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EFFECT OF CALCIUM TARTARATE AND SODIUM BICARBONATE AS INTERNAL GELLING AGENT ON ENTRAPMENT OF METRONIDAZOLE IN CALCIUM PECTINATE BEADS

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ABSTRACT

Ionotropic gelation, a characteristic property of polysaccharides has become one of the easy and simple ways of encapsulating variety of drugs, enzymes, and proteins. The conventionally cross-linked polysaccharide beads have difficulty for entrapment of water soluble and low molecular weight drugs. For the internally crosslinked beads were prepared with calcium tartrate and sodium bicarbonate was dispersed in drug-pectin solution, then the beads prepared using calcium chloride as surface crosslinking agent. The purpose of present work was to study the effect of internal gelling agents in the entrapment efficiency of Metronidazole in calcium pectinate beads, which is the low molecular weight and water-soluble drug. The beads containing drug and/or calcium tartrate / sodium bicarbonate were prepared in 1% and 5% w/v calcium chloride solution. Particle size, swelling ratio, SEM, DSC, and In-vitro drug release were evaluated with the obtained beads. Beads containing sodium bicarbonate showed more entrapment than calcium tartrate. The calcium tartrate containing beads showed faster swelling rate and drug release.

KEYWORDS: Internal crosslinking, calcium tartrate, calcium carbonate, entrapment.

INTRODUCTION

The crosslinked beads of pectin, sodium alginate, gellan, xanthan gum and other related polysaccharides have been used for design of conventional as well as novel oral drug delivery

systems.[1-5] However, such ionically crosslinked beads showed poor entrapment for the water soluble and low molecular weight drugs as compared to insoluble and large molecular weight drugs. Drug entrapment properties of crosslinked beads are not only governed by the solubility and charge on drug molecule but concentration of polysaccharide, valency and concentration of crosslinking agent, curing time of beads and uniformity of crosslinking in the beads. Beads produced by crosslinking of sodium alginate with Sr^{2+} and Ba^{2+} showed low entrapment of nicardipine and delayed disintegration in alkaline medium as compared to calcium alginate beads.[5-10] Zinc ions had more retarding effect on ketoprofen release from crosslinked pectin beads as compared to calcium pectinate.[11] We have already reported the effect of core and suraface crosslinking of calcium pectinate beads on entrapment and drug release of a water-soluble drug, metronidazole.[13] The use of calcium carbonate as core crosslinking agent in calcium pectinate beads improved metronidazole content by 10-20%.

The purpose of the present investigation was to study effects of calcium tartarate and sodium bicarbonate on the entrapment of metrozidazole in pectinate beads. Calcium tartrate and sodium bicarbonate were used as source of divalent ion calcium and monovalent ion sodium respectively. The beads were evaluated for micromeritic properties, entrapment efficiency, surface topography and thermal properties, swelling study and dissolution study.

MATERIALS

Pectin (LM-104 AS) was the generous gift from CPKelco Pvt. Ltd. (Mumbai. India). Metronidazole was obtained as gifted sample by Aarti drugs Ltd. (Ahmedabad, India). Calcium tartrate and sodium bicarbonate, Sisco Research Lab. Pvt. Ltd. (Mumbai, India) was purchased. All other chemicals used were of analytical reagent grade.

METHODS

Preparation of metronidazole loaded calcium pectinate beads

The 10 ml pectin solutions of different concentrations were prepared by dissolving LM pectin in distilled water with gentle agitation Table I. Beads containing internally dispersed cross linking agent were prepared by dispersing the 400 mg of metronidazole and 10 mg of calcium tartrate or sodium bicarbonate in pectin solutions with constant stirring for 2 min for uniform distribution**.** The resultant dispersion was extruded through 18G (1.2 - mm diameter) needle drop wise into 60 ml of stirred calcium chloride solution (1% and 5% w/v) at room temperature. The extrusion flow rate was approximately 4 ml/min, and then the beads formed

were allowed to remain in the stirred solution for 10 min for curing time. The beads were filtered and washed with distilled water and dried at room temp for 24 hours.[12]

Particle Size

The mean diameter of beads was determined by using a stereomicroscope (Carl Zeiss, Germany) attached with a digital camera (Watec, Wat-202, Japan). The captured images were analyzed using Biovis Image Plus software (Expert Tech Vision, India). About 20 particles of each batch were analyzed and average diameter and different surface factors such as circulatory factor, elongation, roundness and perimeter ratio was determined.

