Acta Poloniae Pharmaceutica – Drug Research, Vol. 69 No. 4 pp. 751–761, 2012 ISSN 0001-6837
Polish Pharmaceutical Society

PREPARATION AND STATISTICAL OPTIMIZATION OF ALGINATE BASED STOMACH SPECIFIC FLOATING MICROCAPSULES OF SIMVASTATIN

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Abstract: The present study involves preparation and characterization of floating microcapsules with simvastatin as model drug for prolongation of gastric residence time. The main objective is to improve solubility of simvastatin β-CD complex (1:2) by co-precipitation method and then to deliver the same in sustained release dosage form. Sustained-release simvastatin microcapsules were prepared by the ionic gelation technique, using carbopol 941 as swellable floating polymer. A 3^3 full factorial design was used to study the effect of polymer concentration, drug complex and sodium alginate by plotting main effect plot and 3D surface plots. The formed microcapsules were subjected to various evaluation tests such as drug encapsulation efficiency, *in vitro* drug release and surface morphology by scanning electron microscopy. Powdered X-ray diffractometry and FTIR were used to investigate the complexation of simvastatin in the microcapsules. As the carbopol 941 is self swellable polymer, immediate floating was observed. The *in vitro* release studies and floating behavior were performed in HCl buffer of pH 1.2. The release profile and dissolution kinetic showed that drug release from the microcapsules follows zero order kinetics. It was concluded from the present investigation that porous carbopol 941 microcapsules are promising sustained release system as well as stomach specific carriers for delivery of simvastatin.

Keywords: floating microcapsules, simvastatin, *in vitro* release, sustained drug release, main effect plot, contour plots

Microcapsules are small particles that contain an active agent or core material surrounded by a coating or shell. At present, there is no universally accepted size range that particles must have in order to be classified as microcapsules. However, many workers classify capsules smaller than 1 μm as nanocapsules and capsules larger than 1000 μm as microcapsules. Commercial microcapsules typically have a diameter between 3 and 800 μm and contain 10–90 weight percent core (1).

Floating drug delivery systems (FDDS) or hydrodynamically balanced systems (HBS) are among the several approaches that have been developed in order to increase the gastric residence time (GRT) of dosage forms (2–4). It has been suggested to prepare a suitable dosage forms for the drugs that have narrow absorption window. These dosage forms prolong the gastric residence time enabling an extended absorption phase for the local treatment of drugs. Floating drug delivery systems provide better bioavailability for the drugs that are unstable in intestinal or colonic environment (5, 6).

Alginates are non-toxic, biodegradable, linear co-polymers composed of L-glucuronic and D-mannuronic acid residues. They are widely used in food and pharmaceutical industries. Calcium alginate is rapidly formed by gelation of alginic acid in the presence of calcium ions. Alginate beads had been developed as floating dosage forms to prolong the GRT (7). Ionic gelation method consists in dropping or spraying a sodium alginate solution into a calcium chloride solution producing microcapsules. The divalent calcium ions cross-link the alginate, forming gelled droplets. These gel droplets can be permanently cross-linked by addition to a polylysine solution. Variations on this method with different polymers have been developed (8–11).

The purpose of present study is to improve the bioavailability of simvastatin, a 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-COA) reductase inhibitor with a very low aqueous solubility, which is enhanced by simvastatin β-cyclodextrin (β-CD) complex, in addition, release retarding polymer was also incorporated into the microspheres to achieve a prolonged action.

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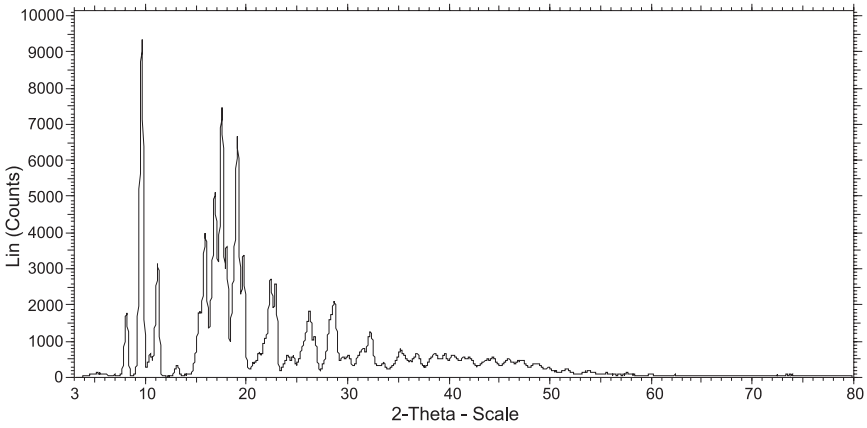

