AAPS PharmSciTech, Vol. 12, No. 2, June 2011 (© 2011)
DOI: 10.1208/s12249-011-9626-x

Research Article

Theme: Advanced Technologies for Oral Controlled Release
Guest Editors: Michael Repka, Joseph Reo, Linda Felton, and Stephen Howard

Design and *In Vitro* Performance Evaluation of Purified Microparticles of Pravastatin Sodium for Intestinal Delivery

Yogesh Garg[1] and Kamla Pathak[1,2]

Received 3 November 2010; accepted 27 April 2011; published online 19 May 2011

Abstract. The purpose of research was to develop a mucoadhesive multiparticulate sustained drug delivery system of pravastatin sodium, a highly water-soluble and poorly bioavailable drug, unstable at gastric pH. Mucoadhesive microparticles were formulated using eudragit S100 and ethyl cellulose as mucoadhesive polymers. End-step modification of w/o/o double emulsion solvent diffusion method was attempted to improve the purity of the product, that can affect the dose calculations of sustained release formulations and hence bioavailability. Microparticles formed were discrete, free flowing, and exhibited good mucoadhesive properties. DSC and DRS showed stable character of drug in microparticles and absence of drug polymer interaction. The drug to polymer ratio and surfactant concentration had significant effect on mean particle size, drug release, and entrapment efficiency. Microparticles made with drug: eudragit S100 ratio of 1:3 (F6) exhibited maximum entrapment efficiency of 72.7% and *ex vivo* mucoadhesion time of 4.15 h. *In vitro* permeation studies on goat intestinal mucosa demonstrated a flux rate (1,243 μg/cm^2/h) that was 169 times higher than the flux of pure drug. The gastric instability problem was overcome by formulating the optimized microparticles as enteric-coated capsules that provided a sustained delivery of the highly water-soluble drug for 12 h beyond the gastric region. The release mechanism was identified as fickian diffusion (n=0.4137) for the optimized formulation F6. Conclusively, a drug delivery system was successfully developed that showed delayed and sustained release up to 12 h and could be potentially useful to overcome poor bioavailability problems associated with pravastatin sodium.

KEY WORDS: enteric-coated capsules; modified w/o/o double emulsion solvent diffusion method; mucoadhesive microparticles; pravastatin sodium; sustained release.

INTRODUCTION

In order to develop oral drug delivery system, it is necessary to optimize both the residence time of the system in the GI tract and the release rate of the active ingredients from the system. Oral multiple-unit dosage forms such as microcapsules and microspheres have received much attention as modified/controlled drug delivery systems. However the success of these oral multiunit dosage forms is limited owing to their short residence time at the site of absorption. It will therefore be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes. This can be achieved by coupling the bioadhesive characteristics to microcapsules and developing bioadhesive microcapsules. Bioadhesive microcapsules have advantages such as efficient absorption and enhanced bioavailability of drugs owing to high surface to volume ratio, a much more intimate contact to mucus layer and specific targeting of drugs to the absorption site (1).

Microencapsulation, using mucoadhesive polymer is an extensively studied technique that is used to prolong the residence time of dosage form in the gastrointestinal tract and release the loaded drug in sustained manner (2).Most of the microencapsulation techniques have been used for sustaining and controlling the release of lipophilic drugs, since hydrophilic drugs usually demonstrate low loading efficiency (3). Conventional oil in water solvent diffusion and evaporation method may not be useful for water-soluble drugs, due to low loading efficiency, resulting from partitioning of drug into the continuous phase. To reduce the partitioning of a drug into the continuous phase, various methods such as chemical modification, modification of the continuous phase or dispersed phase of the emulsion have been employed (4). In this study, modified water-in-oil-in-oil (w/o/o) double emulsion solvent diffusion method has been used to improve the quality of product obtained by conventional method.

Pravastatin sodium, a hydrophilic drug was selected for its formulation as multiunit oral drug delivery system that can potentially minimize first-pass metabolism and consequently enhance the bioavailability. Pravastatin sodium is HMG CoA

[1] Department of Pharmaceutics, Rajiv Academy for Pharmacy, N.H. #2, P.O. Chattikkara, Mathura, 281001, India.
[2] To whom correspondence should be addressed. (e-mail: kamla_rap@yahoo.co.in)

inhibitor used as antihyperlipidemic agent, has a short biological half-life and undergoes extensive first-pass metabolism (5) so that its bioavailability is as low as 17%. It is a Biopharmaceutical Classification System (BCS) class 3 drug, that has highly solubility, low permeability, and is unstable in gastric pH. All these factors have guided the researchers to develop a therapeutically efficacious formulation of the drug. Attempts to overcome its low bioavailability have been made by formulating the drug as mucoadhesive bilayered buccal tablet using carrageenan gum as the base matrix (6) as the potential alternative route for the administration of pravastatin sodium. However, oral route has always been the preferred route of administration; therefore, the approach was to develop an oral drug delivery system that could deliver the gastric acid labile drug beyond gastric pH by means of an enteric-coated capsule system. Additionally, a multiunit mucoadhesive formulation is proposed with the aim to improve both low-permeability characteristics and for sustainment of the drug release.

Mucoadhesive polymers selected were eudragit S100 and ethyl cellulose. Eudragit S100 is a biocompatible polymer with low toxicity. It is a long-chain, high-molecular-weight anionic polymer that has property to swell by absorbing water and adheres to the mucosa through strong hydrogen bonding groups (–OH, –COOH). This is to provide a longer contact time for drug transport across the mucosal membrane, before the formulation is cleared by the mucosal surface. On the other hand, ethyl cellulose is a long-chain, high-molecular-weight polymer that has high flexibility and tensile strength to penetrate mucous membrane and it becomes sticky by absorbing water and adheres to mucosa though nonspecific, non-covalent interaction (7). Thus by using two different classes of mucoadhesive polymers, eudragit S100 (provides bonding-mediated adhesion) and ethylcellulose (provides hydration-mediated adhesion), microparticles of pravastatin sodium were developed to identify the formulation with best performance characteristics that could be formulated as enteric-coated capsule to deliver the drug to the small intestine for a prolonged period of time.

MATERIALS

Pravastatin sodium (PS) was a kind gift from Cadila Pharmaceuticals Ltd., Ahemdabad, India. Eudragit S100 (ES100) was purchased from Degussa India Pvt. Ltd., Germany; ethyl cellulose was purchased from S. D. Fine Chemicals Ltd., Mumbai, india. Dichloromethane, ethanol (95% *v/v*), tween80, span80, *n*-hexane, and other reagents were of analytical grade.

