1 cm^2) was cut into small pieces and added to 100 ml of phosphate buffer of pH 7.4 for complete swelling at 37° C for 24h. The swollen membranes were crushed in a glass mortar with pestle. The solution was then heated gently for 2 h to extract the drug completely and centrifuged using a table-top centrifuge(R-8C DX Remi, India) at 3000 rpm for 10 min to remove polymeric debris. The clear supernatant solution was analysed for drug content using UV spectrophotometer (LabIndia-UV3000+)(λ_{max}) at 255nm. The average of three determinations was considered. The % encapsulation efficiency was calculated by the following Equation 2.

$$\% \text{ Encapsulation efficiency} = \left(\frac{\text{Actual loading}}{\text{Theoretical loading}} \right) x100 \quad ---(2)$$

In-Vitro release study

In vitro drug release study was performed by using tablet dissolution tester (LabIndia, Mumbai, India). The membranes of 4.0 cm^2 area were mounted for release study. The amount of drug released was determined by withdrawing 10 ml samples at a specific time intervals for12 h. The volume withdrawn was replaced with an equal volume of fresh buffer solution; the samples were analysed in a UV spectrophotometer (LabIndia, Mumbai, India) (λ_{max}) at 255 nm.

X-Ray Diffraction

X-Ray Diffractions of the plain PVA-g-MMA membrane and drug loaded PVA-g-MMA hydrogel membranes were carried on a Shimadzu Lab-XRD-6000X diffractometer [Japan], using Nickel-filtered Cu Kα radiation [λ=0.154 nm] at 40 kV and 50 mA in the 2theta range of 0-50^0.

SEM analysis

SEM images of pure grafted Hydrogel membrane and Drug loaded grafted hydrogel membranes were recorded using a JSM 6400 SEM (JEOL Ltd., Akishima, Tokyo, Japan) at 100 and 80 magnification. Working distance of 39 mm was maintained and the acceleration voltage used was 20 kV, with the secondary electron image (SEM) as a detector.

Results and Discussion

The different formulations of PVA, MMA, Drug and crosslinker variations have shown as M1-M9 codes and are incorporated in Table.2

Table 2: Film composition obtained from PVA-g-MMA mixture with Drug

Code	Polymer (w%)	Monomer (w%)	Drug (gm)	Crosslinker (ml)
M-1	100	-	0.1	0.1
M-2	90	10	0.1	0.1
M-3	80	20	0.1	0.1
M-4	70	30	0.1	0.1
M-5	90	10	0.1	0.1
M-6	90	10	0.2	0.1
M-7	90	10	0.3	0.1
M-8	90	10	0.1	0.2
M-9	90	10	0.1	0.3

FTIR Spectral Analysis

The grafting of MMA on PVA molecules was verified by FT-IR spectra of PVA, MMA-g-PVA and drug loaded MMA-g-PVA as shown in Figure 1. The spectra of both PVA and grafted PVA show a characteristic broad absorption band of the hydroxyl group around 3500-3150cm^{-1}. This attributed to the O-H bond stretching vibration [42-44] of PVA. The spectrum of the grafted PVA exhibits a strong absorption band at 1730cm^{-1}, which is absent in spectrum of pure PVA. The peak near 1730cm^{-1} may be associated with C=O stretching vibration of an ester group [45,46] from MMA. The appearance of a new peak at 1730cm^{-1} in the resulted copolymer provides strong evidence of grafting.

FT–IR spectrums of Levofloxacin alone and in combination with graft polymer were studied. FT-IR spectrum of the Levofloxacin and the drug-polymer mixture have characteristic bands at 1723 cm^{-1} (carbonyl group), 1884 cm^{-1} (carbonyl group of quinolone moiety), 2935 cm^{-1} (aromatic C–H stretching), and 3275.5 cm^{-1}(O-H group of carboxyl moiety) indicating that Levofloxacin is not involved in any chemical reactions with the polymers used.