Figure 1. X-ray diffractometry of pure simvastatin

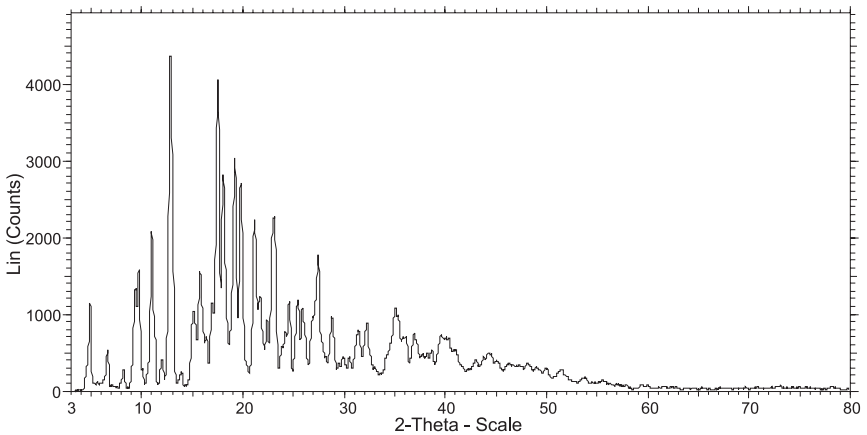

Figure 2. X-ray diffractometry of simvastatin β-CD complex (1:2)

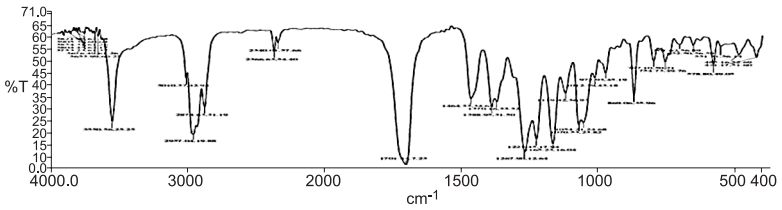

Figure 3. FTIR spectrum of pure simvastatin

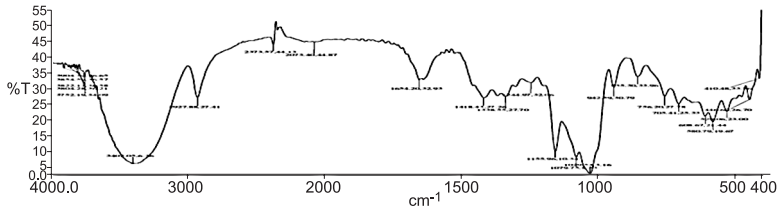

Figure 4. FTIR spectrum of β-CD

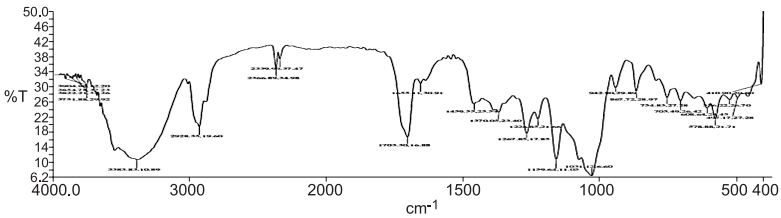

Fig 5: FTIR spectrum of simvastatin β-CD complex (1:2)

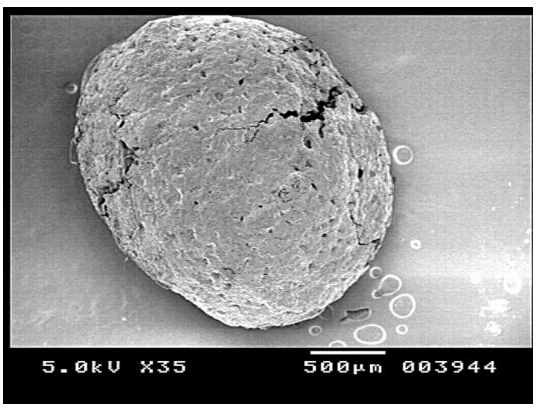

Figure 6. Scanning electron microscopy of optimized batch F2

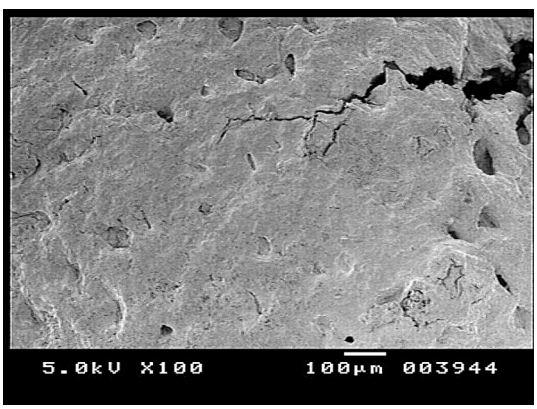

Figure 7. Scanning electron microscopy of optimized batch F2 (surface view)

EXPERIMENTAL

Materials

Simvastatin was a gift sample from Ranbaxy Labs., Gurgaon, India. Carbopol 941 (Lubrizol Lab. Pvt. Ltd. Mumbai, India), sodium alginate and calcium chloride (Cental Drug House, New Delhi India) were procured from commercial sources. All other reagents used were of analytical grade.

