PREPARATION OF POLY (METHACRYLAMIDE-CO-ACRYLIC ACID) MICROSPHERES FOR CONTROLLED RELEASE OF TRIPROLIDINE HYDROCHLORIDE

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ABSTRACT

pH-Sensitive poly (methacrylamide-*co*-acrylic acid) microspheres loaded with Triprolidine hydrochloride, water-soluble drug have been prepared by the dispersion polymerization method. Triprolidinine Hydrochloride (TPH) an antibiotic drug, has been loaded in situ into the cross-linked poly (MAAm-*co*-AA) particles. Plain as well as drug loaded microparticles have been characterized by differential scanning calorimetry (DSC), X-Ray Diffraction (X-RD) and Scanning Electron Microscopy (SEM). DSC and X-RD studies have indicated a molecular level dispersion of the drug *in situ* loading and SEM pictures have shown the formation of spherical and oval-shaped particles. *In vitro* release of TPH from the cross-linked poly (MAAM-*co*-AA) particles has been carried out in both 7.4 pH and 1.2 pH buffer medium. Both encapsulation efficiency and release patterns are found to depend on the nature of the cross-linking agent, amount of cross-linking agent and the amount of drug loaded. *In vitro* release studies indicated the release of TPH up to 10 hours.

KEY WORDS: Methacrylamide, Acrylic acid, dispersion polymerization, microspheres, *In vitro* release studies.

1. INTRODUCTION

The success of using synthetic polymers as biomaterials (Ramesh Babu, 2006; Agnihotri, 2004; Hiratania, 2004; Kim, 1994; Nivasu, 2004), primarily relies on their wide range of mechanical properties and transformation processes that allow a variety of shapes to be easily obtained at low production costs. On the contrary, biological polymers possess good biocompatibility although their mechanical properties are often poor. In this sense, functionalized crosslinked polymers have attracted attention as carrier networks in a wide variety of medical and biological applications such as affinity, immobilization technologies and drug-delivery systems (Nivasu, 2004; Ramesh Babu, 2007; Edman, 1980; Mallikarjuna Reddy, 2008), though the necessity of preserving biological properties complicates their process ability. The most frequently used polymers for the production of particles used in the CR of drugs are acrylic derivatives. In order to increase the hydrophilicity of the particle surface, attempts have been made to employ copolymerization of alkylmethacrylate with various acrylic acid derivatives such as acrylamide and acrylic acid (Lakshmi Narayana Reddy, 2010; Schwarz and Mehnert, 1997). The pH-sensitivity of hydrogels is due to the presence of weakly acidic and/or basic functional groups on the polymer backbone. Their water-uptake properties are attributed to the ionization of functional groups, which depends upon the pH and ionic strength of the external medium where the hydrogel is placed, thus making the system pH-sensitive. Particularly, synthetic polymers like poly (methyl methacrylate) (Shantha and Harding, 2000), poly(acrylic acid) (Bettini, 1995), poly(N,N-iso-propylacrylamide) (Bettini, 1995), and natural polysaccharide such as chitosan (Ramakissoon-Ganorkar, 1999) have been used as pH-sensitive drug delivery systems.

In the last decade, the design of polymeric matrices for biomedical applications has been extensively investigated, thus leading to new concept in the treatment of human diseases. Although numerous reports have already been published on the swelling behavior of pH sensitive polymeric matrices but a thorough survey of the literature reveals that copolymeric system involving methacrylamide and acrylic acid has not been taken into consideration for antibiotic drug delivery. Methacrylamide is a water-soluble monomer that has thermosensitivity (Qu, 2000), biocompatibility (Avoce, 2003), and is used to prepare drug-release devices by attacking hydrophobic monomers to hydrophilic matrices (Kissel, 2001). Similarly, polyacrylic acid is known to be a good mucoadhesive and may increase the transit time of formulation (Krishna Rao, 2006). The polymers, composed of acrylic acid, have the ability to absorb a

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large amount of water and are used in many applications including ion exchange resins, personal hygiene products, membranes for hemodialysis, ultra filtration, and controlled release devices (Hornof, 2003; Am Ende and Peppas, 1996; Gudeman and Peppas, 1995 ; Elliott, 2004; Buchholar and Grahan, 1998; Krisnaiah, 1998).

We report here *in vitro* CR data for TPH, which is known as a antibiotic drug, through the copolymeric matrices of microspheres prepared from MAAm and acrylic acid in different compositions. The polymeric matrices are also characterized. The effect of acrylic acid content, crosslinking agent, and drug concentration on the release rates of TPH has been investigated.