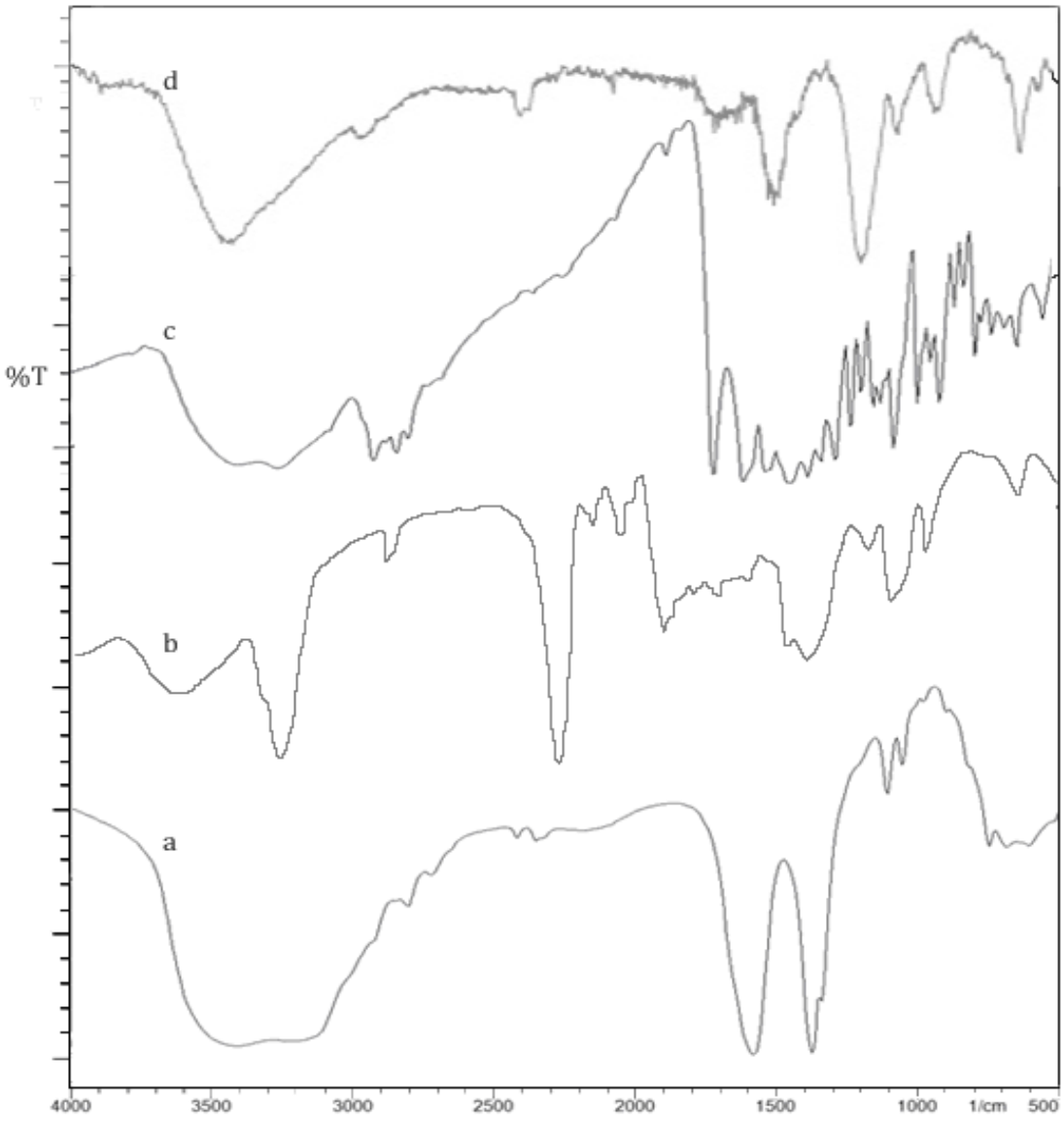


Figure 1: FT-IR spectra of Drug loaded Hydrogel Membrane (a), Grafted PVA(b), Pure LH (c), PVA (d).

Thermo Gravimetric Analysis (TGA)

Thermo Gravimetric analysis (TGA) of pure PVA (a) and grafted hydrogel membranes (b) are shown in Figure 2. The TGA of pure PVA shows a weight loss in two stages. The first stage occurs below 100^0C and shows 10% loss in weight. This may correspond to the loss of adsorbed and bounded water. The second stage of weight loss starts at 210^0C and continues up to 420^0C during which there is around 90% weight loss due to the degradation of PVA. If it is clearly observed, we can also find two stages of degradation in case of graft copolymers also. The first one corresponding to 15% of weight loss at about 110^0C-250^0C, which may attributed to the degradation of the ungrafted PVA. The second distinct weight loss is observed between 310^0C–480^0C with about 80% weight loss and theses can be attributed to the grafted copolymer. It is evident that grafting MMA onto PVA could augment the thermal stability of pure PVA. Due to the presence of MMA, the copolymer exhibits enhanced hydrophobic character compare to pure PVA.

