RESEARCH ARTICLE

Preparation and evaluation of mucoadhesive simvastatin microcapsules using orifice gelation technique

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Preparation and characterization of Simvastatin/ Hydroxy propyl beta cyclodextrin (HPBCD) (SV/HPBCD) binary systems by co-grinding technique and formulating the binary system in oral mucoadhesive microcapsules by using hydrophilic sodium alginate (SA) and another plant seed mucilage dillenia (obtained from *Dillenia indica*, Family, Dilleniaceae) using orifice gelation technique and systematically evaluating *in vitro* by using scanning electron microscopy (SEM), fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), and X-ray diffractometer (XRD). The microcapsules were smooth and elegant in appearance showed no visible cracks as confirmed by SEM; and extended drug release of 72.682% upto 12 hours in phosphate buffer of pH 6.8; showing particle size within the range of 371.5-457 μm, and less angle of repose, Hausner's ratio and Carr's consolidation index; and showed encapsulation efficiency of 63.068 ± 0.002 to 99.083 ± 0.017%. The *in vitro* release data of optimized batch of microcapsules were plotted in various kinetic equations to understand the mechanisms and kinetics of drug release, which followed zero order kinetics and value of "*n*," is calculated to be 0.505 and drug release was diffusion controlled. The *in vivo* antihyperlipidemic activity of formulations in mice was carried out developing hyperlipidemia in mice and then administering the optimized formulations orally, and the formulation showed promising results.

Key words: *Co-grinding, dillenia, microcapsules, orifice gelation, simvastatin*

INTRODUCTION

Oral route serves as the most convenient route for drug delivery.[1] An ideal dosage regimen in the drug therapy of every disease is the one which maintains the desired therapeutic concentration of drug in the plasma for entire duration of treatment. An ideal oral controlled drug delivery system is one which delivers the drug at a predetermined rate, locally or systematically for a specified period of time.[2] Simvastatin (SV) is a cholesterol lowering agent that is derived synthetically from a fermentation product of *Aspergillus terreus*[3] and is widely used to treat hypercholesterolemia. The drug is an inactive lactone and is converted to corresponding β, δ-dihydroxy acid in liver by cytochrome P450 (CYP) 3A after oral administration.[4,5] The drug (SV) is practically insoluble in water and poorly absorbed from the gastro intestinal (GI) tract,[6,7] having a very less half life of 2 hours.[8] Several methods like use of solid dispersion,[9,10] use of complexing agents,[11] are tried to increase the solubility of the drug (SV) in water. Previously it was reported that SV forms inclusion complexes with Hydroxyl Propyl Beta Cyclodextrin (HPBCD),[11] thereby increasing the solubility of SV in water. Polymeric microparticles are recommended for drug release into oral, nasal, pulmonary areas as these systems provide extended release and also protect the drug from degradation and gastric metabolism.[12] In order to prepare microcapsules, several polymers can be used.[13]

The present study is mainly based on the preparation of the binary mixtures of SV with HPBCD in a 1:1 ratio and then encapsulating this binary system in the form of microcapsules using the orifice gelation technique by

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Access this article online

Quick Response Code:

Website: www.asiapharmaceutics.info

DOI: 10.4103/0973-8398.100152

using different polymeric combinations of Sodium alginate and a fruit mucilage named as "Dillenia" obtained from *Dillenia indica Linn.*, Family-Dilleniaceae.[14,15] The prepared microcapsules were evaluated *in vitro* and *in vivo*.

MATERIALS AND METHODS

Simvastatin (SV) was gifted by Glenmark Pharmaceuticals (Mumbai, India); Hydroxyl Propyl Beta Cyclodextrin (HPBCD) and Beta Cyclodextrin (BCD) was gifted by Roquette (Lestrem, France); Sodium Alginate (SA) was purchased from Loba Chemicals; Dillenia (D) was locally available in Ranchi, Jharkhand. All other chemicals used were of analytical reagent grade purity.

Extraction of dillenia

The seeds of the fruits of *Dillenia indica Linn.* (Family-Dilleniaceae) were boiled in sufficient quantities of distilled water and 90% ethanol was added to the precipitate the mucilage.[16] The solution was filtered and precipitate was air dried to remove the traces of alcohol and water. The dried mucilage "Dillenia (D)" was powdered using a glass mortar -pestle and kept in an air tight container.

Phase solubility studies of SV

The phase solubility study as per the method described by Connors *et al*.[17] of the SV was carried out by using HPBCD. An excess of SV was added to 50 ml volumetric flasks containing 25 ml phosphate buffer of pH 6.8, with successively increasing quantities of (0, 2, 4, 6, 8, 10, 12 mM) of HPBCD.[7] Flasks were sealed and brought to solubility equilibrium at room temperature after shaking for 72 hours. After equilibrium, the content of each flask was filtered through a millipore membrane (0.45 μm) and appropriately diluted with methanol and determined spectrophotometrically the amount of dissolved SV, at 238 nm (using Shimadzu UV-1800). The phase solubility diagram was plotted as total dissolved drug against total HPBCD concentration as given in Table 1.