METHODS

Preparation of Mucoadhesive Microparticles

Microparticles were prepared by modified w/o/o double emulsion solvent diffusion method using different drug to polymer ratios and varying concentration of surfactant in external oil phase, shown in Table I. For preparation of microparticles, requisite quantities of pravastatin sodium and mucoadhesive polymer(s) were dissolved in 10 ml of the mixed solvent system, consisting of ethanol and dichloromethane in 1:4 ratio. The initial w/o emulsion was prepared by adding 2 ml of water containing 1.5% *v/v* of tween 80 to the drug–polymer solution while stirring, using a magnetic stirrer at 200 rpm for 5 min. This primary w/o emulsion was slowly added to 100 ml of corn oil, the secondary oil phase containing span 80 (0.5% and 1% *v/v*) while stirring at 200 rpm. After 2 h, 5 ml of *n*-hexane was added to harden the microparticles and the stirring was continued for further 1 h. The microparticles were collected by filtration and washed with 50 ml of *n*-hexane and dried in oven for 30 min. The dried microparticles were rewashed with three portions of 50 ml of water and dried in oven for 30 min at 50°C. Washings of microparticles with water, was the additional end step to the method reported by Lee *et al.* (4), that was aimed at getting a product free from un-entrapped drug. The dried product was stored in desiccator under vacuum for 48 h to ensure complete removal of solvents.

EVALUATION OF MICROPARTICLES

Percentage Entrapment Efficiency, Yield, and Drug Loading

Ten milligrams of the drug-loaded microparticles was crushed, accurately weighed and extracted with 10 ml of phosphate buffer, pH 6.4. The extract was filtered though micropore filter (22 μm), adequately diluted and the absorbance was read spectrophotometrically (PharmaSpec 1700, Shimadzu, Japan) at 239 nm. The amount of drug was calculated using calibration curve ($y=0.055x+0.054$; $r^2=0.9998$). Intraday precision study conducted for 12 h demonstrated relative standard deviation (%RSD) of less than 2% indicating the drug to be stable during the entire period and that the analytical method to be reliable. The entrapment efficiency was calculated as percent ratio of actual drug content to theoretical drug content. Percent yield was determined by dividing the weight of microparticles actually produced by the total expected weight of drug and polymer. Percent drug loading was calculated by dividing the weight of drug with the weight of microparticles.

Particle Size and Rheological Properties

The average particle size of microparticles was determined by optical microscopy method using calibrated ocular micrometer (Erma, Tokyo, Japan). The microparticles mounted on a glass slide were placed on mechanical stage. The microscope eye piece was fitted with a calibrated ocular micrometer and number of microparticles in different size ranges was counted. The data was used for the calculation of average particle size of microparticles. The bulk density of the microparticles was determined by tapped density apparatus (Hicon Enterprises, New Delhi) and the true density was determined by liquid displacement method using ethanol (95% *v/v*) as displacing liquid. The density determinations were used for calculation of Hausner's ratio and Carr's index, and hence for analysis of the rheological properties. Additionally, the rheological properties were also analyzed by angle of repose determinations using fixed funnel method (8).

Table I. Experimental Design for Formulation of Mucoadhesive Microspheres of Pravastatin Sodium

Formulation code	Polymer used	Drug:polymer (by weight)	Concentration of surfactant in external oil phase (by volume)
F1	Eudragit S100	1:1	0.5
F2		1:1	1.0
F3		1:2	0.5
F4		1:2	1.0
F5		1:3	0.5
F6		1:3	1.0
F7	Ethylcellulose	1:1	0.5
F8		1:1	1.0
F9		1:2	0.5
F10		1:2	1.0
F11		1:3	0.5
F12		1:3	1.0

Swelling Index

An accurately weighed amount of microparticles (100 mg) was suspended in 10 ml of phosphate buffer, pH 6.4 and allowed to swell; after 12 h, microparticles were again weighed and the percentage swelling (S) of microparticles was calculated by using following equation (9)

$$S(\%) = \left(\mathrm{Ws} - \frac{\mathrm{Wo}}{\mathrm{Ws}}\right) \times 100$$

Where Wo is weight of microparticles before swelling and Ws is weight of microparticles after swelling.

In Vitro Wash-Off Test for Mucoadhesion

A freshly cut small intestinal tissue obtained from local abattoir within 1 h of killing of the goat, was cleaned by washing with isotonic saline solution. Jejunum was separated and soaked in receptor medium (phosphate buffer, pH 6.4). This tissue represents a significant portion of the overall gastrointestinal tract and is therefore a good representative of the target tissue for orally administered bioadhesive drug delivery systems. Therefore, for experimentation, a piece of jejunum mucosa (2×3 cm) was mounted onto glass slide (2×1 cm) with cyanoacrylate glue. An accurate weight of microparticles (50 mg) was placed on mucosal surface. The glass slides were put in the grooves of the USP tablet disintegrating test apparatus (HICON, Grover Enterprises, New Delhi, India) and regular up and down movement was given in a beaker containing phosphate buffer pH 6.4. The duration for complete washing of microparticles from goat intestinal mucosa was recorded and averaged from three determinations.

In Vitro Drug Release

The microparticles equivalent to 30 mg of drug were filled in hard gelatin capsules (no. 2) and coated with 1% *w/v* solution of cellulose acetate phthalate (CAP) by dip-coating method. The coating was accomplished by dipping the filled capsules thrice in CAP solution and air drying after each coating step successively. The release studies were carried out in 0.1 N HCl, pH 1.2 for 2 h followed by phosphate buffer, pH 6.4 at 37±0.5°C for next 10 h using USP type I dissolution test apparatus using fabricated basket mesh # 120. The dissolution was done at 100 rpm at 37±0.5°C. Samples were withdrawn from the vessel at appropriate time intervals, replaced with fresh dissolution media and analyzed spectrophotometrically. Percent of pravastatin sodium dissolved at various time intervals was calculated from the regression equation generated from suitably constructed calibration curve. Data obtained from *in vitro* release studies were fitted to various kinetics equations to find out the mechanism of pravastatin sodium release from the microparticles by PCP disso software 2.0v, Pune, India. The kinetic models used were zero order, first order, Higuchi, Peppas, and Hixson–Crowell model.

In Vitro Permeation

The permeability of pravastatin sodium from the microparticles was determined across goat intestinal mucosa using Franz diffusion cell with a diffusional area of 1.76 cm^2 and receptor volume of 11 ml. A freshly cut small intestinal tissue of goat obtained from local abattoir within 1 h of killing, the animal was cleaned by washing with isotonic saline solution. The jejunum part was separated and soaked in receptor medium (phosphate buffer, pH 7.4) for 8 h. Jejunum mucosa was used as barrier membrane and mounted between the donor and receptor compartments. An amount of microparticles equivalent to 10 mg of the drug, in order to ensure sink conditions during the experiment, was placed on the membrane surface in the donor compartment that was sealed from the atmosphere with an aluminum foil. During the experiment, the solution in the receptor side was kept at 37±1°C and it was stirred at 100 rpm with Teflon-coated magnetic stirring bar. After application of the test formulation on the donor side, 1 ml aliquots was collected from receptor side at designated time intervals and replaced by the same volume of fresh receptor solution to maintain sink condition and constant volume. The sample was analyzed spectrophotometrically at 239 nm and cumulative amount permeated from microparticles was determined (n=3).The *in vitro* permeation profiles were used to derive permeation parameters.