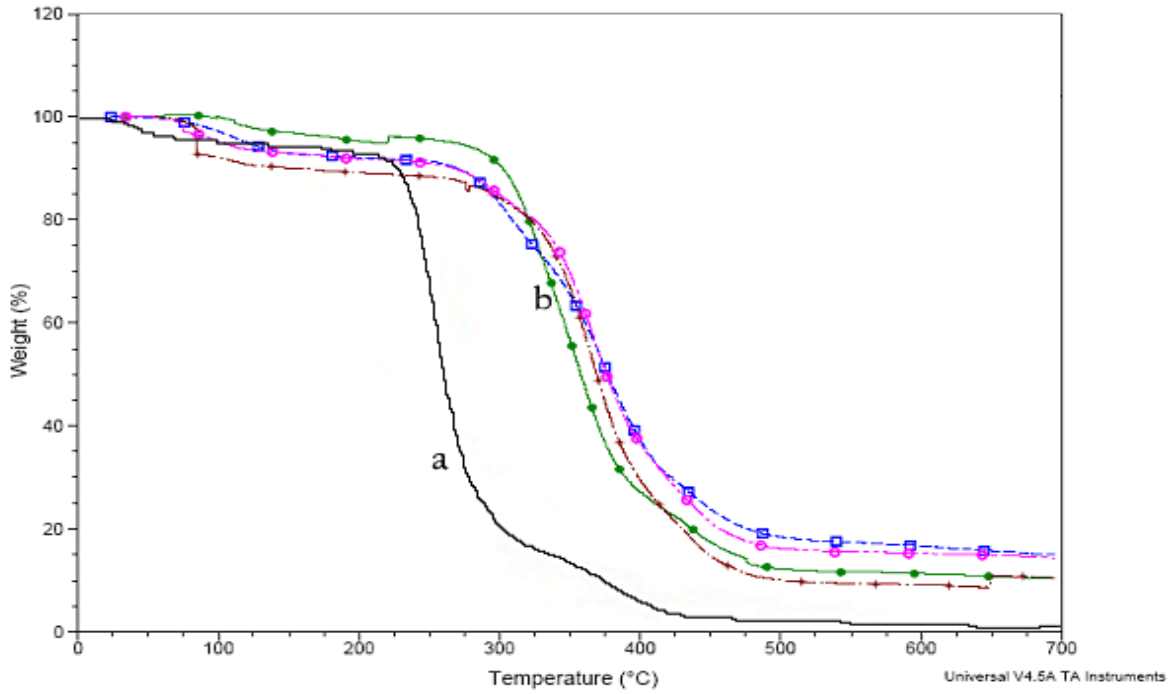


Figure 2: Thermo Gravimetric thermograms of pure PVA (a) and different ratios of PVA and MMA graft hydrogel membranes (b)

Mechanical Properties

The values of tensile strength for different grafted copolymer membranes are given in Table 3. From table it is clear that the improved mechanical strength of the grafted membranes compared to pure PVA membrane was confirmed by tensile strength (TS) measurement. The NP-8 membrane made of pure PVA showed TS of 2.12kg/cm^2, while, the grafted PVA membranes shown higher TS value (3.9 Kg/cm^2). This may be due to the formation of large number of links among the polymer chains as a result of grafting, thereby increasing strength of the Polymer membranes. Among the membranes, TS increased with an increase in concentration of GA, indicating an increased strength of matrix with increasing cross-linking.

Table: 3 The value of Tensile strength for different grafted hydrogel membranes

Code	Tensile Strength
M-1	2.12±0.02
M-2	2.71±0.03
M-3	3.12±0.12
M-4	4.21±0.40
M-5	2.54±0.03
M-6	2.63±0.10
M-7	2.92±0.20
M-8	3.92±0.62
M-9	4.08±0.30

X-Ray Diffraction

X-RD study helps to find the crystallinity of drug in the grafted hydrogel membrane. The X-Ray diffractograms of pristine Levofloxine hemihydrate (a) Plain grafted hydrogel membrane (b) and Levofloxine hemihydrate loaded graft hydrogel membrane(c) are presented in Figure 3.

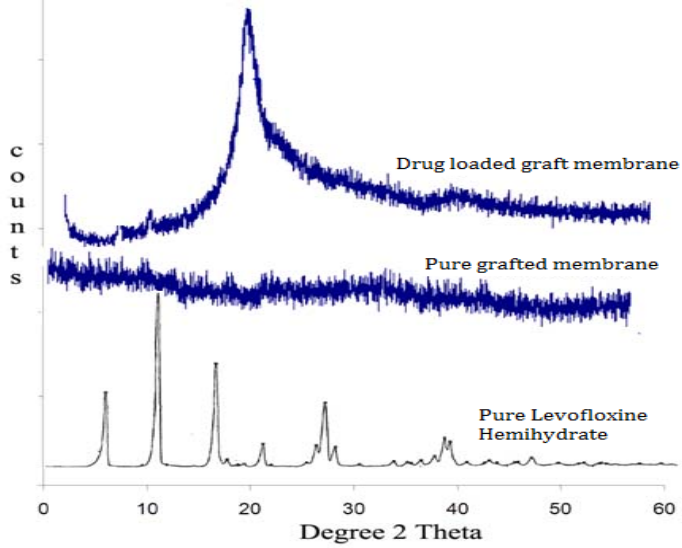


Figure 3: X-Rd spectra of Plain LH, Pure graft hydrogel Membrane and Drug loaded Hydrogel Membrane.

