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Synthesis and Characterization of Sterculia Gum Based pH Responsive Drug Delivery System for Use in Colon Cancer

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The present study deals with the modification of sterculia gum to develop the novel colon specific delivery system for use in colon cancer. The sterculia and acrylic acid based hydrogels were synthesized and characterized with FTIR, SEMs, TGA and swelling behavior. Swelling studies of the hydrogels were carried out as a function of reaction parameters such as monomer concentration, initiator concentration, amount of sterculia gum and crosslinker concentration and nature of swelling mediums. Swelling kinetics of the hydrogels and *in vitro* release dynamics of anticancer model drug methotrexate from the hydrogels were studied to evaluate the swelling mechanism and drug release mechanism from the drug-loaded hydrogels. The values of diffusion exponent for the release of drug were 0.883, 0.910 and 0.787 in distilled water, pH 2.2 buffer and pH 7.4 buffer, respectively. The release of drug from the polymer matrix occurred through a non-Fickian type diffusion mechanism.

Keywords: Hydrogels, drug delivery system, colon cancer.

1 Introduction

The colon is susceptible to a number of disorders including ulcerative colitis, Crohn's disease, irritable bowel syndrome and carcinomas (1), but colorectal cancer is very common and a major cause of mortality and morbidity of the present era (2). Systemic administration of colon anticancer drugs is associated with a number of side effects. Therefore, it needs some targeted drug delivery system to deliver the drug to the colon which would ensure relief from these side effects along with the direct delivery of drug to the colon in a controlled manner.

A number of polymer-based drug delivery devices and carriers have been proposed for efficient therapy. Among all these drug delivery devices, hydrogels, specially based on polysaccharides, have attracted considerable attention as an excellent candidate for controlled release of therapeutic agents. Hydrogels are three-dimensional polymeric networks that swell quickly by imbibing a large amount of water or de-swell in response to changes in their external environment. The volume phase transitions, in response pH, make these materials suitable to deliver the drug to the colon (3–5).

In most of the cases, polysaccharide-based formulations are non-toxic, safe, biocompatible, biodegradable, abundant and colon specific (6). However, polysaccharides alone are not suitable materials to develop the drug delivery systems due to their substantial swelling and rapid enzymatic degradation in biological fluids (7). Graft copolymerization of polysaccharides with synthetic monomers is a powerful technique to modify the properties of polysaccharides and make them advanced materials for use in drug delivery (8, 9).

The grafting of vinylic monomers onto polysaccharides has improved the biocompatibility and bioadhesion of the polymer matrix (10). Incorporation of acrylic acid (AAc) onto the polysaccharide backbone has made the polymer networks pH-sensitive (11, 12) which have been used for colon specific drug delivery (13, 14). The composition of the polymer matrix affects the swelling of the hydrogels and release of drug from the hydrogels. As the concentration of the AAc increases in the polymer matrix, the crosslinking density increases, which decreases the swelling of the hydrogels (15). Poly(acrylic acid) based hydrogels have a special bioadhesive property that makes them stick to the mucosal lining of the small intestine, which after swelling, releases the loaded drug (16). The grafting has also overcome some difficulties (controlling viscosity and short stability time) associated with the polysaccharide gums based formulations (17). A higher content of AAc in arabic gum results in networks with higher crosslink density and a lower swelling rate (18). The release of drug from the polymer matrix occurred in a controlled manner with an increase in grafting ratio (19). Sterculia gum has been evaluated as a controlled-release matrix

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and has shown more superior muco-adhesion than guar gum (20).

Sterculia gum, is a medicinally important naturally occurring polysaccharide, composed of galacturonic acid, beta-D-galactose, glucuronic acid, L-rhamnose, and other residues (21). Major structural features of sterculia gum consists of two structural regions (that is A and B). The region A composed of repetitive 4-linked O-α-D-galactopyranosyluronic acid-(1$\rightarrow$2)-L-rhamnopyranose units bearing β-D-glucopyranosyluronic acid residues at O-3 of galacturonic acid and undefined side chains at O-4 of rhamnose residues; and region B composed of branched trisaccharide units that are probably not present in repetitive sequences (22–24). Sterculia gum exudes from the tree *Sterculia urens*, belongs to the family 'Sterculiaceae' and is commonly known as karaya. (25). It has been used in the treatment of diarrhea (26). It possesses mucoadhesive and bulk laxative properties (20, 27) which can be exploited to develop the drug delivery system for the colon.

It is worthy to mention here that the model anticancer drug, that is methotrexate, is an antimetabolite and antifolate drug. It is a potent inhibitor of the dihydrofolate reductase enzyme, which is essential for DNA synthesis and cell growth (28). Its higher concentration in the blood plasma may cause hepatic and renal toxicity in addition to other side effects (29, 30).

In view of the pharmacological importance of sterculia gum, drug delivery devices based on hydrogels and side effects associated with the higher plasma concentration of the anticancer drugs, sterculia gum, if suitably tailored to prepare the hydrogels, can act as the potential candidates for colon specific drug delivery in a controlled manner. Modification of the sterculia gum to develop the hydrogels is reported little in the literature and the potential of sterculia gum to develop the drug delivery systems has not been explored. Therefore, the present study is an attempt to modify sterculia gum with acrylic acid (AAc) by using N,N-MBAAm as a crosslinker and ammonium persulfate (APS) as initiator, for developing the novel hydrogels for use as drug delivery devices. The polymeric networks were characterized with SEMs, FTIR, TGA and swelling studies. Swelling behavior of the hydrogels has been studied as a function of various reaction parameters and, pH and salt concentration of the swelling media. Swelling kinetics of the hydrogels and *in vitro* release dynamics of model drug (methotrexate) from the drug loaded hydrogels in a different release medium have been carried out for the evaluation of the mechanism of swelling and diffusion coefficients.

2 Experimental

2.1 Materials and Method

Acrylic acid (AAc) was obtained from Merck-Schuchardt, Germany. Ammonium persulphate (APS) was obtained from Qualigens Fine Chemicals, Mumbai, India and N,N′-methylenebisacrylamide (NN-MBAAm) was obtained from Sisco Research Laboratory Pvt. Ltd., Mumbai, India. Methotrexate was obtained from Cipla Ltd., Verna, Goa, India. Sterculia gum was obtained froman herbal medical store.

2.2 Synthesis of Sterculia-*cl*-poly (AAc) Polymers

The reaction was carried out with 1 g of sterculia gum, a definite concentration of APS, a definite concentration of monomer and crosslinker in 10 mL distilled water taken in a test tube at 65°C temperature for 2 h. The polymers thus formed were stirred for 2 h in distilled water and for 2 h in ethanol, to remove the soluble fractions in the polymers and were then dried in an oven at 40°C. These polymers were named as [sterculia-*cl*-poly(AAc)]. The optimum reaction parameters were evaluated for the synthesis of sterculia-*cl*-poly(AAc) by varying [AAc] (from 0.291 to 1.457 mol/L), [APS] from (4.386 to 21.930 mMol/L), an amount of sterculia gum from 0.2 g to 1.0 g and

[N,N′-MBAAm] (from 6.486 to 32.432mMol/L) (Table 1). These reaction parameters were evaluated on the basis of the amount of water uptake by the hydrogels after 24 h swelling in distilled water, and shape and structural integrity maintained by the hydrogels after swelling. The optimum reaction conditions for the synthesis of sterculia-*cl*-poly(AAc) were obtained as [AAc] = 1.166 mol/L, [APS] = 13.158 mMol/L, sterculia gum = 0.8 g and [N,N′-MBAm] = 6.486 mMol/L. Further, sterculia-*cl*-poly(AAc) hydrogels were synthesized at the optimum reaction conditions and were used to study the swelling kinetics of hydrogels and release dynamics of drug from the hydrogels in different pH buffer.

2.3 Characterization

Sterculia gum and sterculia-*cl*-poly(AAc) polymers were characterized by the Scanning Electron Micrography (SEM), Fourier Transform Infrared Spectroscopy (FTIR) Thermogravimetric analysis (TGA) and swelling studies. To investigate and compare the surface morphology of sterculia and sterculia-*cl*-poly(AAc) hydrogels, SEMs were taken on Jeol Steroscan 150 Microscope. FTIR spectra of sterculia and sterculia-*cl*-poly(AAc) polymers were recorded in KBr pellets on Nicolet 5700FTIR (THERMO). The TGA of sterculia and the sterculia-*cl*-poly(AAc) were carried out on a Perkin-Elmer (Pyris Diamond) apparatus DTA-DTG-TG in air (200ml/min) at a heating rate of 10°C/min

Swelling kinetics of the hydrogels were carried out in triplicate by a gravimetric method (31). The known weight of polymers were taken and immersed in excess distilled water for different time intervals at 37°C and then the polymers were removed, wiped with tissue paper to remove the excess of solvent, and weighed immediately. The difference in weight has given the amount of water uptake by the polymers after definite time intervals. Swelling behavior of the polymeric networks was studied as a function of monomers concentration, initiator concentration, amount of sterculia

Table 1. Optimum reaction parameters for the synthesis of sterculia-*cl*-poly(AAc) hydrogels prepared at 65°C for 2 h .

S. No.	*[AAc] (Mol/L)*	*[APS] (mMol/L)*	*Sterculia (g)*	*[NN-MBAAm] (mMol/L)*	*Amount of water uptake after 24 h (per g of gel)*
1	**0.291**	4.386	1.0 g	6.486	28.90 ± 2.53
2	**0.583**	4.386	1.0 g	6.486	24.17 ± 1.18
3	**0.874**	4.386	1.0 g	6.486	17.34 ± 2.09
4	**1.166**	4.386	1.0 g	6.486	**18.77 ± 0.73**
5	**1.457**	4.386	1.0 g	6.486	8.00 ± 2.67
6	1.166	**4.386**	1.0 g	6.486	18.77 ± 0.73
7	1.166	**8.772**	1.0 g	6.486	9.41 ± 1.81
8	1.166	**13.158**	1.0 g	6.486	**12.59 ± 2.38**
9	1.166	**17.544**	1.0 g	6.486	8.08 ± 0.46
10	1.166	**21.930**	1.0 g	6.486	10.13 ± 0.31
11	1.166	13.158	**0.2 g**	6.486	6.78 ± 0.62
12	1.166	13.158	**0.4 g**	6.486	6.89 ± 0.71
13	1.166	13.158	**0.6 g**	6.486	7.50 ± .79
14	1.166	13.158	**0.8 g**	6.486	**10.13 ± 0.59**
15	1.166	13.158	**1.0 g**	6.486	12.59 ± 2.38
16	1.166	13.158	0.8 g	**6.486**	**10.13 ± 0.59**
17	1.166	13.158	0.8 g	**12.973**	5.15 ± 0.27
18	1.166	13.158	0.8 g	**19.459**	5.84 ± 0.47
19	1.166	13.158	0.8 g	**25.945**	6.08 ± 1.19
20	1.166	13.158	0.8 g	**32.432**	5.52 ± 0.54

and crosslinker concentration. The effect of pH and [NaCl] on swelling kinetics was also studied.

