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Design and characterization of mucoadhesive microspheres of novel NSAID drug using algino-eudragit RS100 system

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ABSTRACT

Mucoadhesive microspheres of aceclofenac was formulated using combination of algino-eudragit RS100 system by ionic gelation technique. The prepared microspheres were evaluated for various parameters like percentage yield, particle size, flow property, surface study, ex-vivo mucoadhesive study, in vitro drug release, etc. It was found that all formulations showed improved flow behavior as compared to pure drug, it was observed that on increasing the polymer concentration of formulations the entrapment efficiency and particle size were increased. The surface morphology study by SEM indicated that microspheres were spherical with rough outer surface. There was no interaction between the drug and the polymers, as studied by FTIR study. In vitro drug release study showed that on increasing polymer concentration the drug release of all the formulations were gradually decreased and the formulations followed zero order kinetics. Ex- vivo mucoadhesion study depicts that when the polymer concentration was increased the mucoadhesion nature was also increased. Therefore, it can be concluded that aceclofenac loaded algino-eudragit RS100 microspheres can be formulated for sustained drug delivery of aceclofenac.

Keywords: Aceclofenac, algino-eudragit RS100, sustained release, ionic- gelation method.

INTRODUCTION

A novel non-steroidal anti-inflammatory drug (NSAID), that is Aceclofenac(AC) is a phenyl acetic acid derivative [2-(2',6'-dichlorophenyl)amino] phenylacetoxyacetic acid], is used for symptomatic treatment of pain and inflammation[1]. It has higher anti-inflammatory effect than

conventional NSAIDs and act by blocking the action of cyclo-oxygenase, which is produced by prostaglandins. As acaelofenac has short biological half-life (about 4 h), therefore frequent administration and gastrointestinal disturbances makes it an ideal candidate for sustained release dosage form. Commonly seen drawback of sustained release formulations is its inability to increase residence time of formulation in the gastrointestinal tract (GIT). The residence time of a dosage form is typically short in gastric region, during fasting condition it is not more than an hour and it is also common for dosage forms to transit rapidly through the small intestine for not more than 3h[2]. Thus, this frequent gastrointestinal (GI) transit phenomenon may ultimately lead to reduction in the extent of absorption of various drugs. It is known that most of the drugs are absorbed from small intestine or some specific segments of intestine; it is therefore advantageous to develop a mucoadhesive dosage forms, which can remain intact in intestinal region for a longer period of time, to extend the residence time. Various techniques have been developed in this aspect and one of them is to use mucoadhesive system for oral drug delivery[3]. Mucoadhesive system can be formed by using mucoadhesive polymers, which are hydrophilic molecules that contain various Hydrogen bond formation groups like, -OH, -COOH, amides, etc. [4]. Strong anionic charges containing carboxylic groups [5] as they usually have high molecular weight, i.e. > 10000[6] they have sufficient flexibility to penetrate the mucus network or tissue cervices [7]. Usually natural polymers like proteins and polysaccharides are given preferences to formulate sustain release system. Alginate [8] a naturally occurring biocompatible and biodegradable linear polysaccharide extracted from brown seaweed has been commonly used, due to its good mucoadhesive property. It is a derived polysaccharide block composed of regions of sequential β-D-mannuronic acid monomers (M-blocks), regions of α-L-guluronic acid (G-blocks), and regions of interspersed M and G units [9]. Most of the commercially available alginates are in the form of the salt, i.e. sodium alginate. The unique property of sodium alginate is the transformation from sol to hydrogel with more than 95% of water molecules physically held inside [10] which is of important for the maintenance of bioavailability by providing an aqueous environment to the entrapped substances. When alginate reacts with calcium ions, it undergoes gelation in aqueous solution due to binding of calcium ions with G-blocks of adjacent alginate chains creating ionic inter-chain bridges. The ionic gelation [11] technique was selected to prepare microspheres due to its simplicity, low cost and its high entrapment rates achieved with poorly water soluble drug. The present investigation is aimed at using sodium alginate in combination with Eudragit RS 100 for sustained drug delivery of water insoluble drug for microsphere to get study of drug release profile from microspheres formulation. Sodium alginate on exposure to dissolution fluids gets swelled & forms a viscous gel layer that sustained the drug release, where as Eudragit RS 100 being water insoluble polymer retards drug release. So the objective of the present study was to develop mucoadhesive system of aceclofenac for sustained release and evaluate the effect of polymer concentration on drug release kinetics and also to improve the flow behaviour of pure drug.

