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pH-RESPONSIVE SEMI- INTERPENETRATING POLYMERIC HYDROGELS MICROSPHERES OF CHITOSAN AND POLY VINYL ALCOHOL FOR *IN –VITRO* CONTROLLED RELEASE OF CLARITHROMYCIN

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ABSTRACT

pH – responsive biodegradable semi- interpenetrating polymeric network (semi- IPN) hydrogels microspheres based on Chitosan and PVA were prepared and Characterized for the controlled drug release investigations. Glutraldehyde was used as a crosslinking agent. The equilibrium swelling Characteristics were investigated for the hydrogel microspheres at 37^0C in buffer solution at pH 2.1 (simulated gastric fluid, SGF) and pH 7.4 simulated intestinal fluid, SIF). Clarithromycin was entrapped in the hydrogels for stomach-specific drug delivery system for controlled release of drug for eradication of Helicobacter pylori (H. pylori); in vitro release profiles of the drug were established at 37^0 C at pH 2.1 and pH 7.4. FTIR, SEM was used to characterize hydrogels.

Key words: Chitosan, Microspheres, pH-Sensitive, *In vitro* release.

INTRODUCTION

Controlled release (CR) of drugs using polymers is a well-established technology, wherein polymers are judiciously Chosen to encapsulate drug and to monitor its release from the device in a predesigned and controllable manner. The release of active drug depends on the nature of the Chosen polymer matrix and the type of devices used of the many types of devices used in the literature, micro/nanoparticles derived from the biopolymers have attracted much attention in recent years as the potentional drug delivery devices because of their inherent advantages over the conventional type dosage forms.

Among many polymeric systems investigated in CR applications, the prime attention has been focused on natural, synthetic as well as combination of both types of polymers

Natural polymers like sodium alginate, Chitosan and methyl cellulose have been the preferred polymers because of their biocompatibility and biodegradability. However, there are some synthetic polymers that exhibit biocompatibility under the physiological conditions used in CR studies.

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.

Chitosan (CS) is a cationic bioadhesive, biocompatible and biodegradable polymer obtained by alkaline deacetylation of Chitn and hence, is widely used in developing CR devices. Since Chitosan is made by deacetylation of Chitin, the term degree of deacetylation (DDA) is used to Characterize Chitosan. DDA gives the proportion of monomeric units of which the acetylic groups that has been removed, indicating the proportion of free amino groups on the polymer.

Pure Chitosan is too fragile and easy to dissolve in acid conditions, thus restricting its applicability. To improve Chitosan properties for controlled drug delivery, semi- interpenetrating polymer networks (semi-IPNs) of CS with hydrophilic polymers have been reported.

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The mucoadhesive properties of chitosan have been illustrated by its ability to adhere to porcine gastric mucosa in vitro, and hence it could be useful for in site-specific drug delivery. Important mechanism of action was suggested to be ionic interactions between positively charged amino groups in chitosan and the negatively charged mucus gel layer in addition to adhesion by hydration/ due to molecular attractive forces formed by electrostatic interaction between positively charged chitosan and negatively charged mucosal surfaces. The interactions are strong at acidic and slightly acidic pH levels, at which the charge density of chitosan is high. Increase in molecular weight of chitosan results in stronger adhesion. These properties may be attributed to strong hydrogen bonding groups like –OH, –COOH, strong charges ,high molecular weight sufficient chain flexibility, a surface energy properties favoring spreading into mucus.

Poly (vinyl alcohol), PVA, is a non-toxic, water-soluble synthetic polymer and has good physical and Chemical properties and microspheres-forming ability. The use of this polymer is important in many applications such as controlled drug delivery systems, membrane preparation, recycling of polymers and packaging. Studies on the mechanism of dissolution and Changes in crystallinity and swelling behaviour of PVA and its physical gel-forming capabilities, have been carried out. Various crosslinking agents such as formaldehyde and glutaraldehyde have been used for this purpose. The crosslinked Chitosan can be used as a pH-sensitive hydrogel that swells in acidic solutions due to protonation of free amino groups and Chitosan hydrogels have been widely used in CR of drugs in stomach via oral route.

Clarithromycin was Chosen as a model drug for encapsulation in the polymer matrix and to study the in vitro release in the acidic medium as well as basic medium. Formation of interpolymer complexes of Chitosan with polyvinyl alcohol and development of IPN microspheres by crosslinking using glutaraldehyde (GA) has been discussed in this study.

