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# Studies on Pectin as a Potential Carrier in Colonic Drug Delivery

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## Abstract

The feasibility of new microencapsulated cores for colon specific delivery of drug was studied using, diclofenac potassium (DP), a NSAID, as a model anti-inflammatory drug. The polysaccharide pectin was reacted with sodium alginate in the presence of calcium chloride to form microcapsules with a polyelectrolyte complex membrane. Microcapsules were evaluated for physical characteristics such as size, shape, surface features, strength and flexibility of the membrane, viscosity and encapsulation efficiency. Microcapsules were also evaluated for in vitro study containing rat caecal content and ulcer study. It was found that pectin-alginate microcapsules, prepared with 0.5% w/v of the encapsulating polymer and 2.5 % w/v of sodium alginate were best suited for the colon specific delivery. These microcapsules were further modified to attain a higher specificity. The key factor found to affect membrane formation was the pectin molecular weight. These results indicate that the microcapsules with the greatest promise for success are those produced with high and low molecular weight polymers. Increasing the alginate concentration from 1.5 to 2.5% w/v further retarded the drug release. Good colon specificity was achieved at 2.5% w/v concentration of sodium alginate for pectin microcapsules. Selected formulations were also studied for ulcer study. Pure DP showed ulcer index of 30.8 and whereas no ulcer was found in the case of microcapsules.

**Keywords** – Diclofenac potassium, Pectin, Colon, Microcapsules.

## Introduction

A polymeric delayed-release colon specific delivery system was investigated with Diclofenac potassium (DP), a NSAID, as a model anti-inflammatory drug. We expected that this drug would benefit from the proposed system, first, because DP is particularly well absorbed in the colon (Gleiter *et al.*, 1989) and, second because its release in the gastric cavity is avoided thus occurrence of local side effects like gastric discomfort, ulceration, bleeding, etc is minimized. Also DP undergoes an intramolecular cyclisation in an acidic medium (Racz, 1989), causing inactivation of the compound in the gastric juices.

Colon specific drug delivery system has been the focus of increasing interest for the last decade. This is mainly due to recently recognized importance of this region of the gastrointestinal tract, not only for local but also for systemic therapy. Conventional rectal delivery of dosage forms (suppositories and enemas) as alternatives are not always effective since a high variability in the distribution of these forms is observed (Wood *et al.*, 1985). Suppositories are only effective in the rectum because of the confined spread and enemas solution can only effective topical local action to the sigmoidal and descending colon (Rhodes *et al.*, 1985). There are two main classes of bacterial enzymes, the azo reductase and

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polysaccharides, which are in sufficient quantity as to be exploited in colonic drug targeting. Based on this idea, different natural and synthetic polymers have been evaluated for susceptibility of being cleaved by these bacterial enzymes (Friend, 1991) and thus for their use as major constituents for colon specific drug delivery systems (Bronsted *et al.*, 1992). The practical use of these polymers is however limited by some concerns about their safety and also about the control of their decomposition rate. Promising alternative polymers are natural polysaccharides such as chitosan, pectin, guar gum, dextran, amylose, and chondroitin sulphate which are liable to hydrolysis of their glycosidic bonds in the colon. The only inconvenience of these polymers is their high solubility in gastrointestinal fluids this implies the need of crosslinking to assess their integrity until they reach colonic region (Robinstein *et al.*, 1992 and Ashford *et al.*, 1994). Hence, this study looks in to the feasibility of pectin alginate microcapsules for colon specific drug delivery system.

## Materials and Methods

DP was obtained as a gift sample from Amolic organics Ltd., Mumbai, India. Pectin was obtained as a gift sample from G.S. Chemical Testing Lab, India. Analytical grade acetone, sodium nitrite, sodium hydroxide, potassium chloride, calcium chloride and glacial acetic acid were purchased from s.d. fine chemical Ltd., Boiser, India. Sodium alginate was purchased from s.d.fine chimical Ltd., Tamilnadu, India.