MATERIALS AND METHODS

Materials

Aceclofenac(AC) was a gift sample from Cadila Pharmaceuticals Ltd., Ahmedabad, India. Sodium alginate (viscosity ≈ 3500 cps) was a gift sample from Signet Chemical Co, India. Eudragit RS 100 was purchased from Loba Chem, Pvt. Ltd., India. Calcium chloride was

purchased from Loba Chem, Pvt. Ltd., India. All other chemicals used were of analytical reagent grade.

Preparation of aceclofenac microspheres with sodium alginate and Eudragit RS 100 system

Microspheres containing aceclofenac were prepared employing sodium alginate by ionic gelation method. Sodium alginate was dissolved in sufficient quantity of distilled water to form a homogeneous polymer solution. When sodium alginate was uniformly mixed, then specified quantity of Eudragit RS 100 was added to it and mixed by the magnetic stirrer. Finally, Core material aceclofenac was added to the polymers solution and mixed thoroughly to form a smooth viscous dispersion The resulting dispersion was then added drop wise by using 24 G needle in 500 ml of 5% calcium chloride solution under continuous stirring at 200 rpm. The stirring was continued for 30 minutes to make the dispersion as fine as possible to produce spherical microspheres, the reaction was allowed for proceeding for about 30 minutes. Then the mixture was filtered and product was dried at 40°C for 12 hour. The microspheres along with coat composition are listed in Table-1.

Characterization of mucoadhesive microsphers

Percentage Yield [12]

The percent yield of each batch of microspheres was determined on weight basis with respect to the initial weight of material; the data are described in Table-2.

Particle Size Analysis: The optical microscopy method was used to determine the particle size of prepared microspheres [13] In this method, the diameter of 100 microspheres was determined and from it the mean diameter was calculated. All readings were taken in triplicate.

Table -1Composition of microspheres

Formul^{n} code	Aceclofenac (g)	Sodium alginate (g)	Eudragit RS 100 (g)	Curing time (min)	Cross-linking agent ($CaCl_2$ % w/v)
F1	1	0.5	0.5	30	5
F2	1	0.5	1	30	5
F3	1	0.5	1.5	30	5
F4	1	0.5	2	30	5
F5	1	0.5	2.5	30	5
F6	1	0.5	3	30	5
F7	1	0.5	3.5	30	5
F8	1	0.5	4.0	30	5

Drug Entrapment: 100 mg of the formulation was taken in 50 ml of phosphate buffer of 6.8 pH in a volumetric flask and then stirred for 30 minutes in sonicator at 125W(Imeco Sonifier, Imeco Ultrasonics, India.) then volume was made up to 100 ml with 6.8 phosphate buffer and again stirred for 1 hour and kept overnight for 24 hours to extract the drug from microspheres. The filtrate was collected by passing through 0.45µ filter and then desired dilutions were made and the absorbance of resulting solution was measured at 273 nm using UV-Visible spectrophotometer (UV- 2450 Shimadzu.Japan) against blank. This study was conducted in triplicate and values are depicted in Figure-1. The drug entrapment was calculated by using the formula given below,

% Drug entrapment = (Calculated drug content/Theoretical drug content)x100

Micromeritic Study:

Flow Properties:

The flow property of prepared microspheres were studied by determining the parameters like angle of repose, bulk density, tapped density, Carr's index and Hausner ratio [14].Bulk density and tapped density [15] were determined by Bulk density apparatus (Electrolab India) and angle of repose was calculated by fixed base cone method. All the readings were taken three times.