Preparation of microcapsules

The method used for the preparation of microcapsules adopted is orifice gelation technique.[18] Binary system of HPBCD/SV was prepared by co-grinding technique,[19] where mixing of "SV" in a ratio of 1:1 with HPBCD in a glass mortar for 30 minutes, and stored in a dessicator. Then this binary system was mixed with SA and Dillenia separately for formulating different batches of microcapsules as stated in Table 2. The physical mixtures (PM) (of the SV along with HPBCD, SA, and Dillenia) was analyzed separately for any possible drug-excipient interactions. The binary system of HPBCD/SV was triturated with SA and Dillenia in a glass mortar pestle and dispersed this mixture in distilled water using magnetic stirrer for 30 min at 200 r.p.m, and allowed to form uniform slurry named as "SVDHP". A 3% Calcium Chloride solution was prepared and filtered separately and

Table 1: Solubility of Simvastatin with respect to HPBCD

Concentration of HPBCD (mM)	Concentration of simvastatin (mM)
0	0.0554
2	0.152
4	0.2522
6	0.3515
8	0.45294
10	0.551078

HPBCD: Hydroxy Propyl Beta Cyclodextrin

Table 2: Composition of formulations

Formulation code	Drug (Carvedilol) (mg): HPBCD	Polymers (Ratio by parts)
SD1	1:1	Sodium alginate: Dillenia (2:1)
SD2	1:1	Sodium alginate: Dillenia (1:1)
SD3	1:1	Sodium alginate: Dillenia (1:2)
SHPSAI	1:1	Sodium alginate (1000 mg)

HPBCD: Hydroxy Propyl Beta Cyclodextrin

to this solution the slurry "SVDHP" was added dropwise through a 10 ml syringe having needle of size no.26 G. The microcapsules formed were allowed to remain in the calcium chloride solution for 30 min to complete the curing reaction. The formed microcapsules were filtered and washed with millipore water to remove any traces of calcium chloride from the microcapsule surfaces and dried the microcapsules in open air and kept in a desiccator. Microcapsules devoid of "Dillenia" were also prepared, which was named as SHPSAI to see the effect of Dillenia in retarding the drug release.

Characterization of microcapsules

Determination of yield of production

The production yields[20] of microspheres of various batches were calculated using the weight of finally dried microspheres with respect to the initial total quantity of the drug and polymer used for preparation. Percent production yields were calculated as per the formula below:

$$\text{Production yield} = \frac{\text{Practical mass (microspheres)}}{\text{Theoretical mass (polymer + drug)}} \times 100 \quad (1)$$

Determination percentage encapsulation efficiency

Percentage encapsulation efficiency is the percentage of drug encapsulated in the microcapsules related to the initial quantity of the drug used in the formulation. One hundred milligrams of microcapsules were taken and crushed in a glass mortar-pestle. In a 100 ml volumetric flask, the grounded microcapsule powder was mixed with methanol to make up the volume upto 100 ml and placed the whole system in a sonicator for 30 min to get the maximum extraction

of SV in the solvent. The sample so obtained were filtered to obtain clear solution and assayed for the drug content spectrophotometrically at 238 nm. Percent encapsulation efficiency[20] was determined using the formula below.

$$\text{Percentage encapsulation efficiency} = \frac{\text{Actual drug content (mg)}}{\text{Practical drug content (mg)}} \times 100 \quad (2)$$

Micromeritic properties of microcapsules

Particle size

The particle size of the dried microcapsules was measured using a stage micrometer scale by optical microscopy method. Around 100 nos. of dry microcapsules were placed on a clean glass slide and a few drops of liquid paraffin were added and covered with a glass slide, and observed under a compound microscope using stage and ocular micrometer.[21,22]

Determination of bulk density

The bulk density of the formulations was determined by using the following formula,[20]

$$\text{Bulk density} = \frac{\text{Sample weight}}{\text{Sample volume}} \quad (3)$$

Determination of tapped density

Tapped density is used to investigate packing properties of microcapsules into capsules. The tapped density was measured by employing the conventional tapping method using a 10 ml measuring cylinder and the number of tappings was 100 as sufficient to bring a plateau condition.

Tapped density was calculated using the following formula:[20]

$$\text{Tapped density} = \frac{\text{Weight of the microspheres}}{\text{Volume of microcapsules after 100 tapings}} \quad (4)$$

Determination of carr's consolidation index

It is indirect measurement of bulk density, size and shape, surface area, moisture content, and cohesiveness of materials since all of them can influence the consolidation index. It is also called as compressibility index. It is denoted by Ci and is calculated using the formula below.[20]

$$Ci = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Bulk density}} \times 100 \quad (5)$$

A Carr's index less than 15% is referred to as very good flow, 16-26% is good, 27-35% is fairly good, and > 35% are considered as poor.[23]

Determination of hausner's ratio

It is another parameter for measuring flowability of the microcapsules. It is calculated using the following formula,[20]

$$\text{Hausner's ratio} = \frac{\text{Volume before tapping}}{\text{Volume after tapping}} \quad (6)$$

Determination of angle of repose

Angle of repose of the microcapsules was determined by passing the microcapsules through the glass funnel on a horizontal surface.[20] The height (h) of the heap formed was measured and the radius (r) of the cone base was also observed and calculated. The angle of repose (θ) was calculated as follows:

$$\Theta = \tan^{-1} H/R \quad (7)$$

Percentage of swelling of microcapsules

Swelling rate of the microcapsules was measured as a function of water uptake. The formulations were placed in phosphate buffer of pH 6.8 at room temperature for a time period of 10 hours. At different time intervals, the microcapsules were taken out and gently pressed with a tissue paper to remove the excess liquid and then weighed. The percentage swelling of the microcapsules was determined by using the following formula as below,[22]

$$\%\,\text{Water uptake} = 100 \times \frac{\text{Weight of wet microcapsules} - \text{Weight of dry microcapsules}}{\text{Weight of dry microcapsules}} \quad (8)$$

Mucoadhesion properties of the microcapsule

Bioadhesive strength of the microcapsules was measured on a modified physical balance using the method described by Gupta *et al*.[24] Carbopol 934P was taken as standard to compare the mucoadhesivity of microcapsules. The microcapsules were sandwiched between two mucosal surfaces of rat intestine. The intestines were placed on two oppositely placed platforms of two slides, one hanged to the left pan of balance and other placed on a water bath at the base. Then weights were placed to the right pan of the balance in ascending order starting from lower weights. After each addition of weights, allowed to stand for 1 min, and the next weight was then added and this process was continued till the two mucosal surfaces detached from one another on the left side of pan and thus the detachment force required to separate two glass slides was measured. This process was repeated for the microcapsules too.