2.4 Release Dynamics of the Model Drug

The loading of a drug into hydrogels was carried out by a swelling equilibrium method. The hydrogels were allowed to swell in the drug solution of a known concentration for 24 h at 37°C and then dried to obtain the release device. *In vitro* release studies of the drug were carried out by placing dried and loaded samples in a definite volume of a releasing medium at 37°C (31). The amount of methotrexate released was measured spectrophotometrically in distilled water, pH 2.2 buffer and pH 7.4 buffers after every 30 min in each case. The absorbance of the solution of methotrexate was measured at λ_{max} 302 nm.

Calibration graphs were prepared in a different pH solution to determine the concentration of the drug release from the drug loaded hydrogels in a different pH solution. In this procedure, the absorbance of a number of standard solutions of the reference substance at concentrations encompassing the sample concentrations were measured on the UV Visible Spectrophotometer (Cary 100 Bio, Varian) at λ_{max} 302 nm and calibration graphs were constructed. The concentration of the drug (methotrexate) in the sample solution was read from the graph as the concentration corresponding to the absorbance of the solution. Three calibration graphs were made in distilled water, a pH 2.2 buffer and a pH 7.4 buffer to determine the amount of drug release from the drug loaded polymeric matrix in these mediums.

Buffer solutions of a different pH were prepared to study swelling of polymers and the release dynamics of the model drugs in a different pH solution. A buffer solution of pH 2.2 was prepared by taking 50 mL of 0.2M KCl and 7.8 ml of 0.2 N HCl in a volumetric flask to make a 200 ml volume with distilled water. A buffer solution of pH 7.4 was prepared by taking 50 mL of 0.2M KH_2PO_4 and 39.1 mL of 0.2 N NaOH in a volumetric flask to make a volume of 200 ml with distilled water (32).

2.5 Mechanism of Swelling and Drug Release from Polymer Matrix

Swelling of the polymers and the drug release profiles from the drug loaded polymer have been classified into three types of diffusion mechanisms on the basis of a relative

Table 2. Thermogravimetric analysis of sterculia, sterculia-*cl*-poly(AAc).

			Decomposition temp (°C) at every 10% weight loss									
Sample	*IDT (°C)*	*FDT (°C)*	*10*	*20*	*30*	*40*	*50*	*60*	*70*	*80*	*90*	*100*
Sterculia	243	717	83	240	258	271	283	311	405	484	546	717 (3.72% residue)
Sterculia-*cl*-poly(AAc)	227	525	140	245	276	305	340	388	430	476	501	525 (2.72% residue)

Table 3. Results of diffusion exponent 'n', gel characteristic constant 'k' and various diffusion coefficients for the swelling kinetics of sterculia-*cl*-poly(AAc) hydrogels in different medium at 37°C.

S. No.	Parameter	Diffusion exponent 'n'	Gel Characteristic Constant 'k' $\times 10^2$	Diffusion coefficients (cm^2/min)		
				Initial $D_i \times 10^4$	Average $D_A \times 10^4$	Late time $D_L \times 10^4$
Effect of [AAc]						
1	0.291 Mol/L	0.596	1.592	3.032	5.586	0.492
2	0.583 Mol/L	0.570	1.925	4.106	7.592	0.673
3	0.874 Mol/L	0.569	2.178	4.822	7.800	0.753
4	1.166 Mol/L	0.561	2.183	7.492	12.930	1.207
5	1.457 Mol/L	0.452	5.433	9.701	17.091	1.731
Effect of [APS]						
1	4.386 mMol/L	0.561	2.183	7.492	12.930	1.207
2	8.772 mMol/L	0.480	3.388	6.859	15.291	1.264
3	13.158 mMol/L	0.458	3.741	4.854	10.860	0.884
4	17.544 mMol/L	0.458	3.802	5.117	11.894	0.968
5	21.930 mMol/L	0.486	3.177	5.008	11.369	0.923
Effect of amount of sterculia gum						
1	0.2 g	0.611	1.702	9.605	16.680	1.485
2	0.4 g	0.633	1.374	11.557	20.146	1.797
3	0.6 g	0.611	1.528	11.046	19.746	1.758
4	0.8 g	0.594	1.652	10.905	21.227	1.801
5	1.0 g	0.458	3.741	4.854	10.860	0.884
Effect of [NN-MBAAm]						
1	6.486 mMol/L	0.594	1.652	10.905	21.227	1.801
2	12.973 mMol/L	0.496	3.132	11.100	23.726	1.990
3	19.459 mMol/L	0.498	3.262	12.214	23.940	2.119
4	25.945 mMol/L	0.529	3.002	15.774	26.526	2.575
5	32.432 mMol/L	0.473	4.281	17.175	32.323	3.025
Effect of pH						
1	Distilled water	0.594	1.652	10.905	21.227	1.801
2	pH 2.2 Buffer	0.488	2.296	12.028	24.714	2.129
3	pH 7.4 Buffer	0.593	1.479	9.469	19.442	1.635
Effect of [NaCl]						
1	Distilled water	0.594	1.652	10.905	21.227	1.801
2	0.9% NaCl	0.493	3.521	13.757	26.287	2.366

rate of diffusion of water into a polymer matrix and rate of polymer chain relaxation, (33–36). The mechanisms of swelling and drug release have been discussed in detailed in our earlier study (31). In the case of water uptake, the weight gain, Ms, is described by the Equations 1:

$$M_s = kt^n \quad (1)$$

Where k and n are constant. Normal Fickian diffusion is characterized by n = 0.5, while Case II diffusion by n = 1.0. A value of n between 0.5 and 1.0 indicates a mixture of Fickian and Case II diffusion, which is usually called non-Fickian or anomalous diffusion. Ritger and Peppas showed that the above power law expression could be used for the evaluation of drug release from swellable systems (35, 36). In this case, M_t/M_∞ replacse M_s in the above equation to give Equation 2. For cylindrical shaped hydrogels, the initial diffusion coefficients (D_i), average diffusion coefficient D_A and late diffusion coefficients (D_L) has been calculated from

Table 4. Results of diffusion exponent 'n', gel characteristic constant 'k' and various diffusion coefficients for the release of methotrexate from drug loaded sterculia-*cl*-poly(AAc) hydrogels in different medium at 37°C.

S. No.	Drug releasing medium	Diffusion exponent 'n'	Gel Characteristic Constant 'k' $\times 10^2$	Diffusion coefficients		
				Initial $D_i \times 10^4$	Average $D_A \times 10^4$	Late time $D_L \times 10^4$
1	Distilled water	0.883	0.3909	17.648	19.276	2.192
2	pH 2.2 Buffer	0.910	0.308	15.169	18.278	1.933
3	pH 7.4 Buffer	0.787	0.579	13.116	19.106	1.843

Equations 3, 4, and 5, respectively .

$$\frac{M_t}{M_\infty} = kt^n \quad (2)$$

$$\frac{M_t}{M_\infty} = 4\left(\frac{Dit}{\pi \ell^2}\right)^{0.5} \quad (3)$$

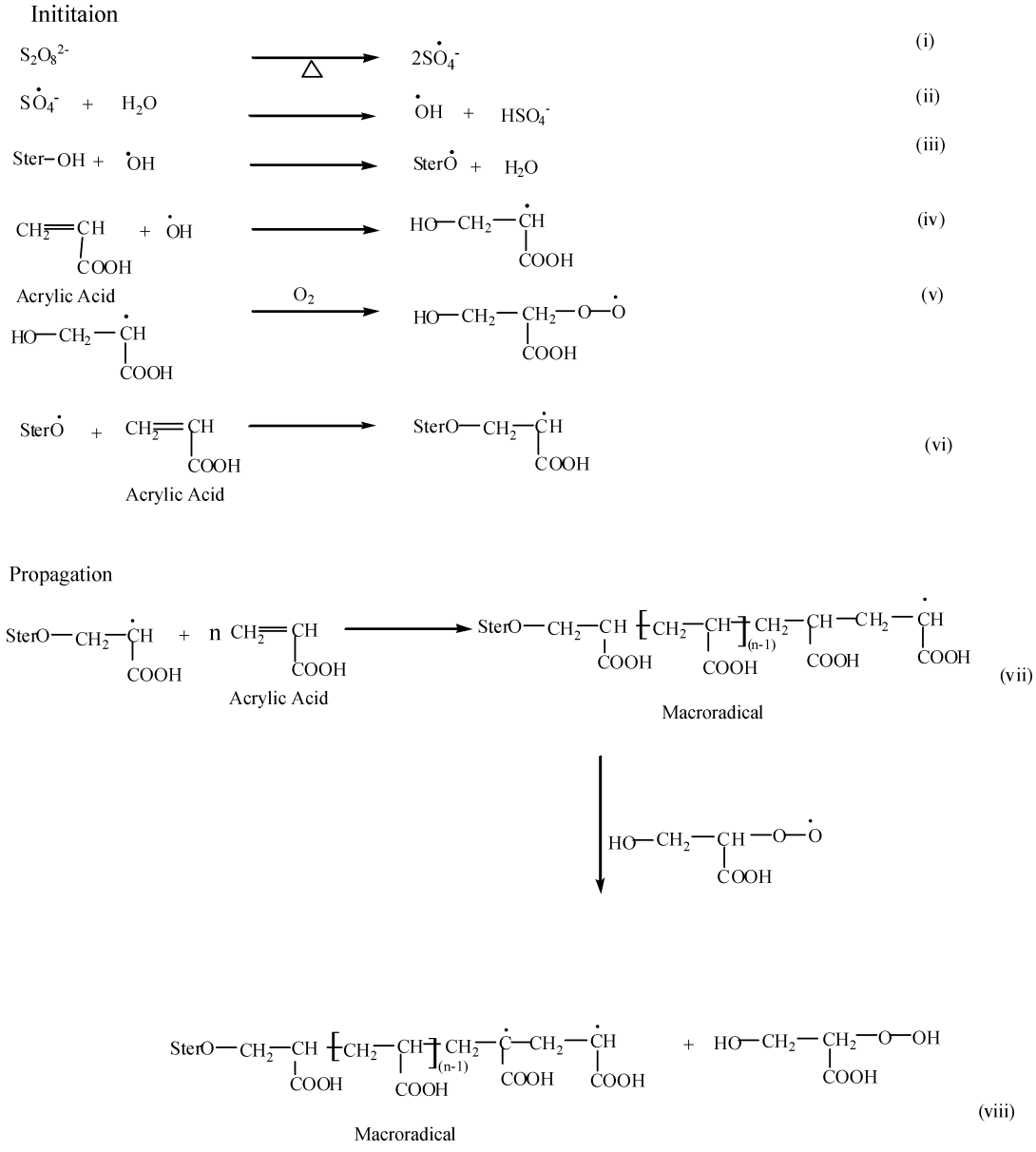
$$D_A = \frac{0.049\ell^2}{t^{1/2}} \quad (4)$$