2. EXPERIMENTAL

Materials

The monomers Methacrylamide and Acrylic Acid, the crosslinks N, N-methylene *bis*-acrylamide, the initiators Sodium Lauryl Sulfate, Potassium per sulphate were purchased from s.d.fine chemicals, Mumbai, India. Triprolidine Hydrochloride was given as gift sample from waksman salesmen pharmaceuticals, Anantapur. The monomer MMAm was distilled in methanol to remove the inhibitor, while AAc was vaccum distilled at 47°C /7mm Hg. Double distilled water was used throughout the studies.

Synthesis of poly (Methacrylamide-co-Acrylic Acid) microspheres

Sodium Lauryl sulfate (1g) was dissolved in 75mL of water taken in a three necked round bottom flask equipped with a mechanical stirrer, a condenser and a gas inlet to maintain the inert nitrogen atmosphere. The flask was immersed in an oil bath with a thermostatic control to maintain the desired temperature accurate to ±0.1°C. The solution was stirred at 800 rpm speed until it became clear and 100 mg of potassium per sulfate was added. Required amount of MAAm, AA, the crosslinking agent NNMBA and Triprolidine hydrochloride were dissolved separately in 25mL of water. This mixture was added to the reaction mixture drop wise using a dropping funnel and the reaction was continued for 8 h at 70°C to obtain the maximum yield. The reaction mixture was taken out after 8 h and added to 1% calcium chloride solution drop wise to break the emulsion. Particles were then isolated by centrifuging the product at the rotor speed of 12,000 rpm, washed with water and dried under vacuum at 40°C for 24 h. The blank microspheres without drug incorporation were prepared by above method.

Loading of Triprolidine Hydrochloride :- Triprolidine Hydrochloride was loaded into polymeric microspheres by two methods. In the first method (method-I), drug was added during *in situ* polymerization, i.e., drug was mixed with monomer, crosslinking agent, initiator, and the mixture was added to the polymerization medium. In the second method (method-II), drug was loaded into polymeric microspheres by keeping the weighed amount of microspheres in methanolic drug solution of known concentration and evaporating methanol under vacuum. During this process, drug in the solvent will absorb into the surface as well as adsorbed onto the microspheres.

Estimation of drug loading and encapsulation efficiency

Loading efficiency of Triprolidine Hydrochloride in the microspheres was determined spectrophotometrically. About 10mg of the drug-loaded microspheres were placed in 10mL of buffer solution and stirred vigorously for 24 h to extract the drug from the microspheres. The solution was filtered and assayed by UV-spectrophotometer (model Labindia-3000$^+$, Mumbai, India) at the fixed λ_{max} value of 200 nm. The results of % drug loading and encapsulation efficiency were calculated, respectively using Eqs. (1) and (2) respectively.

$$\% \text{ Encapsulation efficiency} = \left(\frac{\text{Actual loading}}{\text{Theoretical loading}}\right) \times 100 \quad (1)$$

$$\% \text{ Drug loading} = \left(\frac{\text{Amount of drug in microspheres}}{\text{Amount of microspheres}}\right) \times 100 \quad (2)$$

Conversion of copolymer

The yield of the copolymeric microspheres was determined gravimetrically. After copolymerization, the latex solution was added to 1% calcium chloride solution and centrifuged to isolate the particles from the mixture. The copolymeric microspheres were washed several times successively with distilled water and then with methanol solvents to remove the remaining monomer and initiator, and then dried in a vacuum oven at 50°C till attainment of constant weight. The conversion of monomers was calculated as:

$$\text{Conversion} = (W/M) \times 100 \quad (3)$$

Where W is the weight of the dry copolymer obtained from the latex sample and M is the weight of the monomer taken. The yield of copolymeric microspheres varied between 80 and 85% for various formulations prepared in this study.

In-vitro release studies :- In-vitro release studies have been carried out by performing the dissolution experiments using a tablet dissolution tester (Lab India, Mumbai, India) equipped with eight baskets. Dissolution rates were measured at 37°C under 100 rpm speed. Drug release from the microspheres were studied in both an intestinal (1.2 and 7.4 pH phosphate buffer media) fluids like atmosphere. At regular intervals of time, sample aliquots were withdrawn and analyzed by UV spectrophotometer (Model Labindia-3000$^+$, Mumbai, India) at the fixed λ_{max} value of 200 nm.

