PHARMACEUTICAL TECHNOLOGY

STATISTICAL OPTIMIZATION AND *IN-VITRO* EVALUATION OF HOLLOW MICROCAPSULES OF AN ANTI-HYPERTENSIVE AGENT

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Abstract: The present work attempts to formulate and evaluate hollow microcapsules of an antihypertensive drug - ramipril, which will remain in vicinity of absorption site. The emulsion diffusion solvent evaporation method was employed for preparation of microspheres using Eudragit E100. Glycerol monostearate and sodium lauryl sulfate were used as surfactants, which showed good effect of film integrity. The different proportion of Eudragit E100 and ramipril at varying speed were employed for formulating hollow microspheres using 3^2 full factorial design. The formulated microspheres were subjected to evaluation of various parameters such as particle size analysis using motic microscope, drug loading efficiency and *in vitro* drug release. The main effect plot showed negative impact of polymer concentration and drug complex concentration, whereas positive impact of rotation speed on the % release of drug and drug encapsulation efficiency. The optimized batch of microcapsules was formulated as a hard gelatine capsule dosage form containing loading (plain drug) as well as sustained fraction of drug in form of microcapsules. It was found that dosage form also showed good *in vitro* release profile.

Keywords: ramipril, hollow microcapsules, emulsion diffusion, solvent evaporation, Eudragit E100

Ramipril is angiotensin converting enzyme inhibitor and mainly used in treatment of hypertension (1, 2). Microspheres are discrete multiparticulate delivery systems and are prepared for prolonged drug delivery, to improve bioavailability or stability and to get a site specific drug delivery. Microspheres can also offer advantages like reducing side effects, decreasing dosing frequency and improving patient compliance as well as limiting fluctuation within therapeutic range (3-5). Eudragit polymers are series of acrylate and methacrylate polymers available in different ionic forms. Eudragit E100 is insoluble in aqueous media but is permeable and have pH-dependent release profile (6-8). The aim of this study was to prepare Eudragit microspheres containing ramipril to achieve a sustained drug release profile suitable for peroral administration. The work started by investigating some formulation variables (polymer type, polymer : drug ratio, stirring speed) to obtain spherical particles. Then, the yield of production, particle size distribution, encapsulation efficiency, surface properties and ramipril release rate from microspheres were investigated. The influences of

formulation variables on the microsphere properties were examined by employing full factorial design using Eudragit E100 (0.75, 1.5 g), ramipril complex (0.75, 1.5 g) and stirring speed (900, 1500 rpm)

MATERIALS AND METHODS

Chemicals and reagents

Ramipril was obtained as generous gift sample from Ajantha Pharmaceuticals, Mumbai, Eudragit E 100 was obtained as gift sample from Evonik Industries, Mumbai, glycerol monosterate, dichloromethane, polyvinyl alcohol, Tween 20, sodium lauryl sulfate and all other chemicals used were of analytical grade.

Preparation of floating microspheres of ramipril by emulsion diffusion solvent evaporation technique

Floating microspheres containing ramipril were prepared using emulsion solvent diffusion technique (9, 10). For the preparation of floating