SELECTION OF OPTIMIZED FORMULATION

The optimized microparticulate formulation was selected for visualization and spectral characterization on the basis of performance characteristics.

VISUALIZATION AND SPECTRAL CHARACTERIZATION OF MICROPARTICLES

Scanning Electron Microscopy

Scanning electron photographs of pravastatin sodium, selected microparticles (F6) before and after washing were obtained using a scanning electron microscope (LEO- 435 VP, U.K.). The samples were gold coated by sputter coater E5 100 UK POLARON for 15–20 min and the photomicrographs were taken under various magnifications depending on the sample.

Differential Scanning Calorimetry

Differential scanning calorimetry was done for pravastatin sodium, eudragit S100, physical mixture of PS and ES100 and selected microparticles (F6) using Perkin–Elmer differential scanning calorimeter equipped with liquid nitrogen sub ambient accessory (Perkin–Elmer Pyris diamond, Tokyo, Japan). The instrument was operated under nitrogen pure gas at a rate of 20 ml min^{-1}. Samples were sealed in the alumina pans (TA instruments, Belgium) and heating at a scanning rate of 10°C/min from 20°C to 400°C.

Diffuse Reflectance Spectroscopy

Diffuse reflectance spectroscopy was done for pravastatin sodium, eudragit S100, physical mixture of PS and ES100 and selected microparticles (F6) using Fourier transform infrared Spectrophotometer with diffuse reflectance spectroscopy (DRS) attachment. Samples were diluted with KBr and mounted into the instrument. Spectrum was determined in the range of 400–4,000 cm^{-1} and interpreted for characteristic peaks of respective bonds. Any possible interaction was identified by shift in peak or development of new peak.

RESULTS AND DISCUSSION

Purified Microparticles of Pravastatin Sodium

The microparticles prepared by w/o/o double emulsion solvent diffusion method, when observed by SEM showed microparticles along with some crystalline particles of PS (Fig. 1a). This can be explained by stepwise understanding of microparticle formation. In the first step of emulsification, the water-soluble drug occupied the internal phase of w/o emulsion. Owing to the limited amount of internal aqueous phase forces, some drug gets partitioned into the oily phase of the emulsion. In the next step, when the primary emulsion is added to another quantity of oily phase, the partitioned drug tends to crystallize out, thus reducing the amount of drug entrapped within the microparticles. Thus the product obtained was a mixture of pure drug and microparticles of the drug. In order to obtain pure product, the literature-cited method was modified by washing the product with three portions of distilled water that ensured complete removal of pravastatin sodium crystals. The microparticles were then dried in oven at 55°C for half an hour to get the pure product as discrete microparticles (Fig. 1b) free from PS crystals (Fig. 1c). The drug content of both non-purified microparticles and purified microparticles was analyzed and is tabulated in Table II. Interestingly, the data indicated a reduction of 4–8% in drug content of the produce that is attributable to the purification step used. Thus, emphasis needs to be laid on the purification of microparticles of highly water-soluble drug produced by double emulsion solvent diffusion method, as absence of the same can potentially affect the dose calculations of sustained release formulations and hence bioavailability.

EVALUATION OF MICROPARTICLES

Percent Entrapment Efficiency, Percent Loading, and Percent Yield

Drug loading, percent entrapment efficiency (%EE) and yield were estimated for all formulations and the data is tabulated in Table II. The %EE of prepared microparticles was in the range of 51.60–79.56% while percent drug loading ranged between 25.31% and 55.56%, respectively. Broadly speaking, the PS–ES100 microparticles showed higher %EE and drug loading as compared to pravastatin sodium–ethylcellulose (PS–EC) microparticles. This is attributable to higher solubility of drug in ES100 in comparison to EC and also higher swelling capacity of ES100 due to which it uncoils to an extended structure in the media thus providing a larger surface for entrapment (10). Further, within the PS–ES100 microsphere formulations, the results demonstrated that an increase in drug:polymer ratio resulted in formation of larger-sized microparticles and consequently an increase in the %EE. On the other hand within the same drug: polymer ratio, %EE decreased as the concentration of surfactant increased, that can be attributed to the increased solubility of drug into external oil phase with increase in surfactant concentration, due to which the drug gets partitioned out more rapidly (11). A similar pattern was observed with PS–EC microparticles except for lower numerical values for %EE and percent drug loading. As discussed by Florence and Whitehall (12), when microspheres are prepared by w/o/w emulsion diffusion method, stability of the internal water phase influences both, the loading efficiency of the water-soluble drugs as well as the morphology of the internal structure of microspheres. The yield of various formulations was narrowly ranged (93.99–98.76%), conforming the efficiency of the w/o/o double emulsion method for preparation of microparticles.

Micromeritic Properties

The microparticles were characterized for their micromeritic properties such as particle size, angle of repose, and other derived rheological parameters (Table III). The mean particle size of microparticles increased with increase in polymer concentration and decreased with increase in surfactant concentration. With increased polymer concentration,

