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Research Article

FORMULATION AND EVALUATION OF ENTERIC COATED MICROSPHERES OF KETOPROFEN USING NATURAL POLYMERS FOR COLON DRUG DELIVERY

NEHA SHARMA*, S.L. HARIKUMAR

Department of Pharmaceutics, Rayat & Bahra Institute of Pharmacy, Sahauran, Distt. Mohali, Punjab 140104, India.
Email: missneha1000@yahoo.com

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ABSTRACT

Objective: The aim of the present study was to prepare and evaluate shellac-coated pectin microspheres for the colon targeted delivery of Ketoprofen.

Methods: Pectin microspheres containing ketoprofen were prepared by emulsion dehydration method using different ratios of pectin (1:1 to 1:6), stirring speeds (500-1500 rpm) and emulsifier concentration (1.25% w/v). Shellac-coating of pectin microspheres was performed by emulsion–solvent evaporation technique using different core: coat ratios (1:2 to 1:5). The prepared microspheres were evaluated for surface morphology, percentage yield, particle size, percentage drug entrapment, swellability, flow properties, *in vitro* release studies and stability kinetics.

Results: Pectin microspheres prepared by using drug: polymer ratio 1:3 and 1:4, stirring speed 1000 rpm, and 1.25% w/v concentration of emulsifying agent were selected as optimized formulations. The percentage yield was high for 1:3 and 1:4 ratio pectin microspheres. In case of shellac-coated microspheres, the percentage yield and % drug entrapment efficiency was high for ratio 1:5. Hence, the shellac-coated microspheres having core: coat ratio 1:5 was selected as an optimized formulation. Further six batches of shellac-coated pectin microspheres were prepared using optimized core: coat ratio 1:5. The release profile of ketoprofen from the six batches of shellac-coated microspheres was pH dependent. In SGF of pH 1.2, no measurable drug release observed; however, the significant drug release was observed in colonic fluid (pH 7.4). Stability studies suggested that the formulation is quite stable at 4º C and is the most suitable temperature for storage of prepared micropsheres.

Conclusion: It can be concluded from the present investigation that Shellac-coated pectin microspheres are promising controlled release carriers for colon-targeted delivery of Ketoprofen.

Keywords: Ketoprofen; pectin; microspheres; shellac coating; colon targeting.

INTRODUCTION

In the controlled release area, biodegradable microspheres are one of the most useful devices to deliver materials in an effective, prolonged and safe manner. Biodegardable pectin microspheres offer a novel approach for developing sustained release drug delivery systems that have potential for colonic drug delivery [1]. Colon specific drug delivery is intended to improve the efficacy and reduce side effects by exerting high drug concentrations topically at the disease site. Because of the distal location of the colon in the gastrointestinal (GI) tract, an ideal colon-specific drug delivery system should prevent drug release in the stomach and small intestine, and affects an abrupt onset of drug release upon entry into the colon. This requires a triggering mechanism built in the delivery system responsive to the physiological changes particular to the colon. However, the physiological similarity between the distal small intestine and the proximal colon presents very limited options in selecting an appropriate drug release triggering mechanism [2].

The purpose of designing multiparticulate dosage form is to develop a reliable formulation that has all the advantages of a single unit formulation and yet devoid of the danger of alteration in drug release profile and formulation behaviour due to unit to unit variation, change in gastro-luminal pH and enzyme population. Ketoprofen is one of the most widely used therapeutic substances due to its analgesic, antipyretic and anti-inflammatory properties. Despite the proliferation in development of new non-steroidal anti-inflammatory drugs (NSAIDs), ketoprofen remains one of the most effective 'over-the-counter' drugs in the treatment of rheumatoid arthritis disease. Use of ketoprofen is associated with two major limitations; first, rare, but serious and sometimes fatal, gastrointestinal (GI) side-effects, including ulceration, and hemorrhage, especially in the elderly, and second, poor water solubility [3]. Successful targeted delivery of drugs to the colon via the gastro intestinal tract requires the protection of a drug from degradation, release and/or absorption in the stomach and small intestine and then ensures abrupt or controlled release in the proximal colon. This might be achieved by the use of specially designed drug delivery system (DDS) that can protect the drug during its transfer to colon [4, 5].

The objective of the present investigation was to formulate colon specific enteric coated microspheres of ketoprofen for the treatment of irritable bowel syndrome by utilizing the pH-dependent solubility of Shellac polymers and microbial degradability of Pectin polymers. Ketoprofen-loaded Pectin microspheres were prepared, which is then microencapsulated with Shellac polymer. Shellac polymer shows the solubility at or above pH 7.

MATERIALS AND METHODS

Materials

Ketoprofen was purchased from Allwell Pharmaceuticals, Chandighar. Pectin was obtained from Central Drug House, New Delhi. All other chemicals were of analytical grade and procured from commercial sources.

Method

Preparation of ketoprofen loaded pectin microspheres

The pectin microspheres were prepared by emulsion dehydration technique [5]. Pectin and ketoprofen were dissolved in 20ml of distilled water and stirred overnight to solubilize completely. This drug-polymer solution was dispersed in 50 ml light liquid paraffin containing 1.25% wt/vol span 80 and stirred at 1000 rpm continuously to obtain stable water/oil (w/o) emulsion. The solution was rapidly cooled to 15^{0}C and then 50 ml acetone was added in order to dehydrate the pectin droplets.

This system was maintained under mechanical agitation with propeller stirrer at 1000 rpm at 25^{0}C for 30 minutes to allow the complete solvent evaporation. The Microspheres were washed with acetone, collected and placed in vacuum desiccators overnight and then subjected to characterization.

