WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 13, 539-547. Research Article ISSN 2277– 7105

THE EFFECT OF DIFFERENT CONCENTRATION OF PECTIN SOLUTION IN CALCIUM PECTINATE BEADS ON THE ENTRAPMENT OF INSULIN

Prabakaran V.[1]* and Dr. N. V. Satheesh Madhav[2]

[1]Research Scholar, Jawharlal Nehuru Technological University, Hyderabad.

[2]Director, DIT, Faculty of Pharmacy, Dehradun.

Article Received on
21 August 2017,

Revised on 12 Sept. 2017,
Accepted on 04 Oct. 2017

DOI: 10.20959/wjpr201713-9813

***Corresponding Author**
Prabakaran V.
Research Scholar, Jawharlal Nehuru Technological University, Hyderabad.

ABSTRACT

Multiple unit dosage forms for oral delivery of bioactive agents offer many advantages over single unit dosage forms. Insulin, Encapsulated in a calcium pectinate gel beads by dispersing the insulin in a Solution of pectin, and beads are prepared by dropping the dispersion in calcium chloride, the beads Formed by Inotropic gelation. The externally cross-linked beads are suitable for Water in soluble and cationic drugs and it is limited for water soluble and drugs low Molecular weight. The purpose of present study was checking the entrapment efficiency (EE) and drug release With externally crosslinked beads. The EE increased up to 39% to 93% by using $CaCo_3$ as a internal cross linking agent with lower concentration of CaCl2 (1%). The EE decreased with increasing the concentration of $CaCl_2$ (5%) up to 14%. The beads where studied for micromeritic properties, SEM, DSC, Swelling and in-vitro studies in acidic medium. SEM micrographs revealed the Leaching of drug and tightness of gel. The drug release was found to be depending on the solubility of drug with no effect of gel characteristics of pectin.

KEYWORDS: Inotropic Gelling, Calcium Pectinate Beads, Entrapment.

INTRODUCTION

Pectin is a cell wall structural carbohydrate in higher plants. Pectin contains large amount of poly (D-galacturonic acid bonded via α-1, 4 glycosidic linkages, in this the carboxyl groups are partially in the methylester form. The degree of esterification (DE) & degree of amidation(DA) determine the content of carboxylic acid in pectin Chain. Pectin with greater than 50% DE is considered to be high ester (HE) pectin, while pectin less than 50% DE is

considered to be low ester (LE) pectin. Calcium ions are essential for gelation of LE pectin, which is determined by the DE and DA of LE pectin. [1,2,3] The exact arrangement of acid and methyl ester groups along the pectin Molecule controls the pectin behavior as a gelling agent. Univalent salts of low DE pectin are highly water soluble and forms gel only at extremely low solution pH or in the presence of Divalent cation, such beads to be satisfactory for water insoluble and cationic drugs and its limited applications for water soluble and low molecular weight drugs. The dug Entrapment and its release from cross-linked polysaccharide are depend on properties polymer, additives and cross linking agent as well as nature of drug. Binding of calcium ion in to poly galacturonate molecule through egg-box complexes with poly saccharide chains in analogous 2_1 conformatin. The amount of calcium ions added in to the pectin gels has impact on gel strength of the gels and the release of incorporated drugs. the presence of calcium ions with the concentration up to optimal amount decrease the release of drug from beads with low DE pectin It can be postulated that the incorporation of the water soluble drug in beads was not significant, as their low encapsulation and fast drug release, governed by the pore size and inherent solubility of drug in a matrix system, due to presence of the aqueous environment.

The purpose of present research work was to study effect of different concentration of $cacl_2$ on the entrapment, drug release and swelling properties of pectin beads. Insulin a high molecular weight drug, widely used as an diabetic patients. The beads were evaluated for micromeritic properties, entrapment efficiency surface topography, and differential scanning calorimetry, swelling study and Permeation study.