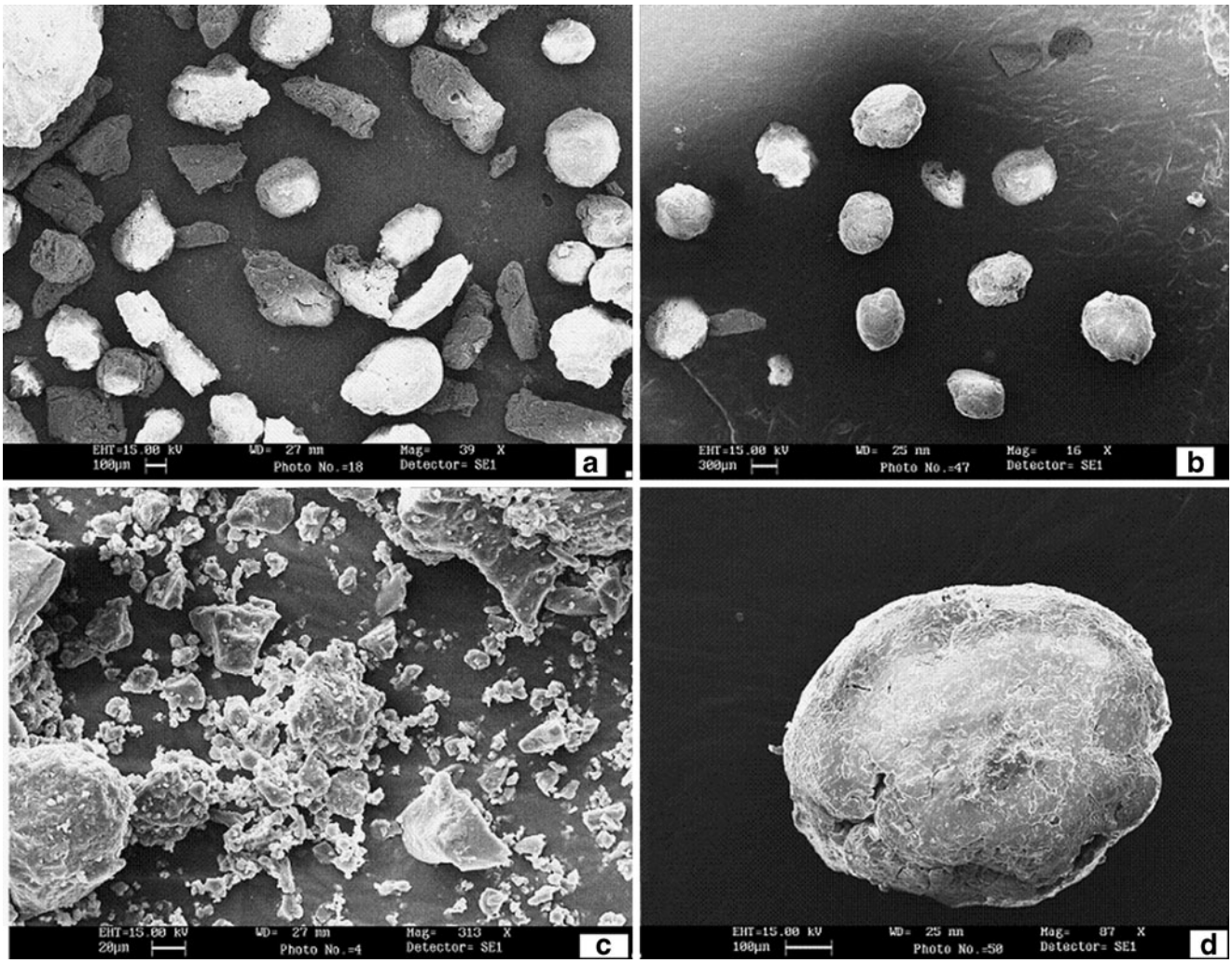

Fig. 1. Scanning electron micrographs of **a** microparticles along with residual drug crystals, **b** Purified microparticles, **c** drug particles, and **d** enlarged view of microparticle showing surface morphology

the viscosity of the medium increased, resulting in enhanced interfacial tension that led to a decrease in the shearing efficiency and resulted in formation of larger-sized microparticles (13). On the other hand, increasing concentration of surfactant lowered the interfacial tension, enhanced due to increase in polymer concentration and prevented droplet coalescence that led to reduction in size of microparticles (14). Thus for a given drug: polymer ratio, an increase in

Table II. Drug Content Before and After Purification, Percent Entrapment Efficiency, Percent Drug Loading, and Percent Yield Data of Microspheres of Pravastatin Sodium. The Values Reported are mean±s.d. (n=3)

Formulation code	Drug content (%)		Percent entrapment efficiency	Percent loading	Percent yield
	Before purification	After purification			
F1	59.78±0.34	55.86±0.23	60.08±1.81	55.55±0.34	89.99±0.5
F2	57.81±0.11	51.60±0.57	58.50±1.60	53.56±0.11	93.35±0.19
F3	76.23±0.05	69.28±0.30	75.13±1.22	34.88±0.05	95.05±0.32
F4	62.45±0.08	60.48±0.40	65.28±0.84	33.68±0.08	98.76±0.40
F5	85.06±0.02	79.56±0.66	79.56±0.66	26.36±0.02	94.80±0.10
F6	78.18±0.02	72.72±0.17	72.72±0.17	25.32±0.02	98.07±0.08
F7	66.50±0.10	60.08±1.81	55.86±0.23	51.77±0.10	96.57±0.20
F8	63.60±0.07	58.50±1.60	51.60±0.57	51.43±0.07	97.20±0.14
F9	79.43±0.03	75.13±1.22	69.28±0.30	34.85±0.03	95.57±0.12
F10	68.13±0.05	65.28±0.84	60.48±0.40	33.48±0.05	96.68±0.10
F11	81.56±0.02	74.48±0.73	74.48±0.73	25.63±0.02	97.52±0.06
F12	72.81±0.30	65.88±1.44	65.88±1.44	25.31±0.30	98.45±0.04

surfactant resulted in decrease in particle size for both set of formulations. Owing to these interrelated parameters the mean particle size of microparticles was in the range of 178–252 μm. The particle size of microparticles is a very important parameter, since it affects drug release and pharmacokinetics and processing. All the formulations were free flowing as measured by angle of repose that ranged between 20°–27°. The free flowing property was due to discrete, almost uniform-sized microparticles, as visualized by scanning electron microscopy. Other valuable derived rheological properties that will ensure easy handling of formulations are tabulated in Table III.

Mucoadhesive Character

The mucoadhesive property of the microparticles was evaluated by swelling studies and *in vitro* wash of test. All microparticles swelled in phosphate buffer pH 6.4 with a characteristic swelling pattern. Poor initial swelling in the first 5 h was followed by relatively higher swelling till the end of test period for PS–EC microparticles. On the other hand, gradual swelling was observed for PS–ES100 microparticles during the entire period of study. Quantitative assessment of swelling reported as percent swelling was found to be in the range of 28.57–43.25 for PS–EC microparticles and 42.62–70.39 for PS–ES100 microparticles (Table IV). Higher swelling of PS–ES100 microparticles than PS–EC microparticles can be attributed to greater ability of polymeric chains of ES 100 to uncoil into an extended structure, facilitating interpenetration and entanglement and consequently allowing binding groups to come together (15). The time period for which microparticles adhere to intestinal mucosa was estimated by *in vitro* wash-off test that is another useful assessment of mucoadhesive character. The microparticles made with ES100 demonstrated higher mucoadhesion time in comparison to those made with ES. Thus F6 made using ES100 showed highest mucoadhesion time of 4.18 h amongst all formulations tested and the least was exhibited by F7 (0.77 h) made with least drug:EC ratio. Mucoadhesion of pH sensitive mucoadhesive polymer ES 100 is favored when majority of carboxylic groups are in unionized form that occurs at pH below its pKa. Thus at pH 6.4, due to the strong electrostatic attraction between ES100 and mucin (10), highest mucoadhesion time was recorded for microparticles made with drug: ES100 (1:3). On the other hand PS–EC microparticles made with nonionic polymer demonstrated weak mucoadhesive property as mucoadhesion of such polymers is solely based on its ability to hydrate and consequently become sticky. Higher molecular weight of ES100 in comparison to EC is yet another contributing factor that resulted in higher swelling and consequently mucoadhesion of PS–ES100 microparticles rather than PS–EC microparticles (16). It was also observed that irrespective of the type of mucoadhesive polymer, microparticles prepared with highest drug:polymer ratio (F6 and F12) showed highest mucoadhesion time and swelling capacity for the simple reason that water uptake/binding ability of microparticles increases with increase in polymer concentration (1).Thus, the amount of polymer directly affects mucoadhesion and swelling index and consequently ensures prolonged residence time at the absorption site to facilitate intimate contact with the absorption surface and thereby improve and enhance the bioavailability (17).

