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CARBOXYMETHYLCELLULOSE -G - ACRYLAMIDE BLEND CHITOSAN MICROSPHERES FOR CONTROL RELEASE OF CHLORO PHENIRAMINE MALIATE

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ABSTRACT

Semi-interpenetrating polymer network [IPN] microspheres of carboxy methyl cellulose-grafted-acryl amide/Chitosan microspheres were prepared by water-in-oil (W/O) emulsion method. These microspheres were loaded with, Chloro pheniramine maliate and Cross-linked with glutaraldehyde(GA); these microspheres were characterized by differential scanning calorimetry (DSC), scanning electron microscopy (SEM) and laser particle size analyzer. DSC thermograms of Chloro pheniramine maliate.-loaded AAm-CMC/ Chitosan microspheres confirmed the molecular level distribution of Chloro pheniramine maliate in the polymer matrix. SEM of the microspheres suggested the formation of spherical particles. Swelling experiments on the microspheres provided important information on drug diffusion properties. Release data have been analyzed using an empirical equation to understand the nature of transport of drug containing solution through the polymeric matrices. The controlled release characteristic of the matrices for Chloro pheniramine maliate was investigated in pH 7.4 media. Particle size and size distribution of the microspheres was studied by laser light diffraction particle size analyzer. Drug was released in a controlled manner up to 12 h.

KEYWORDS: Carboxy methyl cellulose (CMC), Acryl amide (AAm), Chitosan (CS), Groft polymer, Semi-interpenetrating polymer network [IPN], cross linking, Chloro pheniramine maliate (CPM),controlled release etc.

INTRODUCTION

A well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a given drug. To obtain

maximum therapeutic efficacy, it becomes necessary to deliver the agent to the target tissue in the optimal amount in the right period of time there by causing little toxicity and minimal side effects.[1] There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. One such approach is using microspheres as carriers for drugs.

Over the past decades, blends have been investigated to satisfy the need of specific sectors of polymer industry. Such polymeric blends showed superior performances over the conventional individual polymers and consequently, the range of applications have grown rapidly for such class of materials. In the recent years, carbohydrate and biodegradable polymers have been extensively used to develop the controlled release formulations of drugs having short plasma life.[2-7] Among the various polymers employed, hydrophilic biopolymers are quite suitable in oral applications due to their inherent advantages over the synthetic polymers.[8] The importance of biocompatible and biodegradable polymers is continuously increasing in pharmaceutical applications because of their propensity to form crosslinked three-dimensional network hydrogels that tend to swell in water or biological fluids.[9] Such systems have been considered as a the potential candidate to deliver bioactive molecules, particularly in controlled release applications.[10-12]

The chemical and physical combination methods and properties of multipolymers have been of great practical and academic interest for the controlled release of drugs because they provide a convenient route for the modification of properties to meet specific needs. Among these methods, considerable interest has been given to the development of IPN based drug delivery systems. This would open up new avenues to use IPN in designing the novel controlled release drug release systems.[13-15] A combination of judicially selected natural and synthetic polymers has been found to be useful in enhancing the release of short half-lived drugs under physiological conditions. In order achieve this, the properties of natural and synthetic polymers have been modified by grafting, blending and other means. Grafting of vinyl monomers onto natural polymers such as cellulose has been widely accepted.[16-18]

Chloro pheniramine maliate (CPM) was Chosen as a model drug for encapsulation in the polymer matrix and *in vitro* released studies have been performed by dissolution experiments. Formation of interpolymer complexes of carboxymethylcellulose grafted-acrylamide/Chitosan and development of IPN microspheres by crosslinking using glutaraldehyde (GA) has been discussed in this study.

Experimental

MATERIALS AND METHODS

Carboxymethylcellulose (CMC), Acryl amide (AAm), Chitosan (CS), light paraffin oil, Glutarldehyde (25 % aqueous solution) (GA) were purchased from s.d. Fine Chemicals, Mumbai, India. Tween-80 was purchased from Sigma Chemicals Chloro pheniramine maliate (CPM) was given as gift sample from WaksmanSaleman pharmaceuticals Pvt.Ltd. Anantapur, India.

Preparation of semi IPN microspheres

Varying amount of CarboxyMethyl Cellulose was weighed and dissolved in water by overnight stirring. To this solution different amount of acryl amide and potassium persulphate were added and stirred well. This reaction mixture is polymerized under nitrogen atmosphere for 6 h at 70^{o}C. This polymerized is cooled and polymer was extracted by precipitating the polymer in acetone and precipitated polymer was dried under vacuum for 24 h.