In vitro drug release of microcapsules

A total of 900 ml of phosphate buffer of pH 6.8[7] was taken as dissolution medium for *in vitro* drug release in USP Type-I dissolution apparatus. One hundred milligrams of the microcapsules were taken and filled in a hard gelatin capsule and placed in the basket and started the dissolution at 75 r.p.m and continued the study for a period of 12 hours. Five milliliter of sample was withdrawn after every 0.5,1,2,3,4,5,6,7,8,9,10 ,11,12,13 hours and analyzed spectrophotometrically at 238 nm and calculated the cumulative drug release and calculated the drug release kinetics. Data obtained from *in vitro* release studies were fitted to various kinetic equations to find out the mechanisms of the drug release. The kinetic models used were zero order equation, first order Equation, Higuchi equation, Hixson Crowell Equation, Peppas-Korsmeyer Equation.

Analytical studies of microcapsules

FTIR spectroscopy

Binary System (HPBCD/ CR)–Polymers interactions were studied by FTIR spectroscopy (FTIT Shimadzu 8400S). Also, the spectra for pure drug and drug-loaded microcapsules were recorded. Samples were prepared in KBr disks (2 mg sample in 200 mg of KBr). The scanning was 400-4000 cm^{-1} and the resolution was 2 cm^{-1}.[20]

X-ray powder diffractometry

This technique was carried out to investigate the effect of polymers and complexing agent HPBCD on the characteristics of the drug after formulation. Powdered samples of pure drug, polymers, HPBCD, and microcapsules were irradiated with monochromatized X-rays (Cu-ka) of 30 kV and 15 mA current in a Rigaku analytical XRD (Model Miniflex, Japan). The scanning rate employed was 0.20 min^{-1} of 2θ. The X-ray powder diffractometry (X-RD) patterns of the dug and drug loaded microcapsules were recorded.[19]

DSC studies of the microcapsule

A total of 5 mg weight of samples (pure drug, polymers, optimized microcapsule) were taken to carry out tests in DSC using aluminium sample pans at a scanning speed of 10°C per min form 10°C-200°C to detect any interaction between drug and polymers.[19]

Morphological studies of microcapsules

The optimized batch of microcapsules used for determination of surface morphology were coated by gold sputtering technique and observed using the SEM[19] (Model Jeol Japan; JSM-6390LV).

Determination of zeta potential of microcapsules

The optimized batch of formulation was dispersed in phosphate buffer of pH 6.8 and the surface charge (zeta potential) was measured by laser doppler anemometry using a Zetamaster (Malvern, UK).[12] Also, the zeta potential of the individual polymers and the drug were measured for a comparative study.

In vivo animal studies

Animals

Male Swiss albino mice of 8-weeks old, weight between 20-50 gm were selected for hyperlipidemic studies.[25] They were housed in polypropylene cages with four of them in one cage. They were maintained at a temperature range of 22-24°C with access to standard animal food and clean drinking water. This study was approved by the Institutional Animal Ethical Committee vide letter No. CPCSEA approval no: 621/02/ac/CPCSEA.

Experimental protocol

Hyperlipidemia was induced in mice by oral feeding of high fat cholesterol diet.[26] The high fat diet for inducing hyperlipidemia comprised the following as shown in Table 3. Eighteen mice were randomly divided into three groups of 6 animals each. Group 1 received pure Simvastatin (SV); Group 2 received optimized formulations (at 100 mg/kg body weight[27] via oral route); Group 3 was taken as control group (i.e., feeded with normal filtered water from Aquaguard). For all the three groups initially, before any treatment, the blood was withdrawn from eyes by retro orbital puncture of eyes[28,29] and collected in eppendorff tubes and allowed to clot for 20 minutes undisturbed and centrifuged for 20 min at 3000 rpm. The serum collected was kept in refrigerator before performing any tests. The serum was used for determination for estimation of total cholesterol (TC), high density lipoproteins (HDL), triglycerides (TGs), and low density lipoproteins (LDL) by the use of lab enzymatic test kits (Autospan Liquid Gold, Span Diagnostic Ltd., Surat, Gujrat). All the groups of mice except the normal control were on high fat diet throughout the period of treatment, i.e., for one month. On the 21st day, blood was gain collected from the mice by retro orbital puncture under ether anaesthesia. Blood was collected in eppendorff tubes and repeated the process for determination of TC, HDL, TGs, and LDL. Then for the last one week, the mice were treated with optimized formulations and again the blood was withdrawn and repeated the process to observe the activity of the optimized formulations.

RESULTS AND DISCUSSION

Mucoadhesive simvastatin microcapsules prepared by orifice Gelation technique using natural polymers were found to be free flowing and almost spherical in shape. The preparation method used was advantageous for entrapment of water-insoluble drugs. Moreover by formation of binary system of SV/HPBCD, solubility parameter of drug SV increased.

The phase solubility profile of SV-HPBCD was plotted as given in Figure 1, as total dissolved drug concentration against total HPBCD concentration as given in Table 1. According to Higuchi and Connors, the phase solubility diagram of SV-HPBCD could be classified as A_L type. The curve shows a linear increase in SV solubility as function of HPBCD with a slope of 0.049 ($R^2 = 1.00$) in the concentration range of 0-10 mM investigated. The apparent stability constant $K_{1:1}$ was found to be 1050.388 M^{-1}.

Table 3 : Diet composition for mice for inducing Hyperlipidemia[26]

Ingredients	Amount
Whole wheat	50 gms
Yellow corn	50 gms
Barley	25 gms
Anik spray	38 gms
Butter	50 gms
Calcium chloride	2.5 gms
Salt	2.5 gms
Corn oil	25 gms
Vitamin B_{12}	1 tablet

The production yield of microcapsules varied with different ratios of the polymers. The results are shown in Table 4 . This high yield may be due to the entire polymer available for gelation into crosslinking agent. These studies were done in triplicate.