Coating of ketoprofen loaded pectin microspheres

The ketoprofen loaded pectin microspheres were microencapsulated by emulsion-solvent evaporation technique [6]. The pectin microspheres (100 mg) were dispersed in 20 ml of coating solution prepared by dissolution of different ratios of shellac in ethanol: acetone mixture (2:1). This organic phase was then poured in 40 ml of light liquid paraffin containing 1% wt/vol span 80. The emulsification process was carried out for 2 h at 1000 rpm with mechanical stirrer. The Shellac coated microspheres were collected and rinsed with n-hexane and dried.

Characterization of uncoated and coated pectin microspheres

Particle size analysis

Particle size distribution of the pectin microspheres alone and shellac-coated pectin microspheres were determined by otical microscopy using calibrated ocular eyepiece micrometer. Product dispersed in light liquid paraffin and smear of the dispersion was observed under compound microscope.

The size of 100 microspheres was measured in each case against a calibrated eyepiece in micrometer [7, 8].

Determination of shape and sphericity

Morphological appearance and surface characteristics of the pectin microspheres alone and shellac coated pectin microspheres were studied by dispersing the microspheres in liquid paraffin and observed under light microscope [7, 8].

Scanning Electron Microscopy

The shape and surface morphology of pectin microspheres alone and shellac-coated pectin microspheres were investigated using scanning electron microscopy (SEM). The samples for SEM study were prepared by lightly sprinkling the formulation on a double-adhesive tape stuck to an aluminum stub. The stubs were then coated with gold to a thickness of ~300 Å under an argon atmosphere using a gold sputter module in a high-vaccum evaporator. The coated samples were then randomly scanned and photomicrographs were taken with a scanning electron microscope (Jeol JSM-1600, Tokyo, Japan) [9].

Percentage yield

The percentage yield of various batches of pectin microspheres alone and shellac-coated pectin microspheres were calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for preparation of microspheres and % percentage yield was calculated as per the formula mentioned below:

$$\%yield = \frac{Total\ wt.of\ microparticle}{Total\ wt.of\ drug\ and\ polymer}\ X\ 100$$

Determination of percent drug entrapment

Efficiency of drug entrapment for each batch was calculated in terms of percentage drug entrapment (PDE) as per the following formula:

$$Drug\ Entrapment\ Efficiency\ (\%) = \frac{Experimental\ drug\ content\ (mg)}{Theoretical\ drug\ content\ (mg)} X\ 100$$

Theoretical drug content was determined by calculation assuming that the entire drug presents in the pectin solution used gets entapped in microspheres and no loss occurs at any stage of preparation of microspheres [7].

Swellability/ Degree of swelling

The swelling ability of the pectin microspheres alone and shellac coated pectin microspheres on physiological media was determined by suspending them in the PBS buffer (pH 7.4). Accurately weighed amount (100 mg) of various ketoprofen pectin microspheres and shellac-coated pectin microspheres were placed in enzyme-free simulated intestinal fluid (pH 7.4 Phosphate buffer) in vials and allowed to swell for the required period of time.

The microspheres were periodically removed and blotted with filter paper; then their change in weight (after correcting for drug loss) was measured until attainment of equilibrium. Degree of swelling was then calculated using the following formula [6]:

$$Degree\ of\ swelling = \frac{Wg - Wi}{Wg} X\ 100$$

Where Wi, initial weight of microspheres; and Wg, final weight of microspheres.

Flow properties

Angle of repose

Angle of repose is defined as the maximum angle possible between the surface of the pile of the powder and the horizontal plane. It is calculated by the formula:

$$\tan\theta = \frac{h}{r}$$

$\theta = \tan^{-1}(h/r)$

Where, h = height of pile, r = radius of the base of the pile and θ = angle of repose

***In- Vitro* Drug release study**

The *in vitro* drug release behaviour of pectin microspheres alone and shellac coated pectin microspheres were performed using United States Pharmacopeia (USP) Type II dissolution apparatus (paddle) at 100 rpm. The dissolution medium consisted of simulated gastric fluid (SGF) pH 1.2 (0.1 N HCl). The readings were taken for first 2 h in SGF and for subsequent 8 h in phosphate buffer saline pH 7.4 (900 ml).The temperature of the dissolution medium maintained at 37^{0}C and stirred continuously at 100 rpm on a magnetic stirrer.

Aliquots of 1.0 ml were withdrawn and analysed for the drug content after suitable dilutions by spectrophotometric method. The volume of dissolution medium was replaced with the same volume of fresh buffer after each sampling. Sink conditions were maintained throughout the experiment. The release studies were conducted in triplicate. The cumulative amount of drug released was calculated and plotted against time. All dissolution studies were performed in triplicate [3, 6].

Drug release mechanism and kinetics

In order to establish the mechanism and kinetics of drug release from the microspheres, the experimental data obtained from the in vitro dissolution study was fitted with different kinetic models like zero order (% release vs. t), first order (log % release vs. t), Higuchi's model (% release vs. √t), Korsmeyer and peppas model (ln Q vs. ln t) etc [10, 11].

Korsmeyer's model is widely used; when the release mechanism is not well known or when more than one type of release phenomena could be involved.

Korsmeyer and Peppas equation: $Q = Kt^n$,

Where Q is the fractional drug release in time't'.

K= constant incorporating of structural and geometric characteristics of controlled release device.

n = diffusional release exponent indicative of release mechanism.