3. RESULTS AND DISCUSSION

Differential scanning calorimetry (DSC)

DSC tracings of Plain poly (MAAm-co-AA) microspheres (A) pure Triprolidine Hydrochloride (PTH) drug (B), drug-loaded microspheres (C) and plain microspheres are displayed in Fig.1. The onset-melting peak of TPH was observed at 122.97° C. However, no characteristic peak of TPH was observed in DSC curves of the drug-loaded microspheres, suggesting that drug is molecularly dispersed in the polymer matrix.

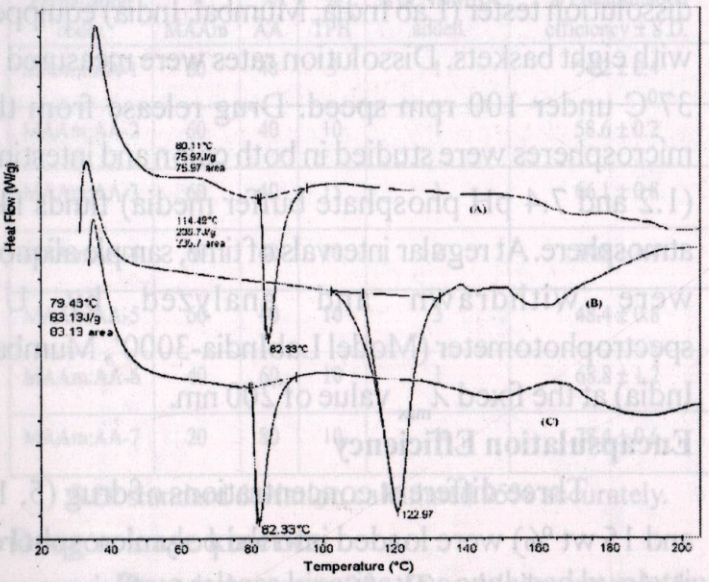


Fig.1: DSC Thermographs of (A) Plain poly (MAAm-*co*-AA) microspheres (B) Pure TPH Drug and (C) Drug loaded poly (MAAm-*co*-AA) microspheres

X-ray diffraction (X-RD) studies

X-RD analysis provide a clew about crystallinity of the drug in the crosslinked microspheres. X-RD patterns recorded for the placebo polymeric microparticles (Fig.2.A), plain TPH (Fig.2.b) and drug-loaded microspheres (Fig.2.c) and are compared in Fig.2. The TPH peaks are observed at 2θ of 15°, 16°, 20°, 21°, 22°, 27°, 28°, 29° and 31° suggesting its crystalline nature. But, these peaks are not found in TPH loaded microspheres and in pristine microspheres indicating that drug is dispersed at a molecular level in the polymer matrix.