The most intensive peaks of Levofloxine hemihydrate are observed at 2θ of 12^0, 17^0 and 29^0 suggesting its crystalline nature as seen in Figure 3 (a). The X-RD patterns of pure grafted hydrogel membrane (Figure 3 (b)) reveal amorphous nature. According to Figure 3 (c) the characteristic peaks of Levofloxine Hemihydrate are found in LH loaded hydrogel membrane with very less intensity only at 2θ of 20^0. This suggests that Levofloxine hemihydrate is dispersed at a molecular level within the grafted hydrogel membrane.

SEM analysis

The SEM Photomicrographs of the membranes of plain membrane (a) and drug loaded (b) taken at a magnification 100X and 80X, respectively and are presented in Figure 4. Analysis of the morphologies of the plain and drug loaded membranes shows that they are smooth and homogeneous, with absence of any micro phase separation. Grafting led to a substantial increase in the surface smoothness; this might be due to the formation of own domains and morphologies at the surface by grafted chains. MMA grafted chains are hydrophilic in nature and hence lead to compatible with PVA matrix, resulting the formation of single phase with a smooth surface, this indicate the good compatibility between the membrane and the drug.

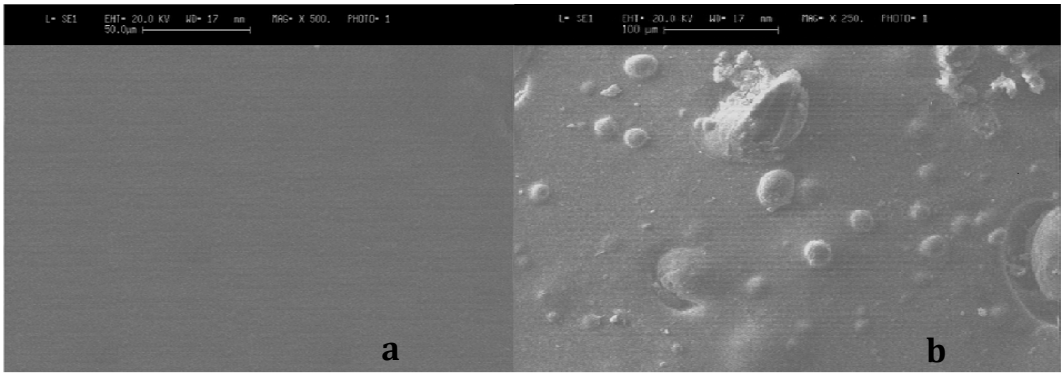


Figure 4: SEM Micrograms of Plain graft hydrogel Membrane (a) and Drug loaded hydrogel Membrane (b)

Swelling studies

The variation of % of swelling ratio of hydrogel membranes with the concentration of MMA (a) and crosslinker (b) were depicted in Figure 5 (a) and (b) respectively. In the present investigation, we employed MMA and GA with different amounts of PVA, for the preparation of these membranes. The membranes swelling properties were influenced by the amount of MMA and crosslinker (GA). As the amount of MMA increases the swelling ratio of hydrogel membrane decreases. This is due to the fact that as the amount of MMA increases in the membrane, hydrophobicity of the membrane could increase slightly due to presence of methyl groups present in MMA. Similarly, In the case of GA crosslinker variation, the swelling ratio decreased with the increase of crosslinker content. This may be due to the formation of rigid network between the polymeric chains as a result of contraction of microvoids of the cross-linked networks and therefore a higher swelling capacity could not be obtained with increase in concentration of crosslinker.