Methods

Preparation of inclusion complex by co-precipitation (12)

Simvastatin and β-CD in 1:2 molar ratio were accurately weighed. Saturated β-CD solutions were prepared with β-CD and water. Then, simvastatin solution in methanol was added slowly and a suspension was formed. The suspension was stirred at 40°C for 30 min and kept stirring at room temperature for 24 h. The obtained masses were filtered through 0.45 µm membrane filter and dried at 40°C in an oven for 24 h. The dried complexes were ground to fine powder and screened through 80-mesh sieve. The formed crystalline powder was kept in a desiccator till further use.

X-ray diffractometry (XRD)

The X-ray diffraction patterns were recorded at room temperature for characterizing the completeness of the complex formation process using a Scintag diffractometer (XGEN-4000, Scintag Corp.,

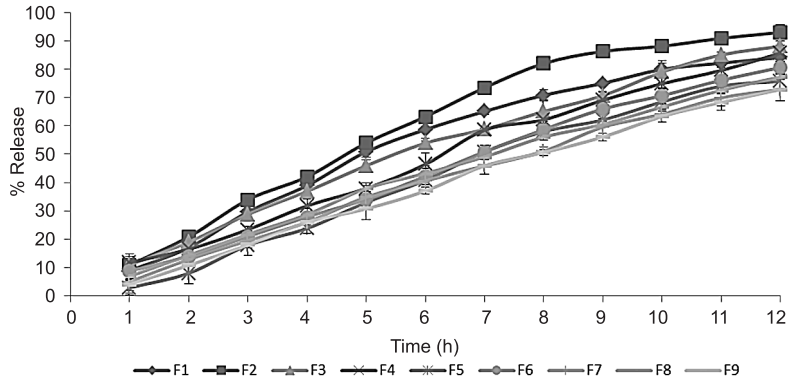

Figure 8. *In vitro* drug release for batches F1–F9

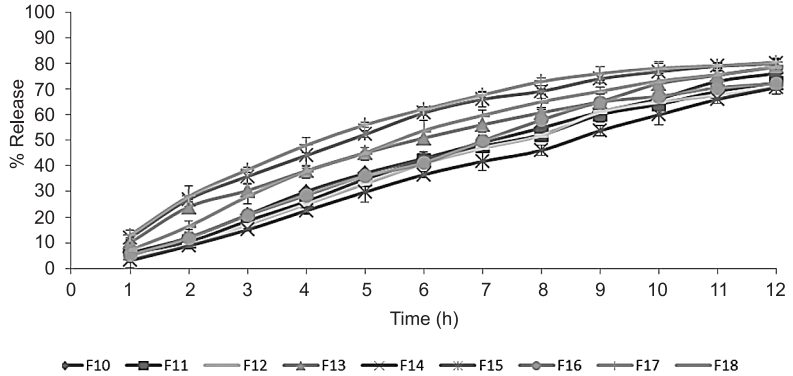

Figure 9. *In vitro* drug release for batches F10–F18

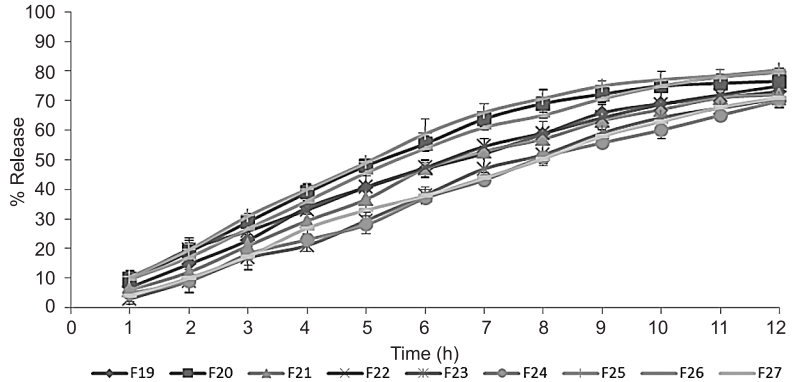

Figure 10. *In vitro* drug release for batches F19–F27

USA). The samples were irradiated with Ni-filtered Cu-Kα radiation at 45 kV voltage and 40 mA current. The scanning rate employed was 2°/min over a diffraction angle of 2 h.