Table IV. Mucoadhesion Time and Percent Swelling of Mucoadhesive Microspheres of Pravastatin Sodium. The Values Reported are mean±s.d. (n=3)

Formulation code	Mucoadhesion time (h)	Percentage swelling at pH 6.4 after 12 h
F1	1.25±0.25	42.62±0.11
F2	1.95±0.09	44.56±0.15
F3	2.08±0.09	56.72±0.23
F4	2.98±0.24	58.27±0.17
F5	3.88±0.09	67.68±0.56
F6	4.15±0.02	70.39±0.18
F7	0.77±0.17	28.57±0.37
F8	0.88±0.22	29.41±0.22
F9	1.61±0.98	38.35±0.09
F10	1.97±0.59	35.64±0.18
F11	2.62±0.21	42.16±0.19
F12	2.79±0.11	43.25±0.16

In Vitro Drug Release

The aims of undertaking the *in vitro* drug release test were to assure that the microparticles of PS are delivered to

Table III. Micromeritic Properties of Mucoadhesive Microspheres of Pravastatin Sodium

Formulation code	Particle size (μm)	True density (g/cc)	Bulk density (g/cc)	Tapped density (g/cc)	Angle of repose (°)	Compressibility index	Hausner's ratio
F1	205±0.45	0.923±0.04	0.339±0.03	0.383±0.03	20.70±0.13	12.36	1.13
F2	190±0.13	0.970±0.17	0.343±0.04	0.369±0.26	24.74±0.24	13.36	1.23
F3	239±1.03	0.870±0.02	0.342±0.05	0.388±0.22	27.94±0.17	12.08	1.03
F4	224±0.64	0.850±0.02	0.343±0.04	0.391±0.10	21.79±0.08	14.01	1.36
F5	252±0.84	0.870±0.05	0.351±0.05	0.394±0.20	23.81±0.14	12.33	1.22
F6	244±0.48	0.920±0.01	0.344±0.01	0.391±0.20	24.67±0.36	13.85	1.19
F7	197±0.18	0.893±0.01	0.361±0.06	0.438±0.07	27.08±0.16	15.61	1.19
F8	178±0.11	0.806±0.04	0.372±0.03	0.431±0.55	20.93±1.75	15.93	1.35
F9	216±1.24	0.860±0.01	0.370±0.05	0.432±0.03	21.93±0.23	16.64	1.32
F10	202±0.95	0.930±0.02	0.371±0.02	0.425±0.01	24.54±1.07	17.24	1.05
F11	239±0.33	0.896±0.21	0.370±0.01	0.450±0.02	23.61±0.64	15.64	1.11
F12	228±0.41	0.980±0.01	0.374±0.05	0.424±0.08	25.12±1.51	15.68	1.17

The values reported are mean±s.d. (n=3)

the predefined target area, and to elucidate the release mechanism for the developed formulation. PS is unstable at gastric pH and therefore the microparticles of the drug were capsulated in hard gelatin capsules, coated with 1% *w/v* CAP and studied for release of drug in pH 1.2 for 2 h followed by release in pH 6.4. ES 100 being a pH-sensitive polymer, is soluble above pH 7.0, thus pH 6.4 was selected for the study because if the microparticles are able to sustain drug release at the borderline pH, these will be able to sustain the release at lower intestinal pH values as well. Another biorelevant consideration is that the transit time of a drug through the absorptive area of the gastrointestinal tract is between 9 and 12 h this includes 2–3 h of gastric residence time (18). Thus the capsulated formulations were initially tested for drug release in the simulated gastric pH for 2 h followed by drug release in simulated intestinal pH.

As observed in Fig. 2, in the initial 2 h, PS was not released in the simulated gastric fluid, thereafter the release was initiated when the pH was changed to simulated intestinal fluid (phosphate buffer, pH 6.4) for the next 10 h. The drug release pattern from all the microparticulate formulations of PS was in a sustained manner, in contrast to the dissolution of pure drug. The release profile of pure drug, after a lag time of 2 h, demonstrated an initial burst release of more than 90% in 2nd to 5th h followed by almost constant release phase till the end of 12th h (99.77%). However, the release of PS from the microparticles made with ES 100 showed a gradual cumulative drug release (% CDR) in the range of 76.8%–89.9% whereas the quantum of drug release from microparticles prepared with EC was of slightly lower order ranging from 75.34%–82.3%. On applying ANOVA followed by Dunett's test a significant difference ($p<0.05$) was observed in the release profiles of all the microparticulate formulations as compared to pure drug suspension. Interestingly, a slight jump was observed in the release profiles at 5th h, for almost all formulations made with EC. This is attributable to the poor affinity of EC for aqueous release phase that did not enable good initial swelling, but with passage of time the swelling characteristics improved that facilitated drug release. Broadly speaking, PS–ES100 microparticles showed higher% CDR compared to PS–EC microparticles due to higher wettability and smaller particle size of the former. It was also observed that drug release from microparticles was directly proportional to the amount of surfactant used and inversely related to the amount of polymer used for formulation.

The release data was modeled for zero order, first order, Higuchi, Peppas, Hixson–Crowell release using PCP Disso2.0v software. The release kinetics of all the formulations best fitted the Higuchi model. The Korsmeyer–Peppas release exponent (n) was analyzed to confirm the release mechanism. The value for n is ≤0.45 for fickian release, >0.45 and <0.89 for non-fickian release, 0.89 for case II release and >0.89 for super case II-type release (19). In the present study, the values of n and the coefficients of determination (r^2) obtained for the release profiles are listed in Table V. The values of n ranged between 0.3147 and 0.5627, indicating both fickian and non-fickian diffusion. Careful analysis of the formulation design revealed that at high level of surfactant in the external oil phase, non-fickian diffusion was observed. The surfactant at 1% level was able to induce surface related behavioral changes in the polymer that led to non-fickian diffusion. This kind of release is characteristic of swelling-controlled system in which the rate of solvent uptake into a polymer (ES100/EC) is largely determined by the rate of swelling and relaxation of the polymer chains. The drug molecules diffuse out through a dissolving gel-like layer formed around the drug during the dissolving process of polymers. Wu *et al.* (20) have observed the similar type of release behavior for highly soluble drug potassium chloride, from sustained release microparticles prepared using EC.