A different weight ratio of chitosan and carboxy methyl cellulose-g-acryl amide was dissolved in the water of certain concentration overnight. The two polymer solutions were mixed and stirred well for proper mixing which lead to miscible polymer solution. A known amount of the Chloro pheniramine maliate was dissolved in 1 mL of Water and is added into the blend polymer solution. The drug loaded blend polymer solution was emulsified into liquid paraffin to form a water-in-oil (w/o) emulsion at 400 rpm using Eurostar (IKA Labortechnik, Germany) high-speed stirrer for 30 min in a separate 500 mL beaker containing 100 mL of light liquid paraffin oil, 2 % (w/v) of Tween-80, 1 mL of 0.1 M HCl and the required amount of GA is added. The microspheres formed were filtered, washed repeatedly with hexane and water to remove the oil as well as excess amount of surfactant and the unreacted GA. These microspheres were dried under vacuum at 40^{0}C and stored in desiccators before further analysis and Characterization. Repeating the above procedure various formulations were prepared by varying Chitosan and CMC-g-AAm, GA and CPM compositions and these are designated as AAm-1 to AAm-9 in **Table-1**.

Swelling studies

Dynamic swelling of CMC-g-AAm blended with chitosan microspheres were prepared using three different concentrations of cross-linker as well as three different drug loadings were

studied in water by mass uptake measurements with time. Swelling experiments performed in 7.4 pH buffer solution. To perform swelling experiments, microspheres were soaked in buffer solution 7.4 pH, several of them were removed from the swelling bottles at different time intervals and blotted carefully with tissue papers (without pressing hard) to remove the surface-adhered buffer solution. The microspheres were then weighed (w1) on an electronic microbalance (ADAM AFP-210L England accurate to ± 0.0001 g). The microspheres were then dried to a constant weight (w2) in an oven maintained at 400C for 5 hours. Swelling experiments were repeated thrice for each sample and average values were used in data analysis. The standard deviations (S.D.) in all cases were < 5 %. The weight % water uptake was calculated as:

$$\%\text{Water uptake} = \left(\frac{Wt\ of\ swollen\ Microspheres(w1) - Wt\ of\ dry\ Microspheres(w2)}{Wt\ of\ dry\ Microspheres(w2)} \right) \quad \text{---- (1)}$$

Determination of Amount of Drug Entrapped

The drug loaded microspheres (10 mg) were pulverized and incubated in 10 ml of 0.02M phosphate buffer (pH = 7.4) at room temperature for 24 h. The suspension was agitated with agitate mortar and filtered through filter paper. The drug solution was assayed spectrophotometrically for CPM content at the wavelength of 262nm. The results of % of drug loading and encapsulation efficiency were calculated using following equations:

$$\%\ \text{Drug loading} = \left(\frac{Amount\ of\ drug\ in\ Microspheres}{Amount\ of\ Microspheres} \right) X100 \text{ -- (2)}$$

$$\%\ \text{Encapsulation efficiency} = \left(\frac{Actual\ loading}{Theoretical\ loading} \right) X100 \text{ -- (3)}$$

In-vitro Release study

Dissolution was carried out using Tablet dissolution tester (Lab India, Mumbai, India) equipped with eight baskets. Dissolution rates were measured at 37±0.5^{o}C at constant speed of 100 rpm. Drug release from the microspheres was studied in 7.4 pH phosphate buffer solutions. At regular intervals of time, sample aliquots were withdrawn and analyzed using UV spectrophotometer (Lab India, Mumbai, India) at the fixed λ_{max} value 262nm. After each sample collection, the same amount of fresh release medium at the same temperature was

added to the release medium to maintain the sink condition. All measurements were carried out in triplicate, and values were plotted with standard deviation errors.

CHARACTE RIZATION TECHNIQUES

Differential Scanning Calorimetric (DSC) studies

Differential scanning calorimetric (DSC) curves were recorded on a TA instruments (Model: STA, Q600 USA). The sample was weighed between 10 to 12mg. The samples were heated from 50^{o} to 400^{o} C at heating rate of 10^{o} C/min in nitrogen atmosphere (flow rate 100 mL/min).