MATERIALS

Pectin (LM-104 AS) was the generous gift from CPKelco Pvt. Ltd. (Mumbai. India) Insulin was purchased from by sigma-Aldrich Ltd. (India). Calcium chloride, Sisco Research Lab. Pvt. Ltd. (Mumbai, India.) were purchased. All other chemicals were of reagent grade.

METHODS

Preparation of Insulin loaded Calcium pectinate beads: Beads were prepared by taking 10 ml of pectin solutions of different concentrations by dissolving LM pectin in distilled water with gentle agitation. Insulin (10 IU) was dispersed in pectin solutions. The resultant dispersions were sprayed by using spray gun in to the calcium chloride solution (1% and 5% w/v) with gentle agitation at room temperature. The drug-loaded beads formed were allowed to stand in the solution for 10 min for curing time, filtered and washed with distilled water.

Then the beads were dried at room temp for 24 hours. Such beads termed as 'externally cross linked beads.

Characterization of the beads

Micromeritic Properties: The mean diameter was determined by using a stereomicroscope (Carl Zeiss, Germany) attached with a digital camera (Watec, Wat-202, Japan). The captured images were analyzed by using Biovis Image Plus software (Expert Tech Vision, India). About 200 particles were analyzed and average diameter and different surface factors such as circulatory factor, elongation, roundness and perimeter ratio was determined.

Encapsulation Efficiency

The weights of dried beads were considered as practical yield of the process. 100 mg of the drug-loaded beads were dissolved in 0.01 N Hydrochloric acid buffer pH 2.0 kept in a round bottom shaker for 24 hrs and the content of insulin was assayed by observing absorption at wavelength 277nm using UV-spectrophotometer Jasco V500 (Japan). The determinations were made in triplicate. The ratio of the actual insulin content in the drug-loaded beads to the theoretical insulin content was termed the encapsulation efficiency (EE).

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

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

Differential Scanning Calorimetry

Thermograms of Insulin, calcium pectinate beads without drug, externally gelled 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.

Surface Topography: Microphotographs of the beads were observed at 50X and 200X magnification using scanning electron microscope Cambridge Stereoscan 120 scanning electron microscope (Cambridge UK) operated with an acceleration voltage of 10 kV. The

beads were mounted on the standard specimen mounting stubs and were coated with a thin layer (20nm) of gold in sputter coater unit (VG Microtech, UK).

Swelling Study: It was carried out by placing beads of each batch in wire basket of USP dissolution apparatus II in a beaker containing 500ml of 0.1 N HCl (pH 1.2) maintained at 37°C. The beads were periodically removed at a predetermined interval and weighed before and during the swelling. Then the swelling ratio was calculated as per following formula,

Swelling ratio = Weight of wet beads/Weight of dry beads

The Permeation study of insulin 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 ^{0}C and stirred at 100 rpm. Samples were collected periodically and replaced with a fresh dissolution medium. Analysis of data was done using 'PCP Disso v2.08' software, India. All the readings were done in triplicate.

RESULTS AND DISCUSSION

Beads were prepared by inotropic gelation, which form intermolecular crosslinking between divalent calcium ions and negatively charged carboxyl group of pectin molecules. Divalent metals establish direct polyanion-cation-polyanion interaction between pairs of carboxylic groups on neighboring helices producing egg-box model.[9]

Primary batches of drug-loaded pectin beads containing 2% pectin produced satisfactory beads and was selected as minimum concentration and maximum pectin concentration was 8% above which the gel was viscous to pump through the spray gun.[10,11] The concentration of calcium chloride solution was kept at 1% and 5 % to ensure relative calcium reactivity.

Particle size had shown significant variation between different formulations. The mean particle size of the beads were between 2.073 ± 0.057 mm and 4.376 ± 0.075 mm. (Data of all batches not shown). 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. In externally crosslinked batches the mean particle size of the beads containing constant drug amount increased with increase in polymer concentration. Using 5% w/v calcium chloride concentration it showed erratic pattern on increase in size of bead.