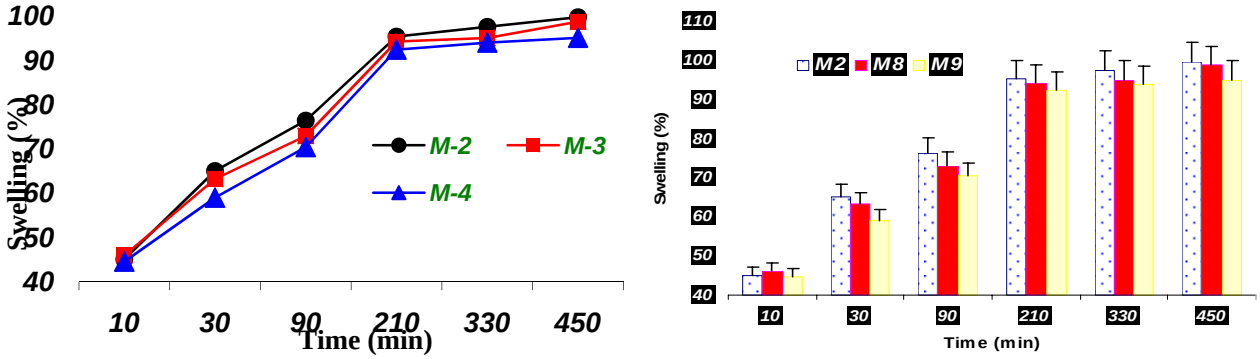


Figure 5: Variation of % swelling ratio with (a) concentration of MMA and (b) crosslinker.

In vitro studies

Table: 4 Results of % Encapsulation Efficiency andRelease Kinetics parameters of Different formulations

Sample code	% of Encapsulation	K	N
M-1	50.50±0.5	0.0412	0.7241
M-2	85.41±0.2	0.0221	0.6780
M-3	79.20±0.7	0.0818	0.8589
M-4	70.01±0.5	0.0210	0.8987
M-5	89.40±0.2	0.6110	0.6407
M-6	90.01±0.5	0.0391	0.6781
M-7	93.10±0.3	0.0151	0.2891
M-8	74.80±0.6	0.0545	0.8450
M-9	67.80±1.2	0.5850	0.7910

Encapsulation efficiency

Results of encapsulation efficiencies are given in Table 4. The % encapsulation efficiency varied depending upon the initial loading of the drug. In general, for formulations M5, M6 and M7, the % encapsulation efficiency increased systematically with increasing drug content of the matrices. In the present study, the highest % encapsulation efficiency of 93.10 was observed for M-7 containing 3 % of Levofloxine Hemihydrate. These are in accordance with results reported in literature [47]. From the study of effect of crosslinking agent on % of encapsulation efficiency, it is observed that with an increase in concentration of crosslinking agent GA (i.e., M7, M8 & M9) in the matrix, the % of encapsulation efficiency (93.10, 74.80 and 67.80) decreased due to lesser free volume space available in the matrix.

Effect of Methyl methacrylate

Effect of MMA content on *in vitro* release of LH was investigated at pH-7.4. *In vitro* release profiles of LH from the formulations prepared with different amounts of MMA (10, 20 and 30%) crosslinked with 0.5 mL GA at 10% of LH loading performed at pH-7.4 are shown in Figure 6 (a). Higher cumulative release rates were observed from formulations prepared with a higher amount of MMA (30%), than those formulations prepared with lower amount of MAA (10 and 20). This increasing trend may be due to the loose crosslinked chains of MMA in the membrane, resulting in an increasing in dimension of the polymer coil, thus a significant increase in molecular volume of the matrix along with the increased swelling of MMA component in the membranes. About 97% of the drug was released in 12hrs at pH-7.4 from the formulations prepared with a higher amount of MMA, whereas only 74% of LH was released in first 12hrs from formulations prepared with lower amount of MMA (10%). It also noticed that a faster drug release was observed from formulations prepared with higher amounts of MMA (M-4). Similar observations were also reported by Venkata Prasad *et al.*, in case of drug release studies on SA-g-AA [48].

Effect of crosslinking agent

% cumulative release versus time curves of membranes M-5, M-8, and M-9 are displayed in Figure 6 (b) for varying amounts of GA (0.1ml, 0.2m and 0.3ml) at a fixed amount of drug (0.1g). The % cumulative release is quite fast and larger at lower amount of GA (0.12ml) (M-5), whereas the release is quite slower at higher amount of GA (i.e., 0.3ml)(M-9). This may be due to the polymeric chains becoming rigid because of the contraction of microvoids, thus decreasing the % cumulative release of LH drug through the membrane.

Effect of drug loading content

Figure 6 (c) Shows the release profile of LH loaded membranes M-5, M-6 and M-7 at different amounts of drug loading (0.1, 0.2 and 0.3g, respectively) at pH-7.4. The release data shows that the membrane containing higher amount of LH (M-7) displayed faster and higher release rates than those formulations containing lower amount of LH. A prolonged release rate was observed in the M-5 membrane because it contains lower amount of drug. Notice that the release rate becomes quite slower at the lower amount of drug in the membrane, due to the availability of more free void spaces through which a lesser number of drug molecules will transport.