As the drug is water insoluble, most of the drug SV got entrapped in the polymer matrix resulting in higher drug content and encapsulation efficiency. Also, the encapsulation efficiency of the drug dependent mainly on the concentration of both sodium alginate and Dillenia and it was found that with equal concentration of both polymers, the encapsulation efficiency also increased. The results are given in Table 4. These studies were done in triplicate.

The microcapsules so produced were uniform in size with a size range of 371.5-457.0 μm as determined by optical microscopy. Moreover from the Table 4, it observed that SD2 is most superior among all the other formulation as the Hausner's ratio, % compressibility index and angle of repose is lowest for SD2 than in comparison to other formulations. Moreover, tapped density and the bulk density of the formulations helps in calculating the Hausner's ratio and % compressibility index. The results are given in Table 5a. These studies were done in triplicate.

The swelling studies, as shown in Figure 2, indicates that with the increase in polymer concentration, the water absorption capacity of the formulations also increases with time, as seen from Table 5b. There is a clear indication of the swollen nature of the microcapsules as seen in Figure 3. It is seen that formulation SD2 shows highest swelling due to optimized combination of polymers.

As observed from Table 4, formulation SD2 shows the highest mucoadhesive property. This may be due to the increase in both polymer concentrations. The results of mucoadhesion are supported by results of assessment of duration of mucoadhesion as cited in Table 4. Figure 4 shows microcapsules adhesioned to the rat intestine of 3 cm^2 area, where length is 3 cm and breadth is 1 cm. Figure 5 shows the mucoadhesion assembly.

The *in vitro* release studies of formulations were carried out in phosphate buffer of pH 6.8. It was observed that greater stress was caused to the polymers at alkaline pH thereby causing release of medicament. The beads were swelled excessively followed by erosion in the buffered alkaline medium. The Dillenia shows the gelling property in alkaline medium which is considered beneficial for sustaining drug release from the microcapsules. The sustained release for a period of 12 hours was obtained from microcapsules due to the hindered diffusion of the drug from the gel matrix of Dillenia formed *in situ*[14] and thereby causing reduced drug release. The release of drugs was retarded with the increase in the polymer ratio. From Figure 5, it is clearly visible that formulation SD2 has the highest property of retarding the drug release upto 12 hours for 72.682%. The results were

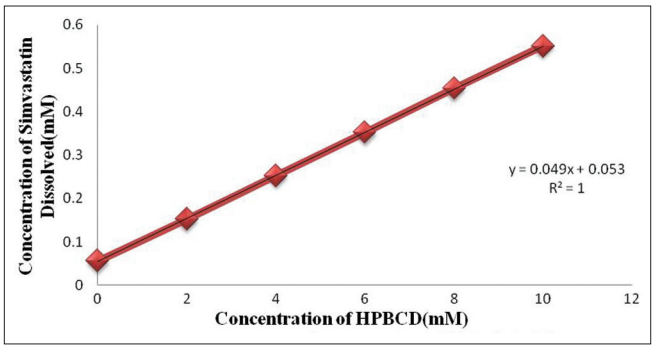

Figure 1: Phase solubility diagram of Simvastatin with HPBCD

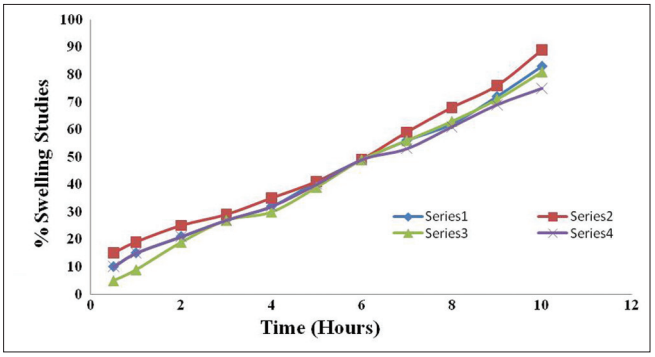

Figure 2: Swelling capacities of the microcapsules

Table 4 : Micromeritic properties along with encapsulation efficiency and assessment of mucoadhesivity of formulations

Formulation code	% encapsulation efficiency (%E.E ± S.D)	% yield	Particle size (μm)	Mucoadhesivity [detachment strength (N/cm²)]	Assessment of duration of mucoadhesionin (min)	% C.I	Hausner's ratio	Angle of repose
SD1	63.068 ± 0.002	99.049	457	0.021255	1.4 ± 0.100	11.88	1.133	11.8483
SD2	99.083 ± 0.017	87.285	371.5	0.02256	1.6 ± 0.100	11.2	1.125	11.237
SD3	72.0132 ± 0.00178	92.98	388.5	0.021909	1.4 ± 0.100	33.36	1.5	12.619322
Shpsai	89.544 ± 0.0017	90.947	310.0	0.002249	1.3 ± 0.100	9.1	1.1	13.815
Carbopol934P	----------	-------	----------	0.0256041	1.5 ± 0.100	---------	----------	----------

compared with formulation SHPSAI (devoid of Dillenia) and it is seen that drug release from SHPSAI is 98.7546% within 8 hours. Thus, Dillenia is highly effective in retarding the drug release.

KINETIC MODELLING OF DRUG RELEASE PROFILES

In order to understand the mechanism and kinetics of *in vitro* drug release, the data were analyzed with various kinetic equations like zero order (% cumulative drug release **Vs** time in hours), first order plot (log of cumulative % drug unreleased vs time), Higuchi model (% cumulative drug released Vs square root of time) and Korsmeyer-Peppas Plot (log % Cumulative drug released vs log of time). Coefficient of correlation values were calculated for the linear curves obtained by regression analysis of the above plots.