$$\frac{M_t}{M_\infty} = 1 - \left(\frac{8}{\pi^2}\right)\exp\left[\frac{(-\pi^2 DLt)}{\ell^2}\right] \quad (5)$$

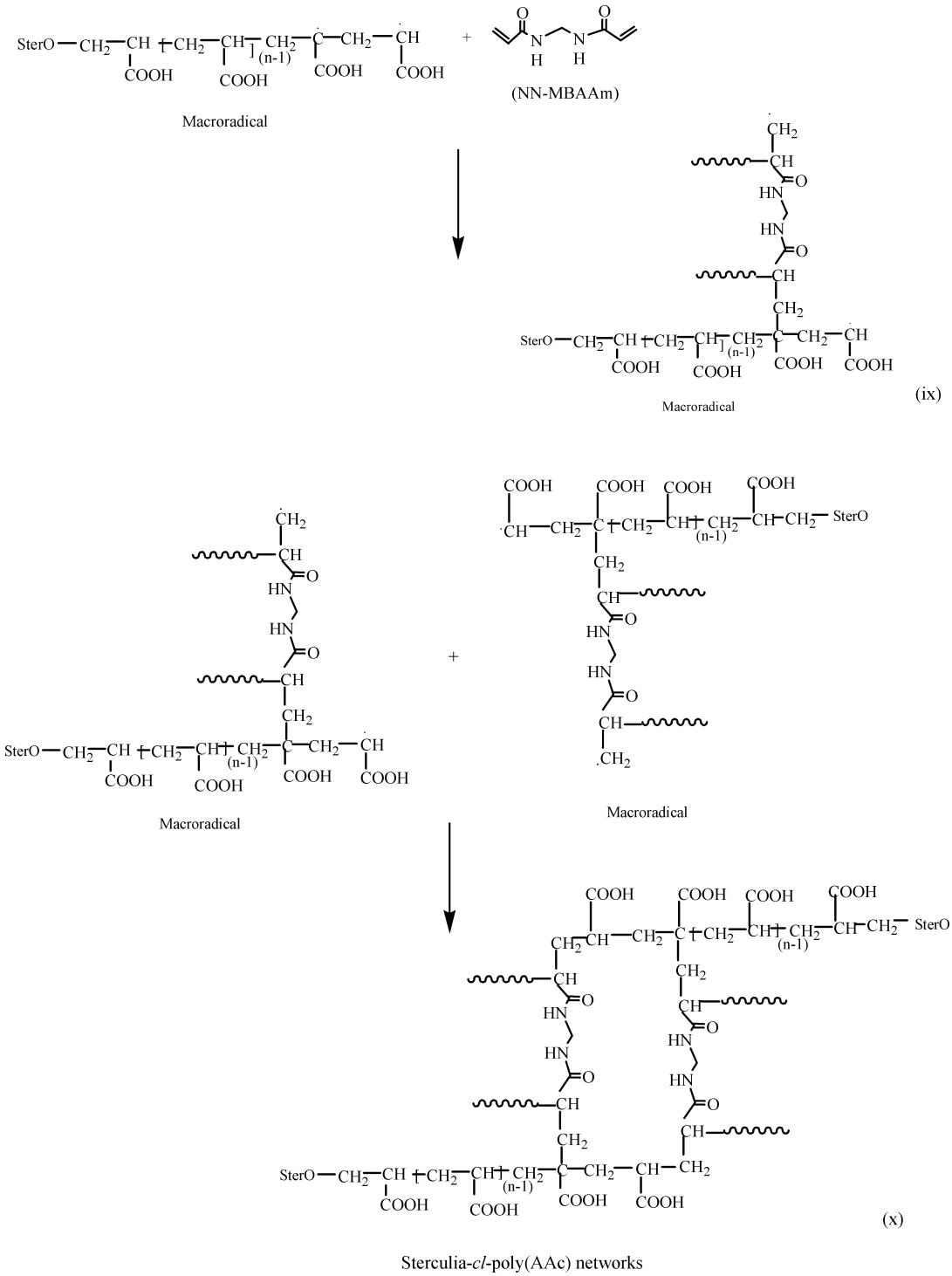
Where M_t/M_∞ is the fractional release of drug in time t, 'k' is the constant characteristic of the drug-polymer system, and 'n' is the diffusion exponent characteristic of the release mechanism. M_t and M_∞ is drug released at time 't' and at equilibrium, respectively, D_i is the initial diffusion coefficient and 'ℓ' is the thickness of the sample. $t^{1/2}$ is the time required for 50% release of drug. The values of diffusion coefficients have been evaluated for the swelling of the polymers and for the release of the drug from the polymer and results are presented in Tables 3 and 4.

3 Results and Discussion

Ammoniumpersulphate is a well known initiator and has been used for graft copolymerization of acrylic acid onto sterculia gum vinyl monomer polymerization. Hydroxyl radical formed from the reaction of $(SO_4)^{\cdot -}$ and water



Sch. 1. Plausible chemically induced mechanism for the synthesis of sterculia-*cl*-poly(AAc) hydrogels (*Continued*).



Sch. 1. (Continued)

has initiated the process of polymerization by generating the free radicals on the sterculia gum and acrylic acid monomer. During propagation, grafting of poly(AAc) onto sterculia gum has occurred and has formed the grafted macroradicals. In the presence of the crosslinker NN-MBAAm (CH_2 =CHCONHCH$_2$ NHCOCH= CH_2), because of its bifunctionality, a new macro-radical get formed that has four reactive sites and these sites can be linked both with the radical on the sterculia gum and poly(AAc). This will lead to the formation of three-dimensional networks which were named as sterculia-*cl*-poly(AAc) hydrogels. The detailed mechanism for the synthesis of sterculia-*cl*-poly(AAc) hydrogels are given in Scheme 1.

Where, $S_2O_8^{2-}$ = Persulphate anion, SO_4^{2-} = Sulphate anion, SterOH = Sterculia, AAc = Acrylic Acid, *cl* = Crosslinking

3.1 Characterization

3.1.1. *Scanning electron micrograph*

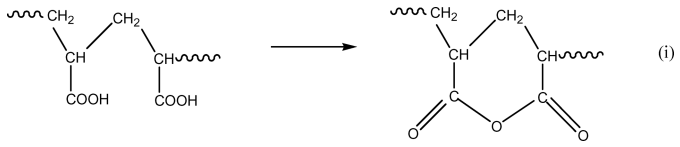
Polymers were characterized by SEMs, FTIR, and TGA. The morphology of sterculia and sterculia-*cl*-poly(AAc) hydrogels has been examined by SEMs which are presented in Figure 1(a-c and d-f), respectively for sterculia and modified sterculia at different magnifications (i.e., ×2500, ×5000 and ×10000), respectively. It has been observed from the SEMs that sterculia has a smooth and homogeneous morphology, whereas modified sterculia has structural heterogeneity. The change in surface morphology of the modified sterculia is clear in the SEMs of the hydrogels and some crosslinked networks have also been observed in the hydrogels which are shown in the SEMs of the hydrogels at different magnifications.

3.1.2. *Fourier Transform Infrared spectroscopy*

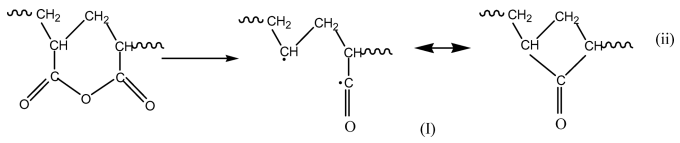
FTIR spectra of polymeric networks have been recorded to study the modification of sterculia and incorporation of poly(AAc) into the networks. FTIR spectra of sterculia and sterculia-*cl*-poly(AAc) were recorded to study the modification of the sterculia and are presented in Figures 2(a and b), respectively. In sterculia-*cl*-poly(AAc) hydrogel, the broad band at 3423.4 cm^{-1} has been observed due to O-H stretching, band at 2926 cm^{-1} due to C–H stretching, band between 2700-2500 cm^{-1} (at 2646.6 cm^{-1}) has been observed due to overtones and combinations of OH in- plane bending and C-O stretching vibrations of –COOH groups of poly(acrylic acid) and peak at 1725.5 cm^{-1} due to C=O stretching vibration of poly(acrylic acid) have been observed apart from usual peaks in the sterculia.