Drug release kinetic parameters of different formulations

Drug-release kinetics was analyzed by plotting the cumulative release data versus time by fitting the data to a simple exponential Equation 3[49]:

$$(M_t/M) = kt^n \quad \text{----------} \quad (3)$$

Where M_t and M represent the fractional drug release at time t, k is a constant characteristic of the drug-polymer system and n is an empirical parameter characterizing the release mechanism. Using the least square procedure, we have calculated the values of n and k for all the formulations and these values are given in Table: 4. If n= 0.5, the drug diffuses and release from the polymer matrix following Fickian diffusion. For n > 0.5, anomalous or non-Fickian drug diffusion occurs. If n = 1, a completely non-Fickian or case-II release kinetics is operative. The intermediary values ranging between 0.5 and 1.0 are attributed to an anomalous type diffusive transport. The values of k increased with increasing of drug LH into the membrane, similarly the n values increased with increasing LH. This indicates the interaction between the membrane and drug as studied from the release kinetics represented by Equation: 3 proposed by Peppas et al. [49]. The values of exponent *n* are found to range between 0.570 to 0.959 at pH-7.4 as calculated from the empirical equation, which indicated that drug release showed the non-Fickian or anomalous transport.

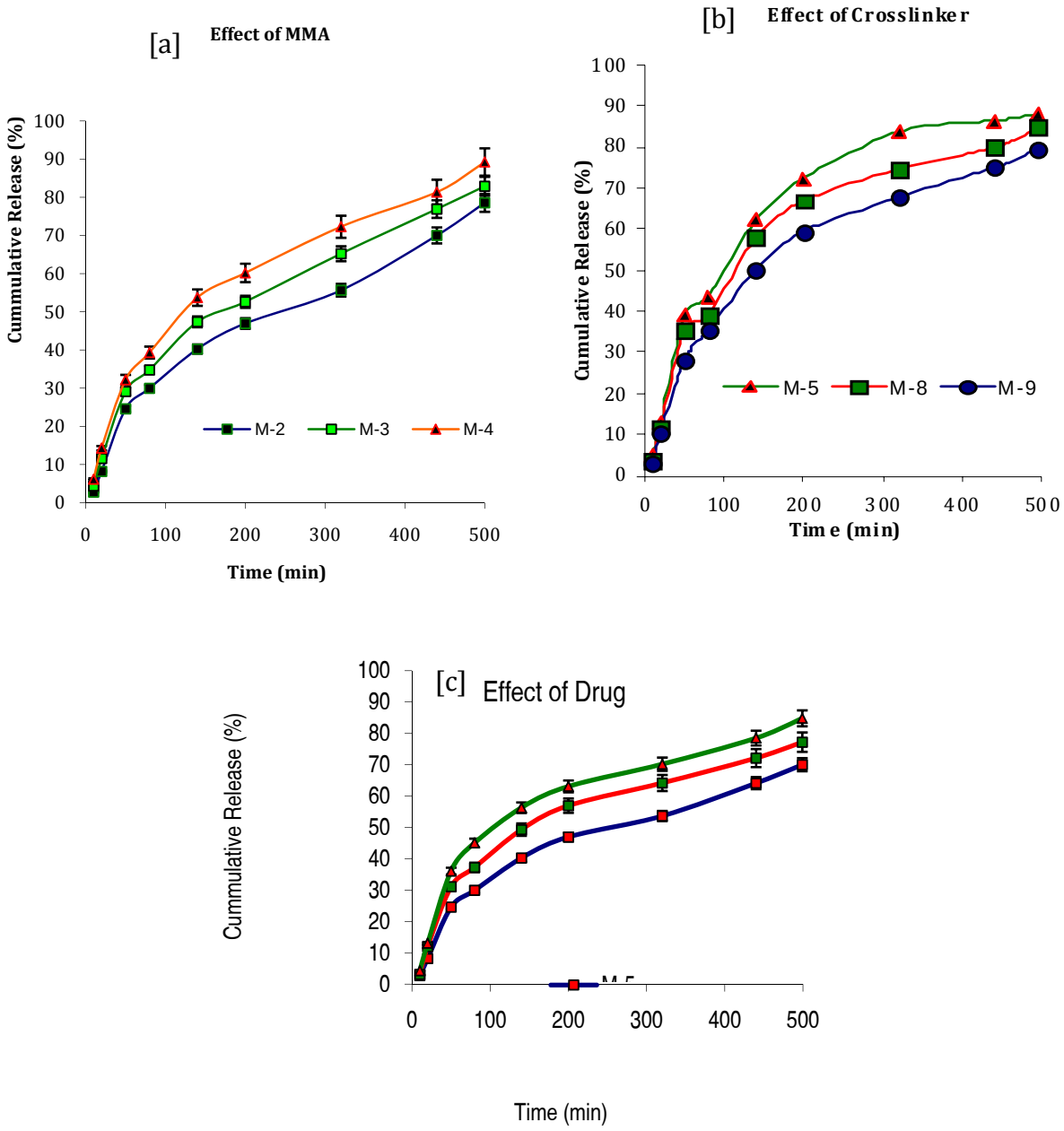


Figure 6: % of Cumulative release of LH through PVA-g-MMA copolymer hydrogel membranes containing different amounts of MMA (a) M2, M3, M4, different amounts of GA (b) M5, M8, M9, and different amount of LH (c) M7, M6, M5

Conclusions

Hydrogel membranes of PVA-g-MMA were prepared and loaded with LH as model drug. TGA analysis of the drug-loaded membranes confirmed that the drug is dispersed in molecular level in the membranes. The morphology characterization showed a good compatibility between the membrane and drug. The results of controlled release tests showed that the amount of Levofloxine release increased with an increase MMA and the amount of drug and decreased with an increase of crosslinker. Thus, we can control the drug release rate through changing some influence factors of the drug loaded membrane. The mechanical property of membrane is also good. By observing all the results the hydrogel membrane under study was found to be a quite promising for controlled release of Levofloxine drug.