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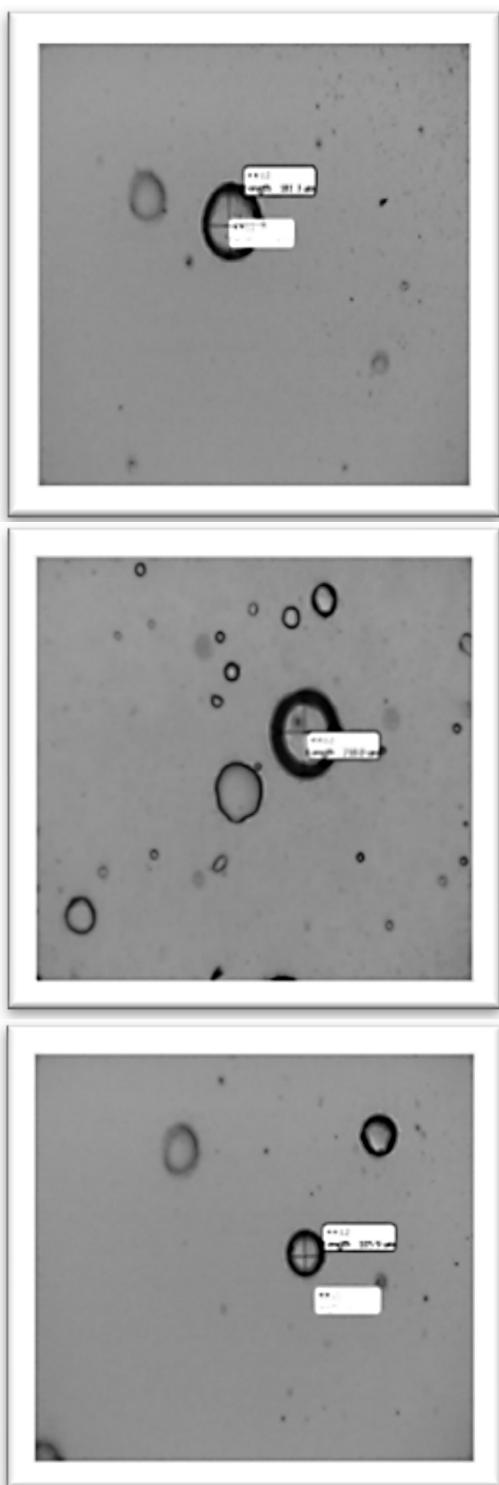
Figure 1. Motic pictures of ramipril hollow microspheres (oil immersion microscopy)

microspheres, employing design of experiment, the release controlling polymer used was Eudragit E100 in varying concentration.

Table 2 provides different proportion of drug complex and polymer concentration used at varying speed for preparation of microspheres. Glycerol monosterate and SLS were added as a stabilizer in the solution of drug and polymer. The following method was adopted for preparation of hollow microspheres.

The required quantity of drug and polymer was dissolved in a mixture of dichloromethane and ethanol (1 : 1, v/v, 80 mL). To this, glycerol mono-sterate (1.5 g) was added. The above mixture was dropped in a solution of polyvinyl alcohol (0.5%, 200 mL) by adding sodium lauryl sulfate (1%). The resultant solution was stirred with a mechanical stir-rer for 1 h at different rotation conditions mentioned in Table 1. The formed floating microspheres were filtered and washed with water, dried at room tem-perature and stored in a desiccator until further use.

Particle size analysis

Particle size analysis was done by oil immersion microscopy (11, 12). Hollowness and release pattern are the basic attraction of the hollow microsphere so these are the very important evaluation parameters and motic images are recorded and shown in Table 2.

Drug encapsulation efficiency

The various batches of the floating micros-pheres were subjected to estimation of drug content (8, 13, 14). The floating microspheres equivalent to 50 mg of ramipril from all batches were accurately weighed and crushed. The powdered microspheres were dissolved in methanol (10 mL) in volumetric flask (100 mL) and made the volume with 1.2 pH buffer. This solution was then filtered through Whatman filter paper No. 44. After filtration, from this solution accurate quantity (10 mL) was taken and diluted up to 100 mL with pH 1.2 buffer. From this solution, accurate volume (2 mL) was pipetted out and diluted up to 10 mL with pH 1.2 buffer and the absorbance was measured at 210 nm against 1.2 pH buffer as a blank. All determinations were done in triplicate. The percentage drug entrapment was calculated as follows:

$$\% \text{ Drug entrapment} = \frac{\text{Calculated drug concentration}}{\text{Theoretical drug concentration}} \times 100$$

Percentage yield

The percentage yield of different formulations was determined by weighing the floating micros-pheres after drying. The percentage yield was calcu-lated as follows.

$$\% \text{ Yield} = \frac{\text{Total weight of floating microspheres}}{\text{Total weight of drug and polymer}} \times 100$$

Table 1. Formulation matrix using Minitab.

Formulation code	Eudragit E100 (g)	Ramipril (g)	Rotation speed (rpm)	% Drug encapsulation efficiency	% Yield
F1	0.75	0.75	1500	76.12 ± 2.32	75.78 ± 2.67
F2	1.50	1.50	1500	52.86 ± 1.56	68.60 ± 3.89
F3	1.5	0.75	900	50.23 ± 3.67	65.90 ± 2.12
F4	0.75	1.50	1500	64.86 ± 3.14	54.65 ± 1.09
F5	1.50	1.50	900	48.16 ± 2.19	67.98 ± 2.92
F6	0.75	0.75	900	72.16 ± 4.56	72.67 ± 1.56
F7	1.50	0.75	1500	68.11 ± 1.56	63.77 ± 1.98
F8	0.75	1.50	900	50.19 ± 2.56	61.89 ± 1.54

± indicates n = 3

Table 2. Mean particle size of different batches of hollow microcapsules.