X-Ray Diffractions (X-RD) studies

X-RD measurement of plain drug, drug-loaded microspheres and plain microspheres were recorded using a Rigaku Geiger flex Diffractometry (Tokyo, Japan) equipped with Ni-filtered Cu Kα radiation (λ=1.548A^{0}). The dried microspheres of uniform thickness were mounted on sample holder, and the patterns were recorded in the range 0 to 50^{0}C at the speed of 5^{0}C/min to know the crystallinity.

Particle size and scanning electron microscopic (SEM) studies

To determine the particle size and size distribution, ~ 100 - 200 microspheres were taken on a glass slide and their sizes were measured using an optical microscope under regular polarized light. SEM micrographs of microspheres were obtained under high resolution (Mag 300X5kv) Using JOEL MODEL JSM 840A, scanning electron microscope (SEM), equipped with phoenix energy dispersive analysis of X-ray (EDAX).

RESULTS AND DISCUSSIONS

Differential scanning calorimetry (DSC) studies

DSC thermo grams of plain AAm-g-CMC /CS microspheres(A),drug loaded- AAm-g-CMC /CS microspheres (B) and plain drug(C) microspheres were recorded using Rheometric Scientific differential scanning calorimeter (Model-DSC SP, UK) and are shown in **Fig: 1** the analysis was performed by heating the samples at the rate of 10^{0}C/min under inert atmosphere. The onset melting peak of chloro pheniramine maliate was observed at 135 ^{o}C. **Fig: I. (c)** However, no characteristic peak of chloro pheniramine maliate was observed in DSC curve of the drug loaded microspheres suggesting that the drug is molecularly dispersed in the polymer matrix.

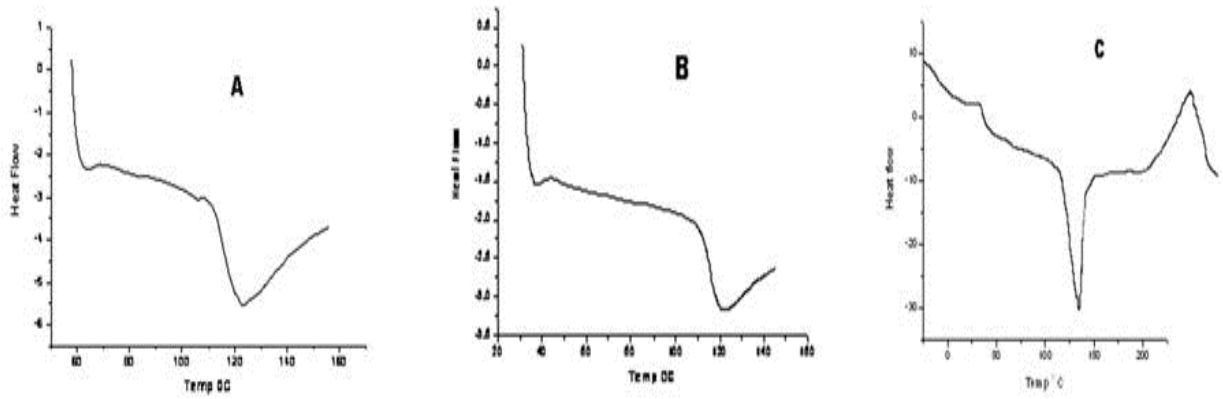

Fig: 1. DSC thermograms of plain AAm-g-CMC /CS microspheres (A),drug loaded-AAm-g-CMC /CS microspheres (B) and plain drug(C).

Fourier Transform Infrared Spectroscopy (FTIR)

The IR spectra of CMC, PAAM, and CMC-g-PAAM are **Fig: 2** shown in Figure 1(a–c), respectively. From the IR spectra of CMC, it showed a broad absorption band at 3444 cm^{-1}, due to the stretching frequency of the AOH group. The band at 2921 cm^{-1} was due to C-H stretching vibration. Appearance of a strong absorption band at 1618 cm^{-1} was due to the presence of COO^- groups. The bands around 1423 and 1326 cm^{-1} were assigned to CH_2 scissoring and OH bending vibration, respectively. In the case of PAAM, a broad absorption band at 3431 cm^{-1} was for the N-H stretching frequency of the NH_2 group. Two strong bands around 1689 and 1647 cm^{-1} were due to amide-I (C═O stretching) and amide-II (NH bending), respectively. The bands around 1400 and 2922 cm^{-1} were for the C-N and C-H stretching vibrations, respectively. Other bands at 1458 and 1323 cm^{-1} were attributed to CH_2 scissoring and CH_2 twisting. For IR spectrum of CMC-g-PAAM, The presence of a broad absorption band at 3434 cm^{-1} was due to the overlap of OH stretching CMC and NH stretching band of PAAM. A band at 1652 cm^{-1} was due to amide-I (C═O stretching) of the amide group of PAAM and the band at 1618 cm^{-1} of CMC and amide-II band of PAAM overlapped with each other and led to a broad band at 1628 cm^{-1}. The presence of a band at 1733 cm^{-1} was due to free acid groups. The bands around 1404 and 2922 cm^{-1} were for the C-N and C-H stretching vibrations, respectively. Other bands at 1458 and 1338 cm^{-1} were attributed to CH_2 scissoring and CH_2 twisting. Also, there was an important peak at 1068cm^{-1} which assigned for the CH-O-CH_2 group resulting from grafting reaction between the hydroxyl group located in anhydroglucose C_2 position and the p-bond of PAAM. The primary