*Preparation of encapsulation solution:* Pectin solution of 0.3, 0.4, and 0.5 % (w/v) were prepared with distilled water. The flasks covered with paraffin and stirred for 2 hrs. The solutions were filtered through a nylon mesh filter and were referred to as one aliquot of pectin solution.

Alginate solutions of 1.5, 2.0, 2.5 % (w/v) were prepared with distilled water containing previously dissolved diclofenac potassium (50mg). Calcium chloride (1.5 % w/v) solution was dissolved in one aliquot of pectin solution by continuous stirring and the resulting solution of pectin and calcium chloride was then used in the encapsulation procedure detailed below.

*Microcapsule formation and drying:* 20ml of alginate solution was loaded into a 20ml syringe fitted with a 23G needlewhich was then attached to the syringe piston. 100ml of pectin and calcium chloride solution was taken in a 500ml beaker and stirred gently. The stirrer was placed during under the syringe so that the needle tip was 6cm above the liquid surface. Alginate + drug solution was extruded at a constant rate of 30 $ml.hr^{-1}$. A reaction time of 20 min was used. After formation of microcapsules the excess of pectin calcium chloride solution was removed and the microcapsules were stirred in distilled water for further 5 min to remove excess salts. Acetone was chosen as the drying agent. After removal of the distilled water, acetone was then removed by filtering through a nylon mesh. The capsules were further air - dried for 24 hrs.

The molecular weight of pectin was reduced by using nitric oxidation reaction. A solution of 0.1% w/v sodium nitrite in distilled water was prepared. Pectin solution was prepared in the concentration twice that of one aliquot and to this sodium nitrite solution was added, with a molar ratio of 0.05 for one aliquot of pectin solution. The resulting nitrite oxidation reaction results in random stoichometric cleavage and was permitted to continue overnight to ensure completion of reaction. Reduction of molecular weight was confirmed by the reduction in the viscosity.

*In vitro evaluation:* Microcapsules, 50mg equivalent to drug, were placed in 100ml amber colored bottle, and contacted with 50ml of the elution medium. The amber colored bottles were water jacketed and maintained at a constant temperature of 37±0.2°C. A buffer change method was used to mimic the gastrointestinal environment. Potassium chloride buffer solution of pH 2.0 was used for the first 2hrs. Then, phosphate buffer of pH 7.0 was used for the next 3 hrs, and finally, rat caecal content medium in phosphate buffer of pH 6.8 was used for the last 4 hrs.

Buffers were changed at the end of each hour. One ml of samples were collected and analyzed for drug content spectrophotometrically at 275nm.

*Preparation of rat caecal content medium:* The susceptibility of microcapsules containing pectin to the enzymatic activity of colonic bacteria was assessed by conducting the drug release studies in pH 6.8 phosphate buffer containing rat caecal contents because of their similarity with to humans with respect to intestinal microflora. The medium was prepared as follows: Male albino rats, weighing 150-200gm and maintained on normal diet were used throughout the study. Thirty minutes before the commencement of drug release studies, two rats were killed by spinal traction. The abdomen were opened, the caecai were traced, ligated at both ends, dissected and immediately transferred into pH 6.8 phosphate buffer, previously bubbled with $CO_2$. The caecal bags were opened; their contents were individually weighed, polled and then suspended in phosphate buffer to give required caecal dilution (1%w/v). As the caecum is naturally anaerobic all these operations were carried out under $CO_2$.

*Drug release studies in rat caecal content medium:* The drug release kinetics from the prepared microcapsules were carried out using in-house fabricated dissolution set up. 50ml of the dissolution medium, taken in a beaker of 100ml capacity was kept in a thermostated water bath (37± 1.0°C) and the entire set up was kept on a magnetic stirrer. Weighed quantities of the microcapsules were added to the medium, the dynamics of which was maintained by a magnetic bead rotated at a speed of 100 rpm. A continuous supply of carbondioxide was facilitated using a bent glass tube immersed in to the dissolution medium. A sampling port plugged with cotton wool was clamped. At predetermined time intervals 1ml of samples were withdrawn and replaced with 1ml of an equal volume of pre-warmed dissolution medium to maintain sink condition throughout the period of study and the experiment was continued for 4 hrs. The samples collected during the study were analyzed spectrophotometrically against the corresponding buffer blanks.