3.1.3. *Thermogravimetric analysis*

The primary thermograms of sterculia gum and sterculia-*cl*-poly(AAc) are presented in Figures 3(a and b), respectively. In each case, weight loss due to entrapped moisture has been ignored and initial decomposition temperature (IDT) has been taken as the temperature where the actual degradation of the polymers started. In the case of sterculia gum**,** initial 13.16% weight loss occurred between 24°C to 100°C, while in the case of the crosslinked polymer, 7% weight loss has been observed. This shows that the sterculia has a more bound water/moisture as compared to the sterculia-*cl*-poly(AAc) matrix. The IDT has been observed at 243°C and 227°C, respectively for sterculia and modified sterculia. A final decomposition temperature (FDT) has been observed at 717°C (3.72% residue) and 525°C (2.72% residue), respectively for sterculia and sterculia-*cl*-poly(AAc) polymer. It is clear from these observations that the thermal stability of the functionalized sterculia decreases. This is further supported by the decomposition temperature at per 10% weight loss which is presented in the Table 2. However, at the early stages, the modification has provided some degree of stability to the polymer. Double stages decomposition mechanisms have been observed from the primary thermograms of sterculia gum and sterculia-*cl*-poly(AAc) polymer, but the second stage of decomposition is not very prominent in a modified polymer matrix. In the case of sterculia-*cl*-poly(AAc) polymers, the actual decomposition stated at 227°C is due to the reason that after losing the associated water due to the moisture, the to dehydration reaction occurring by intramolecular cyclisation of adjacent monomer units to give six-membered anhydride ring structures. The second decomposition stage started at 461°C (after 78.07% weight loss) due to decarboxylation. This may be due to the release of carbon dioxide and water or both, and this reaction has been continued on heating up to 500°C. The temperatures above 200°C, required for intermolecular reaction to occur, probably between -COOH groups are left isolated after random intramolecular dehydration involving adjacent monomer units has occurred at lower temperatures. The intramolecular reaction leads to the formation of six-membered glutaric anhydride type rings as shown Equation (i).



Decarboxylation has also been reported above 200°C. Carbon dioxide evolution, due to anhydride decomposition, can be envisaged as occurring as follows (Equation (ii))



At high temperatures, the postulated intermediate species (I) can be regarded as the source of minor products of degradation, such as carbon monoxide, ketene, ketones and unsaturated compounds, in reactions in which it undergoes fragmentation (37–39).

3.2 Swelling Kinetics of Sterculia-*cl*-poly(AAc) Hydrogels

Swelling of hydrogels is the most important factor for their characterization because a fundamental relationship exists between the swelling of a polymer and the nature of the swelling medium. In order to evaluate the optimum reaction parameters for the synthesis of sterculia-*cl*-poly(AAc) hydrogels, along with structural integrity swelling of the polymers was studied as a function of [AAc], [APS], amount of sterculia and

[NN′-MBAAm] in the polymer matrix.

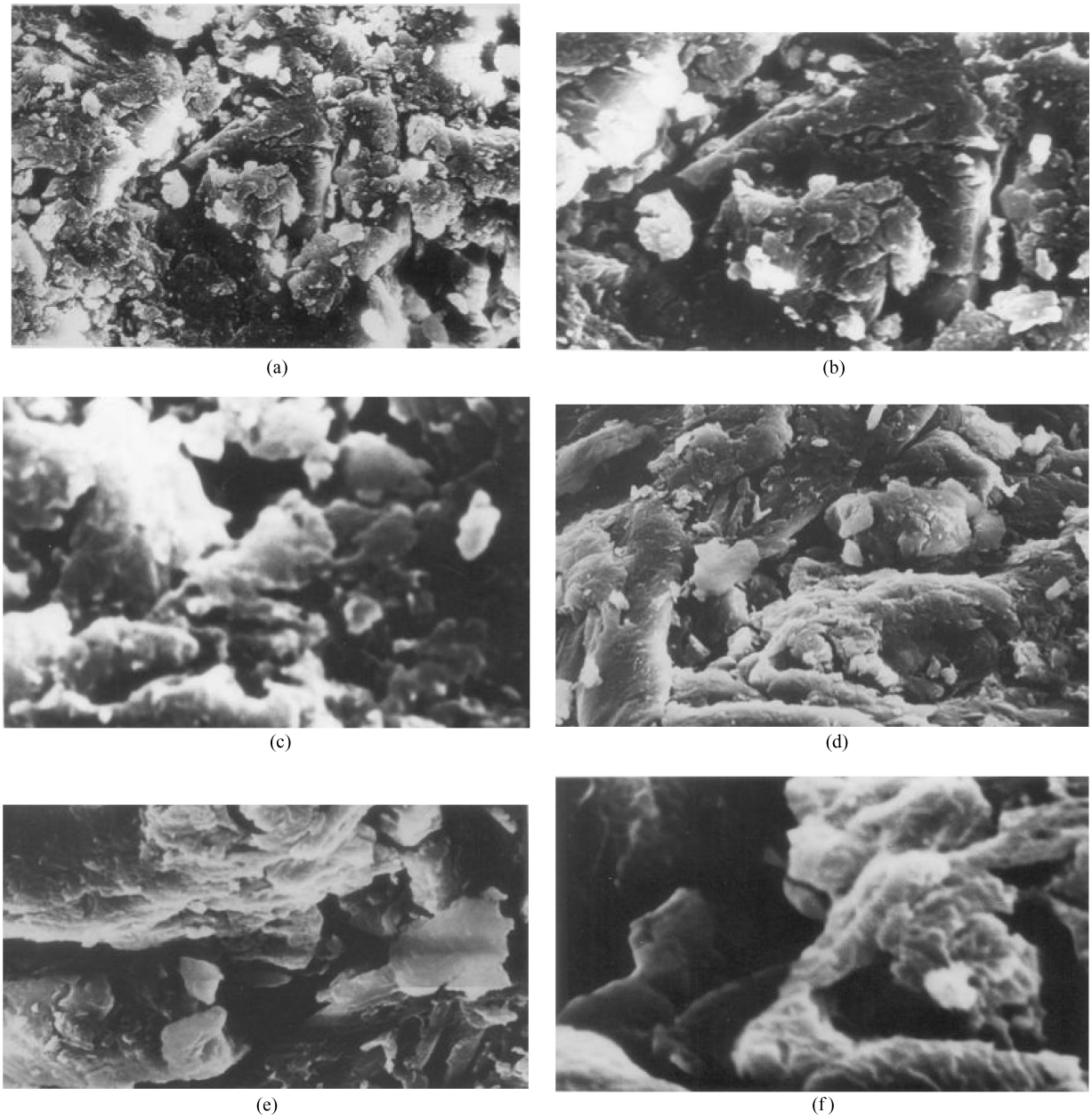


Fig. 1. (a) Scanning electron micrograph of sterculia (Magnification = ×2500) (b) Scanning electron micrograph of sterculia (Magnification = ×5000) (c) Scanning electron micrograph of sterculia (Magnification = ×10,000) (d) Scanning electron micrograph of sterculia-*cl*-poly(AAc) (Magnification = × 2500) (e) Scanning electron micrograph of sterculia-*cl*-poly(AAc) (Magnification = ×5000) (f) Scanning electron micrograph of sterculia-*cl*-poly(AAc) (Magnification = ×10,000).

3.2.1. *Swelling as a function of monomer concentration*

The relationship between the monomer concentration used during the preparation of the polymer matrix and swelling behavior of crosslinked polymers was studied by preparing the polymers with different [AAc]. The polymers were prepared by varying the AAc concentration from 0.291Mol/L to 1.457Mol/L. The swelling of the hydrogels prepared with a different feed monomer concentration was taken and results are presented in Figure 4(1a). It has been observed from the figure that swelling of polymer decreases with increase in monomer concentration during the synthesis of polymers. The swelling of sterculia-*cl*-poly(AAc) hydrogels decreases with increase in feed acrylic acid concentration. This may be due to the fact that cross-linking density increases with an increase in monomer concentration which decreases the pore size of the polymer network. As the concentration of hydrophilic monomers in the gel increases, the swelling should be increased, but in the present case, the network density dominates this factor and results in a decrease in swelling. After 24 h, maximum (18.77 ± 0.73)g/g