Preparation of microcapsules

Microcapsules containing simvastatin were prepared employing sodium alginate in carbopol 941 as coating material. Ionic gelation process (13, 14) that

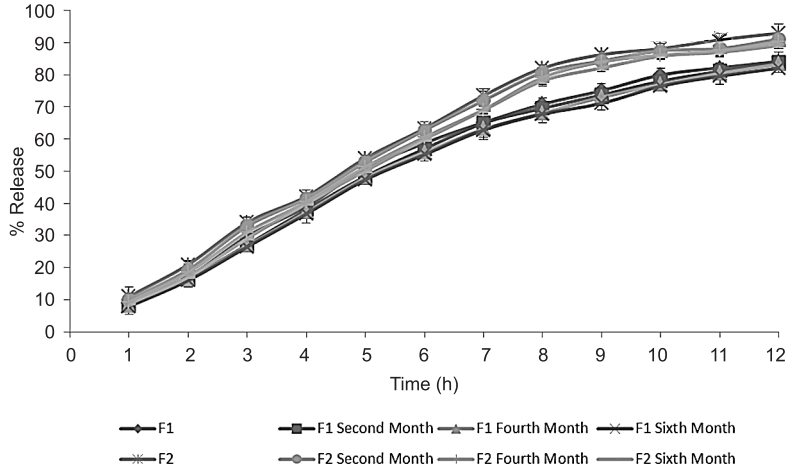

Figure 11. *In vitro* drug release for accelerated stability studies of batches F1 and F2

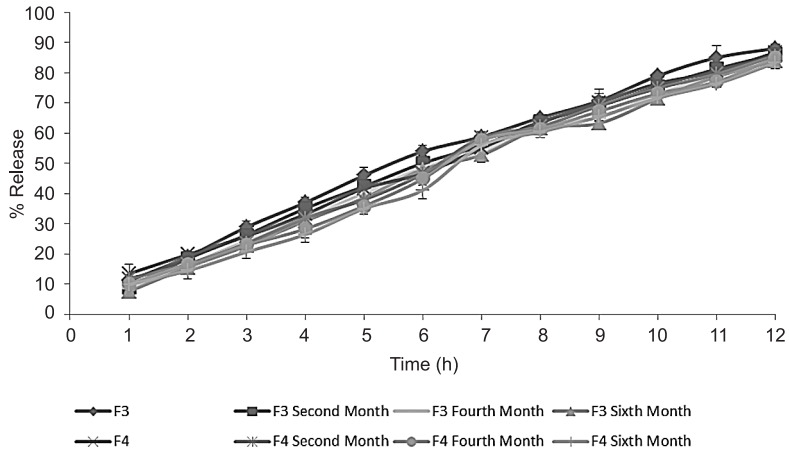

Figure 12. *In-vitro* drug release for accelerated stability studies of batches F3 and F4

has been extensively used to prepare large alginate beads was employed to prepare the microcapsules.

Ionic gelation method

Sodium alginate and polymer carbopol 941 were dissolved in 200 mL of purified water to form a homogeneous polymer solution under magnetic stirring. The active substance, simvastatin β-CD complex was added to the polymer solution and mixed thoroughly with a stirrer to form a viscous dispersion. Table 1 provides the design matrix which was generated using statistical software Minitab®. The resulting dispersion was then added manually dropwise into 100 mL of calcium chloride (10% w/v) solution through a syringe with a needle of 18 gauge maintaining gentle stirring rate of 300–400 rpm. The added droplets were retained in the calcium chloride solution for 1 h under gentle stirring speed of 300 rpm to complete the curing reaction and to produce spherical rigid microcapsules. The microcapsules were collected and the product thus separated was washed repeatedly with water and dried at 45°C for 12 h, then kept in a desiccator till further use.

Characterization of microcapsules

Estimation of simvastatin

Spectrophotometric method based on the measurement of absorbance at 238 nm in HCl acid buffer (pH 1.2). The method was validated for linearity, accuracy, and precision. The method obeyed Beer's law in the concentration range 5–30 µg/mL.

Table 1. Formulation batches of floating microcapsules along-with drug encapsulation efficiency.

Batch code	Carbopol (g)	Sodium alginate (g)	Simvastatin complex (g)	% Drug encapsulation efficiency
F1	6	2	2	59.09 ± 2.98
F2	6	3	6	65.65 ± 2.21
F3	6	3	2	49.65 ± 3.87
F4	4	4	6	53.33 ± 2.35
F5	4	3	2	47.56 ± 1.96
F6	6	4	2	54.67 ± 3.87
F7	2	3	6	46.62 ± 2.74
F8	4	2	6	51.12 ± 2.77
F9	2	2	2	47.50 ± 2.63
F10	2	3	4	50.04 ± 2.54
F11	2	4	6	48.56 ± 2.67
F12	4	4	2	45.67 ± 3.94
F13	6	2	6	57.80 ± 2.74
F14	6	4	6	45.22 ± 3.91
F15	4	2	4	56.54 ± 3.53
F16	4	3	6	46.76 ± 3.06
F17	2	4	2	58.65 ± 3.47
F18	2	2	4	50.55 ± 2.53
F19	6	4	4	46.65 ± 2.97
F20	4	3	4	50.61 ± 3.76
F21	2	4	4	49.88 ± 3.87
F22	4	4	4	51.32 ± 2.76
F23	6	3	4	47.65 ± 3.17
F24	4	2	2	47.54 ± 3.76
F25	2	3	2	57.87 ± 3.24
F26	2	2	6	55.61 ± 3.07
F27	6	2	4	49.09 ± 3.62