MATERIALS AND METHODS

Clarithromycin was purchased from Regain laboratory private ltd hissar (India). High molecular weight chitosan (degree of deacetylation 85% and viscosity 800–2000 cPs) was procured from Central Drug House Pvt. Ltd., New Delhi (IND). Polyvinyl alcohol was obtained from Central Drug House Pvt. Ltd., New Delhi (IND)

Analytical reagent grade glutaraldehyde solution 25% (v/v) and n-hexane Central Drug House Pvt. Ltd., New Delhi (IND) was all purchased from Central Drug House Pvt. Ltd., New Delhi (IND). All the chemicals were used without further purification.

Preparation of IPN Microspheres

IPN hydrogel microspheres of CS and PVA were prepared by Precipitation or coacervation method (Rokhade et al., 2006). Briefly, CS was dissolved in 2% aqueous acetic acid solution by continuously stirring until a homogeneous solution was obtained. 10 wt% PVA was dissolved in water and heated at 80 °C for 4h. After this, PVA was dispersed in CS solution and stirred overnight to obtain a homogeneous solution. To the clear PVA solution, appropriate amounts of glutaraldehyde as cross linking agent. Chitosan particles are prepared by dropping Chitosan solution into an alkaline solution of sodium hydroxide–methanol) through a compressed air nozzle, which produces coacervate droplets. The hardened microspheres were separated by filtration and washed with n-hexane.

Finally, the microspheres were washed with 0.1 M glycine solution to mask the unreacted GA and distilled water to remove the unreacted GA.

The microspheres were vacuum dried at 40^0C for 24 h and stored in a desiccator until further use. Totally, nine formulations were prepared and the assigned formulation codes are given in Table 1.

Scanning Electron Microscopic (SEM) Studies

SEM micrographs of the microspheres were measured using a JEOL model JSM-840A scanning electron microscope (Japan), and micrographs were taken at the required magnification. A 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.

Determination of Amount of Drug Entrapped

Microspheres of known weight (10 mg) were ground to get the powder using an agate mortar, extracted with 50mL of distilled water and sonicated for 60 min. The solution was centrifuged to remove polymeric debris and washed twice to extract the drug completely. The clear solution was analyzed using UV spectrophotometer at λ max of 282nm. The % drug loading and % encapsulation efficiency values were calculated as:

$$\% \text{ Drug loading} = \frac{\text{Weight of drug in microspheres}}{\text{Weight of microspheres}} \times 100$$

$$\% \text{ Encapsulation efficiency} = \frac{\text{Actual drug loading}}{\text{Theoretical drug loading}} \times 100$$

Equilibrium Swelling Studies

The swelling behavior of Ch and Ch/PVA microspheres was measured at 37^0 C in buffer solution of pH 1.2 (SGF) and pH 7.4 (SIF). The pH were precisely Checked by a pH –meter. The swollen weights, after removal of liquid with a tissue paper were determined at certain intervals until equilibrium swelling was attained.

The percent swelling was calculated by the following equation:

$$\% \text{ swelling} = 100 [(Wt - W_0 / W_0]$$

Where, W_0 is the initial weight and W_t is the final weigh to the swelled microspheres at time t.

***In Vitro* Release Kinetics Studies**

Drug releases from the IPN microspheres loaded with Clarithromycin were investigated in pH 1.2 (SGF) and pH 7.4 (SIF). The experiments were performed in a fully automated dissolution test apparatus. A weight quantity of each sample was placed in 500ml of dissolution media maintained at 37^0 C. The concentration of drug release at particular time interval was measured by UV spectrophotometer at λ max 282nm.

RESULTS AND DISCUSSIONS

SEM Investigation

SEM images of the microspheres of different formulations including group of particles as well as individual particles taken at 500×, 2000× and 8000× magnifications as shown in Fig. In few cases, however, some surface-adhered drug particles are seen as revealed by SEM images.

Determination of Amount of Drug Entrapped

In developing an effective formulation, it is important to achieve high encapsulation efficiency (EE), but these data depend on process variables like drug–polymer ratio, IPN blend composition and extent of crosslinking. In the present case, by increasing the amount of PVA, a slight decrease in % EE is observed due to the formation of loose network, thereby leaching more of drug particles during the formulation. The % EE depends upon the extent of crosslinking as the concentration of crosslinking agent increases, EE also increases due to the formation of a rigid network, thereby retaining more drug particles during formulation.