*Size, shape and surface features:* Microcapsules were evaluated for their size, shape and surface features with light microscopy and scanning electron microscopy.

*Strength and flexibility of microcapsule membrane:* Microcapsule strength was determined qualitatively with the aid of light microscope and forceps and was defined as the amount of deformation (i.e. change in diameter) a wet capsule could endure before rupturing. A rating of one (+) indicated that the capsule ruptured with very small pressure applied (< 10% change in diameter), and a rating of four (++++) indicated that the capsule withstood significant deformation before rupturing (to ~ 70% decrease in capsule diameter). The flexibility was also given a qualitative measure and was defined as the amount of deformation that could be applied and still has the membrane return to its original shape. A rating of one (+) indicated that the capsule permanently deformed with very little applied pressure (10% change in diameter) and a rating of four (++++) indicated that the capsule withstood significant deformed (to ~ 70% decrease in capsule diameter). To analyze the strength and flexibility, the capsules were contacted with sodium citrate prior to testing so as to liquefy the core.

*Encapsulation efficiency:* Accurately weighed microcapsules, 50mg equivalent of to drug, were contacted with 50ml of phosphate buffer pH 7.0 in 100ml amber colored vial. This was stirred at 250 rpm using a magnetic stirrer and teflon coated iron bead for 24 hrs. The temperature was maintained at 37±0.2°C. At the end of 24 hrs, the buffer media was filtered to remove the undissolved capsule and core particles, and the filtrate analyzed for diclofenac potassium spectrophotometrically at 275nm. Citration of the capsules, with 0.05M sodium citrate solution for 6 min, to release the remaining DP permitted the calculation of the original DP loading.

$$\text{Encapsulation efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}}$$

$$\text{Theoretical drug content} = \frac{\text{Weight of drug in core}}{\text{Weight of core} + \text{Weight of polymer}}$$

*Viscosity:* The viscosities of the solutions were determined by measuring the time required for the liquid to pass between two marks as it flows by gravity through a vertical capillary tube, known as Oswald viscometer.

*Studies on ulcerogenic effect:* Rats of either sex, weighing between 140-200gms, were used. These rats were fasted for 24hrs, before subjecting them to the study. After 5 hrs of the administration of pure drug ($100mg.kg^{-1}$) and microcapsules, the animals were killed and their abdomen were opened by midline incision. The stomach was spotted, taken out, and cut along greater curvature. It was then washed with running water to see for ulcers in the glandular part of the stomach. The number of ulcers per stomach was noted and the severity of ulcers scored with hand lens (10×).

## Results and Discussion

Microcapsules prepared with 1.5% sodium alginate (control alginate beads) showed an approximately size of 600μm and pectin microcapsules size ranges from 787-1192μm. There was significant rise ($P< 0.01$) in the sizes of pectin microcapsules as compared to control alginate bead.

The SEM photographs of pectin alginate microcapsules are shown in plate 1 and 2. Microcapsules were spherical in wet conditions, but turned to disc shaped after drying. During the drying process, the microcapsules settled under gravity as the surrounding fluid was removed. This caused the capsules to spread out to form a disc shape with a collapsed center (Plate 1). The thickness (vertical dimensions) were reduced to approximately one third of the dry capsule diameter (horizontal dimensions). The pectin membrane itself appeared to be porous in nature (Plate 2).

*Strength and flexibility:* Low concentration pectin formed very thin membranes that were very difficult to see under the microscope. They formed weak capsule membranes (rating of +) that permanently deformed when squeezed to 85% of their original diameter. On the other hand, capsules prepared with high concentration pectin had good strength and flexibility characteristics (rating of +++) as shown in Table-2. The capsules which were coated with reduced molecular weight pectin had very good strength and flexibility (rating of ++++). Microcapsules survived squeezing with forceps to 70% of their original diameter before rupturing. Flexibility was also rated as high for those microcapsules as they were able to regain their spherical form after squeezing, on condition that the membrane had not ruptured. Microcapsules prepared with combination of high and low molecular weight pectin showed more strength and flexibility as compared to microcapsules prepared with high molecular weight polymers (rating ++++).