No.	Formulation code	Particle size (micrometers)
1	F1	206.7 ± 0.74
2	F2	282.6 ± 0.75
3	F3	318.4 ± 6.61
4	F4	338.3 ± 7.14
5	F5	319 ± 5.89
6	F6	213 ± 4.89
7	F7	298 ± 7.99
8	F8	308 ± 5.98

± indicates n = 3
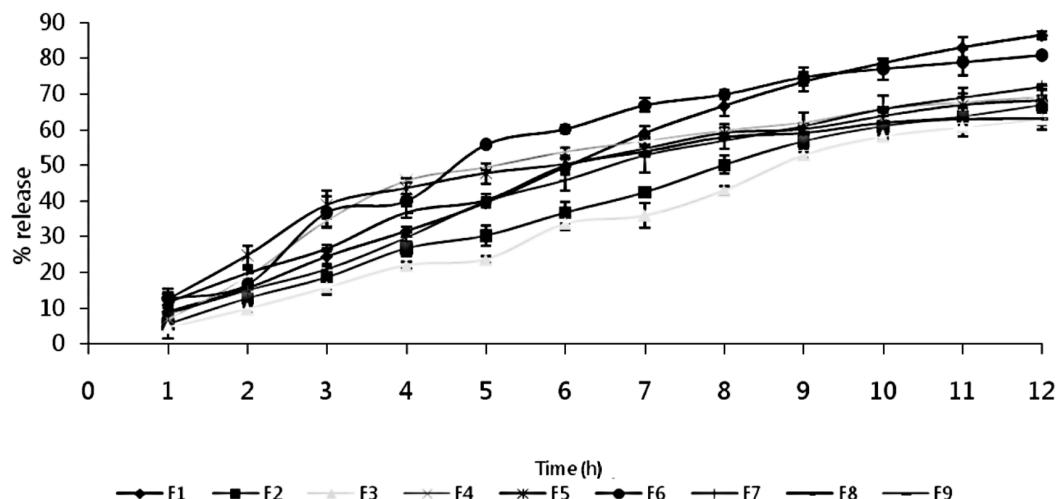
Figure 2. *In-vitro* drug release profile of batches F1 to F8, 1.2 pH buffer

Table 3. Kinetics of drug release from formulated batches F1-F8.

Batch No.	Best fit model	r^2	k
F1	Zero order	0.995	2.109
F2	Zero order	0.996	1.409
F3	Zero order	0.995	-0.990
F4	Higuchi	0.977	2.553
F5	Higuchi	0.989	0.958
F6	Higuchi	0.979	3.161
F7	Zero order	0.988	4.176
F8	Higuchi	0.986	2.324

Table 4. Formulation composition for hard gelatin capsules

No.	Ingradient	Quantity for 20 capsules of weight 150 mg
1.	Ramipril (plain)	100 mg
2.	Ramipril (microspheres)	150 mg
3.	Avicel PH 101	2.0 g
4.	Lactose	750 mg
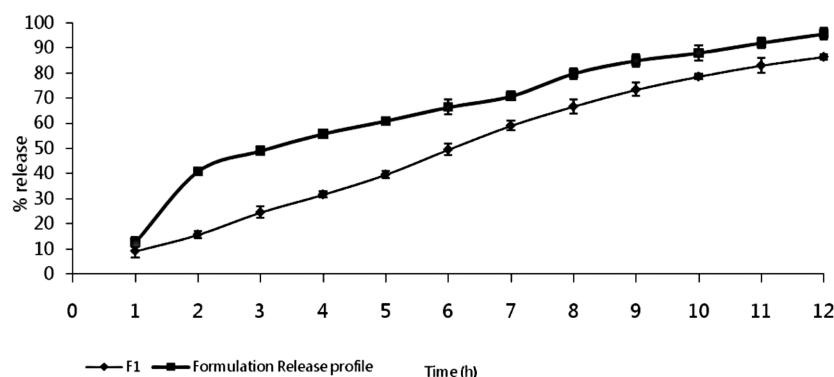
Figure 3. *In vitro* drug release profile of batch F1 and hard gelatin capsule