In-Vitro Drug Release Studies:

Release of aceclofenac from the prepared microspheres was studied in 0.1N HCl and in phosphate buffer pH 6.8 (900ml) using an USP six station dissolution (LAB DISSO 2000) rate testing apparatus with a rotating paddle at 50 rpm and 25 cm depth. A sample of 5 ml was withdrawn at different time intervals and diluted using pH 6.8 phosphate buffer. After suitable dilutions the absorbance was measured at 273 nm using UV- visible spectrophotometer (2450 Shimadzu, Japan) against a blank. The dissolution study was conducted in triplicate [16].

Surface study by Scanning Electron Microscopy(SEM)[17]. The surface morphology especially with respect to surface topography, photomicrography was done with SEM, by using the instrument JSM 5610 LV SEM, JEOL, Japan. The figures of microspheres after SEM analysis with various magnifications are depicted in Figure-2.

FTIR Study: The FTIR study was carried out using Perkin-Elmer FT-IR (spectrum RX). The sample of pure drug(aceclofenac), pure polymers (sodium-alginate and Eudragit) and formulation containing both the drug and polymers were scanned to study the possible interaction between drug and polymers.

Ex-vivo mucoadhesion Study:

The mucoadhesive property of the microspheres was evaluated employing the following method [18]. The test was performed in simulated intestinal fluid (phosphate buffer, pH 6.8). The freshly excised pieces of intestinal mucosa (2 ×3 cm) from goat were mounted onto glass slides with cyano-acrylate glue and about 50 microspheres were spread onto the wet rinsed tissue specimen and immediately thereafter the slides with suitable support were hung onto the arm of a USP tablet disintegrating test apparatus. Then the disintegrating test machine was operated, the tissue specimen was given a slow, regular up and down movement in the test fluid at 37 ^{0}C contained in a one liter vessel, at different time intervals up to 8 h. The machine was stopped and the number of microspheres still adhering to the tissue were counted. The details are highlighted in Figure -5.

RESULTS AND DISCUSSION

The mucoadhesive microspheres of aceclofenac using algino-eudragit RS 100 were prepared by ionic gelation method with calcium chloride as cross linking agent. This method was selected due to its ease of formulation, quick and cost effectiveness. The composition of prepared formulations is represented in Table. 1

Percentage yield : The yield obtained from all the batches was good. The range for % yield was 92.5 ± .04% to 97.8± 2.5% for the prepared microspheres, the result showed a moderate increase in yield. Table-2 depicts the detail data of percentage yield.

Drug Entrapment : The drug entrapment efficiency for various formulations was found to vary between 51.29% to 89.72%. Increase in polymer concentration leads to the formation of larger microspheres, entrapping greater amount of drug, this may be attributed to the greater availability of active calcium binding sites in the polymeric chains and consequently, the greater degree of cross-linking at the time of curing as the quantity of polymer is increased which is depicted in Figure-1.

Particle size analysis: The mean particle size of eight formulations ranged between 0.753mm to 1.3mm. It was found that mean particle size of the microspheres increased with polymer concentration for the formulations F1, F2, F3, F4, F5, F6, F7 and F8. This could be due to the increase in relative viscosity at higher concentrations of polymer and formation of large droplets during addition of the polymer solution to the cross-linking agents. The detail description of which is represented in Figure-1.

Table – 2 Micromeritic study of prepared microspheres

Form[n]	% Yield	Bulk density	Tapped density	Hausner's ratio	Carr's index	Angle of repose
Pure drug	-	0.80 ± 0.25	1.29 ± 0.29	1.67 ± 0.12	38.0 ± 1.56	46.3
F1	92.5 ± 0.04	1.25 ± 0.31	1.6 ± 0.26	1.08 ± 0.85	24.9 ± 0.95	19.1
F2	96.0 ± 0.05	0.55 ± 0.02	0.62 ± 0.01	1.12 ± 0.90	11.2 ± 0.85	18.5
F3	93.5 ± 0.05	0.41 ± 0.01	0.43 ± 0.01	1.04 ± 0.45	4.1 ± 0.07	19.2
F4	93.2 ± 0.07	0.28 ± 0.01	0.31 ± 0.01	1.09 ± 0.55	8.8 ± 0.55	17.5
F5	98.0 ± 0.07	0.27 ± 0.01	0.30 ± 0.01	1.12 ± 0.89	10.8 ± 0.75	15.8
F6	92.6 ± 0.08	0.88 ± 0.75	1.66 ± 0.25	1.88 ± 0.75	7.6 ± 0.45	18.9
F7	96.4 ± 2.3	0.67 ± 0.08	0.78 ± 0.07	1.16 ± 0.34	14.0 ± 0.45	17.3
F8	97.8 ± 2.5	0.68 ± 0.01	0.76 ± 0.01	1.11 ± 0.32	10.5 ± 0.78	18.8