± indicates n = 3

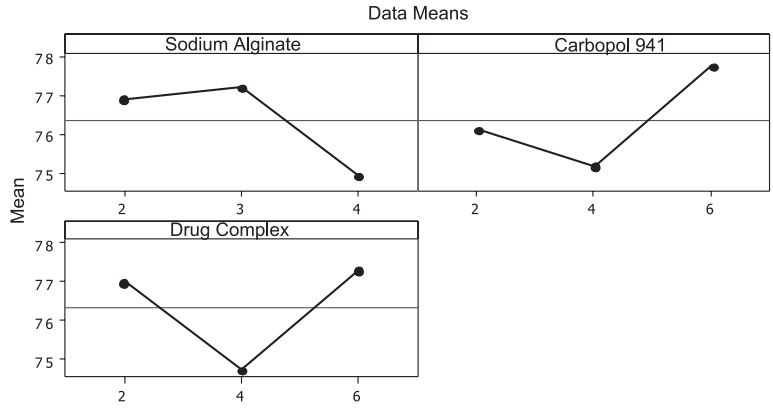

Figure 13. Main effect plot showing effect of sodium slginate, carbopol 941 and drug complex on % release of drug

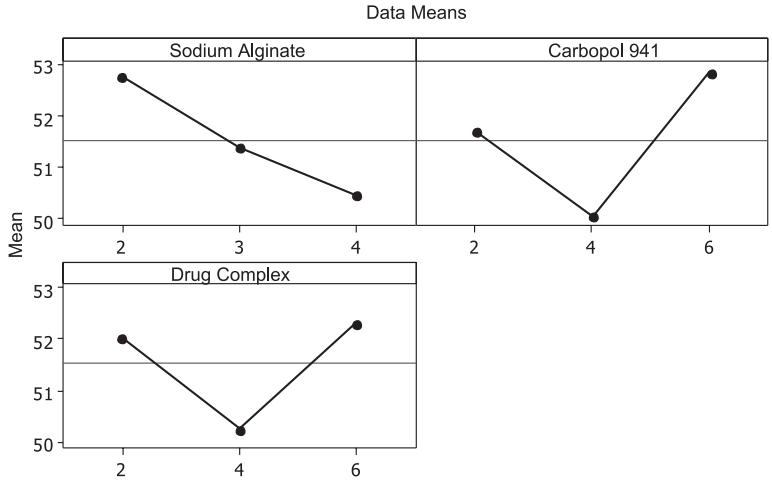

Figure 14. Main effect plot showing effect of sodium alginate, carbopol 941 and drug complex on % drug encapsulation efficiency

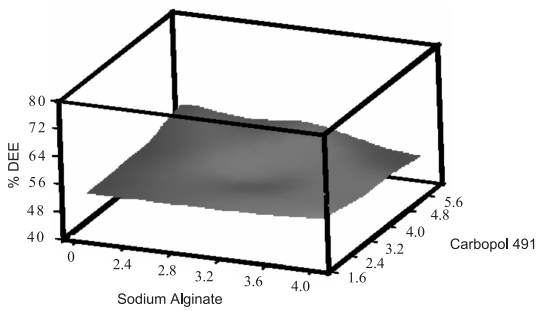

Figure 15. 3D surface plot showing effect of sodium alginate and carbopol 941 on % drug encapsulation efficiency

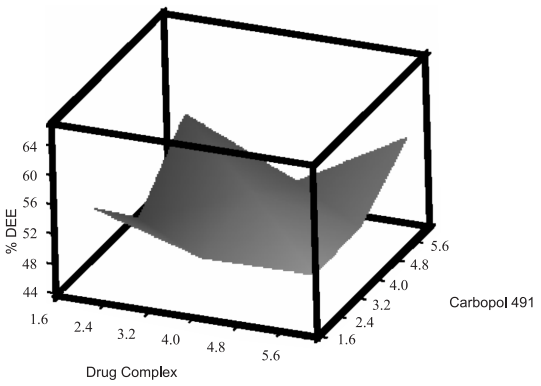

Figure 16. 3D surface plot showing effect of drug complex and carbopol 941 on % drug encapsulation efficiency

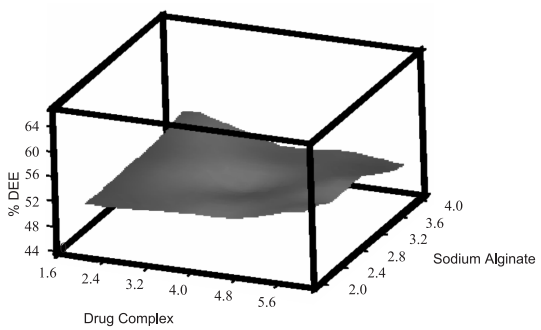

Figure 17. 3D surface plot showing effect of drug complex and sodium alginate on % drug encapsulation efficiency