peaks existed in the CMC-g-PAAm characteristic for the groups of AM, and the shift in the band corresponding to OH group, may suggest formation of ether (>CH-O-CH_2) during the grafting copolymerization. Accordingly, it is apparent that FTIR presented a strong evidence of grafting of PAAM branches onto the polysaccharide backbone; (since homopolymers were removed by solvent extraction)

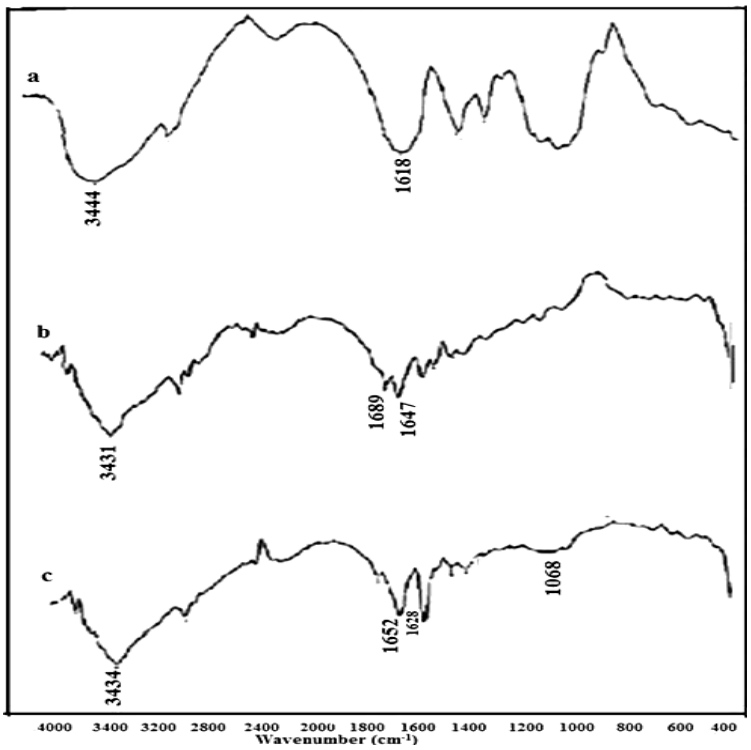

Fig: 2 FTIR of (a) CMC, (b) PAAm, and (c) CMC -g-PAAm.

Scanning electron microscopic (SEM) studies

SEM images of the microspheres were recorded using a scanning electron microscope (Hitachi S520, Japan) at the required magnification. Working distance of 33.5 mm was maintained and the acceleration voltage used was 10 kV with the secondary electron image (SEI) as a detector. **Fig: 3**.shows the cross-sectional SEM micrograph of chloro pheniramine maliate loaded AAm-g-CMC /Chitosan microspheres. Cross-section of the AAm-g-CMC/Chitosan microspheres show corrugated structures that are common with the graft copolymers.