Equilibrium Swelling Studies

Swelling is the most significant Characteristic of hydrogels and it reflects the affinity of the Chemical structure of hydrogels for water and other surrounding fluid. The swelling of Chitosan is normally investigated in neutral medium and in slightly higher pH value. In acidic medium, however Chitosan usually dissolves. For this reason it is essential to obtain Chitosan in a crosslinked form. There are many factors affecting the equilibrium swelling profiles of the hydrogels. This swelling behavior is expected, as PVA is a highly hydrophilic water soluble polymer. As a consequence this hydrophilicity facilitates the entry of swelling fluid into the microspheres to attain higher degree of swelling. The physical nature of Ch/ PVA blend and the hydrogen bond formation restricts the dissolvaion of the PVA during swelling .The same behavior was noticed in the case of crosslinked Ch and Ch/ PVA microspheres from the figure with 25 % glutraldehyde as crosslinker the % equilibrium swelling was directly related to PVA% in the hydrogels. Introduction of glutraldehyde as a crosslinker affected the swelling behavior of both Ch and Ch/ PVA microspheres. Fig. shows the difference in swelling at equilibrium at pH 2.1 and pH 7.4 at 37^0 C for crosslinked Ch hydrogels prepared with varying degree of glutraldehyde. From the figure it can be seen that as the gluraldehyde content increases, the extent of crosslinking increases and consequently the % equilibrium swelling decreases.

Cumulative release measurement

The release pattern of the drug from the polymeric matrices depends mainly on the swelling behavior of these matrices. The following section discusses factors affecting the release profile of clarithromycin, as a model drug, from the prepared Ch and Ch / PVA hydrogels. Following figure shows the release profile of drug form the non- crosslinked Ch and Ch/PVA at pH 7.4 at 37^0 C. In the case of Ch and Ch + PVA, there is an initial burst in the release in the followed by an almost constant release of drug. Also it should be noted that as the PVA content increases in the blend, the release of drug become faster and attain higher value at equilibrium. At the same pH increasing the PVA % in the drug loaded hydrogels microspheres increases the gel hydrophilicity leading to an increase hydrophilicity leading to an increase in the equilibrium swelling and consequently higher value of drug release were attained.

The dependence of the amount of drug release also depend on the glutraldehyde % in the chitosan and Ch/ PVA hydrogels it clearly confirmed that the extent of drug release at equilibrium is inversely related to the degree of crosslinking . The extent of drug release from all the gel irrespective of their composition was much higher in acidic buffer (pH 2.1) than in weakly alkaline one (pH 7.4), because the release is based on the swelling degree of the hydrogels .

The principle mechanism that explains such release is based on the diffusion through the swollen gels. As discussed these hydrogels attained higher value at swelling at pH 2.1 than pH 7.4. Comparing the release behavior at pH 2.1 with that pH 7.4 reveals that pH of the release medium has greater effect on the release value than does the effect of gel composition. This may be attributed to the chemical structure of chitosan where their amino groups are responsible for such pH – sensitivity.

Table 1. Composition of Ch and Ch blend microspheres

Type	Sample code	Chitosan(Ch)		PVA		Glutraldehyde	
		(gm)	%	(gm)	%	(ml)	%
Ch cross linked MS	ChG1	2	100	-	-	0.4	5
	ChG2	2	100	-	-	0.8	10
	ChG3	2	100	-	-	2	25
Cross linked Ch-PVA MS	ChPG1	1	50	1	50	0.4	5
	ChPG2	1	50	1	50	0.8	10
	ChPG3	1	50	1	50	2	25

Table 2. Entrapment efficiency of microspheres

Sample code	Ch		PVA		GA		E_i	E_i	E%
	(gm)	%	(gm)	%	(ml)	%			
ChG1	2	100	-	-	0.4	5	25	19.46	77.84
ChG2	2	100	-	-	0.8	10	25	20.04	80.16
ChG3	2	100	-	-	2	25	25	22.56	90.24
ChPG1	1	50	1	50	0.4	5	25	20.32	81.28
ChPG2	1	50	1	50	0.8	10	25	21.86	87.44
ChPG3	1	50	1	50	2	25	25	21.36	85.44