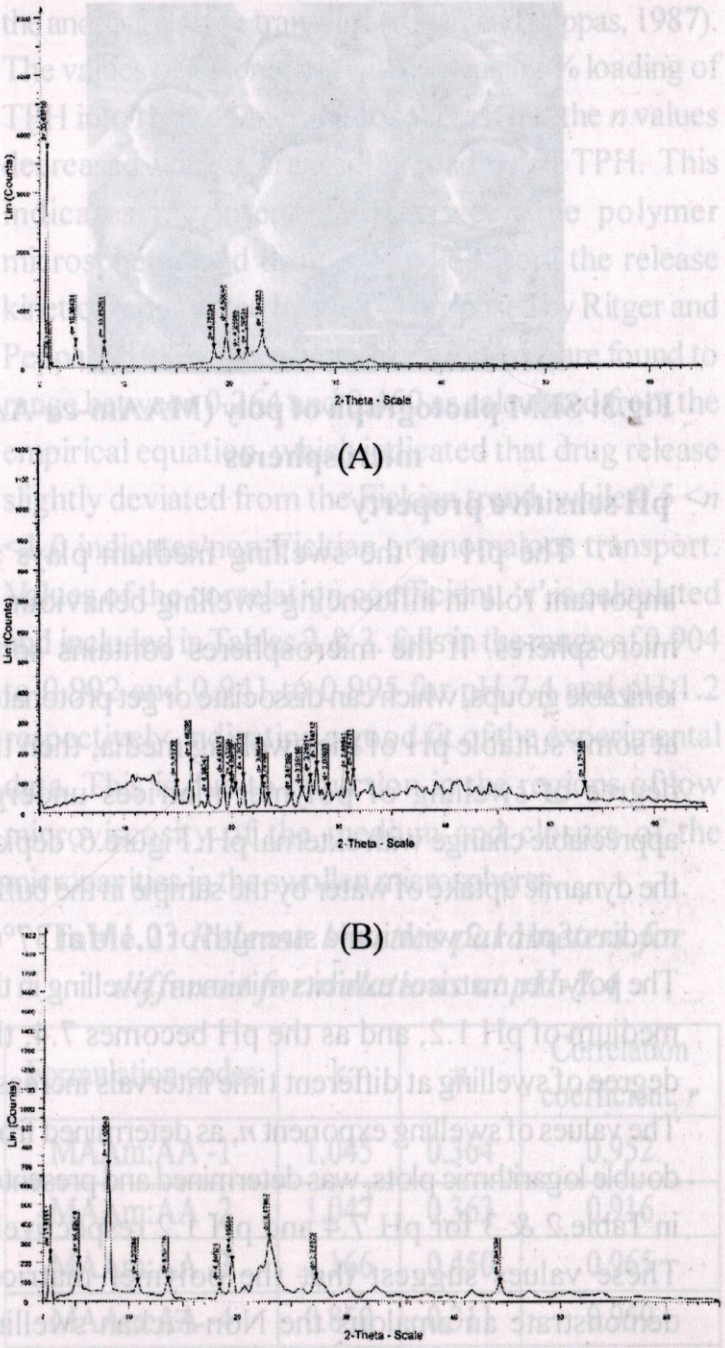


Fig. 2: X-RD of (A) Pure MAAm-AA microspheres (B) Pure TPH drug and (C) Drug loaded MAAm-AA microspheres

Scanning electron microscopic (SEM) studies

Scanning electron microscopy has been used to confirm the formation of spherical structures of the microspheres. SEM micrograms of poly (MAAm-*co*-AA) microspheres are displayed in Fig.3. The microspheres were coated with gold colour and subjected to SEM, which observed the formation structure of the microspheres with hydrophilic shell. The microspheres are spherical in shape and slightly porous in nature. The porous nature of these microspheres might be due to the mechanical state which they are forming.

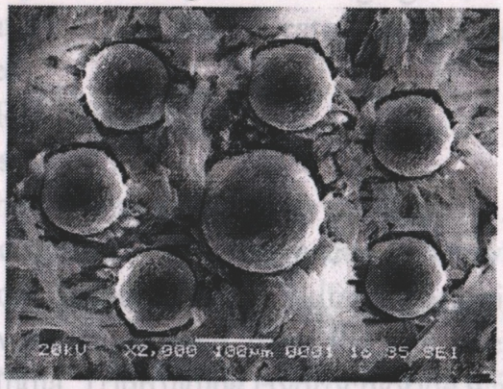


Fig.3: SEM photograph of poly (MAAm-*co*-AA) microspheres

pH sensitive property

The pH of the swelling medium plays an important role in influencing swelling behaviour of microspheres. If the microspheres contains some ionizable groups, which can dissociate or get protonated at some suitable pH of the swelling media, then the degree of swelling of polymer matrices undergo appreciable change with external pH. Figure.6. depicts the dynamic uptake of water by the sample in the buffer media of pH 1.2 with ionic strength of 0.1*M* at 37°C. The polymer matrices exhibits minimum swelling in the medium of pH 1.2, and as the pH becomes 7.4, the degree of swelling at different time intervals increase. The values of swelling exponent *n*, as determined from double logarithmic plots, was determined and presented in Table.2 & 3 for pH 7.4 and pH 1.2 respectively. These values suggest that the polymer matrices demonstrate an amalous the Non-Fickan swelling behaviour in the medium of pH 1.2 and while it shows Fickian behaviour when allowed to swell in the media of pH 7.4. This can be attributed to the fact that when the polymer matrix is allowed to swell in the media of pH 1.2, the -COOH groups present within the network remain almost nonionized, thus imparting almost nonpolyelectrolyte type behaviour to the polymer matrix. Moreover, there exits strong H-bonding interactions between -COOH groups of acrylic acid and -$CONH_2$ groups of methacrylamide, which are present within the network, thus resulting in a compact structure that does not permit much movement of polymeric segments within the microsphere. However, in the medium of pH 7.4, the almost complete ionization of -COOH groups results in extensive chain relaxation due to repulsion among similarly charged -COO^- groups present along the macromolecular chains. Moreover, the ionization also causes an increase in ion osmotic pressure. These two factors are thus responsible for a higher degree of swelling in the medium of pH 7.4. When a dosage form is taken orally, first it goes into the stomach, and after residing there for a definite time, it passes on to the small intestine and finally to the colon. Thus, the dosage form is exposed to media of varying pH during its journey from the mouth to the colon along the GI tract. Therefore, the device has the potential to be used for the colon-targeted drug delivers.