The 'n' value could be used to characterize different release mechanisms as per the description given below:

n = 0.5 [Fickian Diffusion (Higuchi Matrix)]

0.5 < n < 1 [Anomalous transport]

n = 1 [Case II transport (Zero order release)]

n >1 [Super case II transport]

The best-fit model was determined statistically employing comparison of correlation coefficients. The preparation of graphs

and statistical calculations were carried out with the help of Microsoft Excel® software.

Stability studies

The stability studies were performed at temperature of 4^0 C ± 1^0 C in refrigerator , at room or ambient temperature 25 ± 2^0C & 60 ± 5% RH and in incubator 40 ± 2^0C & 75 ± 5% RH for 2 months. The optimized formulation was analyzed for drug content and % drug release [12].

RESULTS AND DISCUSSION

Characterization of Drug and Analytical Study

The model drug selected (ketoprofen) was characterized and analyzed for its physical appearance and solubility, which was complies with the monograph as specified in Indian pharmacopoeia and British pharmacopoeia. UV and IR spectral analysis was done and the drug shows similar data as mentioned in different official publications. By FTIR analysis of pure ketoprofen showed characteristic peaks at 2996 cm^{-1}, 2978 cm^{-1}, 1697 cm^{-1}, 1655 cm^{-1}, 1444 cm^{-1} and 691 cm^{-1} these are almost same as reported in the monograph for ketoprofen.

Drug Polymer Interaction Study

Drug-polymer interaction study by FTIR for pure drug, pectin, shellac and physical mixture of drug with polymers showed that there are no significant changes in the position of the characteristic peaks of drug when mixed with pectin and shellac in Figure 1 to 5, which indicate compatibility of polymers with drug.

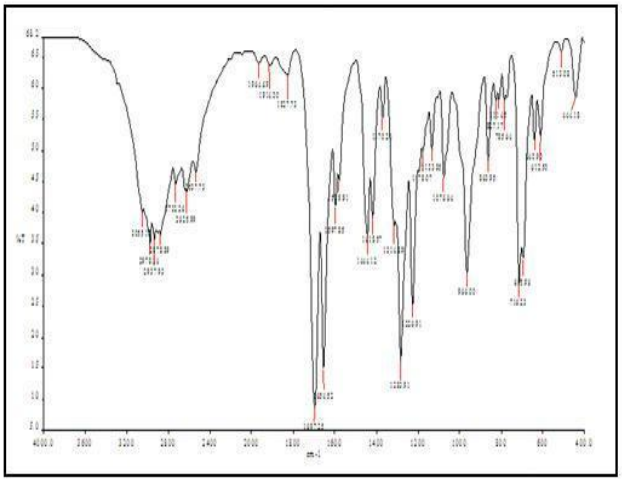


Fig. 1: FTIR Spectra of pure ketoprofen

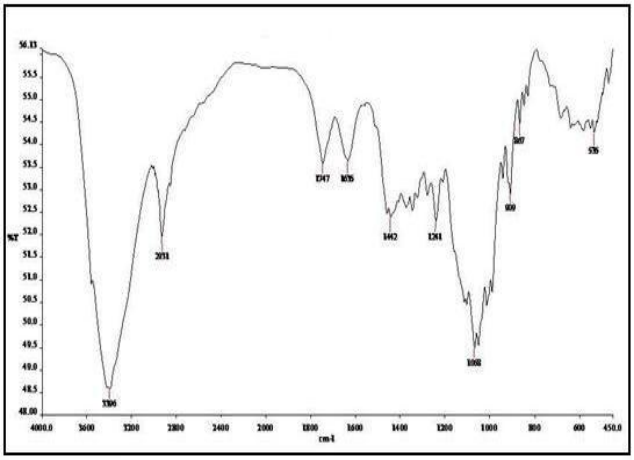


Fig. 2: FTIR Spectra of Pectin

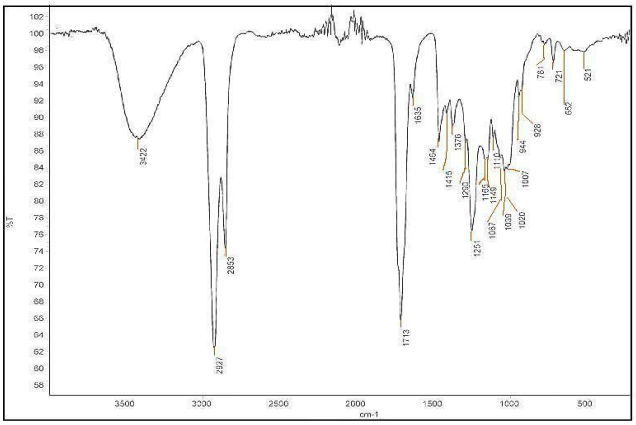


Fig. 3: FTIR Spectra of Shellac

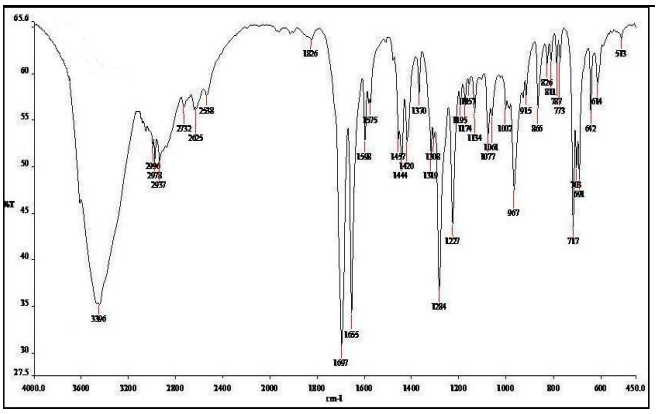


Fig. 4: FTIR Spectra of Ketoprofen-Pectin mixture (1:1)