Thus as observed in Table 6, the formulation SD2, followed zero order kinetic model. The "*n*" values of the Korsmeyer–Peppas plot for the formulation was less than unity, i.e., 0.5, thus indicating that all the formulations showed diffusion controlled mechanism during drug release.

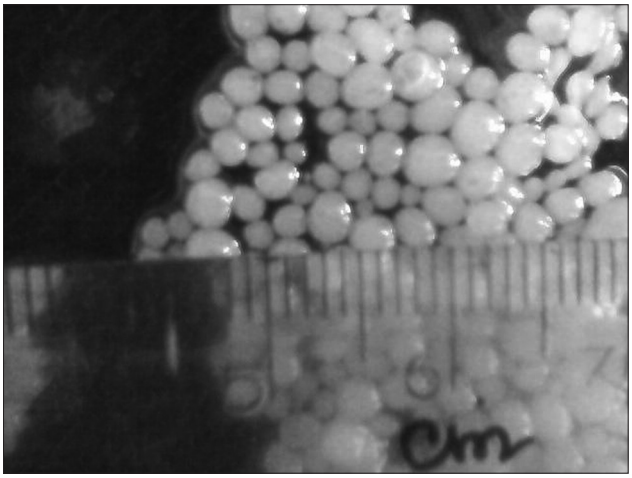

Figure 3: *In vitro* swelling of microcapsules

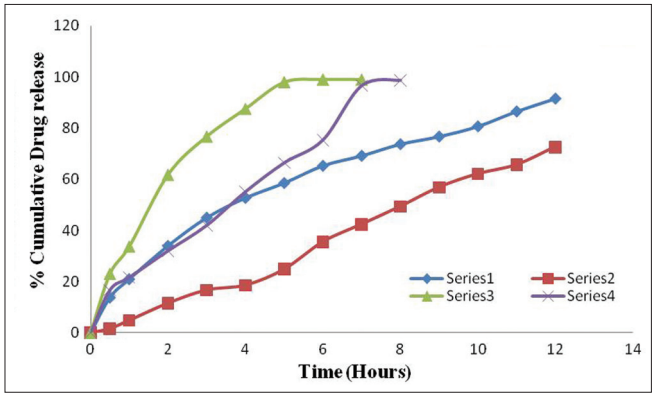

Figure 5: Comparative drug release profile of microcapsules at pH 6.8; SD1 (Series 1), SD2 (Series 2), SD3 (Series 3), SHPSAI (Series 4)

Surface morphology of the optimized microcapsule formulation SD2 is presented in Figure 6. The microca psules are almost spherical and are without any cracks or crevices on the surface although the surfaces are irregular.

As seen from the data presented in Table 7 and in the Figures 7a-d and 7e, the zeta potential of the optimized formulation SD2 was found to be more towards the positive side than in comparison to the pure drug. Moreover, the individual polymers sodium alginate exhibited negative potential, whereas Dilllenia showed less negative potential, which proves that Dillenia helps in zeta potential value towards the positive side. Thus, the formulation shows less negative potential thereby enhancing the mucoadhesion properties, which was already proved earlier.[30,31]

FTIR studies as indicated in Figure 8 showed weak interactions of Simvastatin with HPBCD at 1:1 ratio prepared in the form of microcapsules with different polymers.The spectra shows the characteristic peaks of the carbonyl group in the range of 1600-1800 cm^{-1}, which have disappeared or have lesser intensity than that compared to pure drug indicating that the drug in encapsulated in the cyclodextrin cavity forming an inclusion complex. Based on these results, the C = O group of the lactone ring of Simvastatin might be involved in inclusion complex.[7]

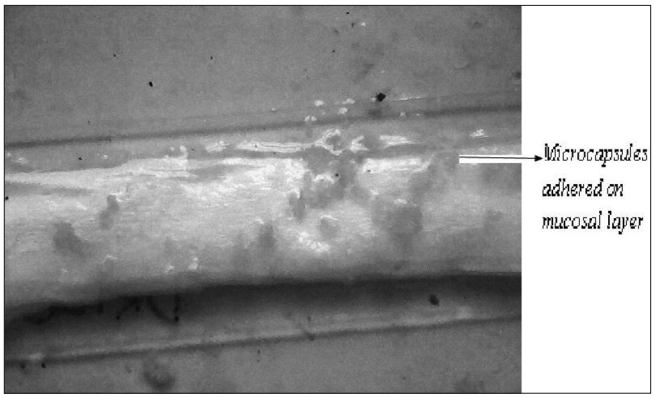

Figure 4: Microcapsules adhesioned onto the mucous surface of the tissue

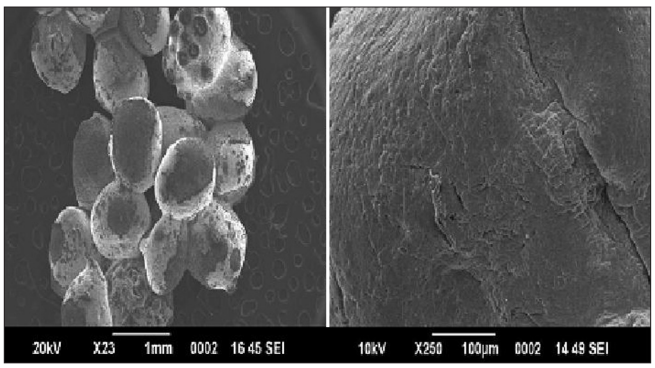

Figure 6: Scanning electron microscopy photograph of SD2

Table 5a: Bulk and tapped density of formulations

Formulation code	Weight taken in (mg)	Bulk volume (ml)	Tapped volume (ml)	Bulk density B_D (g/ml)	Tapped density T_D (g/ml)
SD1	1000	1.7	1.5	0.5882	0.66
SD2	1000	1.8	1.6	0.555	0.625
SD3	1000	0.6	0.4	0.833	1.25
SHPSAI	1000	1.2	1.1	0.666	0.727