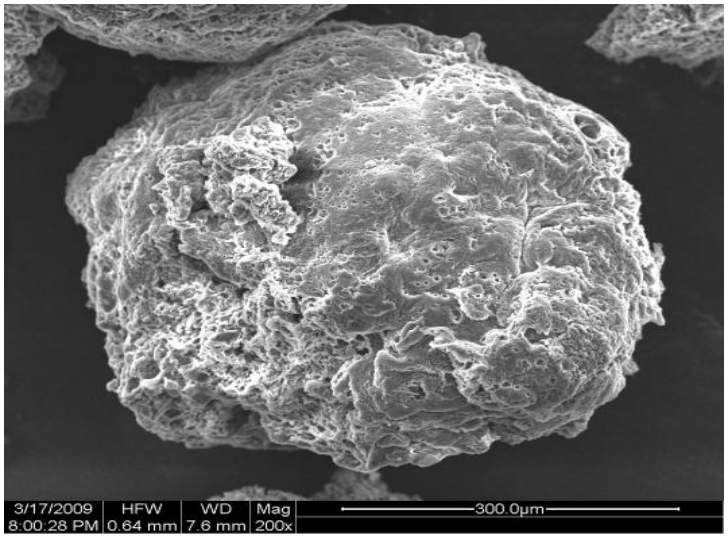

Fig: 3. Scanning electron micrograph of AAm-g-CMC/CS Microspheres.

Particle size analysis

Particle size and size distributions have been analyzed by using a particle size analyzer (Mastersizer 2000, Malvern Instruments, UK). Results of the mean particle size with size distribution curve of the microspheres produced by taking three different amounts of crosslinking agent are presented in **Table.1.** Particle size of different formulations containing different amounts of drug, crosslinking agents and different ratios of CPM are also presented in T**able. I** the particle size of formulations containing different amount of crosslinking agents (GA) i.e. 2.5%, 5%, 7.5% are 168,156 & 112 (AAm-1, AAm-2 &AAm-3)respectively. The particle size decreased with increasing the amount of crosslinking agent due to formation of regid structure and reducing the chain length of the polymer. These results suggest that as the extent of crosslinking increases, the volume mean diameter decreased. On a population basis, particle size distribution is unimodel. Microspheres used in preparing the drug-loaded formulations were selected from a uniform size distribution range as displayed in **Fig: 4.** A narrow size distribution of microspheres was observed with particle size 90-450 µm, but majority of particles is in the size range between 150-220 µm.

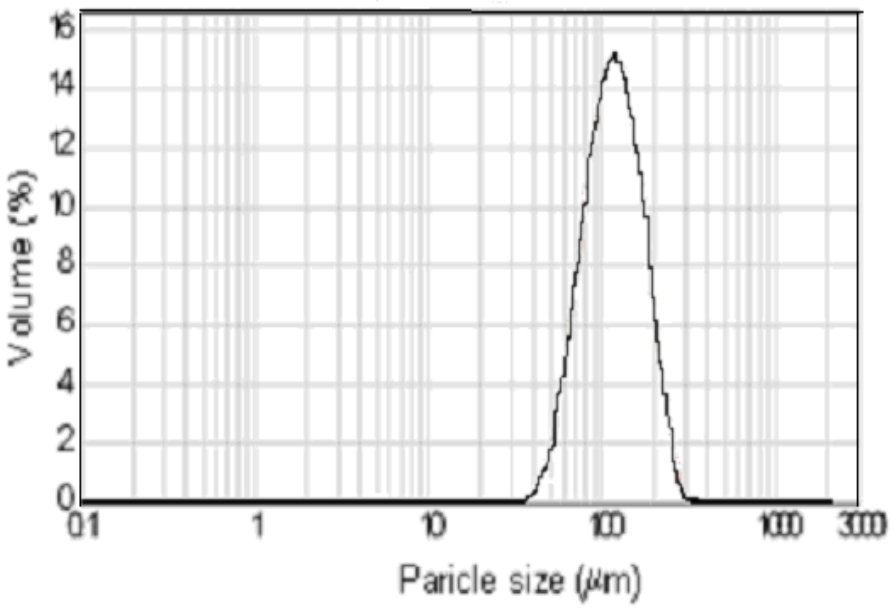

Fig: 4. Particle size distribution curve for AAm-g-CMC/CS Microspheres.

Estimation of drug loading and encapsulation efficiency

Specific amount of dry microspheres were vigorously stirred in a beaker containing 10 mL of dichloromethane to extract the drug from the microspheres. A 10 mL of 7.4-pH phosphate buffer containing 0.02 % Tween-80 was added to the above solution to make the drug soluble and dichloromethane was evaporated with a gentle heating and continuous shaking. The aqueous solution was then filtered and assayed by a UV spectrophotometer (Lab India, Mumbai, India) at the fixed λ_{max} value of 262nm. The results of % chloro pheniramine maliate loading and encapsulation efficiency were calculated using **Eq: (2)** and **(3).** These results are compiled in **Table.1.** The % encapsulation efficiency varied depending upon the initial loading of the drug in general with, for formulations AAM-1, AAm-6, & AAm-7, the % of encapsulation efficiency increased with increasing drug content of the matrices. At higher amount of crosslinking agent i.e. 2.5%, 5%, and 7.5% of GA in the matrix the % encapsulation efficiency decreased.