In-vitro release studies

In-vitro release studies have been carried out by performing the dissolution experiments using a tablet dissolution tester (Lab India, Mumbai, India) equipped with eight baskets. Dissolution rates were measured at 37°C under 100 rpm speed. Drug release from the microspheres were studied in both colon and intestinal (1.2 and 7.4 pH phosphate buffer media) fluids like atmosphere. At regular intervals of time, sample aliquots were withdrawn and analyzed by UV spectrophotometer (Model LabIndia-3000$^+$, Mumbai, India) at the fixed λ_{max} value of 200 nm.

Encapsulation Efficiency

Three different concentrations of drug (5, 10 and 15 wt %) were loaded into the polymicrosphores during crosslinking. The % encapsulation efficiency was also included in Table.1 and it is noticed that these values were increased with increasing drug loading. In the case of MAAm:AA-1, MAAm:AA-2 and MAAm:AA-3 microspheres, the % encapsulation efficiency increases

from 54.6 % to 66.9 % as the drug content increases from 5 to 15 wt %. The % encapsulation efficiency also followed the same trend with an increasing amount of AA in the microspheres. For example, to study the effect of AA in the microspheres [e.g., for microspheres containing different ratios of MAAm and AA with 10 % of TPH (MAAm:AA-2, MAAm:AA-6, MAAm:AA-7)], encapsulation efficiencies were found to be 58.8 %, 70.0 %, and 79.0 %, respectively. Which shows that with increase in AA content in the sphere the encapsulate efficiency also increases. This increase may be due to the presence of –COOH groups, which are responsible for increased hydrophilic nature of the matrix. The effect of crosslinking on size and entrapment efficiency of the microspheres using percentage of crosslinker 1, 2 and 3 wt% containing MAAm: AA microspheres are also represented in Table.1. With increase in degree of crosslinking, the % encapsulation efficiency was decreased, e.g., for microspheres crosslinked with 10, 20, 30 wt% of NNMBA (MAAm: AA-2, MAAm: AA-4 and MAAm:AA-5), entrapment efficiencies were 58.8 %, 56.2 % and 49.2 % respectively. This may be due to the increasing degree of crosslinking, which leads to microspheres becoming more rigid and thus, reducing the free volume space within the polymeric network to yield reduced the percentage of encapsulation efficiency.

Table.1. Results % of encapsulation efficiencies of different formulations

Formulation codes	% MAAm	% AA	% TPH	% of NNMBA added	% Encapsulation efficiency ± S.D.
MAAm:AA-1	60	40	5	1	54.2 ± 0.4
MAAm:AA-2	60	40	10	1	58.6 ± 0.2
MAAm:AA-3	60	40	15	1	66.1 ± 0.8
MAAm:AA-4	60	40	10	2	54.8 ± 1.4
MAAm:AA-5	60	40	10	3	48.4 ± 0.8
MAAm:AA-6	40	60	10	1	68.8 ± 1.2
MAAm:AA-7	20	80	10	1	78.4 ± 0.6

S.D: standard deviation calculated 95% accurately.

Drug release kinetics

Drug release kinetics was analyzed by plotting the cumulative release data *versus* time and by fitting these data to the exponential equation of the type (Ritger and Peppas, 1987).

$$M_t/M\alpha = kt^n \qquad (4)$$

Here, $M_t/M\alpha$ represents 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 squares procedure, we have estimated the values of n and k for all the seven formulations and these values are given in Table.2. If $n = 0.5$, then drug diffuses and releases from the polymer matrix following a Fickian diffusion. For $n > 0.5$, anomalous or non-Fickian type drug diffusion occurs. If $n=1$, a completely non-Fickian or Case II release kinetics is operative. The intermediate values ranging between 0.5 and 1.0 are attributed to the anomalous type transport (Ritger and Peppas, 1987). The values of k increased with increasing % loading of TPH into the polymer microspheres, but the n values decreased with decreased % loading of TPH. This indicates the interaction between the polymer microspheres and drug as studied from the release kinetics represented by Eq. (4) proposed by Ritger and Peppas (1987). The values of exponent n are found to range between 0.264 and 0.450 as calculated from the empirical equation, which indicated that drug release slightly deviated from the Fickian trend, while $0.5 < n < 1.0$ indicates non-Fickian or anomalous transport. Values of the correlation coefficient, 'r' is calculated and included in Tables 2 & 3. falls in the range of 0.904 to 0.992 and 0.941 to 0.995 for pH 7.4 and pH 1.2 respectively, indicating a good fit of the experimental data. This is due to reduction in the regions of low microviscosity of the medium and closure of the microcavities in the swollen microspheres.