In Vitro Permeation

As the mucoadhesive formulation is meant to adhere to the mucin and facilitate drug permeation, therefore apart from drug release, the formulations need to be evaluated for *in vitro* permeation. Goat ileum preparation can be used to study the absorption parameter of actively transported drug PS. The advantage of this methodology and preparation are that it is easy to perform and economical, stability of preparation is high, and importantly, ethically substitutive (21). Accordingly, there are numerous research reports wherein goat jejunum has been utilized to study *in vitro* permeability and hence as a reliable predictor of oral absorption in humans. The *in vitro* permeation profiles (Fig. 3), showed higher permeation of the drug from micro-

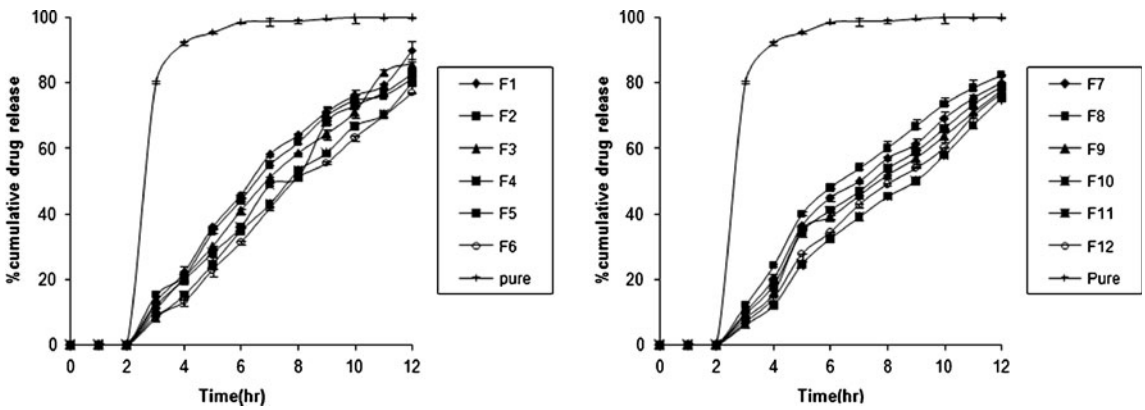

Fig. 2. *In vitro* drug release plots of **a** pravastatin sodium–Eudragit S100 microparticles (F1–F6) and **b** pravastatin sodium–ethylcellulose microparticles (F8–F12) and pure drug in simulated gastric fluid, pH 1.2 for 2 h followed by release in phosphate buffer pH 6.4

Table V. Correlation Coefficients for Model Fitting of the *In Vitro* Release Data of Mucoadhesive Microspheres of Pravastatin Sodium in Phosphate Buffer 7.4

Formulation code	Zero order	First order	Matrix model	Hixson–Crowell	Korsemeyer Peppas (n)
F1	0.8596	0.8896	0.9749	0.9151	0.9112 (0.3817)
F2	0.8572	0.8984	0.9897	0.9011	0.9701 (0.5574)
F3	0.9165	0.8136	0.9889	0.9224	0.9258 (0.3453)
F4	0.9109	0.8605	0.9918	0.9221	0.9575 (0.4661)
F5	0.9676	0.8214	0.9819	0.9634	0.9361 (0.3967)
F6	0.9528	0.8571	0.9894	0.9129	0.9243 (0.4837)
F7	0.9109	0.7986	0.9811	0.9139	0.9785 (0.3147)
F8	0.9195	0.8895	0.9897	0.9129	0.9814 (0.4498)
F9	0.9678	0.8861	0.9895	0.9237	0.9297 (0.3398)
F10	0.9661	0.8871	0.9836	0.9448	0.9582 (0.5627)
F11	0.9679	0.8854	0.9865	0.9334	0.9772 (0.3897)
F12	0.9213	0.8915	0.9842	0.9712	0.9711 (0.5918)

particles than pure drug (F0). This is attributed to the mucoadhesive ability of polymers ES100 and EC that tend to increase the residence time of microparticles on the intestinal mucosa resulting in high permeation than the pure drug. It was observed that PS–ES100 microparticles showed higher permeation than the PS–EC microparticles due to better mucoadhesive properties of ES100 than EC. In their respective groups, F6 and F12 formulated using highest mucoadhesive polymer and surfactant level(s) showed highest permeation. On applying ANOVA followed by Dunett's test a significant difference ($p<0.05$) was observed in the permeation profiles of all the formulations as compared to pure drug.

For the purpose of quantitative comparison, the permeation profiles were analyzed by calculation of various permeation characteristics as tabulated in Table VI. The flux of PS was calculated as 760.63 μg/cm^2/h, which was significantly ($p>0.05$) less than all the formulated mucoadhesive microparticles. Highest flux was recorded for F6, that was 1.6 times higher than the flux of pure drug. Least flux value of 1,110.65 μg/cm^2/h was recorded for formulation F7 (PS–EC microparticle with least surfactant concentration) that was again 1.4 times more than flux rate of pure drug. The definitive role of mucoadhesive polymer in improving the permeability of a BCS class III drug is clearly evidenced. Highest flux value of F6 can be attributed to its strongest mucoadhesive property when compared to rest of formulations and hence has the potential to overcome poor permeability and consequently bioavailability problems of PS.

Selection of Best Formulation

Formulation F6 made with ES100 containing 1% *v*/*v* of surfactant and drug to polymer ratio of 1:3 was selected on the basis of high percentage entrapment efficiency (72.72%), percentage swelling (70.39%), highest mucoadhesion time (4.15 h), highest flux rate (1,243 μg/cm^2/h). Its capsulated formulation demonstrated delayed and sustained drug release till 12 h with diffusion controlled release mechanism. This formulation was subjected to visualization and spectral characterization to elucidate any physical and chemical changes that might have occurred during microparticle formation.

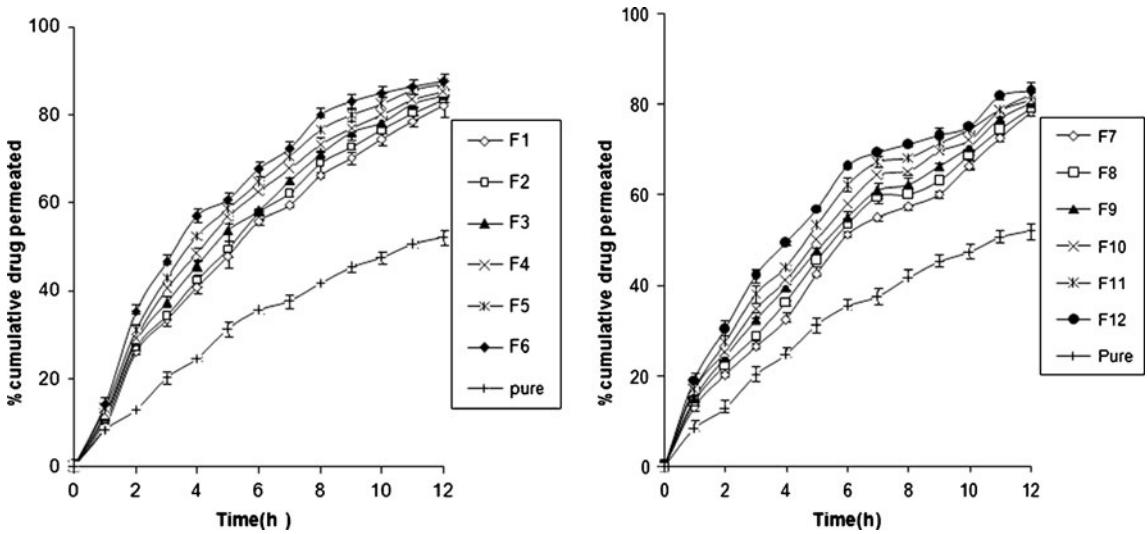

Fig. 3. *In vitro* permeation plots across goat intestinal mucosa of pravastatin sodium–Eudragit S100 microparticles (F1–F6) and pravastatin sodium-ethylcellulose microparticles (F8–F12) and pure drug in phosphate buffer pH 7.4