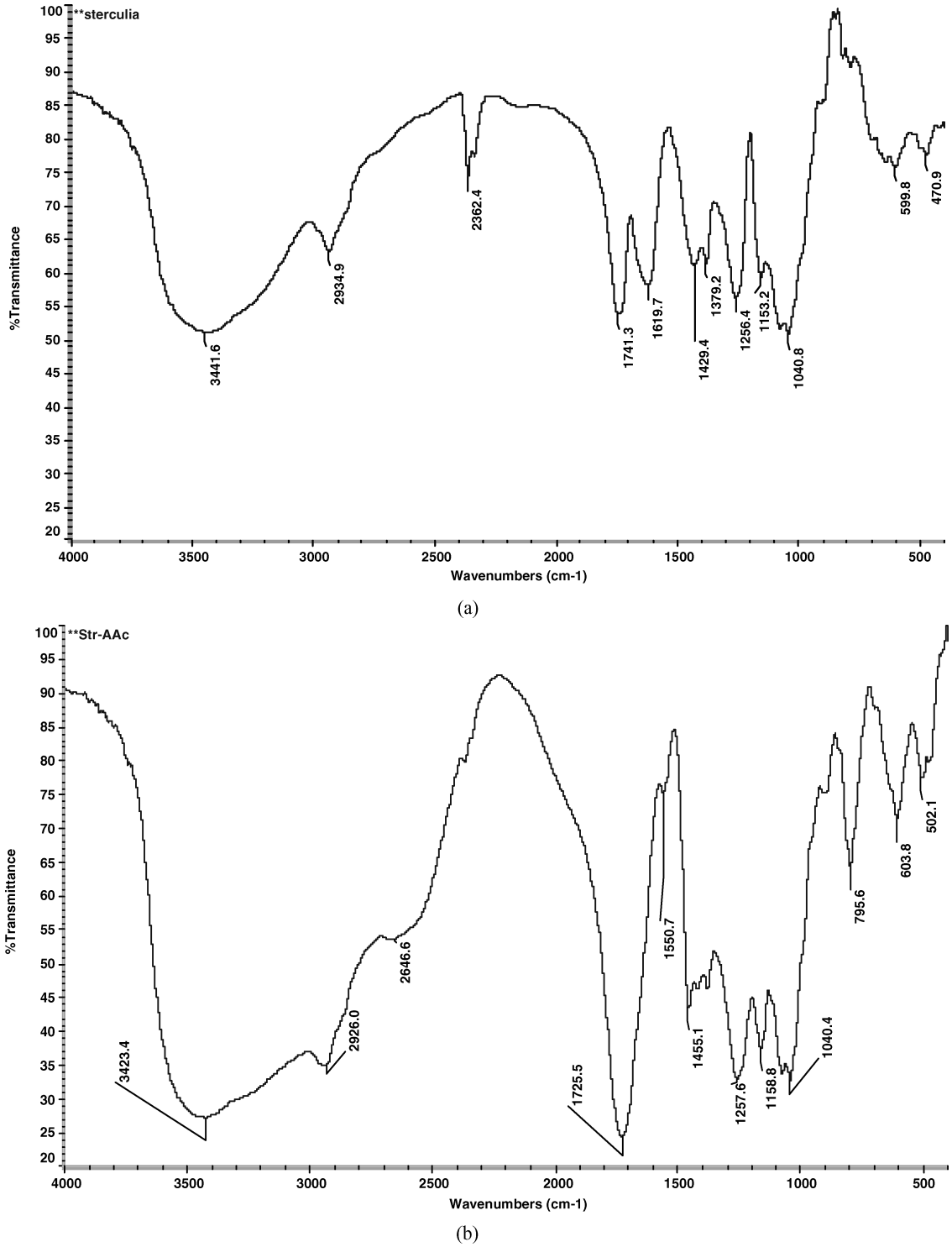


Fig. 2. FTIR spectrum of (a) sterculia gum and (b) sterculia-*cl*-poly(AAc) polymer.

of gel water uptake has occurred for the polymer prepared with 1.166mol/L of monomer concentration. The values of diffusion exponent 'n' and gel characteristic constant 'k' have been evaluated from the slope and intercept of the plot $\ln M_t/M_\infty$ vs. ln t (Figure 4(1b) and the results are presented in the Table 3. The values of diffusion exponent 'n' in most of the cases reflect that the swelling of hydrogels prepared with different monomer concentration occurred through the non-Fickian type diffusion mechanism. When this mechanism is followed for the swelling of hydrogels, the rate of diffusion of water molecules in the hydrogels and rate of relaxation of polymer chains are comparable. The

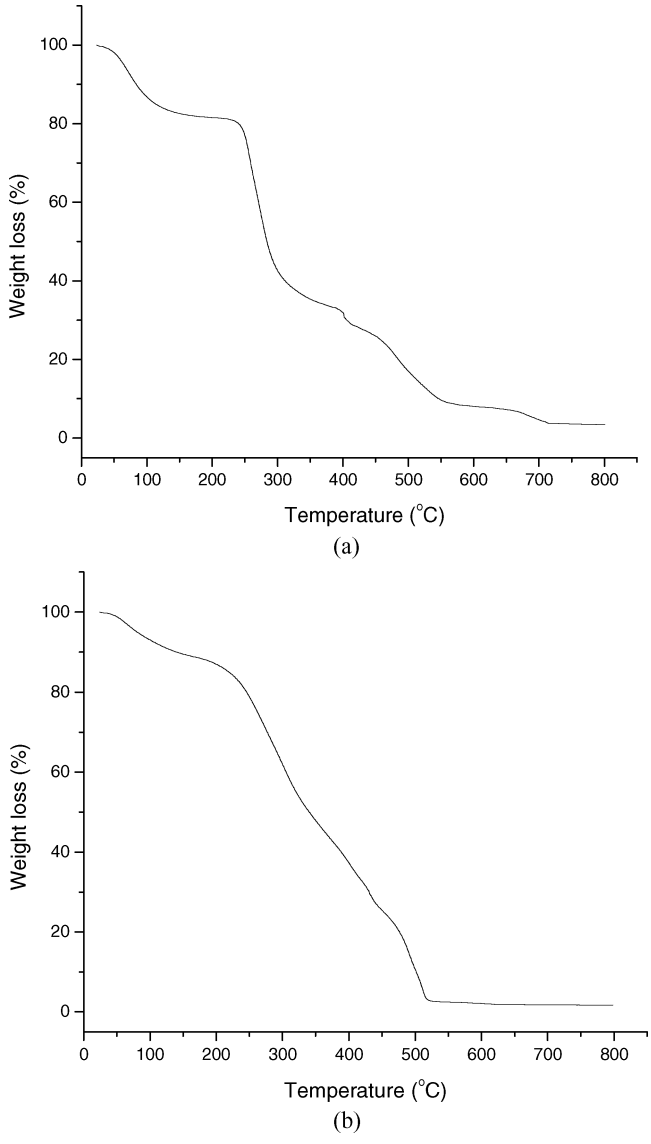


Fig. 3. (a) Primary thermograph of sterculia gum. (b) Primary thermograph of sterculia-*cl*-poly(AAc).

values of the initial and average diffusion coefficients obtained were higher than the late diffusion coefficients which indicate that the initial stages of swelling, the rate of swelling of hydrogels were higher than the latter stages of swelling (Table 3). When a glassy hydrogel is brought into contact with water, water diffuses into the hydrogel and the network spreads out resulting in swelling of the hydrogel. Diffusion involves migration of water into pre-existing or dynamically formed spaces among hydrogel chains. Swelling of the hydrogel involves overall segmental motion resulting, ultimately, in amplified separation between hydrogel chains. With time, polymeric chains relaxation increases with swelling and more and more network expansion occurs. Due to this rate of swelling increases, which is reflects in the higher values of the average diffusion coefficients. However, in the latter stages, when the equilibrium swelling

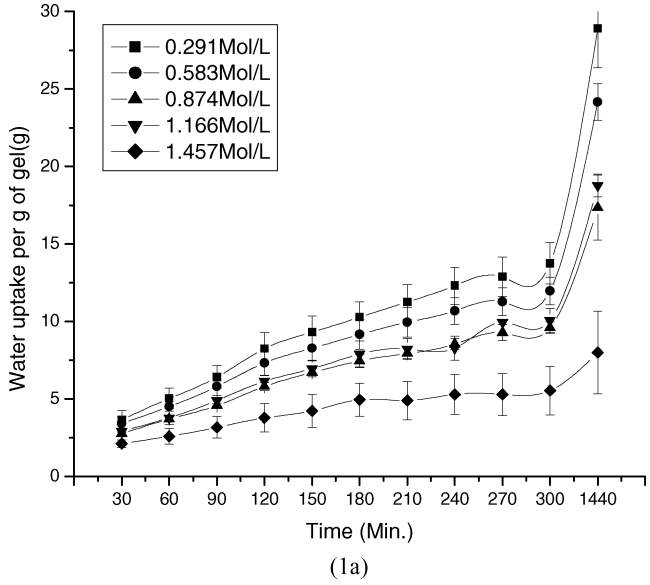


Fig. 4(1a). Effect of [AAc] on swelling kinetics of sterculia-*cl*-poly(AAc) hydrogels in distilled water at 37°C. {Sterculia gum = 1g, [APS] = 4.386 mMol/L, [NN-MBAAm] = 6.486 mMol/L}.

was attained and complete polymeric chain relaxation was about to established, again there was a decrease in rate of swelling, reflecting in the lower value of the late diffusion coefficient of the hydrogels.

3.2.2. *Swelling as a function of initiator concentration*

To observe the effect of initiator concentration on the network formation, the hydrogels were prepared with different [APS] and their swelling was studied thereafter. The [APS]

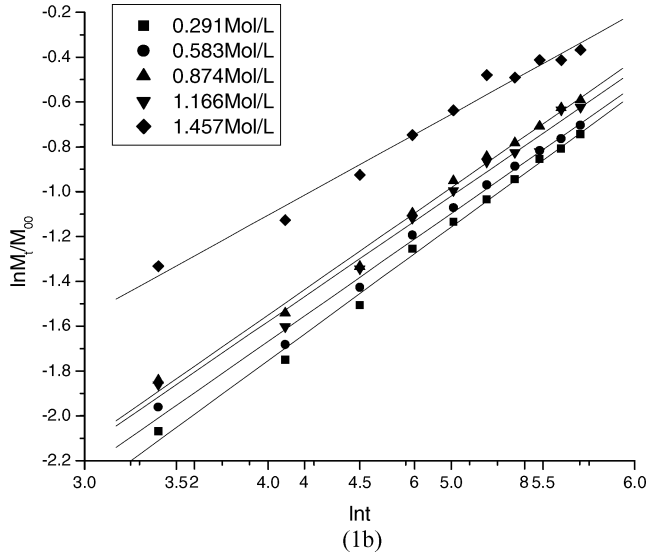


Fig. 4(1b). Plot of $\ln M_t/M_\infty$ versus lnt for the evaluation of diffusion exponent 'n' and gel characteristic constant 'k' for the swelling of sterculia-*cl*-poly(AAc) hydrogels prepared with different [AAc].

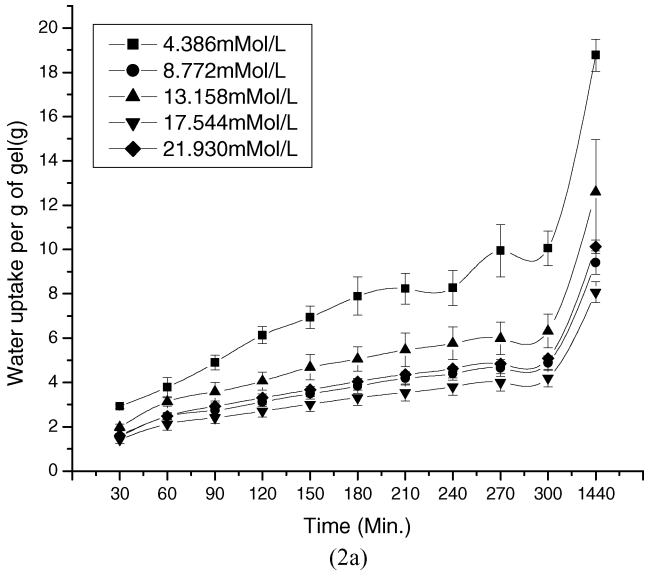


Fig. 4(2a). Effect of [APS] on swelling kinetics of sterculia-*cl*-poly(AAc) hydrogels in distilled water at 37°C. {Sterculia gum = 1g, [AAc] = 1.166 Mol/L,[NN-MBAAm] = 6.486 mMol/L}.