Encapsulation Efficiency

The total weight of dried beads obtained from a batch was considered as practical yield of the process. 100 mg of the drug-loaded beads were dissolved in phosphate buffer pH 7.4 by shaking on rotary shaker (Steelmet Industries, Pune, India) at 200 rpm overnight. The solution was filtered using 0.45 - micron pore size filter and after sufficient dilution with phosphate buffer (pH 7.4), analyzed spectrophotometrically at 320 nm (Jasco V500, Japan). The determinations were made in triplicate. The ratio of the actual metronidazole content in the drug-loaded beads to the theoretical metronidazole content was termed the encapsulation efficiency and calculated by the following equation,

$$\text{Encapsulation Efficiency} = \frac{\text{Amount of encapsulated drug} \times 100}{\text{Amount of added drug}} \quad (1)$$

Encapsulation efficiency difference (EE_{diff}), the difference in encapsulation efficiency of beads prepared using 1% and 5% w/v of calcium chloride solution was calculated.

Surface Topography

The beads were mounted on the standard specimen mounting stubs and were coated with a thin gold-palladium layer (20nm) in sputter coater unit (VG Microtech, UK). Microphotographs of the beads were observed at 50X and 200X magnification using Cambridge Stereoscan 120 scanning electron microscope (Cambridge UK) operated with an acceleration voltage of 10 kV.

Differential Scanning Calorimetry

Thermograms of metronidazole, calcium pectinate beads without drug and drug-loaded beads were obtained using a Mettler- Toledo DSC 821^e (Switzerland) instrument equipped with an

intracooler. Indium standard was used to calibrate the DSC temperature and enthalpy scale. The powder samples were hermetically sealed in perforated aluminum pans and heated at constant rate of 10°C/min over a temperature range of 25 - 300°C. The system was purged with nitrogen gas at the rate of 100 ml/min to maintain inert atmosphere.

Swelling Study

It was carried out in triplicate using three randomly selected beads from each batch. Beads of known weight were placed in wire basket of USP dissolution apparatus II in beaker containing 900ml of 0.1 N HCl (pH 1.2) maintained at 37°C. The beads were periodically removed at predetermined time intervals during study period for 2 hours, drained on tissue paper and weighed. Then the swelling ratio was calculated as per following formula,

Swelling ratio = Weight of wet beads/Weight of dry beads (2)

Dissolution studies

The dissolution of metronidazole loaded calcium pectinate beads was studied using USP 26 Type II dissolution test apparatus (Electrolab TDT-06P, India) containing 900 ml of 0.1 N HCl (pH 1.2) maintained at 37 $\pm$ 0.5^{o} C and stirred at 100 rpm. Samples were collected periodically and replaced with a fresh dissolution medium. After filtration through Whatman filter paper, concentration of metronidazole was determined by spectrophotometrically (Jasco-V500, Japan) at 320 nm. Analysis of data was done using 'PCP Disso v2.08' software, (Poona College of Pharmacy, Pune, India.). All the readings were done in triplicate.

RESULTS AND DISCUSSION

The drug-loaded pectin beads of various pectin concentrations ranging from 3 to 8% w/v were prepared. The crosslinking of beads prepared by dropping drug-pectin dispersion in an aqueous solution of 1% and 5% w/v calcium chloride solution. The beads prepared using 1%w/v ($Cacl_2$) had higher drug entrapment than that prepared using 5%w/v calcium chloride solution. On an average 10 to 20 % increase in drug entrapment was observed in case of sodium bicarbonate as internal gelling agents, than calcium tartrate. The overall effect of internal gelling using calcium tartarate and sodium bicarbonate on drug entrapment was in accordance with that of calcium carbonate already reported us.[12] On the basis of entrapment efficiency batches can be ranked in order of calcium carbonate > sodium bicarbonate > calcium tartarate > only externally cross-linked beads.

Divalent cations forms direct polyanion-cation-polyanion interaction between pairs of carboxylate groups on neighboring helices producing egg-box model.[13] Thus formed intermolecular structure allows loss of water soluble and low molecule weight drugs. Secondly at higher cation concentration the electrostatic repulsion among the carboxylate helices of polysaccharide produces mechanically weak gels.[14] The low entrapment efficiency in the beads produced using 5% calcium chloride solution can be result of thus formed weak gel structure.[12]

The difference in properties of beads obtained using calcium vectors, calcium carbonate and calcium tartrate, indicate that calcium tartrate could have acted differently than the calcium carbonate. The tartrate ions could have competed with calcium ions in the system in that turn decreasing the free ions available for crosslinking with pectin. This may be reason for the lower value of entrapment efficiency and higher value of difference in entrapment efficiency (EE_{diff}) using calcium tartrate.