Table 5b: Swelling studies of simvastatin microcapsules

Time (hours)	% Swelling			
	SD1 (series 1)	SD2 (series 2)	SD3 (series 3)	SHPSAI (series 4)
0.5	10	15	5	10
1	15	19	9	15
2	21	25	19	21
3	27	29	27	27
4	32	35	30	32
5	41	41	39	40
6	49	49	49	49
7	56	59	56	53
8	62	68	63	61
9	72	76	71	69
10	83	89	81	75

Table 6: Release kinetics of simvastatin from microcapsules

Formulation code	Zero order kinetics		First order kinetics		Higuchi kinetics		Hixson crowell kinetics		Korsmeyer-peppas kinetics	
	R^2	k_0	R^2	k_1	R^2	k_H	R^2	k_{HC}	R^2	n
SD1	0.88	6.627	0.976	-0.074	0.982	26.73	0.955	-0.188	0.986	0.505
SD2	0.991	6.272	0.976	-0.074	0.908	22.85	0.955	-0.188	0.986	0.505
SD3	0.872	13.87	0.954	-0.323	0.973	41.79	0.975	-0.570	0.971	0.588
SHPSAI	0.987	11.95	0.794	-0.203	0.946	35.89	0.905	-0.410	0.974	0.669

Table 7: Zeta potential of optimized formulation, polymers, pure drug

Formulation/polymer/pure drug	Zeta potential (mV)
SD2	-25.2
Sodium alginate	-67.3
Dillenia	-17.2
Simvastatin	-39.2
HPBCD	-19.4

The XRD patterns of pure Simvastatin (SV), physical mixture (PM), pure HPBCD and formulation SD2 are illustrated in Figure 9. As seen from Figure 9, the diffraction pattern of the physical mixture has less peak intensities than that of the pure drug and thus indicates that there is possibilities of some interactions between the pure drug and HPBCD.There was no interaction between the drug and other polymers used, which was proved earlier.[15] The diffractogram of SD2 presented a diffractogram quiet similar to that of physical mixture but with much lower intensities and also there was disappearnce of the peaks of the pure drug in SD2.[7]

Table 8: Peak intensities of Simvastatin in XRD patterns of SV-HPBCD systems

2θ	Simvastatin (SV)	HPBCD	SV:HPBCD
			SD2
10.78	106	-----	-----
14.8	112	------	14
15.46	125	------	14
16.390	113	15	19
17.560	220	10	----
18.640	203	-----	-----
23.620	20	------	16
26.26	60	-----	18

The results given in Table 8, depicts the change in nature of the drug when combined with HPBCD and in the formulation SD2.

Differential scanning calorimetry (DSC) can be used for the recognition of inclusion complexes.[34] When the guest molecules are embedded in the cyclodextrin cavity, their melting point, boiling point or sublimation points generally shifted to a different temperature.[35] The thermograms of SV,