Mean ± S.D., n = 3

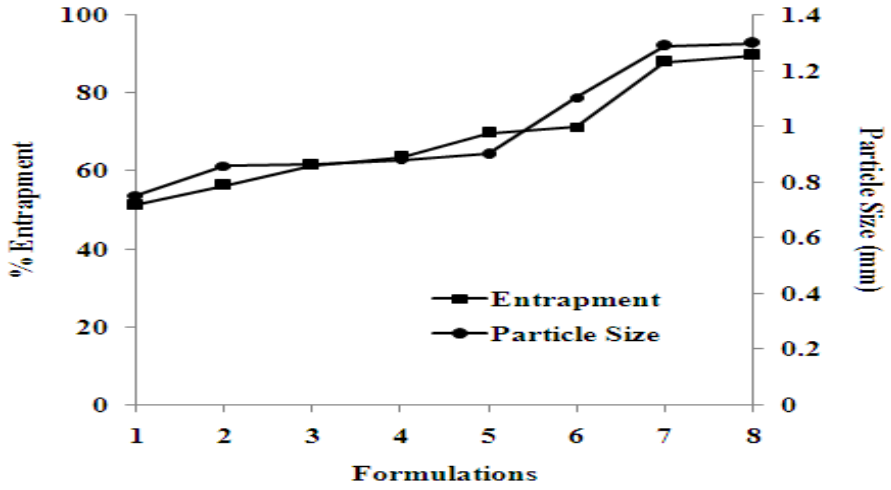

Figure-1

ZERO ORDER PLOT

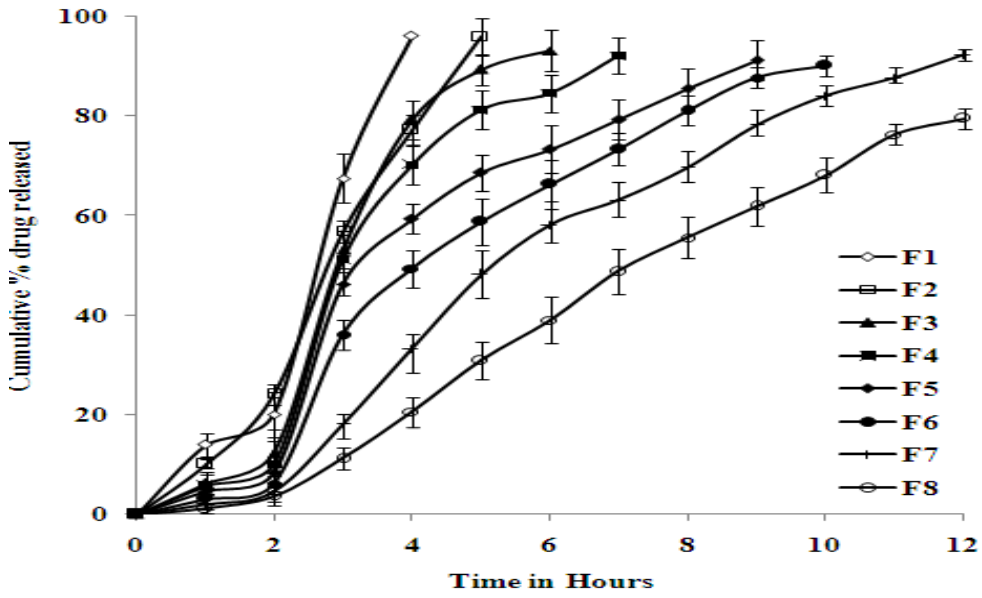

Figure –2 SEM Photographs of prepared microspheres at different magnifications

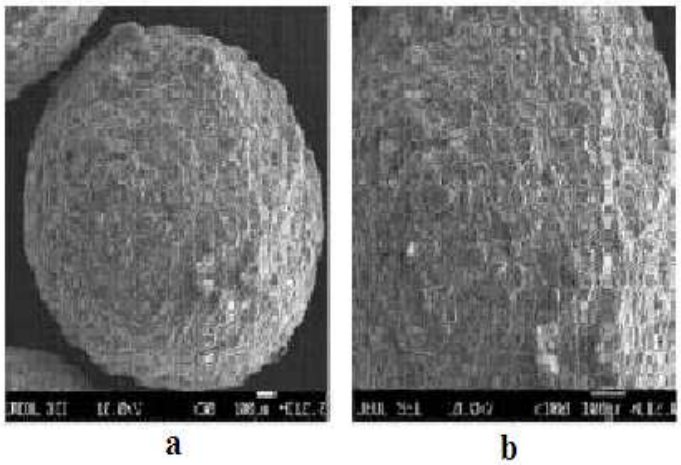

Figure-3

FTIR

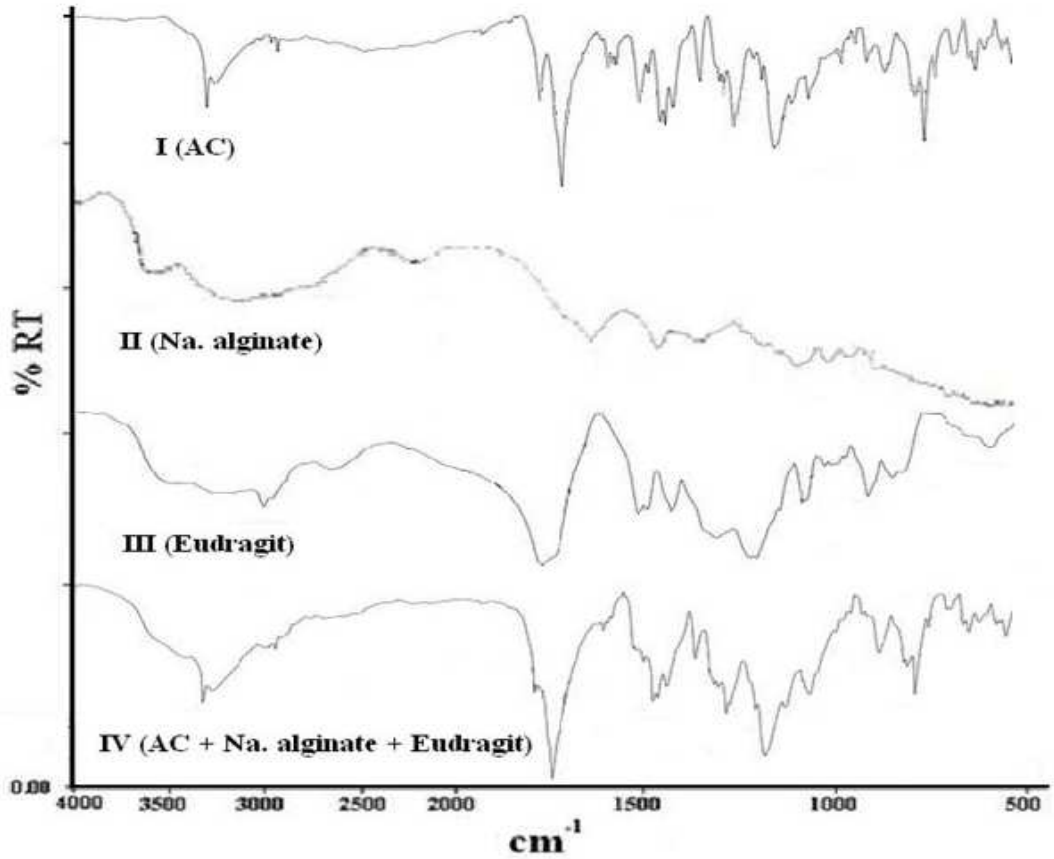

Figure-4

Table -3 Kinetics of drug release from Microsphere formulations

Sl. No.	Formulation	Correlation Coefficients (R^2)				Release exponent (n)
		Zero order	First order	Higuchi model	Korsmeyer model	
1	F1	0.918	0.756	0.732	0.81	1.335
2	F2	0.977	0.756	0.828	0.978	1.534
3	F3	0.931	0.93	0.812	0.877	1.845
4	F4	0.925	0.956	0.846	0.83	1.86
5	F5	0.949	0.973	0.892	0.896	1.981
6	F6	0.918	0.973	0.906	0.925	2.018
7	F7	0.968	0.958	0.909	0.969	2.004
8	F8	0.987	0.957	0.879	0.985	1.881

Mucoadhesion study of prepared microsphere formulations

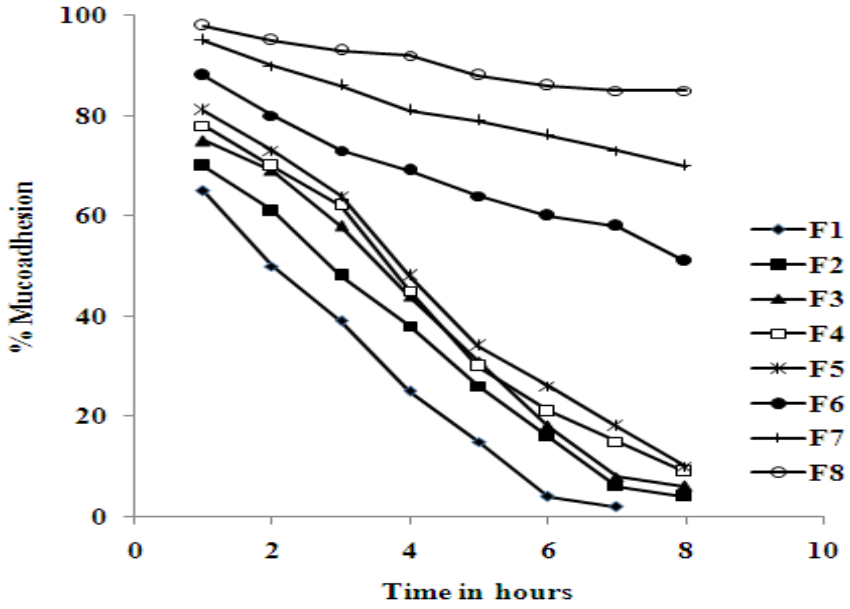