Fig 1. Structure of Clarithromycin

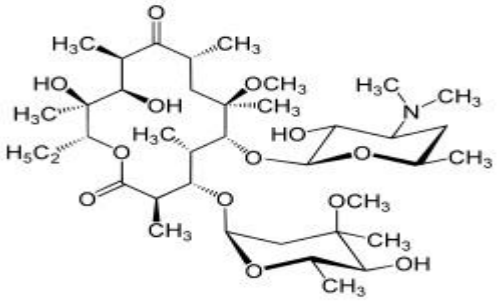


Fig.2. scanning electron microscopy (SEM) photomicrograph of microspheres at 15.00 KX

Fig 3. Swelling % graph of F1, F2, F3

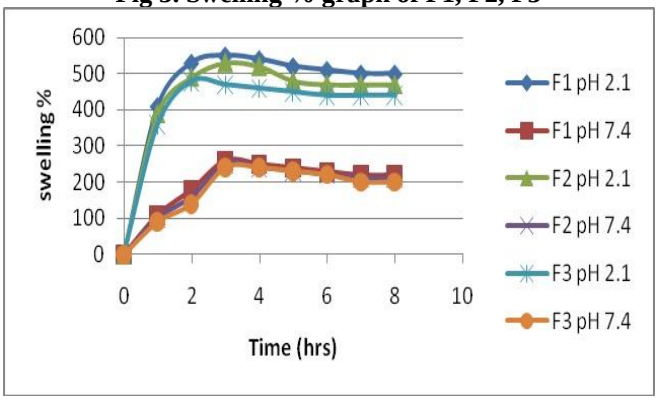


Fig 4. Swelling % graph of F4, F5, F6

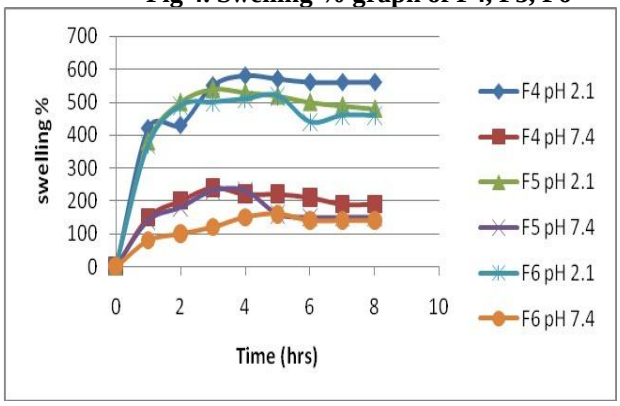


Fig 5. Cumulative release vs time graph of formulation F1, F2 and F3.

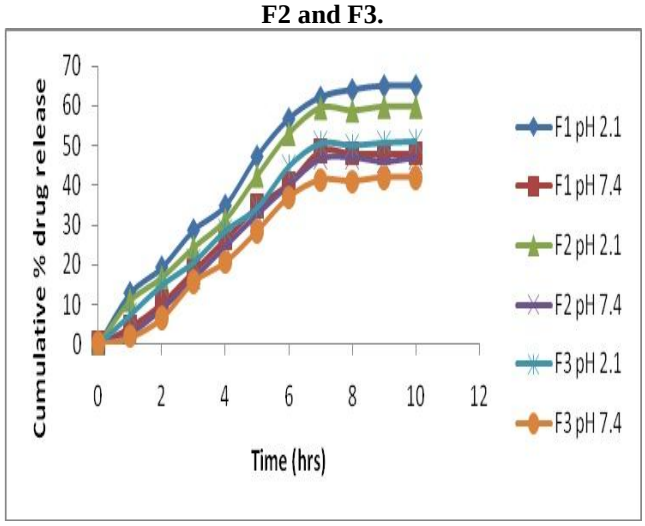
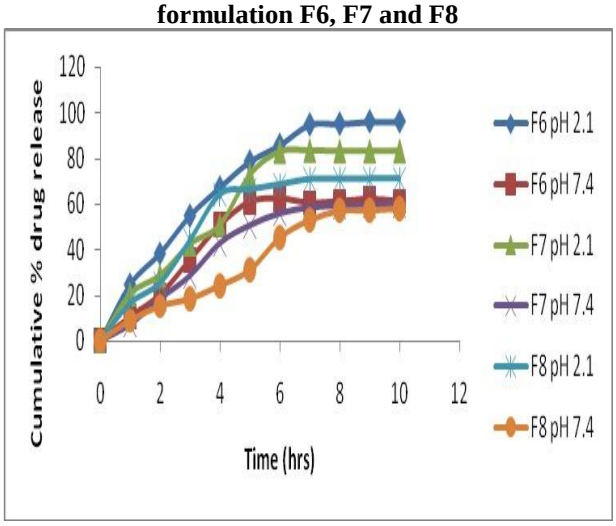