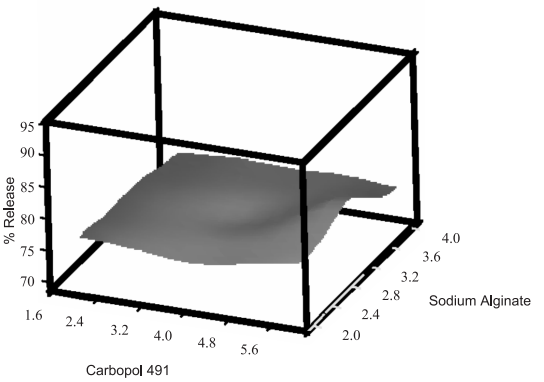

Figure 18. 3D surface plot showing effect of carbopol 941 and sodium alginate on % release of drug

Samples of 10 mg equivalent of microcapsules were taken, crushed and dissolved in 10 mL of methanol. The solution was shaken vigorously for 10 min, then filtered. Equivalent volume of filtrate was diluted to 10 mL with 1.2 pH HCl buffer. The absorbance was measured at 238 nm. The % drug encapsulation efficiency is shown in Table 1.

Microencapsulation efficiency was calculated using the following formula (15): Microencapsula-

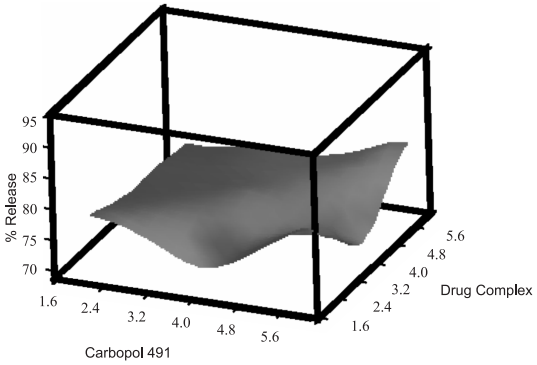

Figure 19. 3D surface plot showing effect of carbopol 941 and drug complex concentration on % release of drug

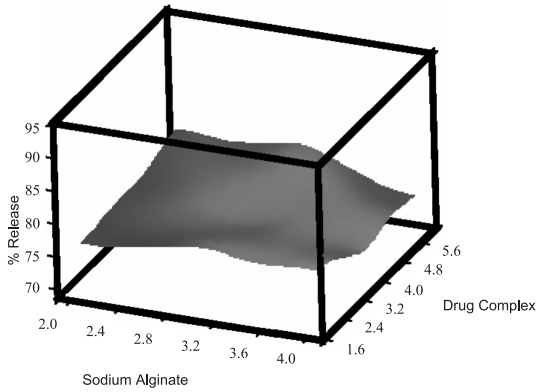

Figure 20. 3D surface plot showing effect of sodium alginate and drug complex concentration on % release of drug

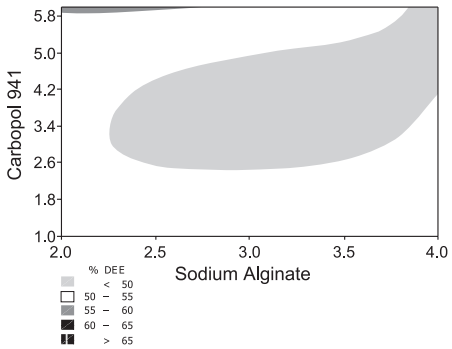

Figure 21. Contour plot showing effect of sodium alginate and carbopol 941 on % drug encapsulation efficiency

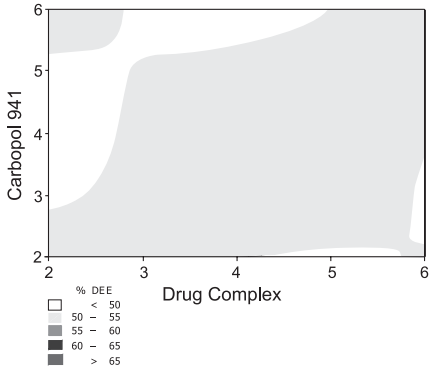

Figure 22. Contour plot showing effect of drug complex and carbopol 941 on % drug encapsulation efficiency

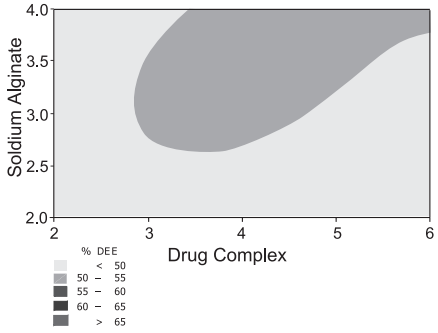

Figure 23. Contour plot showing effect of drug complex and sodium alginate on % drug encapsulation efficiency

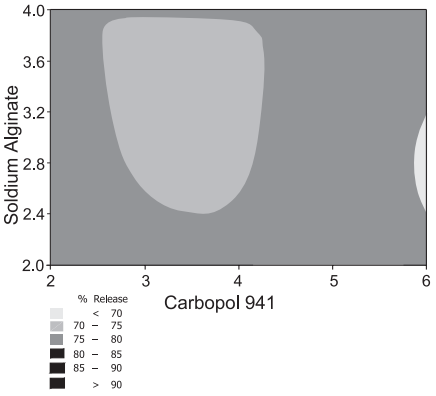

Figure 24. Contour plot showing effect of carbopol 941 and sodium alginate on % release of drug

tion efficiency = (estimated percentage drug content/theoretical percentage drug content) × 100.