Shape and surface characterization of floating microspheres by scanning electron microscopy

The size and surface morphology of floating microspheres were examined by scanning electron microscopy as shown in Figures 4 and 5. These figures illustrate the microphotographs of batch F1. The floating microspheres were spherical with no visible major surface irregularity. Few wrinkles and inward dents at the surface and some crystal shape particles were appeared. It may be due to collapse of floating microspheres during the *in situ* drying process.

***In-vitro* release studies**

In-vitro release of ramipril from floating microspheres was carried out using the USP dissolution test apparatus (Type-II) (15). A weighed amount of floating microspheres equivalent to 50
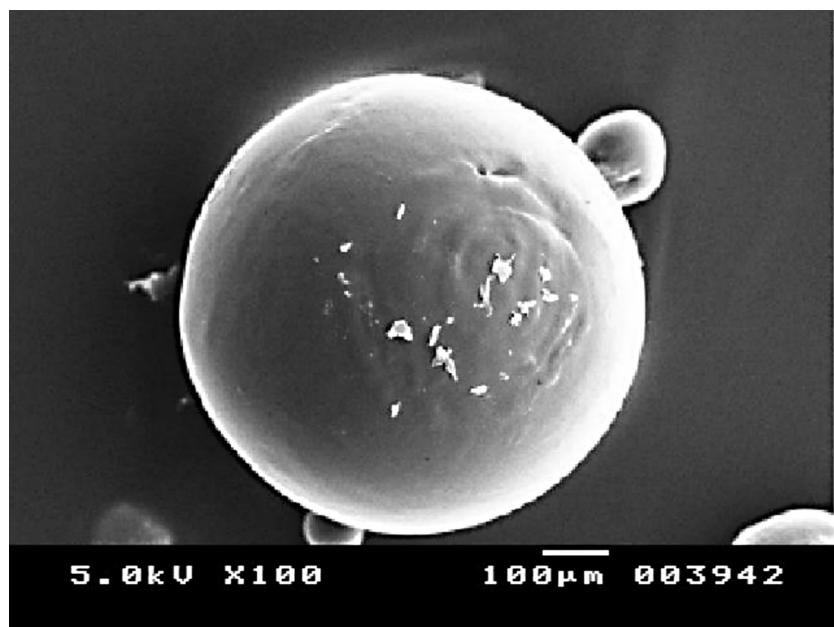
Figure 4. Scanning electron microphotograph of formulation F1
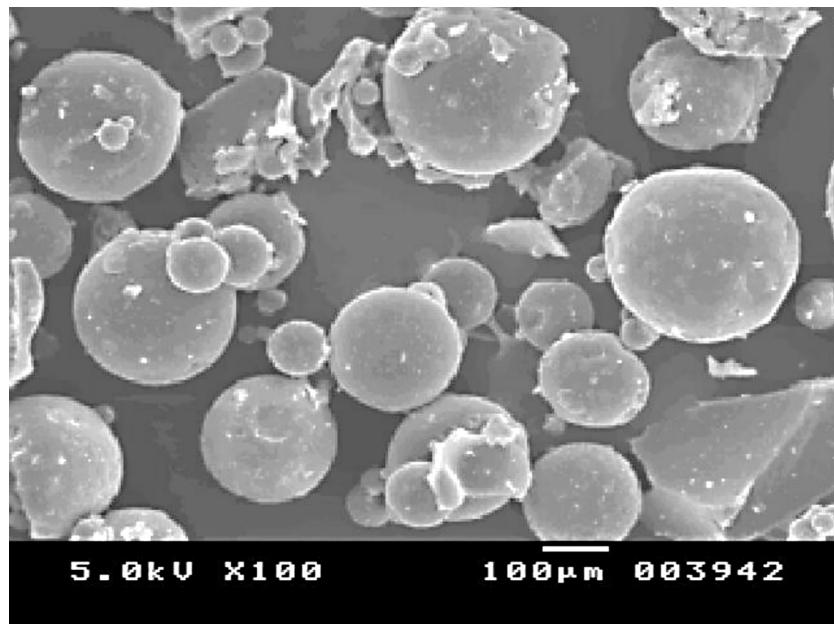
Figure 5. Scanning electron microphotograph of formulation F1