Figure-5

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Micromeritic studies:

Bulk density and Tapped density: For the flow property of powders the inter-particle interaction is very essential [19]. The increase in tapped density compared to individual bulk density for formulated microspheres in various ratios of drug:polymer indicated that microspheres formulation has better flow ability than pure drug. The values are depicted in Table-2. Where the bulk density ranged from 0.27 ± 0.01 to 1.25 ± 0.31 while tapped density ranged from 0.30 ± 0.01 to 1.66 ± 0.25 for the prepared formulation (F1 to F8) in comparison to pure drug whose values for bulk and tapped density were 0.80 ± 0.25 and 1.29 ± 0.29 respectively.

Hausner's ratio*:* Hausner's ratio for pure drug was 1.67±0.12 which clearly indicated poor flow ability but upon formulating to microspheres the ratio obtained showed good flow ability as indicated in Table-2.The value of Hausner's ratio for the formulated microspheres was found to be in range of 1.04±0.45 to 1.88±0.75 .

Carr's index: Carr's index for all eight formulations showed good flow ability when compared to pure drug whose value was 38±1.56% indicating poor flow ability before formulating to microspheres. The range of this data is 4.1±0.07 to 14.0±0.45 for the prepared microspheres, as indicated in Table-2.

Angle of repose: Prepared microspheres showed good flow ability as compared to pure drug, which had angle of repose 46.3^0. A decrease in the angle of repose was observed on formulating microsphere formulation of the drug because of reduction in cohesiveness. The angle of repose was found in between 15.8^0 to19.2^0 for all eight formulations, as indicated in Table-2.