Table.2: *Release kinetics parameters for different formulations at pH-7.4*

Formulation codes	k	*n*	Correlation coefficient, *r*
MAAm:AA -1	1.045	0.364	0.952
MAAm:AA -2	1.047	0.363	0.916
MAAm:AA -3	1.166	0.450	0.965
MAAm:AA -4	0.850	0.311	0.959
MAAm:AA -5	0.828	0.322	0.904
MAAm:AA -6	0.723	0.264	0.992
MAAm:AA -7	0.926	0.335	0.929

Table.3: ***Release kinetics parameters for different formulations at pH-1.2***

Formulation codes	k	*n*	Correlation coefficient, *r*
MAAm:AA -2	0.285	0.966	0.995
MAAm:AA -6	0.283	0.876	0.941
MAAm:AA -7	0.268	0.996	0.967

Effect of Triprolidine hydrochloride

Triprolidine hydrochloride (TPH) is a water-soluble drug and is used in the present research, TPH formulations with loadings ranging up to 62–81% could be achieved at different copolymer compositions. The % encapsulation efficiency data are given in Table.1. Fig.4. displays the drug release characteristics of the formulations containing different amounts of drug with constant amount of crosslinking agent. From Fig.4 it is noticed that the faster the release rates have been observed for formulations containing higher amount of TPH than those microspheres containing lower amount of drug at 1% NNMBA in the matrix. Release data showed that formulations containing higher encapsulation efficiency displayed much faster and higher release rates than those formulations containing the lower encapsulation efficiency. However, a prolonged drug release was observed for formulation containing lower amount of TPH. It is also notice that the release rate becomes quite slower when a lower amount of drug is present in the matrix, probably due to the availability of more free-void spaces through which, lesser number of drug molecules could possibly transport. Generally, drug release through microspheres depend upon the particle size, polymer crystallinity, surface character, molecular weight, polymer composition, swelling ratio, degradation rate, drug binding affinity, rate of hydration, etc. (Ratner, 1996). While *in vitro* release of the drug from the MAAm–*co*-AA drug delivery system in addition to binding affinity of the drug seem to be dominant.

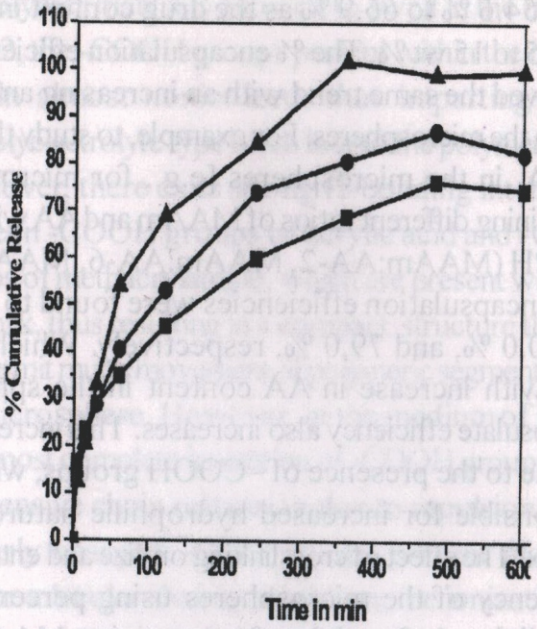


Fig.4. % Cumulative release of triprolidine hydrochloride at constant NNMBA (1%) and monomers (60% MAAm + 40% AA)
Symbols:(■) 5% TPH drug,(●)10 % TPH drug, (▲)15% TPH drug

Effect of crosslinking agent

The % cumulative release data *versus* time plots for varying amounts of NNMBA, i.e., 1, 2 and 3% at the fixed amount of the drug (5%) are displayed in Fig.5. The % cumulative release is quite fast and large at the lower amount, i.e., 1% of NNMBA, whereas the release is quite slower at higher amount, i.e., 3% NNMBA. The cumulative release is also higher at the lower amount of NNMBA, because at higher concentration of NNMBA, the polymeric chains will become rigid due to contraction of microvoids thereby, giving a decrease in % cumulative release of the drug.