Sodium bicarbonate was used as a source of monovalent cation for cross linking. Internal crosslinked beads containing Na^+ have different spatial arrangement during crosslinking than Ca^+ ion. The monovalent cations bind individual carboxylic helices forming closely packed network of gel. The presence of Na^+ could have minimized the electrostatic repulsive of Ca^{2+} ions, forming compact gels with increased entrapment efficiency and decreased EE_{diff} (Table I). The unchanged value of EE_{diff} of beads containing 5% w/v pectin concentration may be attributed to weakening and channeling of gel structure due to loss of carbon dioxide gas. The higher drug entrapment with increase in pectin concentration was also observed.

SEM photographs shown in Fig.1 Reveals surface morphology of dried metronidazole loaded Ca-pectinate beads. The beads were spherical with thick gel coat on the surface. The uniform gel layer with some fine drug crystals on bead surface were observed in batch containing lowest polymer and 1% w/v external cross-linked solution Fig. 1(A). As discussed above, calcium tartarate was not much effective to minimize the drug loss in 5% calcium chloride solution and maximum drug loss was observed in the batch AG5b Fig1 (B). Figure 1(C and C1) shows the carbon dioxide bubbles blanketed by thin polymer film and cracks/ channels formed by escape of gas (Batch AG5a). The gas bubbles entrapped under thick blanket of polymer was observed in the case of beads containing high pectin concentration Fig.1 (D and D1).

The mean particle size of the beads containing calcium tartrate and sodium bicarbonate were 2.073 ± 0.057 mm and 4.376 ± 0.075 mm respectively. The effect of calcium vector on bead size was negligible but sodium vector showed variation in size. The size of sodium bicarbonate containing beads of batch AG3a was smallest amongst the various batches, which may be due to the formation of close packed beads by monovalent ions where as entrapment of carbon dioxide in beads contained higher pectin concentration yielded larger beads. The beads obtained were also evaluated for circulatory factor and roundness. The beads were spherical with circulatory factor in range of 1.2 to 2.0 and roundness in range of 0.35 to 0.8.

DSC thermograms of metronidazole, empty beads and drug loaded internally crosslinked beads of batch AG5 prepared in 5% calcium chloride solution are depicted in Figure 2. Metronidazole have shown melting endotherms at 160.79^{o}C (160.44-167.89^{o}C). Empty pectin beads shown two melting endotherms and drug loaded beads shown three endotherms. The broad endotherm at 115^{o}C in empty, plain and drug loaded beads may be due to water loss, peak intensity of which decreases in internally cross-linked beads. The shift of melting endotherm of drug to higher temperature (192 ^{o}C and 187 ^{o}C for calcium tartrate and sodium bicarbonate respectively) were observed in internally cross-linked beads, which may be attributed to slower heat transfer with increase in hardness of the beads.

For swelling studies the dried beads were immersed in 0.1 N HCl for two hours. The beads containing lower pectin concentration showed some variations in the swelling ratio depending on type of vector but at higher pectin concentration the swelling properties of all the beads were almost same. As shown in fig 3, the beads prepared using 5% w/v calcium chloride solution had lower swelling than those prepared in 1% w/v calcium chloride solution. The lower values of swelling ratio of beads containing sodium carbonate may be attributed to the difficulty in penetration of fluid in the closely packed cross linked beads.

All the beads showed almost complete drug release within 30 to 60 min. in 0.1N HCl (pH 1.2) (Table. II.) A typical drug release profile is shown in Fig. 4. The initial drug release from beads prepared using 5% w/v calcium chloride solution was slow than those prepared using 1% w/v calcium chloride solution. The delayed swelling of beads containing sodium bicarbonate may also be responsible for comparatively slow release of the drug.

Table I: Entrapment efficiency of core cross-linked beads.

Batch	Pectin concentration	Internal gelling Agent		% Encapsulation efficiency		%EE $_{diff}$
		Sodium bi carbonate	Calcium tartrate	1% $CaCl_2$ w/v	5% $CaCl_2$ w/v	
AG3	3	10		58.73 ± 1.5	56.27 ± 1.32	2.46
AG3	3		10	52.48 ± 2.3	49.77 ± 1.23	2.71
AG5	5	10		79.39 ± 2.03	63.70 ± 1.74	15.69
AG5	5		10	75.57 ± 2.48	69.56 ± 1.23	6.07
AG6	6	10		85.12 ± 1.59	83.03 ± 2.09	2.09
AG6	6		10	88.00 ± 1.65	83.67 ± 2.15	4.33
AG7	7	10		87.25 ± 1.86	84.00 ± 1.53	3.25
AG7	7		10	89.25 ± 1.96	84.35 ± 1.09	4.9
AG8	8	10		92.62 ± 1.45	90.25 ± 0.098	2.37
AG8	8		10	87.53 ± 1.53	87.53 ± 1.53	3.4

Table II: Percent release profile of calcium pectinate beads at various time intervals.