Significant differences were observed in the encapsulation efficiency (EE) using different concentrations of pectin, concentrations. EE was in the range of 38 to 92% as shown in Table 3. The encapsulation efficiency of externally gelled beads increased with the increase in the pectin concentration but EE decreased with increasing the calcium chloride concentration 1% to 5%, The calculated Entrapement effiency difference (EE_{diff}) between 1% and 5% $CaCl_2$ was14 to 21 % except batch AG 3 containing lowest polymer content. The increase in EE with increase in pectin concentration at constant drug amount may be attributed to the availability of excess polymer to encapsulate the drug. The reduction in EE of these batches in 5% calcium chloride solution may be attributed to weakening of surface gel strength due to excess of [Ca^{2+}]. The lower value of EE_{diff} for 3% pectin containing batches can be correlated to lower amount of polymer and higher [Ca^{2+}]: polymer ratio, affecting equally in 1% w/v and 5% w/v.

In 3 % pectin there was a quantum increase in percent entrapment efficiency from 38.44 ± 1.2, using 1% w/v $CaCl_2$ solution. Such difference was slight when 8% pectin was used. This was quite opposite to that when using 5% $CaCl_2$ as external crosslinking solution which maintained a static difference throughout using increasing amount of polymer.

This whole phenomenon reflects the physical and chemical interactions that are associated during formation of pectin gel bead. The weak and flexible gel turns strong and rigid as the availability of cations increases with maximum gel strength on utilization of all possible crosslinking sites. When excess of divalent cations are present, they compete for interaction with anionic sites imposing repulsive forces resulting in weakening of gels. [12] In external crosslinked beads, the poor diffusion of large Ca^{2+} in gel structure causes more surface crosslinking as compared towards core giving rise to increased dissolution / diffusion of small and low molecular weight drug through the crosslinked network. . The difference in the EE using different concentration of $CaCl_2$ can be attributed to the formation of soft gel due to presence of excessive calcium, which is supported by decrease in the EE in 5 % $CaCl_2$, in other way showing the dominance of internal crosslinking agent over the external one. While as in 1% $CaCl_2$ the ratio between the calcium ions and the increasing amount polymer played a crucial role, as the polymer concentration increased thereby minimizing the effect of internal crosslinking agent.

SEM photographs and typical surface morphology of dried Insulin loaded Ca-pectinate beads prepared using 2% and 8% pectin in 1 and 5% calcium chloride solution are shown, (Data of

all batches not shown). Batch AG 3-1, externally crosslinked drug loaded beads prepared at low polymer and low calcium chloride concentration yielded soft beads. The bead surface showed prominent thin ridges by 'pull-away' effect producing rough surface. As extent of crosslinking increased with increasing [Ca^{2+}] the surface of beads obtained using 5%w/v calcium chloride solution became smooth, covered with thick gel layer, imparting sphericity, but fine drug crystals appeared on surface due to squeezing of drug solution. The surface of beads containing highest pectin concentration was comparatively smooth in both 1% and 5% w/v calcium chloride solution. As compared to externally cross linked beads, beads that were internally crosslinked crosslinked with calcium carbonate and obtained by using 1% $cacl_2$ were spherical with entrapped drug crystals in thick gel

Swelling of beads was studied by the dried beads were immersed in 0.1 N Hcl for two hours, they swelled due to rehydration .Pectin contains ester, hydroxyl and carboxyl groups that can easily from hydrogen bonding with molecule upon hydration. The maximum swelling was observed with in 20min. Swelling of externally crosslinked beads increased with increase in pectin concentration and comparatively was more in case of beads obtained by using cross linking agent 1% than 5% solution (Fig 8,). Beads of batch AG3-5 showed faster erosion due to weakening of gel strength. Batch AG 5-5 showed maximum swelling and slower erosion among all externally crosslinked pectin batches indicated optimum amount of pectin and [Ca^{2+}] for crosslinking. The beads prepared by using 8% pectin at 1% $CaCl_2$ had less swelling because of in complete cross-linking. The beads by using 8%pectin at 5%$CaCl_2$ beads had slow erosion because stronger gel formation at 5% calcium chloride solution.