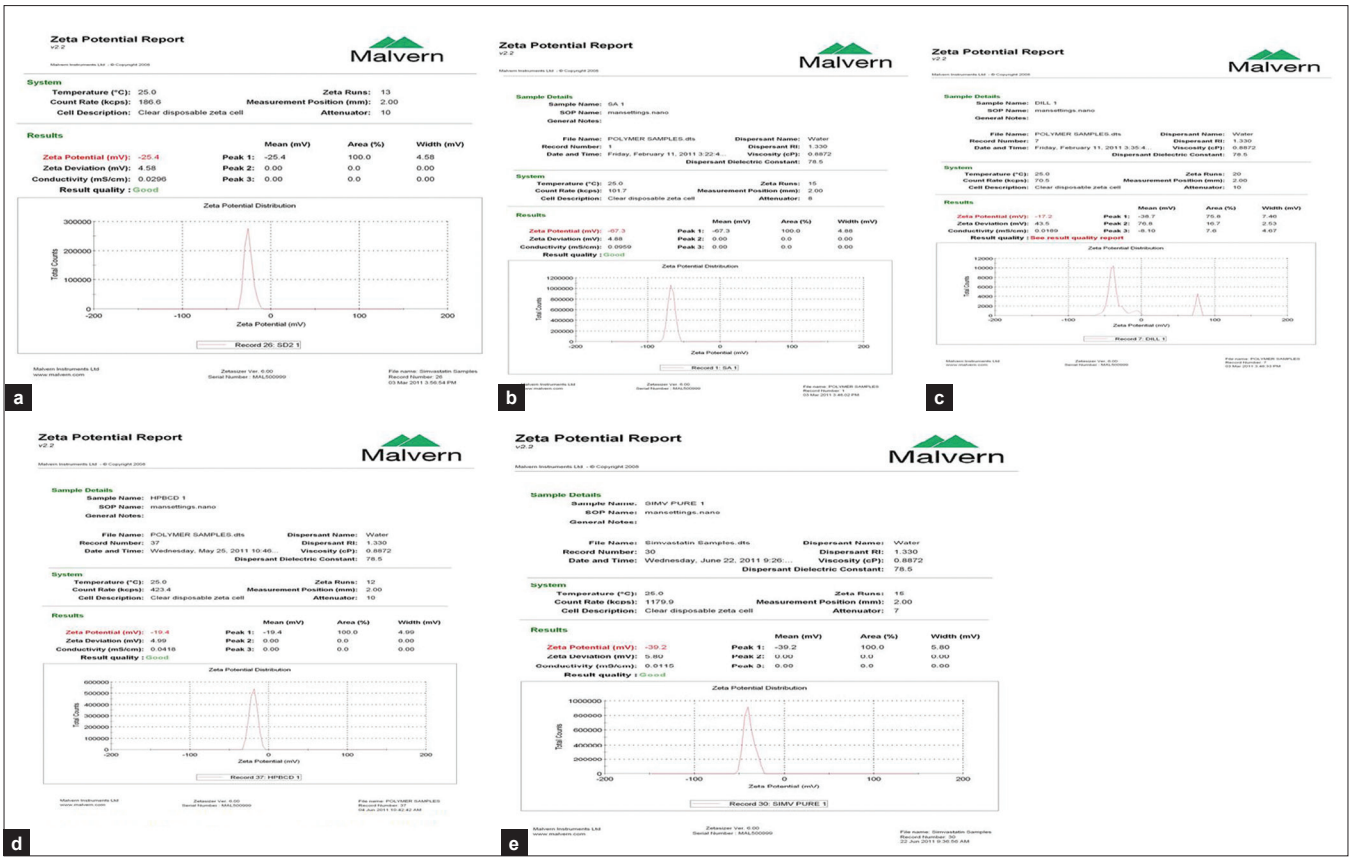

Figure 7: Zeta potential of SD2, Sodium alginate, Dillenia, Pure Simvastatin, HPBCD (a) Zeta potential of SD2 (b) Zeta potential of Sodium Alginate (SA) (c) Zeta potential of Dillenia (d) Zeta potential of Hydroxy propyl beta cyclodextrin (HPBCD) (e) Zeta potential of Pure Simvastatin (SV)

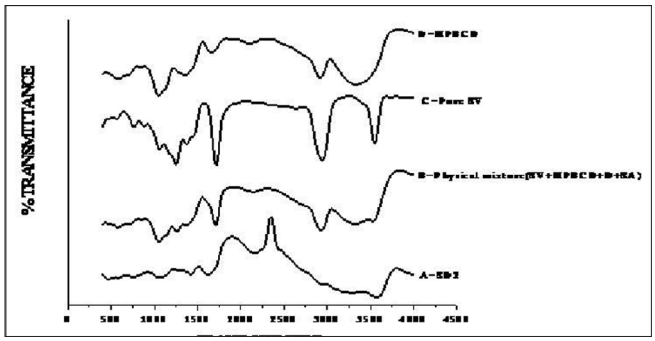

Figure 8: FTIR spectra of SD2 (a), Physical Mixture (b), Pure drug Simvastatin (c), Pure HPBCD (c)

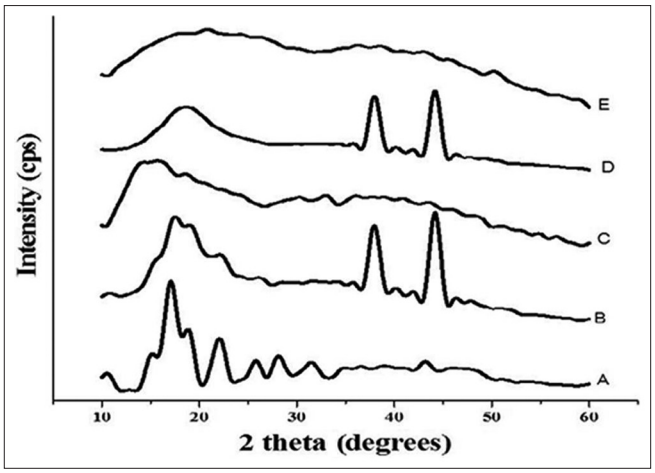

Figure 9: X-ray diffractograms of C=SD2, A=Pure Simvastatin, D=HPBCD, E=Dillenia, B=Physical Mixture(SV+HPBCD+D+SA)

PM, pure HPBCD and SD2 are shown in Figure 10. It shows that drug SV when present in the physical mixture and in case of formulations, shows a broad endotherm of 90.97°C and 89.42°C for SD2. The drug shows a sharp endothermic peak at 140°C but this endothermic peak is completely disappeared in the formulations, indicating the formation of an amorphous inclusion complex, the molecular encapsulation of the drug in the HPBCD cavity.[7,36,37]

The *in vivo* studies were carried out with the optimized formulation SD2 in swiss albino mice to study the effect of microcapsules for a longer and effective antihyperlipidemic effect. For this purpose, the decrease in blood cholesterol was measured using the cholesterol kit. Formulation SD2 was compared with that of pure drug only and control group (without any treatments). The study was carried out for the determinations of TC, TG, LDL, HDL. As seen from Tables 9-12, it is evident that the formulations SD2 is effective the cholesterol levels than in comparison to pure drug, this may be probably due to the complexation of the

Table 9: *In vivo* animal studies for total blood cholesterol

Animal Number	Pure drug group			Control (mg/dl)
	B.T (mg/dl)	A.I.H.LP (mg/dl)	A.T (mg/dl)	
1	90.091	213.986	211.188	89.443
2	90.093	213.988	211.19	89.445
3	90.089	213.988	211.19	89.445
4	90.089	213.984	211.188	89.443
5	90.093	213.984	211.186	89.441
6	90.091	213.986	211.186	89.441
Animal number	**SD2**			**Control (mg/dl)**
	B.T (mg/dl)	**A.I.H.LP (mg/dl)**	**A.T (mg/dl)**	
1	114.685	212.31	160.84	89.443
2	114.687	212.33	160.86	89.445
3	114.687	212.33	160.86	89.445
4	114.685	212.29	160.84	89.443
5	114.683	212.29	160.82	89.441
6	114.683	212.31	160.82	89.441

*B.T: Before treatment, A.I.H.LP: After induction of Hyperlipidemia, A.T: After treatment with formulations