mg of drug were filled into a muslin cloth and tied to the paddle. Dissolution medium used was 900 mL of pH 1.2 buffer maintained at 37 ± 0.5°C and stirred at 50 rpm. At predetermined time intervals, 5 mL samples were withdrawn and replaced with equal amount of pH 1.2 buffer. The collected sam-

ples were filtered and suitably diluted with media and analyzed spectrophotometrically at 210 nm to determine the amount of drug released in the dissolution medium. The *in vitro* drug release is shown in Figure 2.
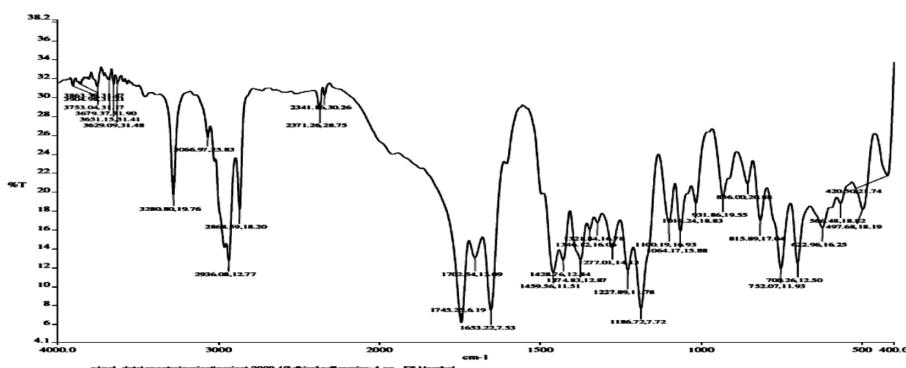
Figure 6. IR-spectrum of ramipril API
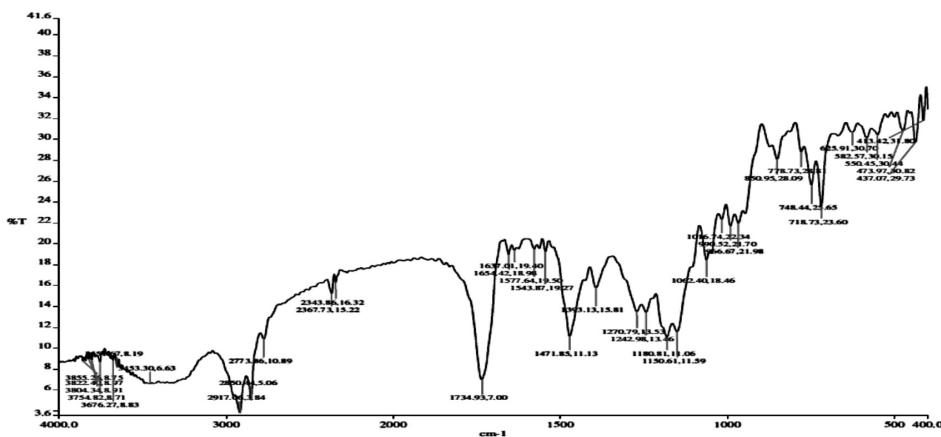
Figure 7. IR spectrum of batch F1
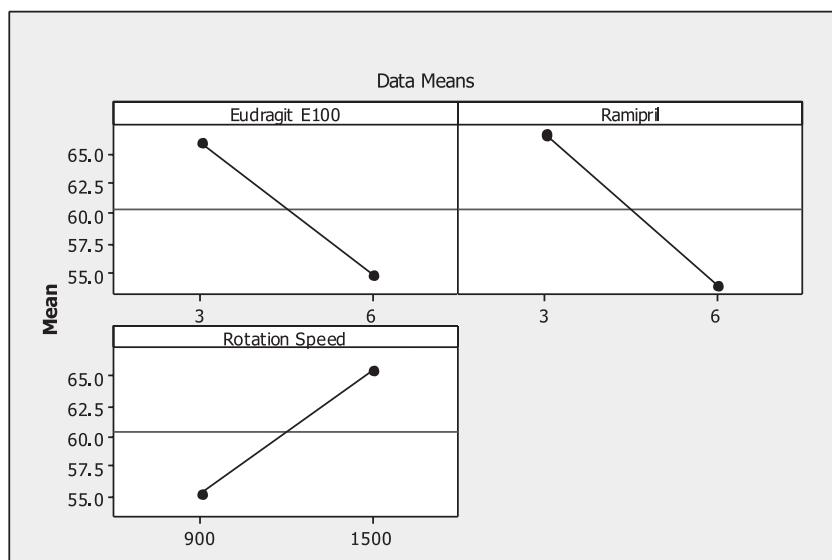
Figure 8. Main effect plot showing effect of Eudragit E100, ramipril and rotation speed on % drug encapsulation efficiency
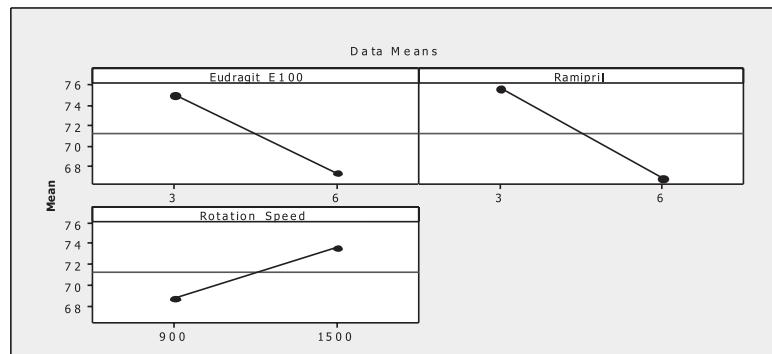
Figure 9. Main effect plot showing effect of Eudragit E100, ramipril and rotation speed on drug release after 12 h
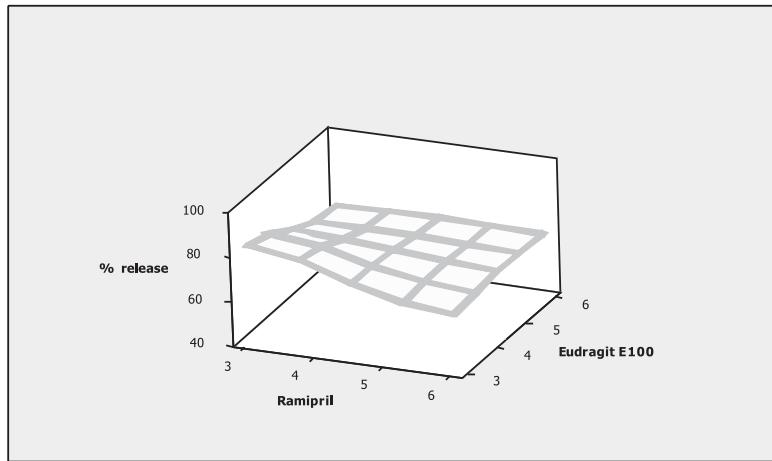