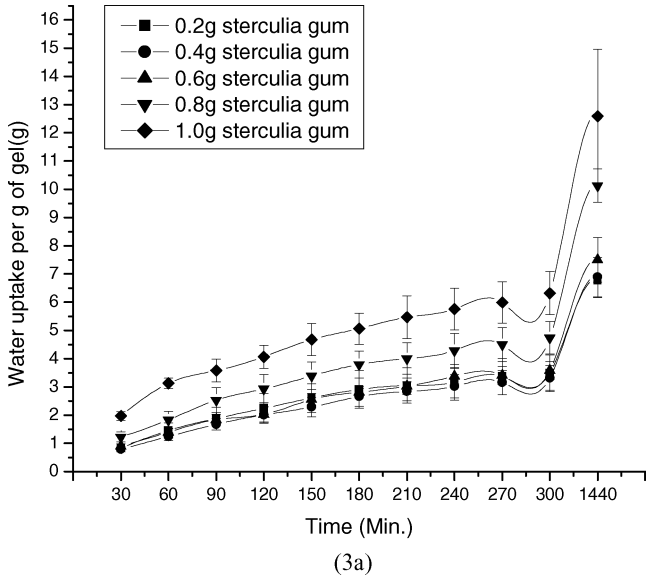


Fig. 4(3a). Effect of amount of sterculia gum on swelling kinetics of sterculia-*cl*-poly(AAc) hydrogels in distilled water at 37°C. {[AAc] = 1.166 Mol/L [APS] = 13.158 mMol/L, [NN-MBAAm] = 6.486mMol/L}.

was varied from 4.386 to 21.930mMol/L during their synthesis. The results are presented in Figure 4(2a)). It has been observed from the results of swelling that the amount of water uptake by the per gram of hydrogels decreases with an increase in [APS]. After 24 h swelling, maximum (12.59 ± 2.38) g water has been taken by the hydrogels prepared with 13.158 mMol/L of [APS]. The values of the diffusion exponent were obtained less than 0.5 and hence, the swelling of the hydrogels occurred through the Fickian type diffusion mechanism when the polymers were prepared with different [APS] (Figure 4(2b), Table 3). In this diffusion mechanism, the rate of polymer chain relaxation is much slower than the rate of diffusion of water through the polymer matrix. The values of the initial and average diffusion coefficients have been obtained higher than late diffusion coefficients which indicate that initially the rate of

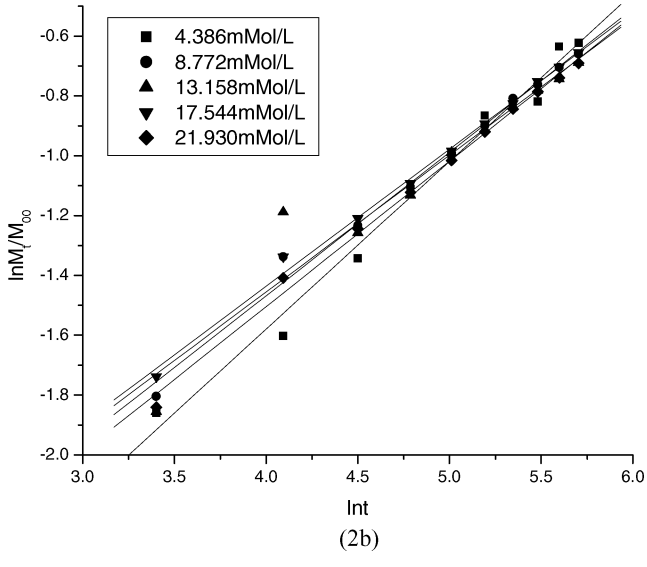


Fig. 4(2b). Plot of $\ln M_t/M_\infty$ vs. lnt for the evaluation of diffusion exponent 'n' and gel characteristic constant 'k' for the swelling of sterculia-*cl*-poly(AAc) hydrogels prepared with different [APS].

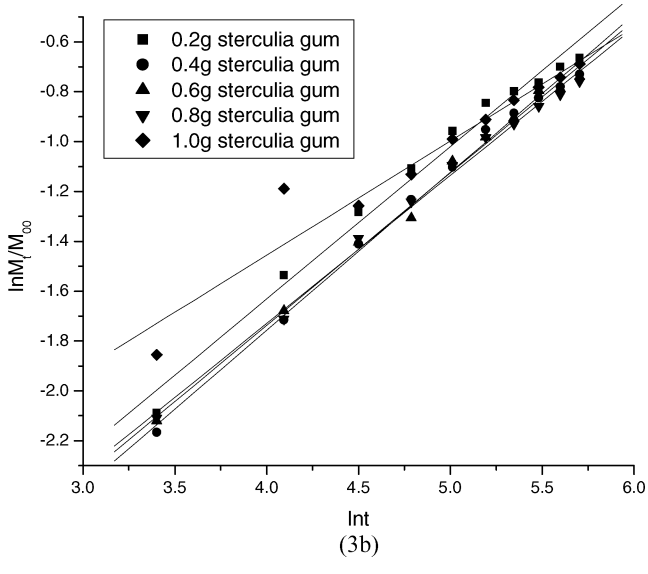


Fig. 4(3b). Plot of $\ln M_t/M_\infty$ vs. lnt for the evaluation of diffusion exponent 'n' and gel characteristic constant 'k' for the swelling of sterculia-*cl*-poly(AAc) hydrogels prepared with a different amount of sterculia gum.

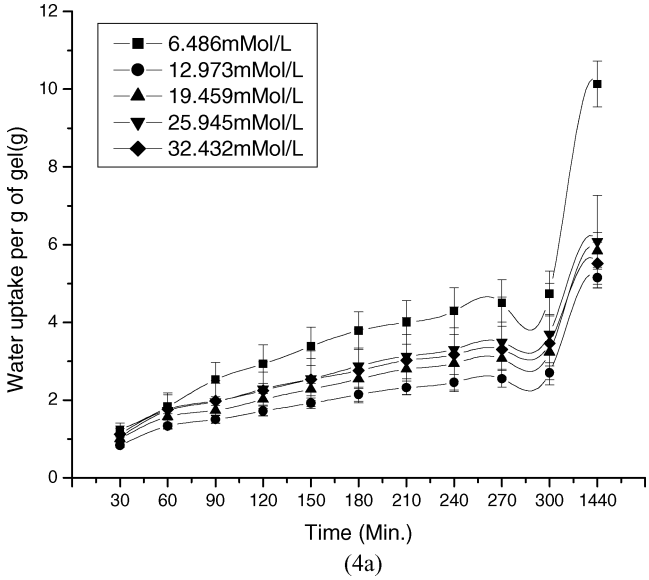


Fig. 4(4a). Effect of [N,N′-MBAAm] on swelling kinetics of sterculia-*cl*-poly(AAc) hydrogels in distilled water at 37°C. {Sterculia gum = 0.8g, [AAc] = 1.166 Mol/L, [APS] = 13.158 mMol/L}.

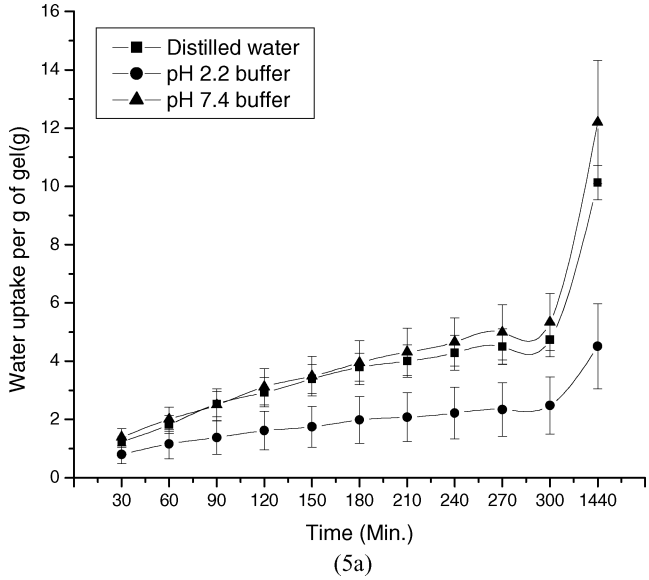


Fig. 4(5a). Effect of pH on swelling kinetics of sterculia-*cl*-poly(AAc) hydrogels at 37°C. {Sterculia gum = 0.8g, [AAc] = 1.166 Mol/L,[APS] = 13.158 mMol/L, [NN-MBAAm] = 6.486 mMol/L}.

swelling of polymer matrix was faster than the latter stages of swelling because of the same reason as cited above.

3.2.3. *Swelling as a function of amount of sterculia gum*

The sterculia-*cl*-poly(AAc) hydrogels were prepared by varying sterculia gum from 0.2 g to 1.0 g. Swelling studies were carried out in distilled water at 37°C and the results are presented in Figure 4(3a). The swelling of the polymers increases with the increase in sterculia contents in the polymer matrix. This is probably due to the reason that a higher degree of gum hydration has occurred which has increased the number of intimate contacts between particles of gum and water and led to high swelling (40). The increase in swelling can also be explained by the fact that as the polymers were prepared with a constant crosslinker amount while increasing the amounts of sterculia, this would result in a lower percentage of crosslinker in the formulation and ultimately

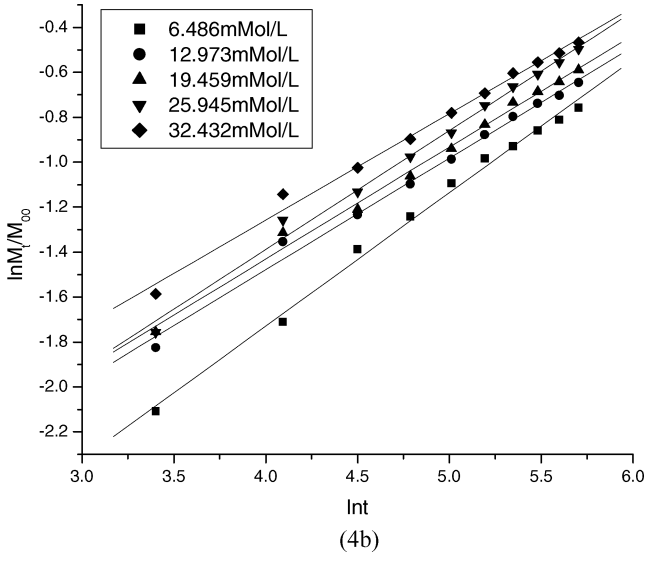


Fig. 4(4b). Plot of $\ln M_t/M_\infty$ vs. lnt for the evaluation of diffusion exponent 'n' and gel characteristic constant 'k' for the swelling of sterculia-*cl*-poly(AAc) hydrogels prepared with different [NN-MBAAm].