Table 10: *In vivo* animal studies for triglycerides

Animal number	Pure drug group			Control (mg/dl)
	B.T (mg/dl)	A.I.H.LP (mg/dl)	A.T (mg/dl)	
1	96.64	176.056	157.746	96.64
2	96.65	176.058	157.748	96.66
3	96.63	176.058	157.748	96.66
4	96.64	176.054	157.746	96.62
5	96.63	176.054	157.744	96.62
6	96.65	176.056	157.744	96.64
Animal number	**SD2**			**Control (mg/dl)**
	B.T (mg/dl)	**A.I.H.LP (mg/dl)**	**A.T (mg/dl)**	
1	109.859	185.915	138.028	96.64
2	109.861	185.917	138.03	96.66
3	109.861	185.917	138.03	96.66
4	109.857	185.913	138.026	96.62
5	109.857	185.913	138.026	96.62
6	109.859	185.915	138.028	96.64
5	115.47	180.2814	133.8026	96.62
6	115.47	180.2816	133.8028	96.64

*B.T: Before treatment, A.I.H.LP: After induction of Hyperlipidemia, A.T: After treatment with formulations

Table 11: *In vivo* animal studies for LDL

Animal number	Pure drug group			Control (mg/dl)
	B.T (mg/dl)	A.I.H.LP (mg/dl)	A.T (mg/dl)	
1	9.264	152.528	141.81	6.332
2	9.266	152.53	141.83	6.334
3	9.266	152.53	141.83	6.334
4	9.262	152.528	141.79	6.332
5	9.262	152.526	141.79	6.33
6	9.264	152.526	141.81	6.33
Animal number	**SD2**			**Control (mg/dl)**
	B.T (mg/dl)	**A.I.H.LP (mg/dl)**	**A.T (mg/dl)**	
1	28.93	143.602	92.912	6.332
2	28.95	143.604	92.914	6.334
3	28.95	143.604	92.914	6.334
4	28.93	143.6	92.912	6.332
5	28.91	143.6	92.91	6.33
6	28.91	143.602	92.91	6.33

*B.T: Before treatment, A.I.H.LP: After induction of Hyperlipidemia, A.T: After treatment with formulations, LDL: low density lipoproteins

Table 12: *In vivo* animal studies for HDL

Animal number	Pure drug group			Control (mg/dl)
	B.T (mg/dl)	A.I.H.LP (mg/dl)	A.T (mg/dl)	
1	62.3167	26.246	37.83	63.785
2	62.3169	26.248	37.85	63.783
3	62.3169	26.248	37.85	63.781
4	62.3165	26.244	37.81	63.781
5	62.3165	26.244	37.81	63.785
6	62.3167	26.246	37.83	63.783
Animal number	**SD2**			**Control (mg/dl)**
	B.T (mg/dl)	**A.I.H.LP (mg/dl)**	**A.T (mg/dl)**	
1	61.583	31.5249	40.3226	63.785
2	61.5838	31.5251	40.3228	63.783
3	61.5834	31.5251	40.3228	63.781
4	61.5834	31.5249	40.3226	63.781
5	61.5838	31.5247	40.3224	63.785
6	61.5836	31.5247	40.3224	63.783

*B.T: Before treatment, A.I.H.LP: After induction of Hyperlipidemia, A.T: After treatment with formulations, HDL: High density lipoproteins

drug with HPBCD, which increases the drug solubility and thereby also increases the bioavailability of the drug.[7] The drug in its pure form shows a poor oral bioavailability of only 15%.[7]

CONCLUSIONS

Microcapsules of Simvastatin were prepared by complexation with HPBCD and thereby including this complex in the polymeric matrix by use of orifice gelation technique resulted in more improved drug delivery. Higher loading efficiency was observed for all the formulations and also the drug release was observed for a period of 12 hours. Thus, the polymer Dillenia showed promising results in retarding the drug release. Moreover, the mucoadhesive studies showed that Dillenia possess sufficient mucoadhesive properties. The polymer did not show any incompatibility reactions with neither drug nor with any other ingredients of the formulation. Morphological analysis by scanning electron microscopy of the formulation showed that the formulations were almost spherical in shape and size. All analytical studies showed that the drug had weakly

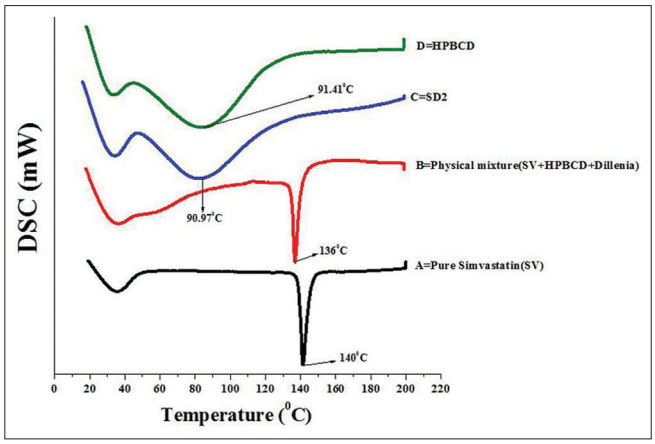

Figure 10: DSC thermograms of pure simvastatin, physical mixture (SV+HPBCD+Dillenia), SD2, and pure HPBCD

interacted with HPBCD thereby leading to change in its solubility characteristics thereby improving its bioavalability and its dissolution profile. The *in vivo* studies showed that the formulation SD2 was effective in controlling the hyperlipidemia for a period of 24 hours by effective controlled release.

ACKNOWLEDGEMENTS

The author acknowledges the Department of Pharmaceutical Sciences and Department of CIF, BIT, Mesra, Ranchi for providing the instrumental facilities for successful completion of this work.

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How to cite this article: Bal T, Murthy PN, Sengupta S. Preparation and evaluation of mucoadhesive simvastatin microcapsules using orifice gelation technique. Asian J Pharm 2012;6:74-84.

Source of Support: Birla Institute of Technology,Mesra,Ranchi.
Conflict of Interest: None declared.