The Permeation studies of the beads were carried out in 0.1N HCl (pH 1.2). All the beads showed almost 80 % drug release within 12 hours. A typical drug release profile and the collective release at different times in the drug release diminished with increasing the pectin concentration with 5% $CaCl_2$. No significant changes with increasing pectin concentration with 1% $CaCl_2$ on drug release.

Table. I: Preparation of insulin loaded calcium pectinate beads.

Batch No.	Drug (mg)	Pectin % w/v	$CaCl_2$ solution % w/v
AG3-1	10 IU	3	1
AG3-2	10 IU	3	1
AG3-5	10 IU	3	5
AG3-6	10 IU	3	5
AG5-1	10 IU	5	1
AG5-2	10 IU	5	1
AG5-5	10 IU	5	5
AG5-6	10 IU	5	5
AG6-1	10 IU	6	1
AG6-2	10 IU	6	1
AG6-5	10 IU	6	5
AG6-6	10 IU	6	5
AG7-1	10 IU	7	1
AG7-2	10 IU	7	1
AG7-5	10 IU	7	5
AG7-6	10 IU	7	5
AG8-1	10 IU	8	1
AG8-2	10 IU	8	1
AG8-5	10 IU	8	5
AG8-6	10 IU	8	5
AG8-7	10 IU	8	5

Table. II: Percent encapsulation efficiency profiles of calcium pectinate beads.

Pectin conc.	% encapsulation efficiency		Difference
	1% $CaCl_2$	5% $CaCl_2$	(% EE_{DIFF})
3%	38.44 ± 1.2	39.52 ± 1.1	1.08
5%	70.38 ± 2.21	54.99 ± 1.02	15.39
6%	75.68 ± 1.89	56.18 ± 3.25	19.5
7%	79.38 ± 1.68	64.88 ± 2.15	14.5
8%	88.24 ± 1.53	67.03 ± 1.86	21.21

EE_{diff}: % Encapsulation efficiency difference.

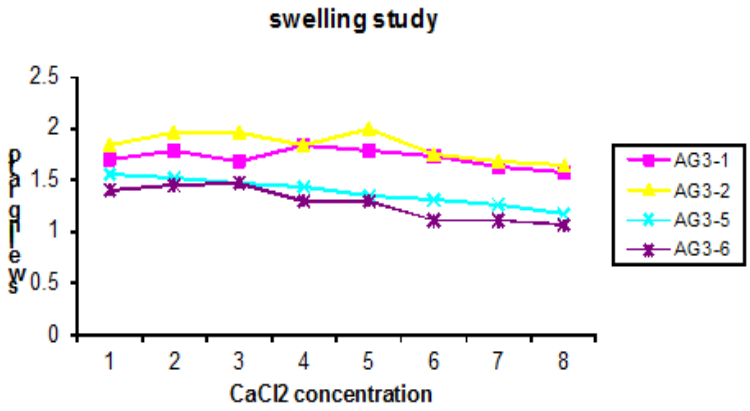

Fig. I: Swelling ratio of Ca- pectinate beads: Batch AG 3.