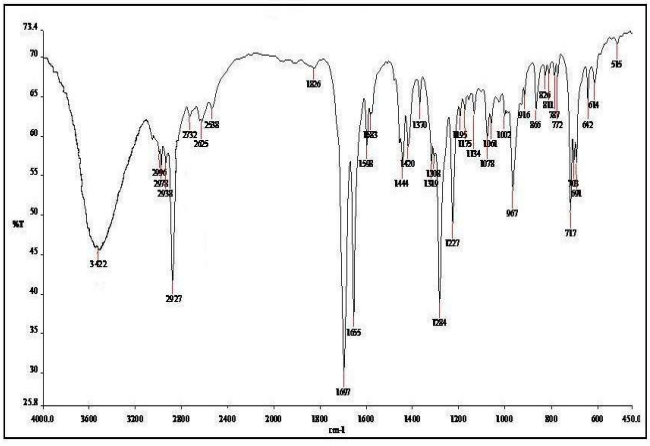


Fig. 5: FTIR Spectra of Ketoprofen-Shellac mixture (1:1)

Optimization and preparation of microspheres

The ketoprofen pectin microspheres were prepared by emulsion-dehydration technique method using pectin in different ratios (1:1 to 1:6) (Table 1). As a part of optimization, preparation of microspheres was tried without the active ingredient (ketoprofen) to observe the vesicle formation and to choose the appropriate parameters to be used in formulation preparation. The optimization study of emulsifying agent selection revealed that the uniform appearing formulations were formed by using 1.25 % w/v span 80 while all the other emulsifying agents showed non uniform formulations with part of its remaining undissolved [13].

From the optimization, batch P-3 and P-4 having 3% w/v and 4% w/v polymer concentration were selected as successful batches, as they showed maximum percentage yield (61.56 ± 0.12% and 61.97 ±

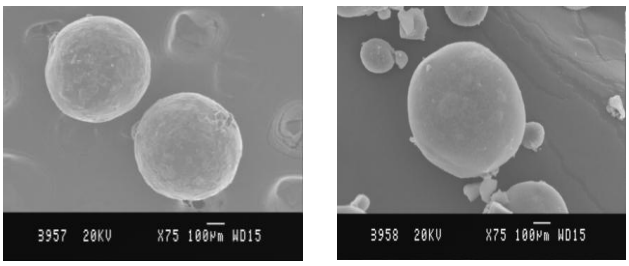
0.04%) among all batches and optimum particle size 134.53 ± 0.91µm (P-3) and 176.20 ± 0.33µm (P-4) at stirring speed of 1000 rpm.

It was also concluded that as the concentration of polymer decreases below 3% w/v, the quantity of polymer become in sufficient to cover ketoprofen particles completely.

Table 1: Effect of different process variables on microspheres formulation

Batch Code	Amount of Pectin Polymer (% w/v)	Span 80 (%w/v)	Stirring speed (rpm)	Appearance	Mean diameter (µm)	Percentage yield (%)
P-1	1	1.25	500	No microspheres formed	---	---
P-2	2	1.25	800	Clumping occurs	---	45.75 ± 0.68
P-3	3	1.25	1000	Homogenous	134.53 ± 0.91	61.56 ± 0.12
P-4	4	1.25	1000	Homogenous	176.20 ± 0.33	61.97 ± 0.04
P-5	5	1.25	1500	Aggregation occurs	211.13 ± 0.21	52.87 ± 0.67
P-6	6	1.25	1500	Aggregation occurs	230.14 ± 0.52	43.86 ± 0.53

The surface morphology of the uncoated pectin microspheres batch P-3 and P-4 containing pectin concentration 3% w/v and 4% w/v was carried out by using SEM studies and it showed that, the batch P-3 (3% w/v) and P-4 (4% w/v) were smooth in surface and spherical in shape (Figure 6).



P-3 P-4

Fig. 6: SEM photograph of uncoated pectin microspheres

Four batches of each formulation P-3 and P-4 containing pectin concentration 3% w/v and 4% w/v were prepared and coated with different ratios of the shellac polymer (1:2 to 1:5) by emulsion–solvent evaporation technique and labelled SP-3A, SP-3B, SP-3C, SP-3D, SP-4A, SP-4B, SP-4C and SP-4D.

Characterization of uncoated pectin microspheres and shellac coated pectin microspheres

Percentage yield

The percentage yield of core pectin microspheres (P-3 and P-4) was found to be 61.56% and 61.97%. The percentage yield of shellac coated microspheres SP-3A to SP-3D was found in range of 69.35% to 78.80% whereas in case of SP-4A to SP-4D shellac coated microspheres it was 69.80% to 82.60% respectively (Table 2).

There is increase in percentage yield of shellac coated microspheres than pectin microspheres alone which may be due to high viscosity of the polymer solution [14] and having more amount of polymer concentration in the formulation.

Particle size analysis

The average particle size of the pectin microspheres P-3 and P-4 was found to be 153.78 ± 0.23µm and 168.63 ± 0.14µm. The particle size of the shellac coated pectin microspheres SP-3A to SP-3D were found to be in range of 162.90 ± 0.01µm to 223.70 ± 1.1µm whereas in case of SP-4A to SP-4D shellac coated pectin microspheres, average particle size was found in range of 188.49 ± 0.29µm to 229.20 ± 0.57µm respectively (Table 2). The shellac coated pectin microspheres, SP-3D (223.70 ± 1.1µm) and SP-4D (229.20 ± 0.57µm) showed increase in particle size of the microspheres than pectin microspheres alone. This was due to increase in relative viscosity, which led to increase in particle size. A higher concentration of polymer produces more viscous dispersion, which formed larger droplets and consequently larger microspheres [15].