Scanning electron microscopy (16)

In order to access surface morphology, the SEM was performed and it was noted that the surface is somewhat irregular with slightly hollowness present in between the crevices, which helps the microcapsules to immediately float on the solvent surface. Figures 6 and 7 give surface and overall view of SEM images. The SEM images reveal that the surface is porous and with minute cracks, which make the microcapsules to float immediately on the surface of water.

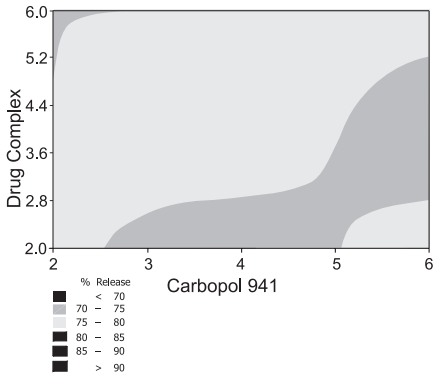

Figure 25. Contour plot showing effect of carbopol 941 and drug complex concentration on % release of drug

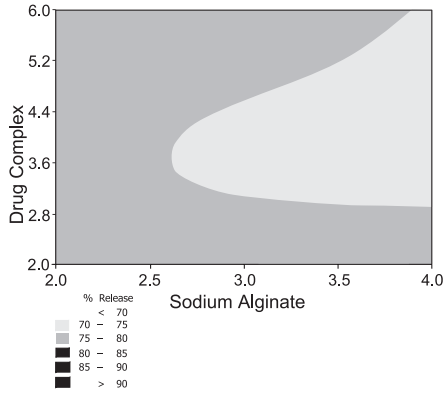

Figure 26. Contour plot showing effect of sodium alginate and drug complex on % release of drug.

Drug release study

The drug release study was performed using USP XXIV paddle apparatus (Electrolab, TDT-06T, Mumbai, India) at 37°C, 6 ± 0.5°C and at 50 rpm using 900 mL of HCl buffer (pH 1.2) as a dissolution medium. Microcapsules equivalent to 45 mg of simvastatin were used for the test. Five milliliters of sample solution was withdrawn at predetermined time intervals, filtered through a 0.45 mm membrane filter, diluted suitably and analyzed spectrophotometrically. An equal amount of fresh dissolution medium was replaced immediately after withdrawal of the test sample. Percentage drug dissolved at different time intervals was calculated using the Lambert-Beer's equation. The average values of % drug release for batches F1 to F27 are shown in Figures 8, 9 and 10.

Floating time (17)

Ten floating microcapsules were placed in 100 mL of HCl acid buffer (pH 1.2). Microcapsules exhibiting floating behavior of microspheres were observed up to 12 h in all batches. All batches showed immediate floating and microcapsules were found in floating condition for 12 h period.

Factorial design

A statistical model incorporating interactive and polynomial terms was used to evaluate the responses:

$$Y= \beta_o+\beta_1X_1+\beta_oX_2+\beta_oX_3+\beta_{12}X_1X_2+\beta_{13}X_1X_3+\beta_{23}X_2X_3+\beta_{123}X_1X_2X_3$$

where, Y is the dependent variable, β_0 is the arithmetic mean response of the 27 runs, and β_i is the estimated coefficient for the factor Xi. The main effects (X1, X2 and X3) represent the average result of changing one factor at a time from its low to high value. The interaction terms (X1X2X3) show how the response changes when 3 factors are simultaneously changed. The polynomial terms ($X_1X_2X_3$) are included to investigate nonlinearity.

In order to give better understanding of effect of various selected excipients, main effect plots are shown in Figures 13 and 14 and 3D surface plots are given in Figures 15–19 and contour plots are given in Figures 20–25.

The above quadratic equation for % release and % drug encapsulation efficiency (DEE) can be rewritten as:

$$\% \text{ Release} = 41.26 - 13.93X_1 + 15.12X_2 + 17.58X_3 + 7.53X_1X_2 + 10.76X_1X_3 - 47.64X_2X_3 + 41.26X_1X_2X_3$$

$$\% \text{ DEE} = 12.27 - 17.59X_1 + 11.01X_2 + 17.03X_3 + 11.85X_1X_2 + 47.31X_1X_3 - 36.02X_2X_3 + 58.57X_1X_2X_3$$

Stability studies

Any formulation coming to market must exhibit desired shelf life so that it can withstand the stress conditions. The main objective of stability studies is to assure that formulated product will remain within its therapeutic limits. ICH has proposed various conditions for performing stability studies (18). Out of all formulated batches F1, F2, F3 and F4 batches were subjected to accelerated stability studies for a period of six months at 40°C and 75% RH in ThermoLabs stability chamber. The *in vitro* release profile after every two months was performed. Percentage drug release in depicted in Figures 11 and 12.