In-vitro drug release study*:* The effect of polymer level on the release of aceclofenac from the microspheres was studied. Formulations F1, F2, F3, F4, F5, F6, F7 and F8 were able to sustain the drug release for around 4,5,6,7, 9, 10,12 hours respectively (Figure- 2). For F1 96.08 % of the drug was released after 4hours, for F2 95.23 % after 5 hours, for F3 93.23% after 6 hours, for F4 92.17 % after7 hours, F5 91.27% after 9 hours, F6 90.17% after 10 hours drug was released, for F7 92.34%of the drug was released after 12 hours, and for F8 79.61% of drug was released after 12 hours. For F7 92.34 % of the drug was released after 12hours. On increasing the quantity of Eudragit RS 100 up to 3.5 % , the release of the drug was too slow and only 92. 34 % of the drug was released after 12 hours. Formulations containing algino-eudragit RS100 microspheres (F1 to F8) were more efficient in sustaining the drug release because Eudragit could form rigid hydrophobic coat. Among all the formulations F7 showed better dissolution profile (more than 90 % drug was released in 12 hours, so it was selected to be the optimized formulation.

The coefficient of determination (R^2) was used as an indicator of the best fitting for each of the models considered (Table- 3). The kinetic data of all the formulations reached higher coefficient of determination (R^2 = 0.918 to 0.987) with the zero order whereas release exponent value (n) ranged from 1.34 to 2.02. From the release exponent in the Korsmeyer-Peppas model, it can be suggested that the mechanism that led to the release of aceclofenac was an anomalous non-Fickian diffusion mechanism leading to the conclusion that a combined release mechanism of drug diffusion and spheres erosion might be appropriate.

Scanning electron microscopy: The scanning electron micrographs (SEM) of the microspheres are shown in Figures3a and 3b. The SEM results revealed that all the aceclofenac loaded microspheres were discrete and spherical in shape with rough outer surface. The surface of the microspheres was rough due to the density of the polymer matrix which in turn justifies its sustained release. The dense network of drug-polymer increases the tortuisity, thus delaying the release of the drug and retarding the penetration of water (penetration of medium) required to make the sphere swell for disintegration.

FTIR: Infrared spectrum was taken in the Perkin-Elmer FT-IR (spectrum RX) by scanning the formulations in potassium bromide discs. The sample of pure drug, pure polymers and the formulations containing both the drug and polymers were scanned and shown in Figure-4. FT-IR spectra showed that no interaction was present between the drug and polymers which indicates the stable nature of aceclofenac in the prepared formulations.

Ex-vivo mucoadhesion study*:* The microspheres consisting of sodium alginate in combination with Eudragit RS 100 exhibited good mucoadhesive property as observed in *In vitro* wash-off test(Figure-5).This may be due to practically insoluble nature of sodium alginate in acidic solution compared to its solubility in simulated intestinal fluid where its hydration and mucoadhesive property were found to increase, which may be due to ionization of carboxyl acid group and other functional groups present in the polymer. This phenomenon increased its solubility which allowed more solvent to penetrate the polymeric coat, produce a viscous gel and increase the mucoadhesion property. It was also observed that algino-eudragit formulations had better mucoadhesive property because combination of both the polymers increases the viscosity in simulated intestinal fluid (pH 6.8), as they produce more viscous gel which further helped in increasing adhesion with intestinal mucosa. It was also observed that after 7h of the study, wash-

off was faster. Thus, it is suggested that the formulations restricted the drug release in stomach, and by adhering to the intestine mucosa for a prolonged period where able to release drug in a sustained manner.

CONCLUSION

The mucoadhesive microspheres of aceclofenac using algino-eudragit RS100 system showed good sustained release system. The prepared microspheres also inhibited the drug release in stomach and got adhered to intestinal region showing drug release by zero order. The physical characterization of the microsphere suggests that on formulating pure drug to microsphere the flow behavior of the drug was improved. So, by this system we can formulate aceclofenac loaded mucoadhesive microspheres for safe and sustained drug delivery.

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