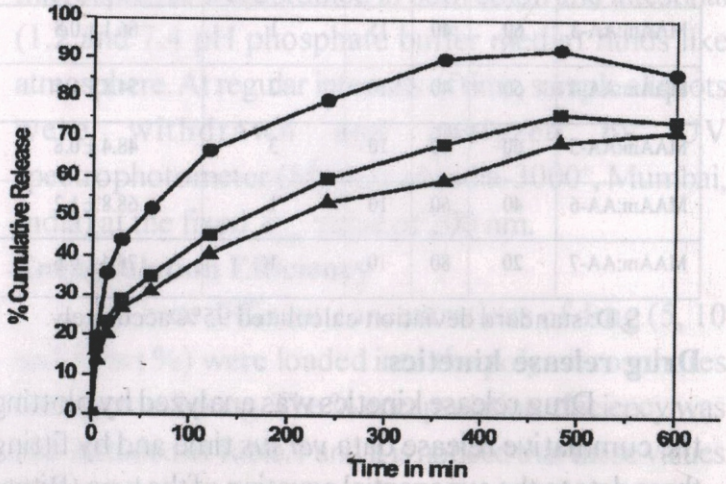


Fig.5. % Cumulative release of triprolidine hydrochloride at constant drug and monomers, Symbols:(●)1% NNMBA, (■)2% NNMBA, (▲)3% NNMBA

Effect of pH

To investigate the effect of pH and ionic strength of the external medium on the swelling of polymer matrices, we have measured the percentage cumulative release in both pH 1.2 and 7.4 media. Cumulative release data presented in Figures 6 and 7 indicate that by increasing the pH from 1.2 to 7.4, a considerable increase in the cumulative release is observed for all microspheres. At higher pH (above the p*Ka* of the microspheres), the -COOH groups may dissociate, increasing the osmotic pressure inside the microspheres, resulting in higher swelling. Cumulative release in both the pH conditions thus, depends upon the extent of crosslinking. At lower crosslinking, the network is loose with a greater hydrodynamic free volume so that the polymer chains can accommodate more solvent molecules, thereby inducing higher swelling and higher cumulative release. However, cumulative release of the microspheres at higher pH depends upon the extent of hydrodynamic free volume, polymer chain relaxation, and availability of hydrophilic functional groups (CONH as in case of ionized polymer) for water to form hydrogen bonds. The release data shown in Figures.6 and 7 obtained in pH 1.2 and 7.4 at the fixed amount of drug (10% TPH) and the fixed amount of crosslinking agent (i.e., 1% of NNMBA) are different because of the differences in the swelling of microspheres in different external media. The percentage cumulative release rate is quite slow in pH 1.2 and whereas, the cumulative release rate is quite fast and large in pH 7.4 media, Note that cumulative release of poly (MAAm–co-AA) microspheres in 1.2 pH media is almost quite slower of the cumulative release observed in 7.4 pH media, which is due to lesser swelling of the beads in 1.2 pH media.

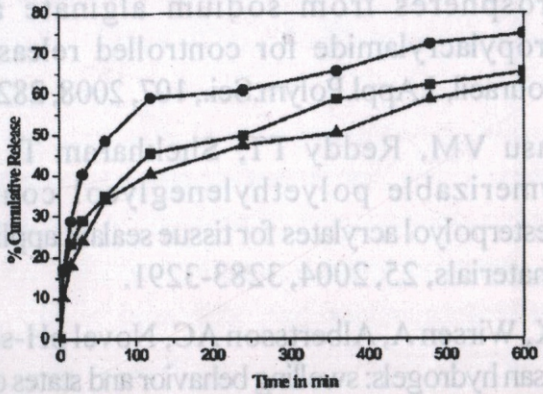


Fig.6. % Cumulative release of triprolidine hydrochloride containing different amount of MAAm-AA at constant pH 1.2 media, Symbols:(▲)MAAm: AA-2, (■)MAAm: AA-6, (●)MAAm: AA-7