Figure 10. Surface plot showing effect of Eudragit E100 and ramipril on % release of ramipril
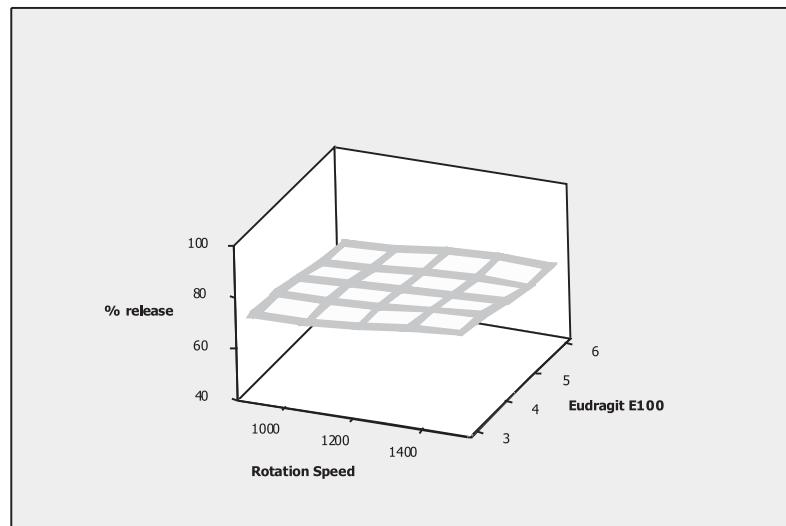