*Viscosity:* Viscosity of pectin (0.5% w/v) was found to be 28.19cps which after nitric oxidation-reduction was found to be 17.56 cps.

*Encapsulation efficiency:* The encapsulation efficiency of microcapsules decreases, as the concentration of polymer increases from 0.3 to 0.5% w/v as shown in Table 2. This might be due to increase in concentration of the polymer. The contribution of the drug to the net weight of an individual microcapsule decreases leading to a decrease in encapsulation efficiency. This could also explain the decrease in drug loading when microcapsules were given various coatings.

Plate-1 SEM photomicrograph of pectin microcapsules at 57X

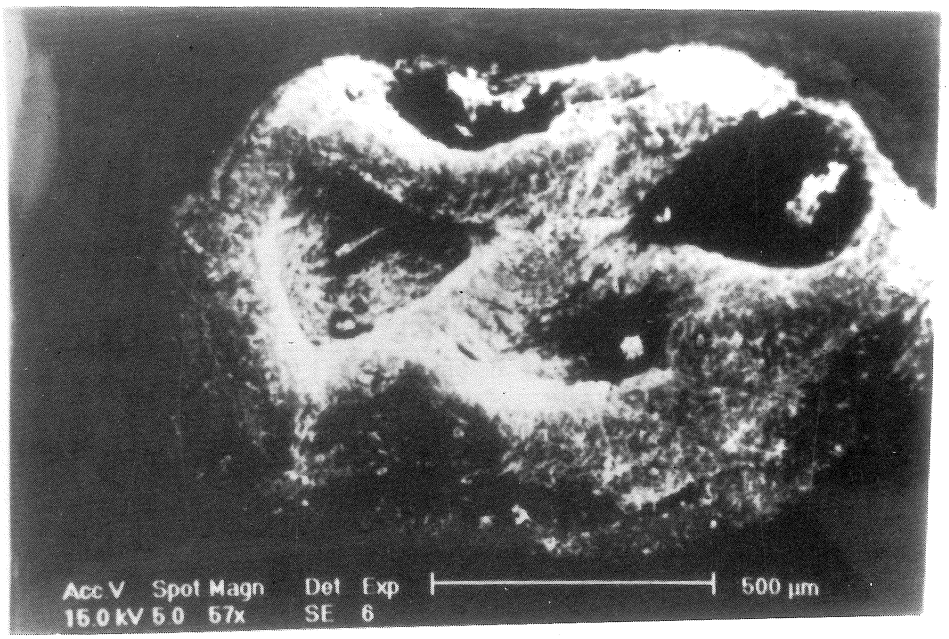


Plate-2 SEM photomicrograph of pectin microcapsules at 386X

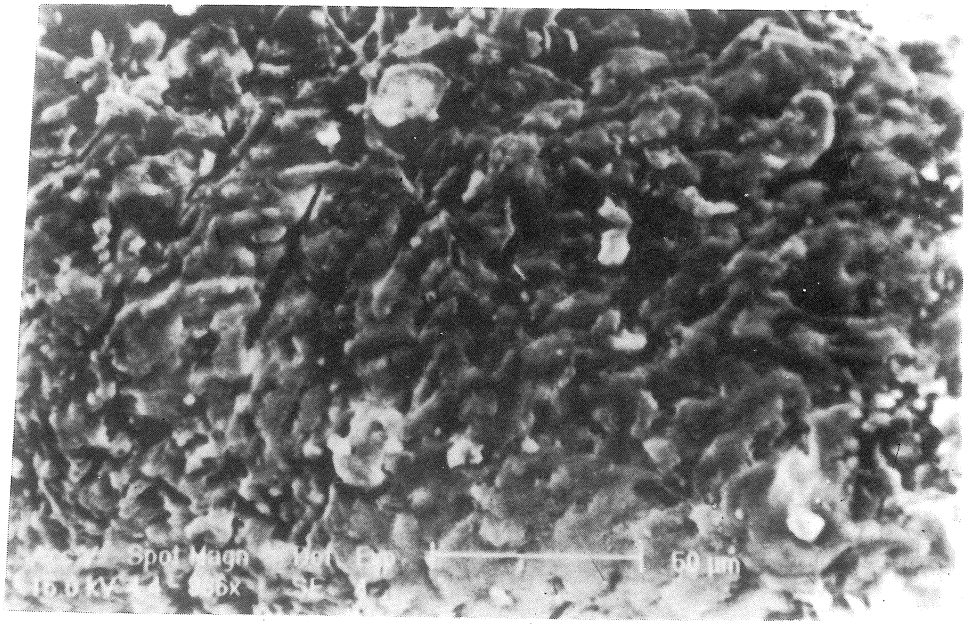

Table 1. Microcapsules were prepared to stud the effect of pectin concentration, sodium alginate concentration and the molecular weight of the encapsulation solution.

| Notation | Microcapsule core | Encapsulation solution | Outer coat |
|---|---|---|---|
| A | DP+ 1.5%Sodium alginate in distilled water | Calcium chloride in distilled water | - |
| $PS_1$ | DP + 1.5% Sodium alginate in distilled water | Calcium chloride + 0.3% pectin in distilled water. | - |
| $PS_2$ | DP + 1.5% Sodium alginate in distilled water | Calcium chloride + 0.4% pectin in distilled water | - |
| $PS_3$ | DP + 1.5% Sodium alginate in distilled water | Calcium chloride + 0.5% pectin in distilled water | - |
| $PS_4$ | DP + 2% Sodium alginate in distilled water | Calcium chloride + 0.5% pectin in distilled water | - |
| $PS_5$ | DP + 2.5% Sodium alginate in distilled water | Calcium chloride + 0.5% pectin in distilled water. | - |
| $PS_{5R}$ | DP + 2.5% Sodium alginate in distilled water | Calcium chloride + 0.5% pectin in distilled water | Calcium chloride+0.5% w/v reduced molecular weight pectin in distilled water. |