Acknowledgment

One of the authors (Mr. M.N. Prabhakar) thanks to University Grants Commission (UGC) New Delhi, India (UGC BSR Sanction letter No: No. F4-3/2006 (BSR)/7-165/2007(BSR) dated 25-09-2012) for providing financial support to carryout this research work.

References

[1]. Gardner C. Drug Targeting: Potentials and Limitations. In: Topics in Pharmaceutical Science, (Eds) D.D. Briemer, P. Speiser, 1983;291.

[2]. Gregoriadis G. Nature, 1977;265:407.

[3]. Gregoriadis G, Senior J, Poste G, (Eds), Targeting of Drugs with Synthetic Systems. NATO-ASI Series (No. 113), Plenum Press, NY, 1986.

[4]. Gregoriadis G, Poste G. (Eds), Targeting of Drugs, Anatomical and Physical Considerations. Plenum Press, NY, 1988.

[5]. Krishna Rao KSV, Kiran Kumar ABV, Madhusudhana Rao K, Subha MCS, Yong-Iii Lee. Polymer Bulletin 2008;61:81.

[6]. Venkata Prasad C, Sudhakar H, Yerri Swamy B, Venkata Reddy G, Reddy CLN, Prabhakar MN, Suryanarayana C, Subha MCS, Chowdoji Rao K. Journal of Applied Polymer Science 2011;121:2717.

[7]. Khanah Mokwena K, Juming Tang. Critical Reviews in Food Science and Nutrition 2012;52:640–650.

[8]. Mallapragada SK, Peppas NA. Dissolution mechanism of semicrystalline poly (vinyl alcohol) in water. J. Polym. Sci., Part B, Polym. Physics 1996;34:1339.

[9]. Paradossi G, Lisi R, Paci M, Crescenzi V. New hydrogels based on poly (vinyl alcohol). J. Polym.Sci. Part A, Polym. Chem. 1996;34:3417.

[10]. Jegal J, Lee K. Nanofilteration membranes based on poly (vinyl alcohol) and ionic polymers. J.Appl. Polym. Sci. 1999;72: 1755-1762.

[11]. Chandy T, and Sharma CP. Prostaglandin E1-immobilized poly (vinyl alcohol) -blended chitosan membranes: Blood compatibility and permeability properties. J. Appl. Polym. Sci. 1992;44:2145-2156.

[12]. Mathew J, Kodama M. Study of blood compatible polymers I. modification of poly (vinyl alcohol). Polym. J. 1992;24:31- 41.

[13]. Saraydin D, Karadag E, Oeztop N, Gueven O. Biomaterials 1994;15:917.

[14]. Rolland, Gibassier D, Sado P, Le Verge R. J. Pharm. Belg. 1986;41:83.

[15]. Kreuter J, Liehl E, Berg U, Soliva M, Speiser PP. Vaccine 1988;6:253.

[16]. Badwan AA, Abumalooh A, Sallam E, Abukalaf A, Jawan OA. Drug Dev. Ind. Pharm. 1985;11:239-256.

[17]. Arıca B, Çalı S, Ka HS, Sargon MF, Hıncal AA, Int. J. Pharm. 2002;242:267-269.