Table.1. Results % of encapsulation efficiency, mean particle size and water uptake of different formulations.

Formulation codes	Ratio of CS: in CMC microspheres	Amount of AAm added (mg)	Amount of CPM loaded(mg)	Amount of GA added (mL)	% Encapsulation efficiency ± S.D.	Mean particle size (μm) ± S.D.	% Water uptake
AAm-1	10:90	10	5	2.5	68.2 ± 0.8	168 ± 5	495
AAm-2	10:90	10	5	5	66.4 ± 1.1	156 ± 6	458
AAm-3	10:90	10	5	7.5	61.5 ± 0.9	112 ± 8	343
AAm-4	20:80	10	5	5	72.6 ± 0.8	160 ± 7	395
AAm-5	30:70	10	5	5	79.8 ± 1.2	185 ± 9	420
AAm-6	10:90	10	10	5	68.5 ± 1.1	158 ± 5	464
AAm-7	10:90	10	15	5	70.9 ± 1.5	155 ± 4	486
AAm-8	10:90	20	5	5	58.2 ± 0.4	178 ±9	491
AAm-9	10:90	30	5	5	49.5 ± 0.6	205 ± 6	518

S.D: standard deviation

Drug release kinetics

Drug release kinetics was analyzed by plotting cumulative release data vs time and by fitting these data to the exponential equation of the type.[19]

$$\left(\frac{M_t}{M_\infty}\right) = kt^n \quad \text{--- (4)}$$

Here, M_t/M_∞ represents the fractional drug released 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 nine formulations and these values are given in **Table.2** At 37^0c. If the value of $n = 0.5$, the drug diffuses and releases out of the polymer matrix following a Fickian diffusion. If $n > 0.5$, anomalous or non-Fickian type drug diffusion occurs. If $n = 1$, a completely non-Fickian or more commonly called **case II** release kinetics is operative. The intermediary values ranging between 0.5-1.0 are attributed to the anomalous type transport.

The values of *k* and *n* have shown a dependence on the extent of crosslinking, % drug loading and AAm content of the matrix. Values of *n* for microspheres prepared by varying the amount of AAm in the polymer microspheres of 10, 20 and 30 % by keeping chloro

pheniramine maliate (5 %) and GA (7.5 mL GA) constant, ranged from 0.278 to 0.727 leading to a shift of transport from Fickian to anomalous type. The chloro pheniramine maliate loaded particles have the *n* values ranging from 0.278 to 0.513 **Table.2**, indicating the shift from errosion type release to a swelling-controlled, non-Fickian mechanism. This could be possibly due to a reduction in the regions of low microviscosity and closure of microcavities in the swollen state. Similar findings have been observed elsewhere, wherein the effect of different polymer ratios on dissolution kinetics was studied. On the other hand, the values of *k* are quite smaller for the drug-loaded microspheres, suggesting their lesser interactions compared to microspheres containing varying amount of chitosan.

Table. 2. Release kinetics parameters of different formulations

Formulation code	*k*	*n*	Correlation coefficient, *r*
AAm-1	0.0032	0. 265	0.970
AAm-2	0.0184	0.278	0.9418
AAm-3	0.0183	0.727	0.9712
AAm-4	0.0115	0.680	0.9303
AAm-5	0.0142	0.540	0.9816
AAm-6	0.0839	0.478	0.9873
AAm-7	0.0137	0.513	0.9642
AAm-8	0.106	0.690	0.9905
AAm-9	0.104	0.611	0.9875

Effect of Acrylamide

Fig: 5 shows the *in vitro* release data of chloro pheniramine maliate from the microspheres particles performed with different ratio of AAm in the polymeric particles. The data shows that higher amount of AAm containing particles have more encapsulation efficiency and also the release studies shown that higher amount of AAm containing particles have shown prolonged release characteristics than the microspheres containing lower amount of AAm. Generally, the drug release pattern depends on many factors like particle size, crystallanity, surface character, molecular weight, polymer composition, swelling ratio, degradation rate, drug binding affinity and the rate of hydration of the polymeric materials, etc.[20] In the release behavior of polymeric system we can consider the binding affinity of drug and polymer swelling property of AAm. A rapid release of more than 98% of drug was observed within 12 h from the microspheres containing lower amount of AAm indicating that interaction between the two polymers.