PECTIN CONC.	CROSSLINKING AGENTS	% RELEASE					
		1% CaCl2			5% CaCl2		
		10min	30min	60min	10min	30min	60min
3%	Sod.bicarbonate	27.21	70.00	81.39	32.68	76.75	87.87
	Cal.tartarate	56.92	83.22	90.29	53.89	81.12	93.56
5%	Sod.bicarbonate	38.01	74.76	81.09	32.27	60.19	65.51
	Cal.tartarate	47.98	74.78	83.61	28.29	66.74	78.97
6%	Sod.bicarbonate	41.56	75.29	80.61	43.83	79.10	87.83
	Cal.tartarate	48.30	77.49	81.99	58.39	81.98	87.51
7%	Sod.bicarbonate	28.02	72.24	82.03	12.25	53.72	70.93
	Cal.tartarate	37.69	65.89	75.13	48.47	70.38	78.01
8%	Sod.bicarbonate	31.15	64.03	71.46	27.37	56.72	69.76
	Cal.tartarate	35.35	67.42	74.33	49.70	66.89	74.91

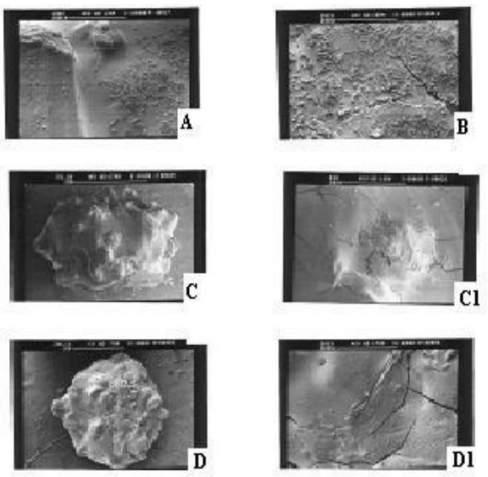

Fig. 1: Scanning electron microscopy of metronidazole loaded calcium pectinate beads at different magnifications. A of batch of batch AG 3-3, B of batch AG 8- 3, CandC1 of batch AG 3-8, D And D1 of batch AG 8-8.