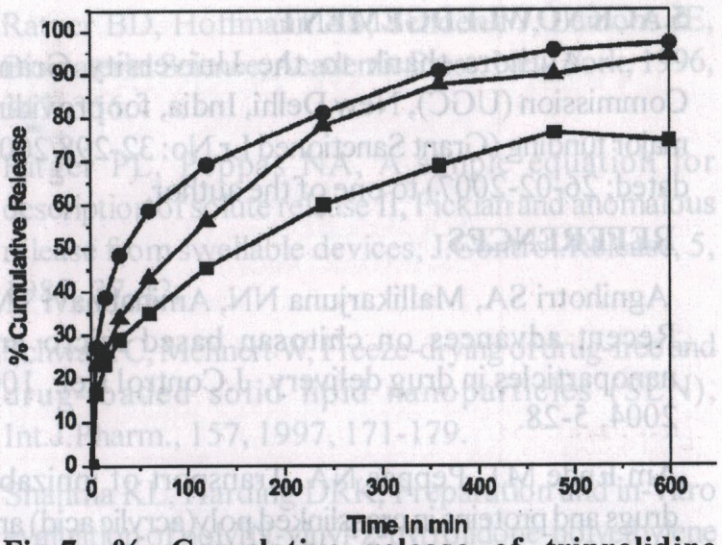
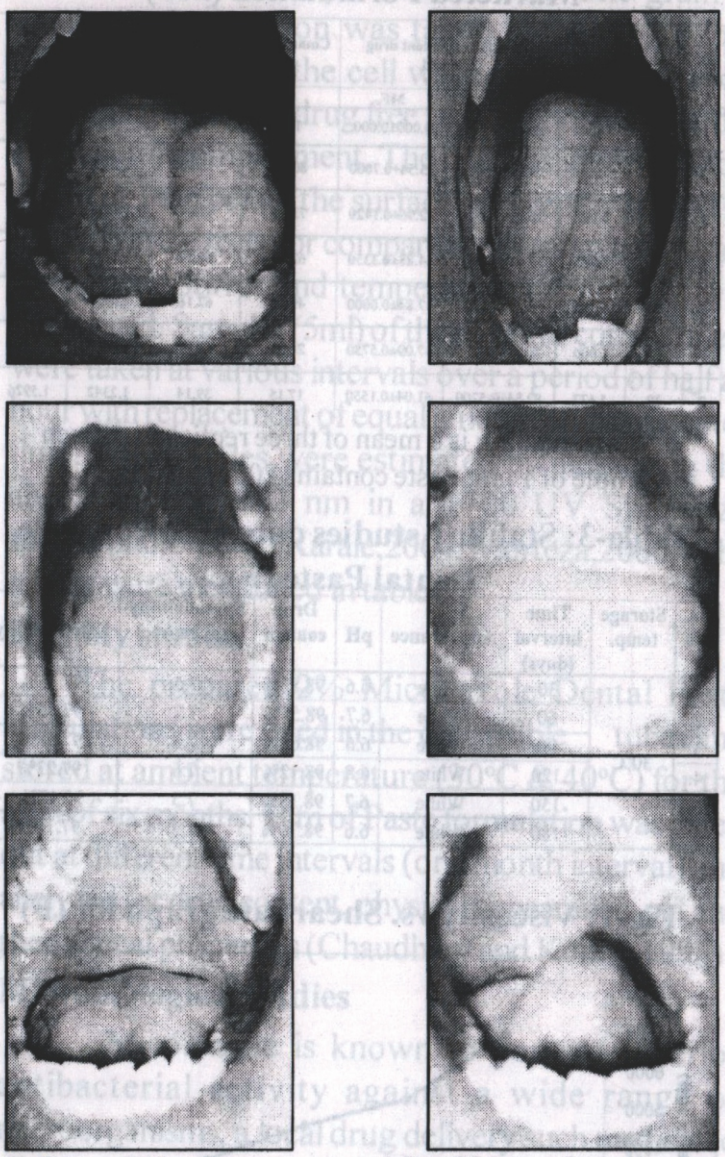


Fig.7. % Cumulative release of triprolidine hydrochloride containing different amount of MAAm-AA at constant pH 7.4 media, Symbols:(■) MAAm:AA-2, (▲)MAAm: AA-6, (●) MAAm: AA-7

4. CONCLUSIONS

The matrices prepared in this study could offer a wide array of release patterns and rates. Depending upon the matrix loading dose and preparation method employed, the release was controlled by the penetration of external medium into the polymer matrix or by drug diffusion into the matrix pores or by both. The matrix prepared with a 1:1 ratio of MAAm to AA, prepared by method-I was deemed to be the most appropriate one to offer a successful CR system. The amount of initiator also influences the water uptake of hydrogels. Differential scanning calorimetry and X-ray diffraction of triprolidine hydrochloride-loaded microspheres have shown a molecular level dispersion of the drug in the matrices. SEM micrographs confirmed the formation of well defined microspheres with distinct spherical shapes. Higher drug loadings and faster release rates have been observed when drug was loaded into microspheres. Sustained and prolonged drug release rates have been observed from the *in situ* drug-loaded microparticles of this study. From the results obtained in above study, it can be concluded that poly (methacrylamide-*co*-acrylic acid) hydrogels undergo a sharp volume phase transition with the change in pH of the swelling medium from an acid to an alkaline one. Finally, the microspheres seem to have potential to be used for controlled drug delivery of triprolidine hydrochloride through oral administration.

Fig-3: Primary Oral mucosal irritation test of one group of Human Volunteers



Before application **After 72 hrs. of application**

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