Fig 6. Cumulative release vs time graph of formulation F6, F7 and F8



CONCLUSION

pH dependent semi IPN hydrogels based on Ch and PVA were prepared and investigated for controlled drug release studies. Glutraldehyde was used as crosslinking agent. The pH responsive behavior of these hydrogels was observed through studying their equilibrium swelling at 37^0C in simulated body fluids pH 2.1and pH 7.4 and the microspheres showed higher equilibrium swelling in SGF pH 2.1than in SIF (pH 7.4) also the equilibrium swelling of the chemically crosslinked Ch/ PVA hyrogels microspheres was found to be directly dependent on the content of PVA and inversely related to the crosslinking content. The in- vitro release profile of drug, as a therapeutic agent, from the hydrogels were also estimated at the same pH value. (pH 2.1 and 7.4) . The amount of drug release at equilibrium was found to be dependent on many factors such as PVA%, crosslinker % and the pH of the medium.

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REFERENCES

1. Agnihotri SA, Mallikarjuna NN & Aminabhavi TM. Recent advances on chitosan-based micro and nanoparticles in drug delivery. *Journal of Controlled Release*, 100, 2004, 5–28.
2. Amiji MM. Permeability and blood compatibility properties of chitosan-poly(ethylene oxide) blend membranes for haemodialysis. *Biomaterials*, 16, 1995, 593–599.
3. Aminabhavi TM, Kulkarni AR, Soppimath KS, Dave AM, & Mehta MH. Applications of sodium alginate beads crosslinked with glutaraldehyde for controlled release of pesticides. *Polymer News*, 24, 1999, 285–286.
4. Shi C, Y Zhu, X Ran, M Wang, Y Su, and T Cheng. Therapeutic potential of chitosan and its derivatives in regenerative medicine, *J. Surgical Res*, 133, 2006, 185–192.
5. Hamman J. Chitosan based polyelectrolyte complexes as potential carrier materials in drug delivery systems," *Mar. Drugs*, 8, 2010, 1305–1322.
6. Risbud MV, AA Hardikar, SV Bhat and RR Bhonde. Ph sensitive freeze-dried chitosan -polyvinyl pyrrolidone hydrogels as controlled release system for antibiotic delivery, *J. Control. Re*., 68, 2000, 23–30.
7. Kim SJ, Park SJ, Kim IY, Shin MS & Kim SI. Electric stimuli responses to poly (vinylalcohol)/chitosan interpenetrating polymer network hydrogel in NaCl solutions. *Journal of Applied Polymer Science*, 86, 1997, 2285–2289.
8. Kim SJ, Shin SR, Lee YM & Kim SI. Swelling characteristics of chitosan and polyacrylonitrile semi-interpenetrating polymer network hydrogels. *Journal of Applied Polymer Science*, 87, 2003, 2011–2015.
9. Korsmeyer RC & Peppas NA. Effect of morphology of hydrophilic polymeric matrices on the diffusion and release of watersoluble drugs. *Journal of Membrane Science*, 9, 1981, 211–227.

10. Soppimath KS, Kulkarni AR, Aminabhavi TM. Controlled release of anti hypertensive drug from the interpenetrating network poly(vinylalcohol)-guar gum hydrogel microspheres. *J. Biomater. Sci. Polym. Ed*, 11, 2000, 27–43.
11. Sperling LH. *Interpenetrating Polymer Networks and Related Materials.* Plenum Press, New York, 1981.
12. Kosmala, JD, Henthorn DB, Peppas LB. Preparation of interpenetrating networks of gelatin and dextran as degradable biomaterials. *Biomaterials*, 21, 2000, 2019–2023.