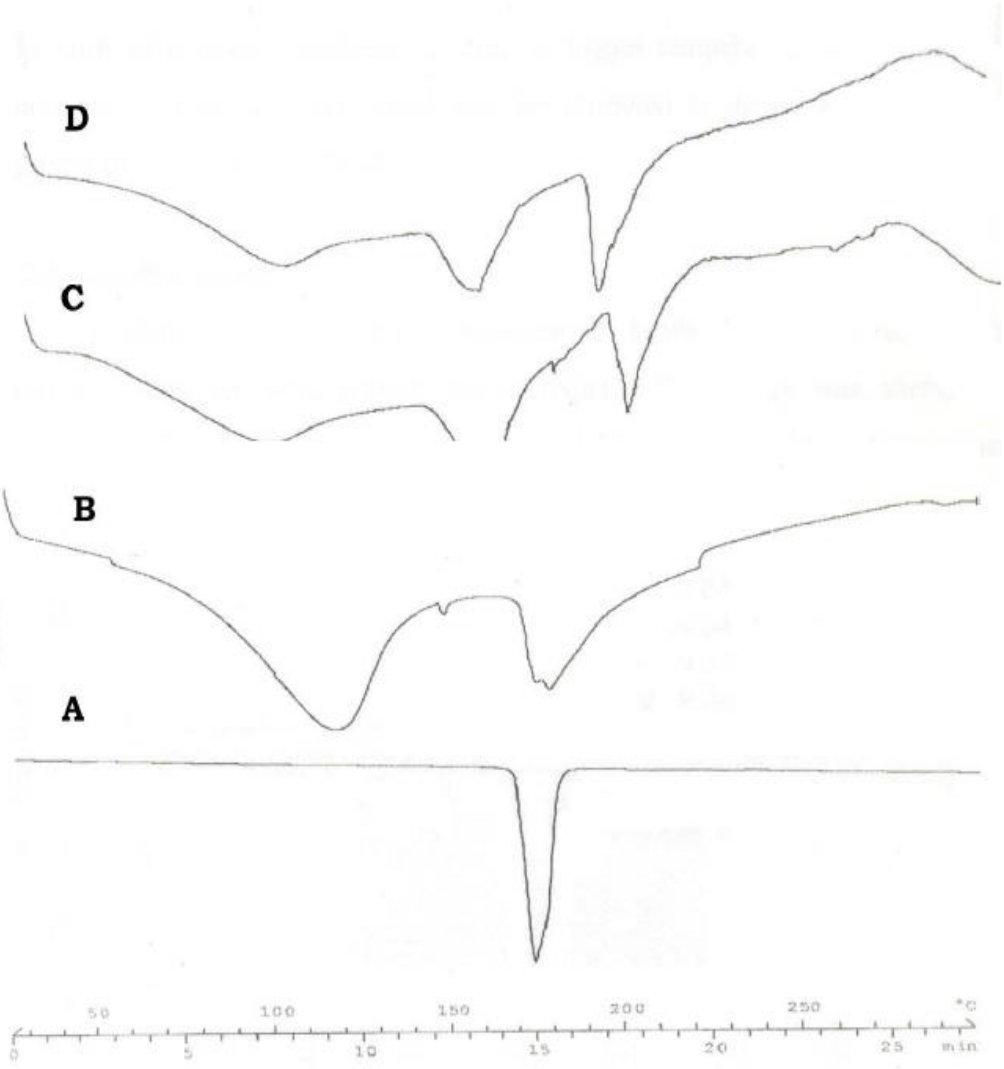

Fig. 2: DSC thermograms of (A) Metronidazole; (B) Empty Ca-alginate beads; (C) Drug-loaded internally crosslinked beads (Calcium tartrate). (D) Drug-loaded internally crosslinked beads (Sodium bicarbonate).

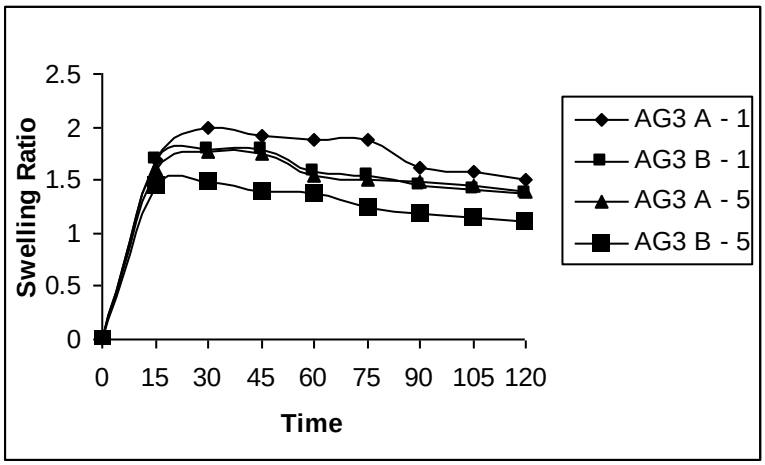

Fig. 3: Swelling ratio of Ca- pectinate beads: Batch AG5.

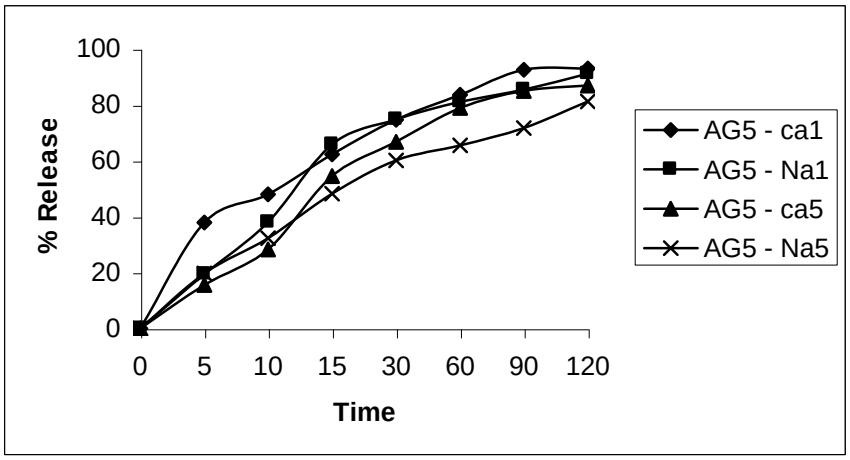

Fig. 4: Effect of internal and external crosslinking on drug release profile of Batch AG-5 with 1% & 5% $CaCl_2$ as external crosslinking agent.

CONCLUSION

From the study it can be concluded that inclusion of internal gelling agent in the internally cross-linked beads would be promising approach to enhance entrapment of water soluble drugs. However, not only ionic vector but also the salt used to provide the cross linking ion determines quality of beads produced.

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