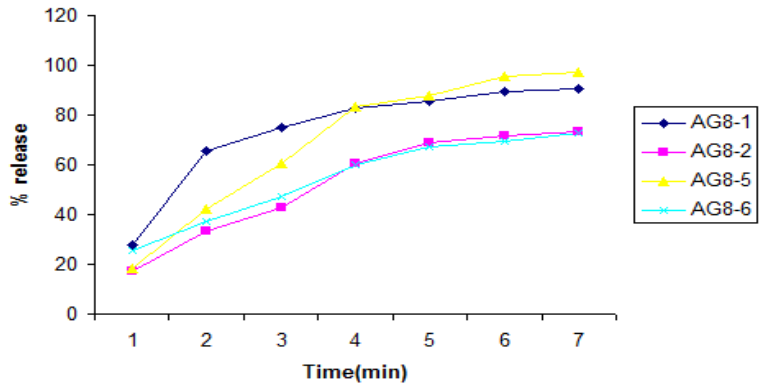

Fig. II: Swelling ratio of Ca- pectinate beads: Batch AG 8.

CONCLUSIONS

Our study has emphasized that crosslinked calcium-pectinate bead by spraying the pectin solution in to calcium chloride solution produces the reproducibility of the particle size and more productivity. The entrapment of insulin in calcium pectinate beads was increased by increase in pectin concentration, but decreased by increasing concentration of calcium chloride solution that was used for peripheral crosslinking. Though in the present study reveals that drug release was dependent on drug solubility and permeability, because the insulin has the high molecular weight. The use of permeation enhancer can modify the permeability of the insulin.

REFERENCES

1. P.Srimornsak, Investigation of pectin as a carrier for oral delivery of proteins using calcium pectinate gel beads, Int. J. Pharm, 1998; 169: 213-220.
2. P.Srimornsak, Effect of calcium concentration, hardening agent and drying condition on release characteristics of oral proteins from calcium pectinate gel beads. Eur. J. Pharm. Sci., 1999; 8: 221-227.
3. S.Sungthongjeen, T.Pitaksuteepong, A. Somsiri, P. Srimornsak,S. Puttipipatkhachorn, Effect of degree of esterification of pectin and calcium amount on drug release from pectin-based matrix tablets, AAPS Pharm Sci. Tech, 2004; 5(1): 9.
4. S.Sungthongjeen,T. Pitaksuteepong, A. Somsiri, P.Srimornsak, Studies on pectins as potential hydrogel matrices for controlled release drug delivery. Drug Dev. Ind. Pharm, 1999; (25): 1271-1276.
5. P.Srimornsak, S. Puttipipatkhachorn, S. Prakongpan, Calcium pectinate gel coated pellets as an alternative carrier to calcium pectinate beads. Int. J. Pharm, 1997; 156: 189-194.

6. Ei – Gibaly, I. Oral delayed release system based on Zn – pectinate gel (ZPG) microparticles as an alternative carrier to calcium pectinate beads for colonic drug delivery. Int. J. Pharm, 2002; 232: 199-211.
7. P.Srimornsak, J. Nunthanid, Calcium pectinate gel beads for controlled release drug delivery: I. Preparation and in vitro studies. Int. J. Pharm, 1998; 160: 207-212.
8. A.Shilpa, S.S. Agrawal, A.R. Ray, Controlled drug delivery from alginate matrix. J. Macromole SciPart C-polymer reviews, 2003; 43(2): 187-221.
9. E. R.Morris, M.G.E. Gothard, M.W.N. Hember, C.E. Manning, G. Robinson, Conformation and rheological transitions of welan, rhamson and acylated gellan. Carbohydrate Polymers, 1996; 30: 165-175.
10. X.D.Liu, D.C. Bao, W.M.Xue, Y.Xiong, W.T.Yu, X.J. Yu, X.J. Ma, Q.Yuan, Preparation of uniform calcium alginate gel beads by membrane emulsification coupled with internal gelation., J. Applied Polymer Sci., 2002; 87: 848-852.
11. D. Poncelet, V. Babak, C. Dulieu, A.A. Picot, Physico-chemical approach to production of alginate beads by emulsification-internal ionotropic gelation. Colloids and Surf. 1999; 155: 171-176.
12. J.Tang, M.A. Tung, Y. Zeng, Compression strength and deformation of gellan gels formed with mono-and divalent cations. Carbohydrate polymers, 1996; 29(1): 11-16.