[18]. Halder A, Mukherjee S, Sa B. J. Microencapsul. 2005;22:67-80.

[19]. Sarmento B, Ferreira D, Veiga F, Ribeiro A. Carbohydr. Polym. 2006;66:1-7.

[20]. Ahmad Z, Pandey R, Sharma S, Khuller GK. Indian J. Chest. Dis. Allied. Sci. 2006;48:171-176.

[21]. Sezer AD, Akbuga J. J. Microencapsul. 1999;16:687-696.

[22]. Janes KA, Alonso MJ. J. Appl. Polym.Sci. 2003;88:2769-2776.

[23]. Yassin AEB, Alsarra IA, Al-Mohizea AM. Sci. Pharm. 2006;74:175-188.

[24]. Srinatha A, Pandit JK, Singh S. Ind. J. Pharm. Sci. 2008;70:16-21.

[25]. Badawi AA, El-Laithy HM, El Qidra RK, El Mofty H, El dally M. Pharm. Res. 2008;31:1040-1049.

[26]. Thimma RT, Tammishetti S, J. Appl. Polym. Sci. 2001;82:3084-3090.

[27]. Reddy T, Tammishetti S. J. Microencapsul. 2002;19:311-318.

[28]. Al-Saidan SM, Krishnaiah YS, Patro SS, Satyanaryana V. AAPS PharmSciTech. 2005;6:14-21.

[29]. Talukdar MM, Kinget R. Int. J. Pharm. 1995;120:63-72.

[30]. Yeole PG, Galgatte UC, Babla IB, Nakhat PD. Ind. J. Pharm. Sci. 2006;68:185-189.

[31]. Maiti S, Ray S, Mandal B, Sarkar S, Sa B, J. Microencapsul, 2007;24:743-756.

[32]. Jain S, Yadav SK, Patil UK, J.Pharm. Tech., 2008;1:374-376.

[33]. Aydin Z, Akbug˘a J, Int. J. Pharm., 1996;137:133-136.

[34]. Sriamornsak P, Nunthanid J., Int. J.Pharm., 1998;160:207-212.

[35]. Patil S, Sharma S, Nimbalkar A, Pawar A, Drug Dev. Ind. Pharm., 2006;32:315-326.

[36]. Parauvathanahalli S, Mishra RB, Acta. Pharmaceutica., 2007;57:413-427.

[37]. Dicaro S, Zocco AM, Cremonini F, Candelli M, Nista EC, Bartolozzi F, Armuzzi A, Cammarota G, Santarelli L, Gasbarrini A, Eur. J. Gastroenterol. Hepat. 2002;14(12): 1309–1312.

[38]. O'Morain C, Eur. Gastroenerol. Rev. 2005;1:56–59.

[39]. Watanabe Y, Aoyama N, Shirasaka D, Maekawa S, Kuroda K, Miki I, Kachi M, Fukuda M, Wambura C, Tamura T, Kasuga M, Digest. Liver Dis., 2003;35:711–715.

[40]. Nista EC, Candelli M, Zocco MA, Cremonini F, Ojetti V, Finizio R, Spada C, Cammarota G, Gasbarrini G, Gasbarrini A, J. Gastroenterol. 2006;101(9):1985–1990.

[41]. Levofloxacin, *the American Society of Health-System Pharmacists*. Retrieved 3 April 2011.

[42]. Chowdhury P, Ali MA, Journal of applied Polymer Science, 2005;97:2335.

[43]. Chowdhury P, Ali MA, Journal of Polymer Matter, 2005;22:227.

[44]. Dyer JR, Application of Absorption Spectroscopy of Organic Compounds (Prentice-hill of India Pvt. Ltd., New Delhi) 1991:36.

[45]. Williams DH, Fleming I, Spectroscopic Methods in Organic Chemistry, 4th Edition (Tata Mc Graw-Hill, New Delhi) 1988:50.

[46]. Ramesh Babu V, Krishna Rao KSV, Sairam M, Vijaya Kumar Naidu B, Hosamani KM, Aminabavi TM, *J. of Appl Polym Sci,* 2006;99:2671.

[47]. Krishna Rao KSV, Vijaya Kumar Naidu B, Subha MCS, Sairam M,. Aminabhavi TM, Carbohydrate Polymers, 2006;66:333.

[48]. Prasad CV, Yerriswamy B, Reddy CLN, Vara Prasad K, Sudhakar P, Subha MCS, Jung IL Song, Chowdoji Rao K, Journal of Polymer Environment, 2012;20:344.

[49]. Ritger PL, Peppas NA, J. Control. Rel. 1987;5:37.