Determination of drug entrapment efficiency

The percentage drug entrapment of pectin microspheres P-3 and P-4 was found to be 43.32 ± 0.26% and 45.16 ± 0.35% with varying polymer ratio 3% w/v and 4% w/v.

The drug entrapment efficiency of the shellac coated pectin microspheres from SP-3A to SP-3D were found to be in range of 52.34 ± 0.56% to 66.70 ± 0.91% whereas in case of SP-4A to SP-4D shellac coated pectin microspheres, it was found in range of 53.59 ± 0.10% to 68.90 ± 0.90% respectively (Table 2).

Table 2: Percentage yield, particle size, percent drug entrapment efficiency, degree of swelling and flow properties of pectin microspheres alone and shellac-coated pectin microspheres

Microspheres formulation	Batch code	Percentage Yield (%)	Particle size (µm)	Entrapment efficiency (%)	Degree of swelling	Flow properties
Pectin microspheres alone	P-3	61.56±0.12	153.78±0.23	43.32±0.26	0.88±0.06	36°40′±0.11
	P-4	61.97±0.04	168.63±0.14	45.16±0.35	1.21±0.12	38°24′±0.21
Shellac coated pectin microspheres	SP-3A	69.35±0.02	162.90±0.01	52.34±0.56	0.03±0.02	24°70′±0.35
	SP-3B	72.90±0.13	185.42±0.02	55.85±0.24	0.05±0.01	24°46′±0.24
	SP-3C	74.53±0.06	197.21±0.01	57.43±0.46	0.09±0.01	22°10′±0.26
	SP-3D	78.80±0.96	223.70±1.1	66.70±0.91	0.12±0.01	20°11′±0.15
	SP-4A	69.80±0.02	188.49±0.29	53.59±0.10	0.04±0.01	26°71′±0.42
	SP-4B	73.62±0.03	198.56±0.10	58.38±0.18	0.08±0.01	25°52′±0.28
	SP-4C	76.45±0.01	210.61±0.45	64.14±0.15	0.11±0.02	22°36′±0.15
	SP-4D	82.60±0.52	229.20±0.57	68.90±0.90	0.13±0.01	20°13′±0.13

Degree of swelling

The pectin microspheres P-3 and P-4 showed significant swelling ($p<0.05$; t-test) when compared with shellac coated pectin microspheres. As pectin is soluble in water, may swell and release the drug in the upper GI tract. No significant ($p<0.05$; t-test) swelling was observed with shellac coated pectin microspheres as compared with pectin microspheres alone, thus ensuring better resistance of

shellac-coated pectin microspheres in the upper GI tract to swelling and preventing subsequent drug release at the non-target site [13] (Table 2).

Flow properties: Angle of repose

Flow properties of the prepared pectin microspheres alone and shellac coated pectin microspheres were characterized by measuring the angle of repose. The values of angle of repose are within the normal acceptable range of 20° to 40°. The pectin microspheres formulation P-3 and P-4 showed highest angle of repose and smaller particle size as compared to shellac coated pectin microspheres formulation SP-3D and SP-4D, which showed lowest angle of repose and larger particle size (Table 2).

***In vitro* drug release studies**

In vitro ketoprofen release study of pectin microspheres alone and shellac coated pectin microspheres was performed in pH progression medium (pH 1.2 to pH 7.4) at 37° C ± 0.5° C. Ketoprofen release from pectin microspheres (P-3 and P-4) in SGF followed the order P-3 > P-4 (Figure 7). The initial higher release of ketoprofen from microspheres might have resulted from the dissolution of drug crystals on the surface of microspheres. The cumulative percentage drug release from shellac based pectin microspheres showed the desired rate, as there was no measurable drug release observed up to 2 h in SGF of pH 1.2 and no drug release occurred below the pH of polymer solubility while at pH 7.4, the significant drug release was observed. The release pattern of ketoprofen from shellac coated pectin microspheres can be understood by protective nature of shellac coating delaying drug release in upper GI tract [13]. Ketoprofen release from the shellac-coated pectin microspheres in SGF followed the order SP-3A > SP-3B > SP-3C > SP-3D and SP-4A > SP-4B > SP-4C > SP-4D (Figure 7 and 8).

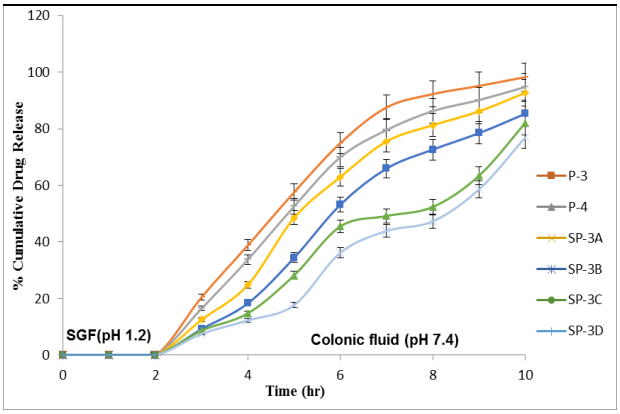


Fig. 7: *In vitro* release profile of uncoated pectin microspheres (P-3 and P-4) and shellac coated microspheres SP-3 (A-D)