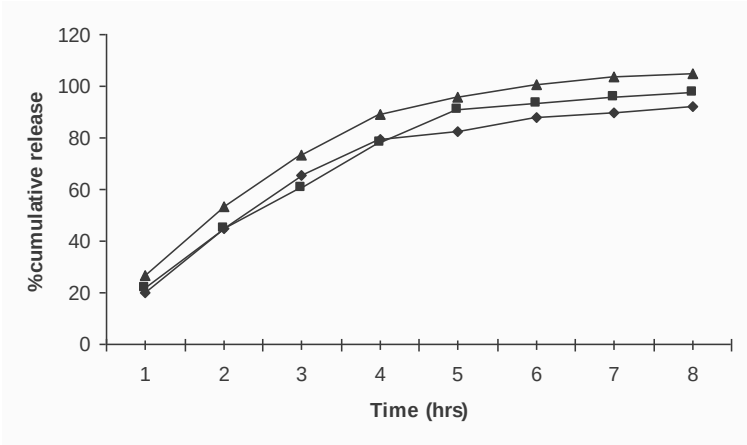

Fig.5. Cumulative % release of chloro pheniramine maliate through AAm-g-CMC/Chitosan microspheres containing different amount of AAm. (Δ) 10 wt. % AAm, (■) 20 wt. % AAm, (♦) 30 wt. % AAm

Effect of crosslinking agent

The % cumulative release data vs time plots for varying amounts of GA i.e. 2.5, 5.0 and 7.5 mL at the fixed amount of the drug (5 %) are displayed in **Fig:6.** The % cumulative release is quite fast and large at the lower amount of GA (i.e., 2.5 mL), whereas the release is quite slower at higher amount of GA (i.e., 7.5 mL). The cumulative release is somewhat smaller when lower amount of GA was used probably because at higher concentration of GA, polymeric chains become rigid due to the contraction of microvoids, thus decreasing %cumulative release of chloro pheniramine maliate through the polymeric matrices. As expected, the release becomes slower at higher amount of GA, but becomes faster at lower amount of GA. As shown in **Fig: 6.**

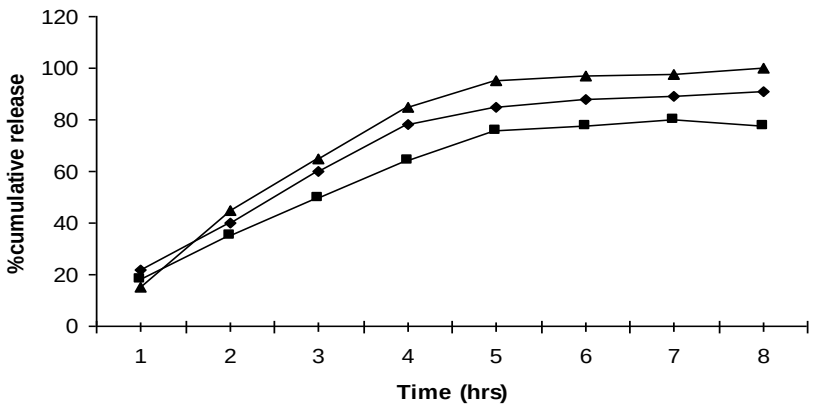

Fig.6. Cumulative % release of chloro pheniramine maliate through AAm–g- CMC/CS microspheres containing different amount of crosslinking agent. (♦) 5mL (Δ) 2.5mL (■) 7.5mL

Effect of percent drug loading

Fig: 7. shows the release profiles of chloro pheniramine maliate loaded poly (AAm-g–CMC/NaAlg) microspheres at different amount of drug loading. Release data showed that formulations containing the highest amount of drug (15 %) displayed fast and higher release rates than those formulations containing a small amount of chloro pheniramine maliate. A prolonged release was observed for the formulation containing lower amount of chloro pheniramine maliate. In other words, with a decreasing amount of drug in the matrix, it is noticed that the release rate becomes quite slower at the lower amount of drug in the matrix, and this is due to the availability of more free void spaces through which lesser number of drug molecules will transport. For all the chloro pheniramine maliate-loaded formulations, the complete release of chloro pheniramine maliate was not observed even after 600 min, but the release rates were around 700 min.