RESULTS AND DISCUSSION

The formed drug complex (1:2) exhibit 9-fold increase in solubility of simvastatin. The XRD studies confirmed the formation of stable complex between drug and β-CD. The FTIR spectra further indicate the lack of interactions between the excipients used.

Table 2. Release kinetics of various formulated batches F1–F27.

Batch	Best fit model	r^2	k
F1	Higuchi	0.978	6.837
F2	Zero order	0.978	7.978
F3	Zero order	0.994	5.492
F4	Zero order	0.996	3.014
F5	Zero order	0.994	-2.155
F6	Zero order	0.998	0.880
F7	Zero order	0.998	0.863
F8	Zero order	0.997	2.206
F9	Zero order	0.999	-0.560
F10	Zero order	0.997	1.473
F11	Higuchi	0.966	5.656
F12	Zero order	0.994	-0.550
F13	Higuchi	0.990	3.034
F14	Zero order	0.999	-1.931
F15	Higuchi	0.990	2.252
F16	Zero order	0.991	1.376
F17	Higuchi	0.989	1.864
F18	Higuchi	0.981	4.092
F19	Zero order	0.987	4.285
F20	Higuchi	0.980	3.611
F21	Zero order	0.988	2.427
F22	Zero order	0.989	6.294
F23	Zero order	0.995	-1.919
F24	Zero order	0.998	-0.794
F25	Higuchi	0.981	3.536
F26	Higuchi	0.980	4.112
F27	Zero order	0.997	-0.294

Table 3. Release kinetics of batches kept for long term stability studies batches F1-F4.

Batches	2^{nd} month		4^{th} month		6^{th} month	
	r^2	k	r^2	k	r^2	k
F1	0.977	4.393	0.978	4.419	0.982	5.403
F2	0.977	4.180	0.979	6.333	0.980	5.547
F3	0.996	4.094	0.997	2.934	0.997	2.014
F4	0.996	3.014	0.996	1.992	0.996	0.769

As carbopol 941 is self swellable polymer, the immediate floating of microcapsules was observed. All the batches exhibited immediate floating when placed in distilled water. The factorial design has started playing vital role in the optimization of the formulation. The main objective of incorporating factorial design is to reduce the time period, identification of various critical variables and also to provide scientific rationale. The full factorial design was set up to access the effect of excipient composition on the % DEE and % release. Ionic orifice gelation method was preferred over other methods due to its simplicity and versatility for formulation of floating microcapsules.

The microcapsules were found to be discrete, spherical, free-flowing and uniform in size. The SEM

photographs indicated that the microcapsules are nearly spherical and completely covered with the coat polymer. The porous nature of the surface helps the microcapsules to float immediately on the surface of water. The microencapsulation efficiency was in the range of 45–65%. Microcapsules with a coat consisting of alginate and a floating polymer exhibited good floating properties. Simvastatin release from the microcapsules was studied in HCl buffer (pH 1.2) for 12 h. As the matrix type of system formed by using carbopol 941 the best release model was found to be zero order release. The little or negligible change of release profile was also observed after the stability study. From the main effect plots (Figs. 13, 14) it was found that there exists negative impact of sodium alginate on both parameters: drug encapsulation efficiency and percentage drug release, whereas positive effect is observed with both drug simvastatin complex and concentration of polymer. In order to support the results shown by main effect plots, 3D surface plots (Figs. 15–20) and contour plots were also plotted. A contour plot is a graphic representation of the relationships among three numeric variables in two dimensions. Two variables are for X and Y axes, and a third variable Z is for contour levels. The contour levels are plotted as curves; the area between curves can be shade coded to indicate interpolated values. As shown in Figures 21–26, the effect of various formulation additives on the % DEE and also on % drug release. Simvastatin release from the microcapsules was slow and depended on the composition of the coat. In order to judge the formulation capability, accelerated stability studies were performed according to ICH guidelines for a period of six month and retest period was set to two months. From the stability studies it was found that there exists little difference between the release profile, so it can be stated that microcapsules passed the stability studies. The release profile of stability batches and release kinetics reveal that there is no significant change seen during the stability studies.

CONCLUSION

Microcapsules containing drug complex with a surrounding coat consisting of alginate and a floating polymer carbopol 941 were prepared by an ionic gelation method. The microcapsules exhibited good floating properties in *in vitro* test. Simvastatin release from prepared microcapsules was slow and extended over longer periods of time and depended on composition of the coat. From main effect plot it was concluded that high concentration of sodium alginate hampers both drug encapsulation efficiency and % release, however, improvement in % drug release was seen with intermediate concentration, whereas high concentration of carbopol and drug complex provides good encapsulation efficiency and high release too. Thus, these microcapsules are suitable for oral sustained release of simvastatin.

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Received: 28. 09. 2011