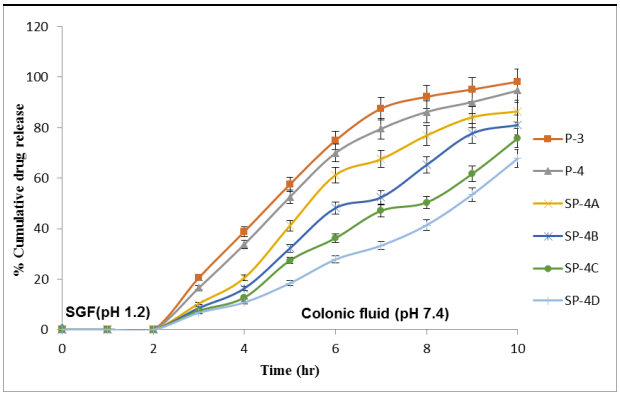


Fig. 8: *In vitro* release profile of uncoated pectin microspheres (P-3 and P-4) and shellac coated microspheres SP-4 (A-D)

On comparing the *in-vitro* drug release data of SP-3(A-D) and SP-4(A-D) formulations in simulated gastro intestinal fluid, the formulations showed significantly ($p<0.05$; t-test) higher release pattern in the initial hour. A high release of drug was observed from the formulation SP-3A and SP-4A which contains 2% concentration of shellac polymer, whereas a less release of drug was observed from the formulation SP-3D and SP-4D which contain 5% concentration of shellac polymer (Figure 9).

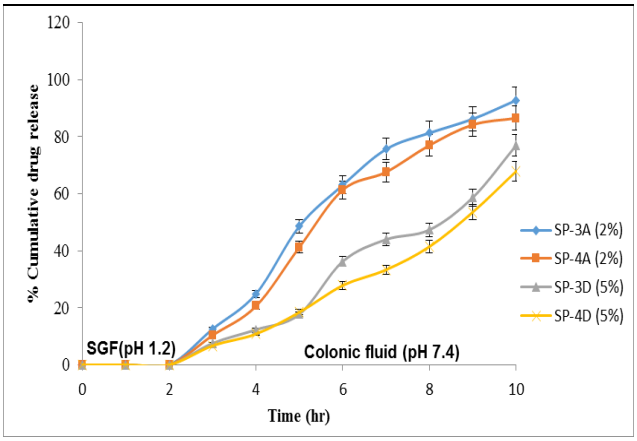


Fig. 9: Comparison of *in vitro* release profile of shellac coated microspheres SP-3A and SP-4A containing 2% polymer concentration with SP-3D and SP-4D containing 5% polymer concentration

These results indicated that formulation with lesser concentration of coating polymer shows significantly ($p<0.05$; t-test) higher drug release. This type of high release in stomach and small intestine is not satisfactory for a formulation, which is supposed to release its content in the colon [16]. The formulation SP-3D and SP-4D, which contain 5% concentration of shellac polymer showed the desired drug release rate in the colonic fluid. Hence, 5% concentration of shellac polymer is selected for the coating of ketoprofen-pectin microspheres and for further evaluation of microspheres.

Six formulations of ketoprofen-pectin microspheres were formulated using 5% coating solution of shellac polymer. These formulations were subjected to evaluation parameters like percentage yield, particle size, surface morphology, drug entrapment efficiency, degree of swelling, flow properties, *in vitro* drug release and stability studies.

Characterization of optimized shellac-coated ketoprofen pectin microspheres

Percentage yield

The percentage yield of different formulations FS-1 to FS-6 was in the range of 71.60 to 87.71% (Table 3). The percentage yield of the microspheres was found to be increased with increase in the polymer concentration in the formulations which signifies the less product loss during preparation of the microspheres.

Particle size analysis

The particle sizes of the optimized shellac coated pectin microspheres were found to be in range of 182.37 to 247.46 µm for formulations FS-1 to FS-6 respectively (Table 3).

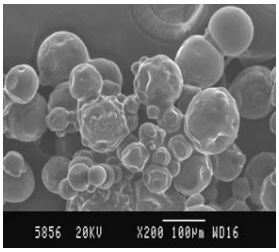
Degree of swelling

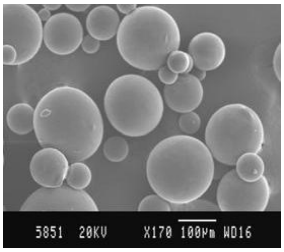
The swelling ability of the microspheres on physiological media was determined and the results were shown in Table 3. No significant ($p<0.05$; t-test) swelling was observed with shellac coated pectin microspheres. Thus ensuring better resistance of shellac coated microspheres in the upper GIT to swelling and preventing subsequent drug release at the non-target site [13].

Surface morphology

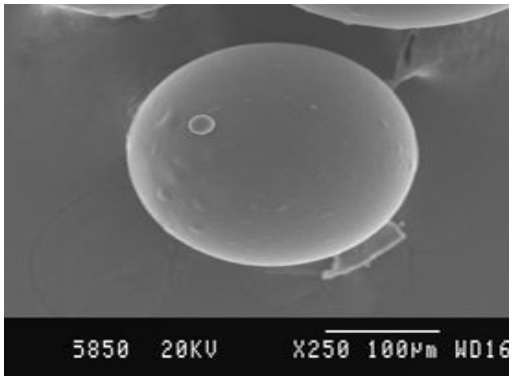
Surface morphology of the microspheres was investigated with a scanning electron microscope. Particle surface of formulations FS-1 was slightly rough surfaced but spherical and FS-2; FS-3 was

smooth, oval and discrete (Figure 10). The smoothness of the surface increased which may be due to increasing ratio of polymer. Very less particulate matter of the drug were seen on the surface of the microspheres indicating uniform distribution of the drug in the polymer network.