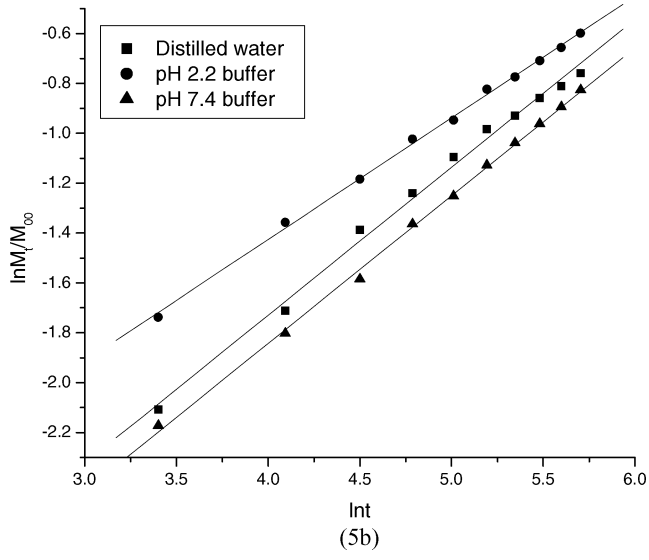
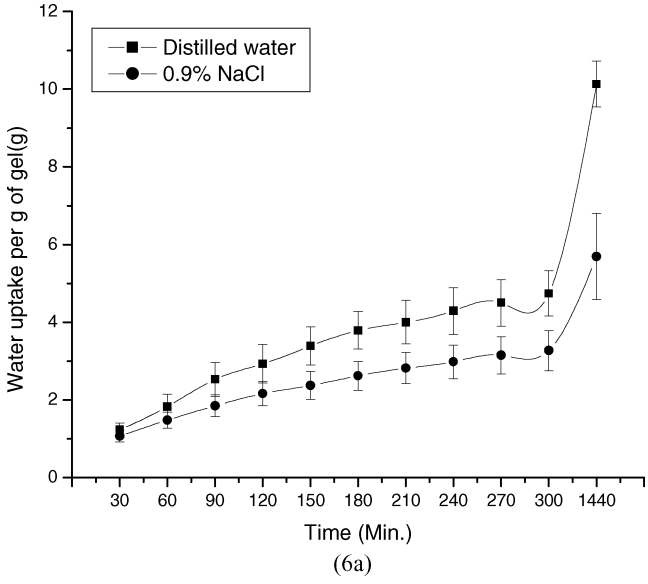


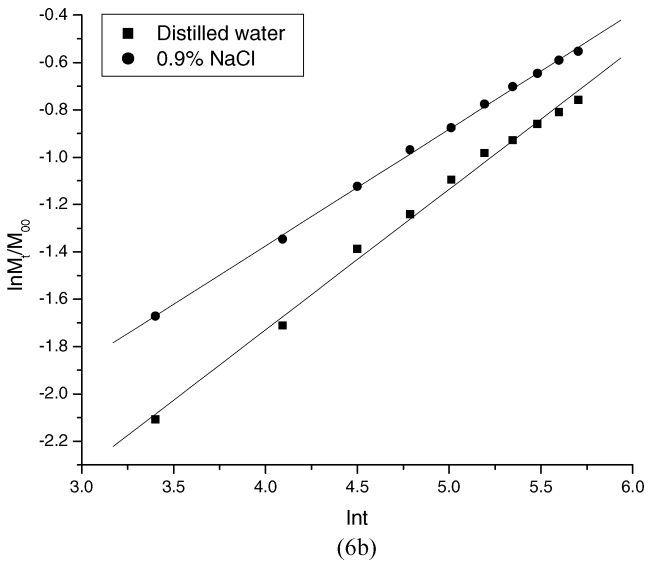
Fig. 4(5b). Plot of $\ln M_t/M_\infty$ vs. lnt for the evaluation of diffusion exponent 'n' and gel characteristic constant 'k' for the swelling of sterculia-*cl*-poly(AAc) hydrogels in different mediums.



(6a)

Fig. 4(6a). Effect of [NaCl] on swelling kinetics of sterculia-*cl*-poly(AAc) hydrogels at 37°C. Sterculia gum = 0.8 g, [AAc] = 1.166 Mol/L, [APS] = 13.158 mMol/L, [NN-MBAAm] = 6.486 mMol/L.

provide another reason for the increase in swelling. After 24 h, maximum (10.13 ± 0.59) g water uptake has occurred for the polymers prepared with 0.8 g sterculia gum. The diffusion of water into hydrogels has occurred through a non-Fickian diffusion mechanism, when polymers were prepared with different sterculia contents (Figure 4(3b). The values of the initial and average diffusion coefficients have been obtained higher than late diffusion coefficients which indicate that initially the rate of swelling of polymer matrix was faster than the latter stages of swelling because of the same reason as cited above (Table 3).



(6b)

Fig. 4(6b). Plot of $\ln M_t/M_\infty$ vs. lnt for the evaluation of diffusion exponent 'n' and gel characteristic constant 'k' for the swelling of sterculia-*cl*-poly(AAc) hydrogels in 0.9% salt solution.

3.2.4. *Swelling as a function of [NN-MBAAm]*

In order to study the effect of [NN-MBAAm] on network formation, the swelling of the hydrogels taken were prepared by varying the crosslinker concentration from 6.486 mMol/L to 32.432 mMol/L. The results of swelling of sterculia-*cl*-poly(AAc) are presented in Figure 4(4a). It has been observed from the figure that swelling decreases with the increase in the concentration of crosslinker in the polymer matrix. This may be due to the fact that more crosslinking concentration causes the higher crosslinking and decreases the pore size in the networks and consequently, has formed the highly crosslinked rigid structure which cannot be expanded and cannot hold a large quantity of water. The maximum amount of water uptake (10.13 ± 0.59) g occurred in the case of the polymer prepared with 6.486 mMol/L of [NN-MBAAm]. In most cases, when the polymers were prepared with a different crosslinker concentration, the values of the diffusion exponent 'n' obtained had less than 0.5 which shows that swelling occurred through Fickian diffusion mechanism Figure 4(4b). The values of the various diffusion coefficients are presented in Table 3. It has been observed from the table that the values obtained for initial and average diffusion coefficients are higher than the late diffusion coefficients which indicate that initially the rate of swelling of polymer matrix was faster than the latter stages of swelling (Table 3).

3.2.5. *Swelling as a function of pH and [NaCl] of the swelling medium*

At the optimum reaction conditions, polymers were synthesized and were used to study the effect of reaction medium on the swelling. The swelling of polymers in different pH and salt solution are presented in Figure 4(5a) and 4(6a), respectively. It has been observed from Figure 4(5a) that more swelling has been observed in a pH 7.4 buffer as compared to a pH 2.2 buffer. After 24 h, a maximum (10.13± 0.59)g, (4.51 ± 1.46) g and (12.20 ± 2.12) g water uptake occurred in distilled water, pH 2.2 buffer, pH 7.4 buffer respectively. Under acidic pH values, most of the carboxylate anions are protonated, so the main anion-anion repulsive forces are eliminated and consequently, swelling values are decreased. At higher values of pH, some of carboxylate groups are ionized and the electrostatic repulsion between COO- groups causes an enhancement of the swelling capacity. The swelling mechanism has been observed non-Fickian in pH 7.4 buffer (Figure 4(5b). To study the effect of salt concentration, swelling of the hydrogels has been carried out in 0.9% NaCl at 37°C and the amount of water uptake by per gram of gel in salt solution has been observed less as compared to the distilled water (Figure 4(6a). In distilled water and 0.9% [NaCl] per gram of the gel, the maximum (10.13 ± 0.59) g, and (5.69 ± 1.11) g water was taken, respectively. The swelling of the sterculia-*cl*-poly(AAc)

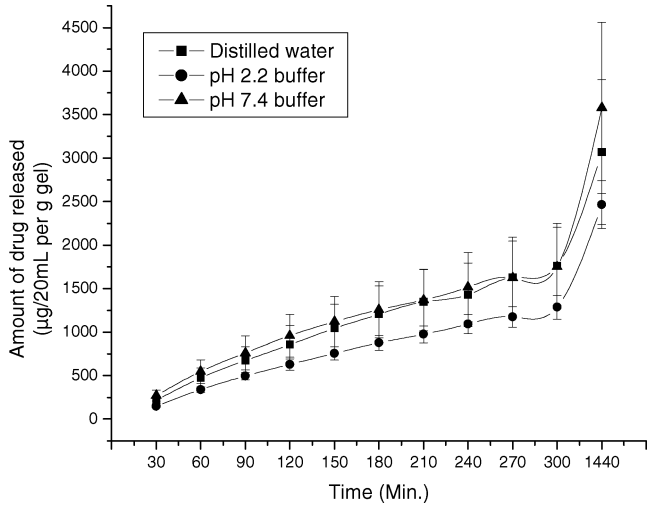


Fig. 5(1). Release profile of methotrexate from drug loaded sterculia-*cl*-poly(AAc) hydrogels in different medium at 37°C. {Sterculia gum = 0.8g, [AAc] = 1.166 Mol/L, [APS] = 13.158 mMol/L, [NN-MBAAm] = 6.486mMol/L}.