Table VI. *In Vitro* Permeation Parameters of Pravastatin Sodium Mucoadhesive Microspheres Across Goat Intestinal Mucosa

Formulation code	Flux (μg/cm²/h)	Enhancement ratio	Q_{8h} (μg/cm²)
F0	740.63	100	15,642
F1	1,166.48	157	23,457
F2	1,185.37	161	24,096
F3	1,192.62	162	24,339
F4	1,208.95	164	24,693
F5	1,234.66	166	24,693
F6	1,243.89	169	24,924
F7	1,110.65	149	24,636
F8	1,125.01	151	25,035
F9	1,140.91	154	25,296
F10	1,152.41	156	25,533
F11	1,169.18	158	26,376
F12	1,180.11	161	26,271

CHARACTERIZATION OF MICROPARTICLES

Scanning Electron Microscopy

The SEM photomicrograph of microparticles showed that the microparticles were almost spherical, globular with dense and rough surface (Fig. 1d). The surface showed numerous folds, depressions and expressions of dried cross-linked polymer as criss-cross lines throughout the surface. This morphology may be attributed to the preference of the polymer to orient itself towards the external oil phase (22). Few pinholes were also present on the surface of microparticles that are the result of rapid escape of volatile solvents used during formulation.

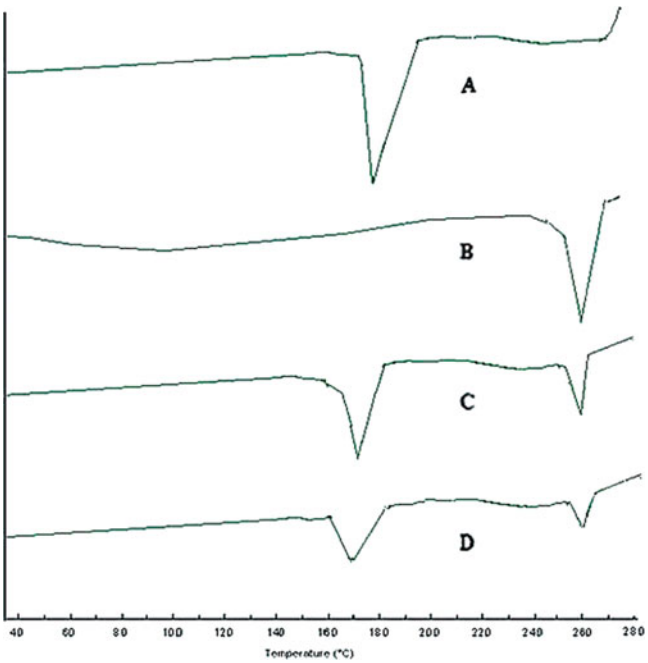

Fig. 4. Differential scanning thermograms of pravastatin sodium (**a**), Eudragit S100 (**b**), physical mixture of pravastatin sodium and Eudragit S100 (**c**), and F6 (**d**)

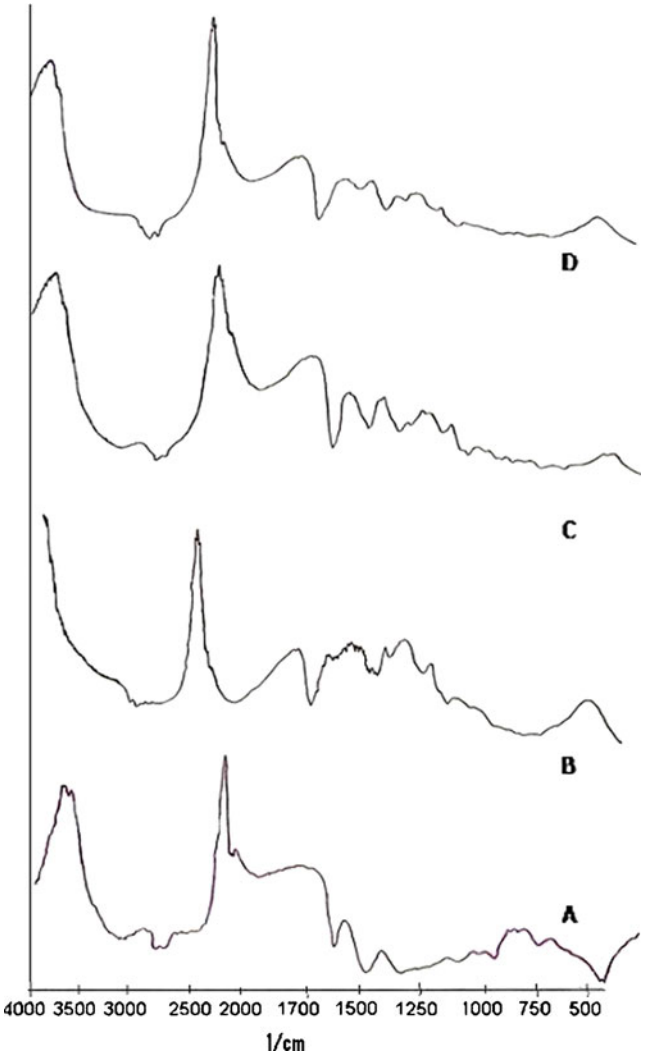

Fig. 5. DRS spectra of Pravastatin sodium (**a**), Eudragit S100 (**b**), physical mixture of pravastatin sodium and Eudragit S100 (**c**), and F6 (**d**)

Differential Scanning Calorimetry

The differential scanning calorimetry (DSC) trace of PS (Fig. 4) was typical of crystalline anhydrous substance exhibiting a sharp endothermic peak at 172°C (ΔH=93.7 μJ/mg) corresponding to melting point of the drug. DSC of ES100 showed sharp endothermic peak at 263°C (ΔH=51.6 μJ/mg) corresponding to its melting point. The thermogram of physical mixture was an additive spectra of the two components and the thermogram of microparticles exhibited endothermic peaks corresponding to the melting peak of PS and ES100. This was indicative of absence of any potential chemical interaction between PS and ES100 during formulation of microparticles that was also confirmed by diffuse reflectance spectroscopy. However, the ΔH value of both PS and ES100 shifted towards lower side when in form of physical mixture (ΔH=73.7 μJ/mg for PS and 39.0 for ES100) that got further reduced when in microparticles (ΔH=63.7 μJ/mg for PS and 35.7) for ES100. This may be a result of change in the heat capacity of polymer as it undergoes transition from the glassy state to

liquid state (23) during microsphere formation. The reduction in the intensity of peaks in microparticles is attributable to higher concentration of polymer and conversion of crystalline form of the drug to its amorphous form (24). The DSC thermograms did not indicate any interaction between the drug and excipients that was further confirmed by diffuse reflectance spectroscopy.