Table 2. Physical characteristics and encapsulations efficiency of the various microcapsules.

| Batch code | Strength[a] | Flexibility[b] | Encapsulation efficiency |
|---|---|---|---|
| A | - | - | 96.19 ± 1.25 |
| $PS_1$ | + | + | 93.20 ± 0.59 |
| $PS_2$ | + | ++ | 87.78 ± 0.61 |
| $PS_3$ | +++ | +++ | 83.61 ± 0.54 |
| $PS_4$ | +++ | +++ | 83.60 ± 0.56 |
| $PS_5$ | +++ | +++ | 78.97 ± 0.76 |
| $PS_{5R}$ | ++++ | ++++ | 75.16 ± 1.39 |

[a] + very weak, ++ weak, +++ strong, ++++ very strong
[b] + very fragile, ++fragile, +++flexible,++++ very flexible
- could no be evaluated

*In-vitro drug release studies:* Control formulation A (1.5% w/v sodium alginate) released 98% of the drug in 9hrs. The microcapsules prepared with higher concentration of pectin (0.5% w/v) released DP slower than the control beads (A). For example, at 5 hrs, the control alginate bead released 68.8% of entrapped DP, whereas the capsules prepared with 0.5% w/v pectin released 50.37 % of DP (Table3). This means the control alginate bead had released 31% of DP in the colon whereas microcapsules prepared with 0.5% w/v pecin released 50 %of DP in the colon

because colonic transit time is 20-30hrs. As the pectin concentration was increased from 0.1 to 0.5% w/v, enteric nature of the microcapsules was also increased.
The colon specificity increases, as the sodium alginate concentration was increased from 1.5 to 2.5%w/v. The microcapsules prepared with 0.5% w/v pectin and 2.5%w/v of sodium alginate released 35.22 % of DP at the end of 5hrs. This showed better colon specificity, since 65 % of drug it releases into the colon (Table4). This increased colon specificity might be due to increase in the alginate concentration which would create better cross linking and reduce membrane permeability. At higher alginate concentrations, a better surface for membrane formation might have been created due to tighter packing of the alginate beads.