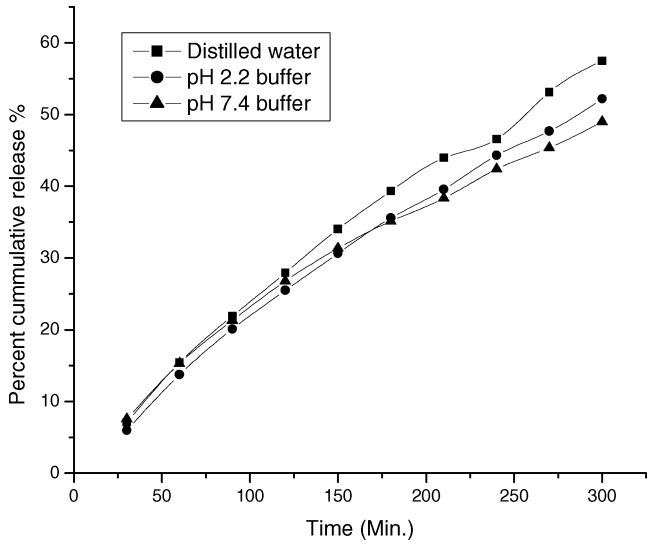


Fig. 5(2). Percentage of total drug release from drug loaded sterculia-*cl*-poly(AAc) hydrogels in different medium at 37°C.

hydrogel in 0.9% NaCl solution was substantially decreased compared to the distilled water. This is due to a screening effect of the additional cations causing a non-perfect anion-anion electrostatic repulsion, leading to a decreased osmotic pressure (ionic pressure) difference between the hydrogel network and the external solution (41).

The values of the gel characteristic constant are different in different swelling mediums, which indicate that the polymer matrix shows different behavior in these mediums. In a salt solution, the hydrogel shows a Fickian type diffusion mechanism (Figure 4(6b). The values of the initial diffusion coefficient, average diffusion coefficient and late diffusion coefficients for the swelling of sterculia-cl-poly(AAc) hydrogels are presented in Table 3. In each swelling medium, values of the initial and average diffusion coefficients have been observed as higher than the late diffusion coefficient. It means that in the early stages, the rate of diffusion of water molecule in the polymer matrix was more rapid than the late stages of diffusion.

3.3 *In Vitro* Release Dynamics of Methotrexate

A *in vitro* release profile of methotrexate from per gram of the drug loaded hydrogels has been studied in distilled water, pH 2.2 buffer and pH 7.4 buffer and the results are presented in Figure 5(1). It has been observed from the figure that the amount of drug released from the per gram of the gel is higher in pH 7.4 and in distilled water than in pH 2.2 buffer. 50% of the total release of the drug in distilled water, pH 2.2 buffer and in pH 7.4 buffer, respectively occurred in 252, 289 and 315 min (Figure 5(2). From the slope and intercept of the plot of ln M_t/M_∞ vs. ln t, the diffusion exponent 'n' and gel characteristic constant 'k' has been obtained for the release of methotrexate from the hydrogels (Figure 5(3). The diffusion exponent 'n' have 0.883, 0.910 and 0.787 values and the gel characteristic constant 'k' have 0.3909×10^{-2}, 0.308×10^{-2} and 0.579×10^{-2} values in distilled water, pH 2.2 buffer and pH 7.4 buffer. It is clear from the values of the 'n' that the release of drug from the hydrogels occurred through a non-Fickian type diffusion mechanism in all three mediums. The values of the diffusion coefficients for the release of drug from the hydrogels in a different pH buffer are obtained from the Figures 5(4) and 5(5), and are presented in Table 4. The values obtained for the initial and average diffusion coefficients are higher than the late diffusion coefficient. These values show that at

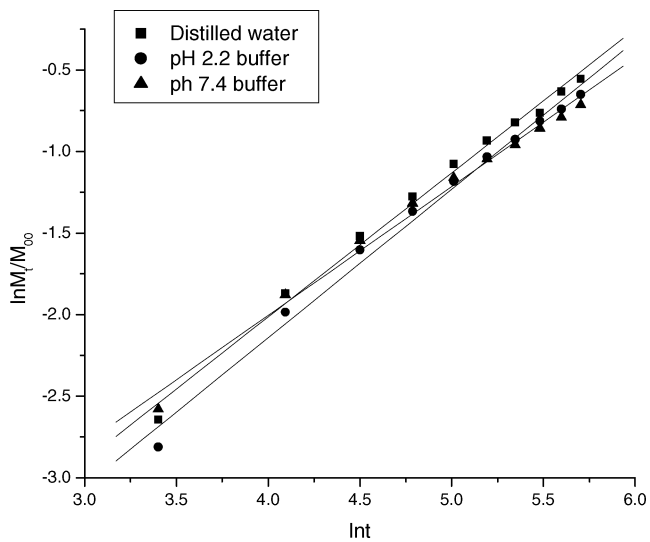


Fig. 5(3). Plot for evaluation of diffusion exponent 'n' and gel characteristic constant 'k' for the drug release from sterculia-*cl*-poly(AAc) hydrogels in different medium at 37°C.

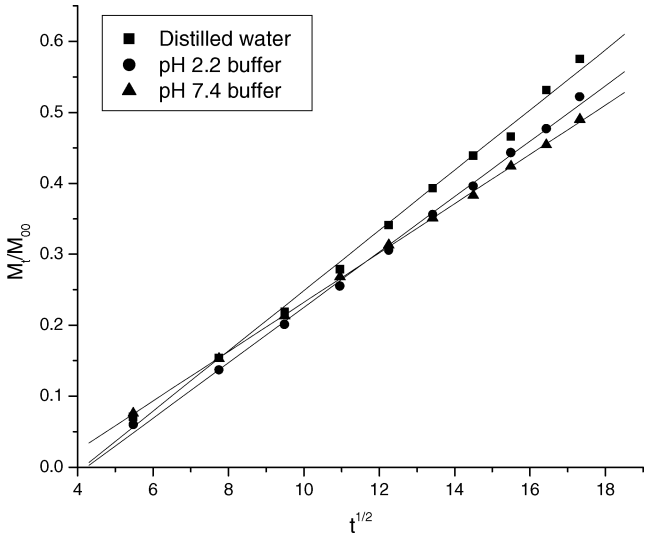


Fig. 5(4). Plot of M_t/M_∞ vs. $t^{1/2}$ for the evaluation of initial diffusion coefficient (D_i) for the drug release from sterculia-*cl*-poly(AAc) hydrogels in different medium at 37°C.

the early stages, the rate of diffusion of drug from the polymer is higher than the rate of diffusion in the later stages.

Chen, Liu and Zhuo (42) have determined the biodegradability of konjac glucomannan and acrylic acid hydrogels by enzymatic hydrolysis carried out in pH 7.4 buffer solution at 37°C with Cellulase 0240. To test the specificity to enzymatic degradation, pancreatin, which occurs in the upper gastrointestinal tract, has been used as positive control, and a buffer solution without enzymes has been used as negative control. It has been observed that gel could not degraded in a pH 7.4 buffer solution, or by pancreatin, but it couldbe degraded by Cellulase E0240, which contains

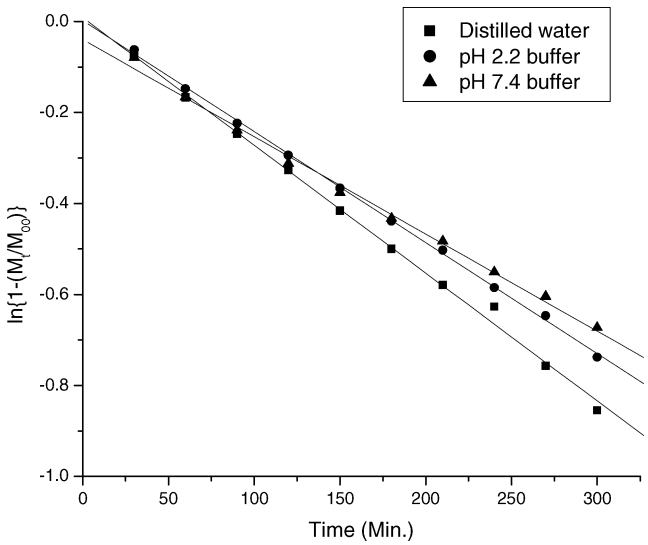


Fig. 5(5). Plot of $\ln\{1-(M_t/M_\infty)\}$ vs. time for the evaluation of late diffusion coefficient (D_L) for the drug release from sterculia-*cl*-poly(AAc) hydrogels in different medium at 37°C.

β-glycosidases, and gels have retained the biodegradability characters of konjac glucomannan (43). They have also observed that the release rate of the drug from the polymer increases with the increase in concentration of Cellulase E0240. In other similar *in vivo* biodegradability studies, hydrophobically modified hydrogels have been observed as stable in the stomach, but degradable by an anerobes present in the colon. The extent of degradation has been considerably related to the equilibrium degree of swelling. The factors influencing the swelling degree have been shown to influence the *in vivo* degradation of the gels (44). Using the same analogy, we can say that sterculia gum-acrylic acid based hydrogels can also be degraded in the colon and can be used for the colon cancer drug delivery system.

4 Conclusions

It is concluded from the foregone discussion that composition of hydrogels, synthetic reaction conditions and the nature of swelling medium affects the swelling of hydrogels prepared from the modification of sterculia with poly(acrylic acid). The increase in feed monomers concentration and increase in crosslinker concentration during the synthesis of polymers have increased the network density in the hydrogels, which has decreased the swelling of the hydrogels. An increase in pH of the swelling medium enhanced the swelling of the hydrogels. An increase in salt concentration in the swelling medium decreased the swelling of the hydrogels. It has also been concluded from the swelling in a different pH buffer that the sterculia-*cl*-poly(AAc) hydrogels are pH responsive because more water uptake has been observed in 7.4pH buffer as compared to the 2.2 pH buffer. Therefore, the hydrogels can be used for delivery of drug to the colon. It is also concluded from the drug release dynamics that drug release from the hydrogels occurred through a non-Fickian type diffusion mechanism in all three mediums. In this mechanism, the rate of diffusion of water molecules in the hydrogels and rate of relaxation of polymer chains are comparable. Further, values of the diffusion coefficients reflect that in the early stages the rate of release of drug from the polymer has been observed to be higher than in the latter stages and drug release has occurred in a controlled manner. Hence, these hydrogels can be exploited for developing the controlled and sustained drug delivery systems anticancer drug to the colon.

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