Diffuse Reflectance Spectroscopy

DRS were used to identify any potential chemical interaction that might have occurred during microsphere formation. DRS spectrum of pure PS showed characteristic peaks corresponding to O–H stretching (3,450 cm^{-1}), C–O stretching at 1,575 cm^{-1} and C=O stretching at 1,760 cm^{-1}(Fig. 5). ES100 showed characteristic O–H stretching bond at 1,733 cm^{-1}. Similar peaks are also observed in the corresponding physical mixture and microparticles. Absence of any new peak in microsphere indicated that PS did not undergo any chemical changes during the formation of microparticles. Some weak absorption peaks shifted to lower wave number suggesting the formation of hydrogen bond between the carbonyl groups of PS and hydroxyl groups of ES100.

CONCLUSION

The modification of w/o/o double emulsion solvent diffusion method resulted in production of microparticles free from residual crystalline drug particles as visualized by SEM. The mucoadhesive microparticles made with Eudragit S100 containing 1% *v/v* of surfactant and drug to polymer ratio of 1:3 were selected as best formulation, that were formulated as enteric-coated capsule to avoid the drug release at gastric pH. The formulated system showed delayed and sustained release up to 12 h and the system is potentially useful to overcome poor permeability and consequently bioavailability problems associated with pravastatin sodium.

ACKNOWLEDGMENTS

The authors wish to thanks IIT Delhi, India for providing SEM and DSC facilities.

REFERENCES

1. Patil DA, Patil GB, Deshmukh PK, Belgamwar *VS*, Fursule RA. Chitosan coated mucoadhesive multiparticulate drug delivery system for gliclazide. Asian J Pharm. 2009;2:18–25.
2. Chun MK, Cho CS, Choi HK. Mucoadhesive microparticles prepared by interpolymer complexation and solvent diffusion method. Int J Pharm. 2005;288:295–303.
3. Rao KM, Senapati P, Das MK. Formulation and *in vitro* evaluation of ethyl cellulose microparticles containing zidovudine. J Microencapsul. 2005;22:863–76.
4. Lee JH, Park TG, Choi HK. Effect of formulation and processing variables on the characteristics of microparticles for water soluble drugs prepared by w/o/o double emulsion solvent diffusion method. Int J Pharm. 2000;196:75–83.
5. Maffat AC, Osselton MD, Widdop B. Clarke's analysis of drugs and poisons. 3rd ed. Great Britain: Pharmaceutical Press; 2004.
6. Shidhaye SS, Thakkar PV, Dand NM, Kadam VJ. Buccal drug delivery of pravastatin sodium. AAPS Pharm Sci Tech. 2010;11:416–24.
7. Patil SB, Murthy RSR, Mahajan HS, Wagh RD, Gattany SG. Mucoadhesive polymers: means of improving drug delivery. Pharma Times. 2006;38:25–8.
8. Martin A, Swarbrick J, Cammarrata A. Physical pharmacy-physical chemical principles in pharmaceutical sciences. 4th ed. New Delhi: B I Waverly Pvt; 2008.
9. Nagda C, Chotai NP, Patel U, Patel S, Soni T, Patel P, *et al.* Preparation and characterization of spray-dried mucoadhesive microparticles of aceclofenac. Drug Dev Ind Pharm. 2009;35:1155–66.
10. Harikaranpakdee S, Lipipun V, Sutanthavibul N, Ritthidej GC. Spray dried mucoadhesive microparticles: preparation and transport though nasal cell monolayer. AAPS Pharm Sci Tech. 2008;7:1–15.
11. Lu H, Zhang Y, Zhang G, Gao S, Duxin S, Zhong Y. Optimization of the drug incorporation into the polymer matrix and modeling of drug release. Int J Pharm. 2008;351:244–9.
12. Florence AT, Whitehill D. Some features of breakdown in water-in-oil-in-water multiple emulsions. J Colloid Interface Sci. 1981;79:243–56.
13. Gattani YS, Kawticwar PS, Sakarkar DM. Formulation and evaluation of gastro retentive multiparticulate drug delivery system of aceclofenac. Int J Chem Tech Res. 2009;1:1–10.
14. Choudhary PK, Khar M, Chauhan CS. Cellulose acetate microparticles as floating depot system to increase gastric retention of antidiabetic drug: Formulation, characterization and *in vitro- in vivo* evaluation. Drug Dev Ind Pharm. 2008;34:349–54.
15. Smart JD. The basics and underlying mechanisms of mucoadhesion. Adv Drug Deliv Rev. 2005;57:1556–68.
16. Ganini E, Rassu G, Cissu M, Giunchedi P. Spray dried microparticles based on methyl pyrolidone chitosan as a new carrier for nasal administration of metclopramide. Eur J Pharm Biopharm. 2008;68:245–52.
17. Das MK, Senapati PC. Furesomide loaded alginate microspheres prepared by ionic cross- linking technique: morphology and release characteristics. Indian J Pharmaceut Sci. 2008;70:77–84.
18. Uchida T, Nagareya N, Sakakibara S, *et al.* Preparation and characterization of polylactic acid microspheres containing bovine insulin by w/o/w emulsion solvent evaporation method. Chem Pharm Bull. 1997;45:1539–43.
19. Mhamanga KS, Parfitt N, Nyamuzhiwa T, Haidula H, Walker RB. The evaluation of eudragit microcapsules manufactured by solvent evaporation using USP apparatus 1. Disso Technol. 2009;16:15–22.
20. Wu PC, Huang YB, Chang JI, Tsai MN, Tsai YH. Preparation and evaluation of sustained release microparticles of potassium chloride prepared with ethylcellulose. Int J Pharm. 2003;260:115–21.
21. Tembhurne SV, Sakarkar DM. Transport of glucose in isolated goat ileum preparation: an alternative research tool for absorption study Indian. J Physiol Pharmacol. 2008;52(2):211–3.
22. Chaddha H, Deluca PP. Effect of polytyrosine on the hydrophobicity of hydroxyethyl starch microparticles. Pharmaceut Dev Tech. 1998;3:597–606.
23. Settle F. Handbook of instrumental techniques for analytical chemistry (1st edn). Singapore: Pearson Education Pvt Ltd; 2004.
24. Paradkar A, Ambike AA, Mahadik KR. Characterization of curcumin—PVP solid dispersion obtained by spray drying. Int J Pharm. 2004;271:281–6.