Table 3. Effect of pectin concentration on in vitro release of microcapsules

| Time | A | $PS_1$ | $PS_2$ | $PS_3$ |
|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 |
| 1 | 4.12 | 2.097 | 1.16 | 0.07 |
| 2 | 6.14 | 3.96 | 2.25 | 1.78 |
| 3 | 20.33 | 19.24 | 16.81 | 14.87 |
| 4 | 49.4 | 47.29 | 41.05 | 37.78 |
| 5 | 68.8 | 65.45 | 58.57 | 50.37 |
| 6 | 78.15 | 70.11 | 72.13 | 75.4 |
| 7 | 90.3 | 74.93 | 76.15 | 78.51 |
| 8 | 97.5 | 79.75 | 83 | 83.95 |
| 9 | 98 | 83.95 | 85.8 | 88.14 |

Table. 4 Effect of sodium alginate and combination of high and low molecular weight polymer on in vitro release of microcapsules.

| Time | $PS_4$ | $PS_5$ | $PS_{5R}$ |
|---|---|---|---|
| 0 | 0 | 0 | 0 |
| 1 | 0 | 0 | 0 |
| 2 | 0.85 | 0 | 0 |
| 3 | 11.6 | 0.17 | 17.37 |
| 4 | 26.87 | 21.11 | 22.98 |
| 5 | 41.37 | 35.22 | 32.48 |
| 6 | 78.35 | 80.37 | 51.3 |
| 7 | 84.1 | 86.12 | 53.48 |
| 8 | 87.83 | 91.1 | 63.27 |
| 9 | 92.5 | 94.35 | 68.56 |

For the last four hours, the dissolution testing was carried out in pH 6.8 phosphate buffer containing rat caecal content. It was observed that the drug release in the last four hours was increased to a greater extent as compared to the previous hours. Microcapsules, swollen with phosphate buffer (pH 7.0) used during the previous hours, started disintegrating and released the drug rapidly. This probably was due to the action of caecal enzymes liberated by the caecal microflora. Pectin is natural polysaccharide that degrade by the caecal microflora. Hence, the caecal enzymes could also degrade the membrane formed by these polymers. Once the membrane barrier is eliminated, there is not much retardation of drug diffusion because the alginate core is already swollen and this result with a rapid drug release. These microcapsules were further modified to attain higher colon specificity. The significant decrease in capsule membrane permeability to diffusion of DP can be attributed to a combination of high and low

molecular weight polymers. It is likely that the contacting of microcapsules prepared with high molecular weight pectin with solution of reduced molecular weight pectin allowed the shorter chain lengths of pectin to penetrate into are as the larger chain lengths could not. This greater penetration would have produced a thicker and stronger membrane and created a greater barrier to diffusion. Microcapsules prepared with combination of high and low molecular weight polymer had released 18.02% of entrapped drug at the end of 5 hours. This showed that it released 82% of drug into the colon.

*Ulcer study:* It was found that ulcer index was 30.8, which was significantly high in the case of DP whereas no ulcer was observed in case of microcapsules. This indicates that gastric toxicity of DP was very high but when it was coated with polymers, its toxicity was significantly reduced.

*Conclusions:* This work has demonstrated that pectin-alginate membranes may be used as a vehicle for colon specific drug delivery system. The extrusion method used provides a quick and effective method of producing membranes, and can be scaled up with existing technology. The key factor found to affect membrane formation was the pectin molecular weight. These results indicate that microcapsules with the greatest promise for success are those produced with either high molecular weight pectin or combination of high and low molecular weight pectin.

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