FS-1 FS-2



FS-3

Fig. 10: Scanning electron photomicrographs of Formulation FS-1, FS-2 and FS-3

Table 3: Percentage yield, Particle size, Percent drug entrapment, Degree of swelling and Angle of repose of optimized shellac coated ketoprofen pectin microspheres

Formulation code	Percentage yield (%)	Particle size (µm)	% Drug entrapment	Degree of swelling	Angle of repose
FS-1	71.60 ± 0.1	182.37 ± 0.5	63.85 ± 0.16	0.03 ± 0.01	20°13′ ± 0.8
FS-2	75.5 ± 0.45	200.53 ± 0.6	65.29 ± 0.70	0.09 ± 0.02	21°42′± 0.49
FS-3	78.80 ± 0.9	223.7 ± 1.1	66.70 ± 0.91	0.12 ± 0.01	24°70′± 0.35
FS-4	82.60 ± 0.5	229.2 ± 0.57	68.90 ± 0.90	0.13 ± 0.01	26°71′± 0.42
FS-5	84.52 ± 0.5	242.72 ± 0.8	75.38 ± 0.46	0.17 ± 0.01	29°24′± 0.24
FS-6	87.71 ± 0.2	247.46 ± 0.4	76.00 ± 0.09	0.23 ± 0.01	34°99′± 0.11

Table 4: Kinetic values from *in vitro* release profile of all shellac coated microspheres

Formulation Code	Zero Order r^2	First Order r^2	Higuchi r^2	Hixon Crowel r^2	Korsemeyer and Peppas N	Release order and Main Transport Mechanism
FS-1	0.977	0.799	0.921	0.881	1.044	Zero order, Super case II
FS-2	0.987	0.903	0.958	0.949	1.125	Zero order, Super case II
FS-3	0.970	0.889	0.930	0.927	1.147	Zero order, Super case II
FS-4	0.975	0.908	0.923	0.935	1.124	Zero order, Super case II
FS-5	0.977	0.913	0.924	0.939	1.145	Zero order, Super case II
FS-6	0.932	0.849	0.864	0.880	1.122	Zero order, Super case II

Determination of drug entrapment efficiency

As the polymer concentration was increased, the entrapment efficiency increased from 63.85% to 76.00% for formulations FS-1 to FS-6 respectively (Table 3). The results indicated that polymer concentration plays a major role in drug entrapment efficiency.

Flow properties: Angle of repose

All the formulations showed improved flow properties, as compared to the pure drug. The results were shown in Table 3. The values of angle of repose were between 20^0 to 40^0, indicates good flow properties and all the batches of microspheres were found to fit in respect of flowability.

***In vitro* drug release studies**

In vitro Ketoprofen release study of shellac coated microspheres was performed in pH progression medium (pH 1.2 to pH 7.4) at 37^0 C ± 0.5^0 C.

The cumualtive percent drug release after 8 h was found to be 92.30%, 85.30%, 76.90%, 67.83%, 65.80% and 62.6% for FS-1 to FS-6 respectively (Figure 11).

The cumulative percentage drug release from shellac based microspheres showed the desired rate, as there was no measurables drug release observed up to 2 h in SGF of pH 1.2 and no drug release occurred below the pH of polymer solubility while at pH 7.4, the significant drug release was observed.

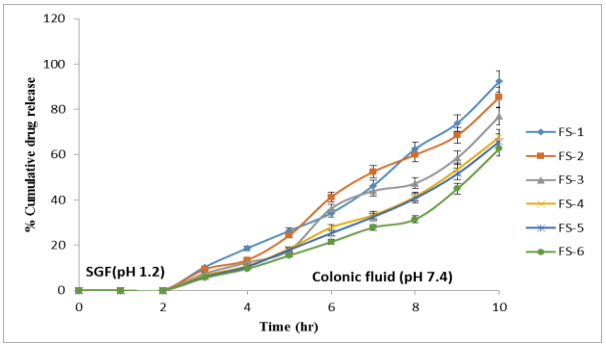


Fig. 11: % Cumulative drug release from ketoprofen microspheres formulation FS-1 to FS-6

While in colonic fluid maximum drug release was observed due to dissolution of shellac coat at pH 7.4 and the pectin microspheres were degraded on exposure to the colonic fluid and results in higher percentage of drug release. The decrease in drug release in formulation FS-6 was due the more the amount of polymer, thus more amount of drug remained entrapped in the polymer matrix.

Kinetic modelling

Based on the highest regression values (r), the best fit model for all the six formulations (FS-1 to FS-6) was zero order which indicated the drug release was in sustained way and also showed that the concentration was independent of drug release (Table 4). Further Korsmeyer and Peppas equation resulted into the values of n >1, which appears to indicate that the release from the prepared microspheres was by Super Case II transport.

Stability studies

Stability studies of the prepared microspheres were carried out by storing the formulation FS-3 at 4^0 C ± 1^0 C in refrigerator, at 40^0 C ± 2^0 C, 75% RH ± 5% RH in humidity control oven and room temperature and humidity (25^0C ± 2^0C/60 ± 5% RH) for a period of three months (acc. to ICH guidelines). Two parameter's namely residual percent drug content and *in vitro* release studies of a selected formulation (FS-3) were carried out at the end of the month.