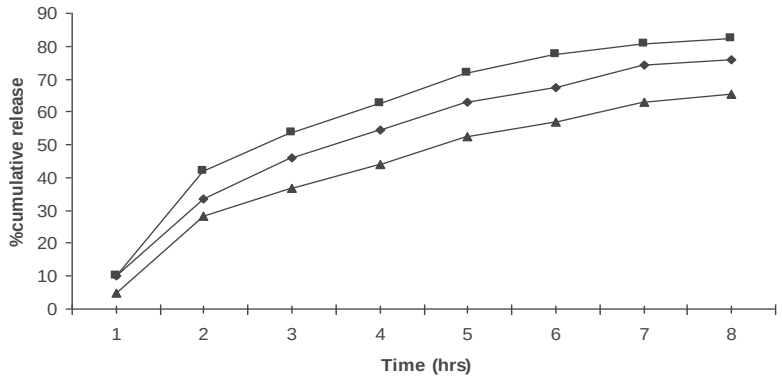

Fig: 7. Cumulative % release of chloro pheniramine maliate through AAm-g-CMC /CS microspheres containing different amount of drug at 37^{0}C. (♦) 10 wt. % drug (■) 20 wt. % drug (Δ) 30 wt. % drugs

Effect of chitosan percent

Effect of chitosan content was studied at constant loading of drug. The release trends of AAm-g-CMC /CS microspheres prepared with different amounts of CS are displayed in **Fig: 8.** it is noticed that during dissolution experiments, the microspheres have shown systematic swollen trends with decreasing amount of CS probably due to the formation of loosely crosslinked net work chains of CS. As the amount of CS increases, cumulative release decreased due to lesser swelling of the CS chains than CMC. This could be because as the amount of CS increases in semi-IPN matrix, the hydrophobicity of the overall matrix

increases, thereby decreasing the release rate for drug. Thus, a regaining –type response of polymeric chains is possible due to the stresses induced by the surrounding solvent media during the dissolution step, resulting in decrease of chain dimension (radius of gyration) of the semi-IPN polymer, this will further decrease the molecular volume of the hydrated polymer due to decreased swelling of CS component of the semi-IPN matrix, there by reducing the free volume spaces of the matrix.

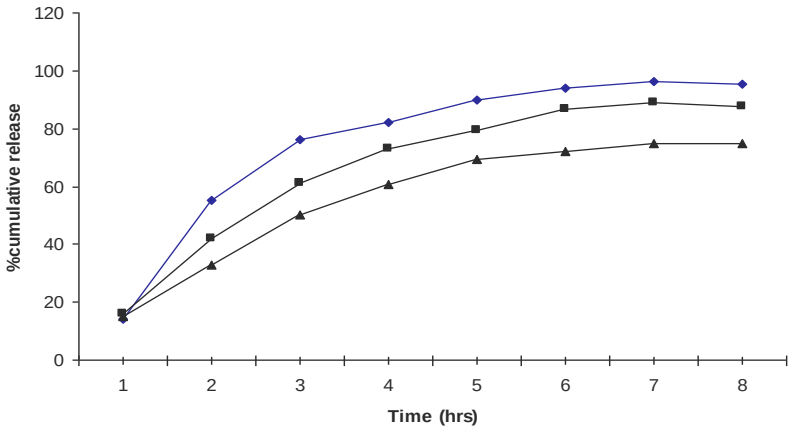

Fig: 8. Cumulative % release of chloro pheniramine maliate through AAm-g-CMC/CS microspheres containing different amount of CS at 37^0c. (♦) 10 wt. % CS (■) 20 wt. % CS, (∆) 30 wt. % CS

CONCLUSIONS

Carbohydrate polymeric grafted microspheres of acrylamide grafted on carboxy methylcellulose and blended with chitosan were prepared and characterized by differential scanning calorimetry, scanning electron microscopy and particle size distribution. DSC thermograms have confirmed the uniform molecular distribution of the drug molecules in the microspheres. SEM micrographs exhibited a spherical morphology of the prepared microspheres. The drug was release in a controlled manner. The swelling studies of microspheres have shown that with an increasing amount of CS in the microspheres, water uptake has decreased. This effect is correlated with the release rates of the drug though the microspheres containing different amount of CS. The microspheres have lower densities and hence, these could be retained in the gastric environment for more than 12 hrs which would help to improve the bioavailability of Chloro pheniramine maleate.

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