It was observed that the formulation FS-3 stored at 4^0 C ± 1^0 C showed very closest drug content and *in vitro* release values to the previous data of FS-3 (Figure 12). Thus, it can be concluded that 4^0C ± 1^0 C is the most suitable temperature for storage shellac-coated ketoprofen loaded microspheres.

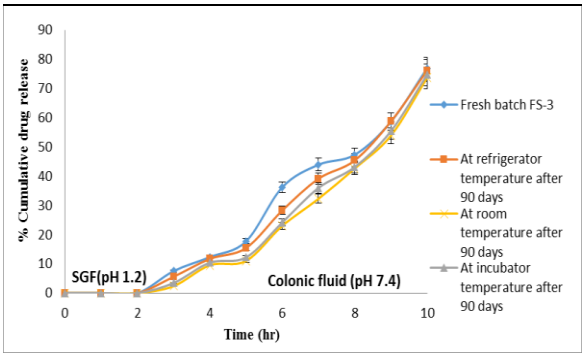


Fig. 12: Stability profile of shellac coated ketoprofen microspheres formulation FS-3

CONCLUSION

In the present investigation colon specific enteric coated microspheres of ketoprofen were prepared and were evaluated for different parameters. The uncoated pectin microspheres of ketoprofen showed decreased particle size, percentage yield and drug entrapment efficiency than shellac coated pectin microspheres. Shellac-coated pectin microspheres showed no swelling as compared to uncoated pectin microspheres, thus ensuring better resistance of shellac coated microspheres in the upper GIT to swelling and preventing subsequent drug release at the non-target site. The shellac-coated pectin microspheres showed the desired drug release rate, as there was no measurable drug release observed up to 2 hours in SGF (pH 1.2), while in SIF (simulated intestinal fluid, pH 6.8), the drug release was quite insignificant up to 4 hours. In the colonic fluid maximum drug release was observed due to dissolution of the shellac coat at pH 7.4. Thus the experimental results demonstrated that, the shellac coated pectin microspheres have the potential to be used as a drug carrier for an effective colon targeted drug delivery system.

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REFERENCES

1. Dashora A., Jain C.P. Development and characterization of pectin-prednisolone microspheres for colon targeted delivery. Int.J ChemTech Res. 2009; 1(3): 751-757.
2. Yang. L. Biorelevant dissolution testing of colon-specific delivery systems activated by colonic microflora. J. Control. Release. 2008; 125: 77-86.
3. Ahmed D.E., Nafadi M.M., Fatahalla F.A. Formulation of a fast-dissolving ketoprofen tablet using freeze-drying in blisters technique. Informa Healthcare. 2006, 32: 437-438.
4. Jose S. Multiparticulate System for Colon Targeted Delivery of Ondansetron. Indian J Pharm Sci. 2010; 72(1): 58-64.
5. Esposito E., Cortesi R., Luca G., Nastruzzi C. Pectin based microspheres: a preformulatory study. Ann N Y Acad Sci. 2001; 944: 160-179.
6. Lorenzo-Lamosa M.L., Remunan-Lopez C., Vila-Jato J.L., Alonso M.J. Design of microencapsulated chitosan microspheres for colonic drug delivery. J. Control. Release. 1998; 52: 109-118.
7. Kavitha D., Sowjanya J.N., Panaganti S. Pharmacosomes: an emerging vesicular system. Int J Pharm Sci Rev and Res. 2010; 5: 168-170.
8. Nikwade S., Kulkarni P., Jathar S, Bajaj A. Development of time and pH dependent controlled release colon specific delivery of tinidazole. DARU J Pharm Sci. 2008; 16(3): 119-27.
9. Desai K.G.H. Preparation and Characteristics of High Amylose Corn Starch/ Pectin Blend Microparticles: A Technical Note. AAPS Pharm Sci Tech. 2005; 1-21.
10. Saparia B., Murthy R.S.R. Preparation and evaluation of chloroquine phosphate microspheres using cross linked gelatin for long term drug delivery. Indian J. Pharm. Sci. 2002; 64(1): 48-52.
11. Nicolas G., Marc P., Bernard M., Gaelle R., Dominique C., Martine L.M. Study of release kinetics of small and high molecular weight substances dispersed into spray-dried ethylcellulose microspheres. J. Control. Release. 2002; 84: 125–135.
12. Abd-Elbary A., El-laithy M.I., and Tadros H.M. Sucrose stearate-based proniosome- derived niosomes for the nebulisable delivery of cromolyn sodium. Int. J. Pharm. "Article in press"; 2008.
13. Paharia A., Yadav A.K., Rai G., Jain S.K., Pancholi S.S., Agrawal G.P. Eudragit-coated pectin microspheres of 5- Fluorouracil for colon targeting. AAPS Pharm Sci Tech. 2007; 8(1): 1-7.
14. Dash S.K., Khan A.S., Das S.R., Padhan A., Rout D., Behera B.C. Formulation and *in-vitro* evaluation of sustained released glibenclamide microspheres. Int. J. Pharm. Sci. Res. 2012; 3: 1433-1443.
15. Pongpaibul Y., Price J.C., Whitworth C.W. Preparation and evaluation of controlled release indomethacin microspheres. Drug Dev. Ind. Pharm. 1984; 410: 1597-1616.
16. Kushwaha P., Fareed S., Nanda S., Mishra A. Design and fabrication of tramadol Hcl loaded multiparticulate colon targeted drug delivery system. J. Chem. Pharma. Res. 2011; 3(5): 584-595.