Figure 11. Surface plot showing effect of Eudragit E100 and rotation speed on % release of ramipril
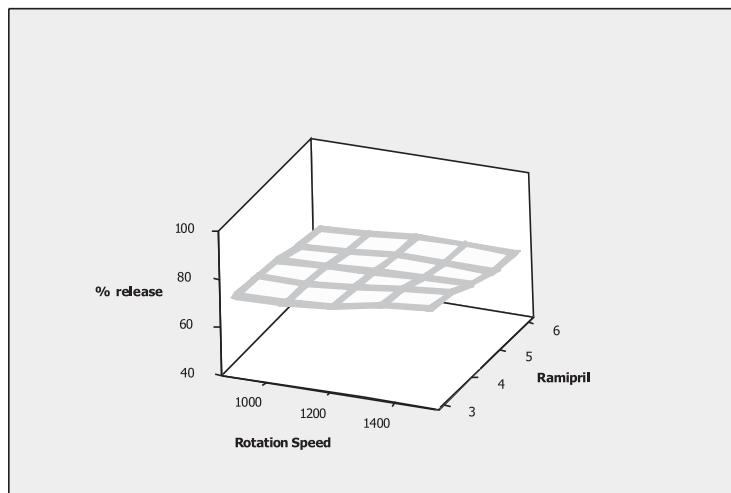
Figure 12. Surface plot showing effect of ramipril concentration and rotation speed on % release of ramipril
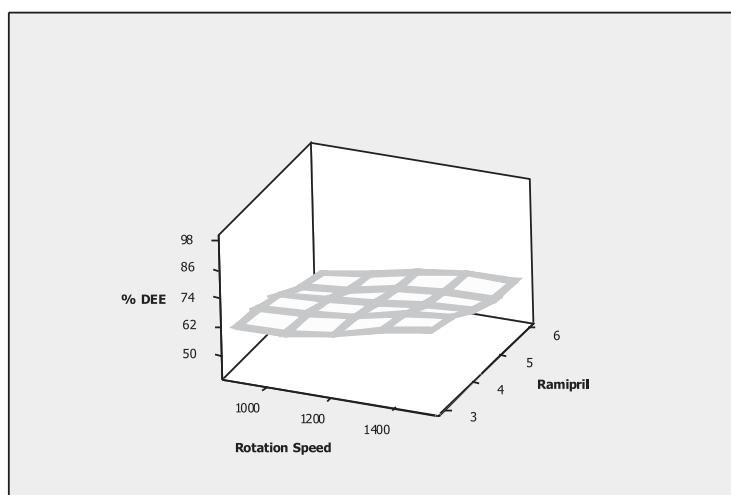
Figure 13. Surface plot showing effect of ramipril concentration and rotation speed on % DEE (drug encapsulation efficiency)
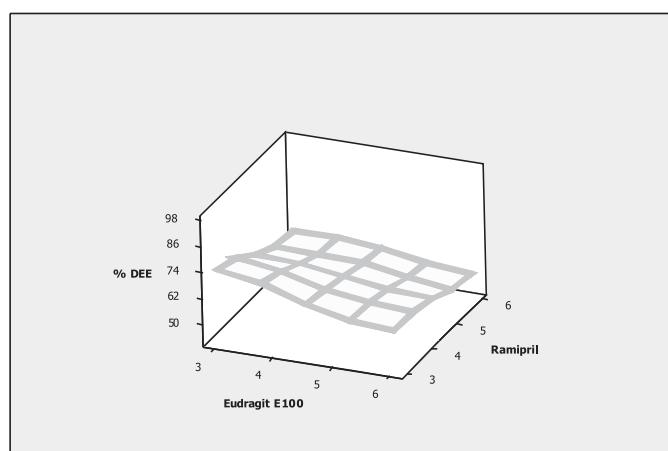
Figure 14. Surface plot showing effect of ramipril and Eudragit E100 on % DEE (drug encapsulation efficiency)
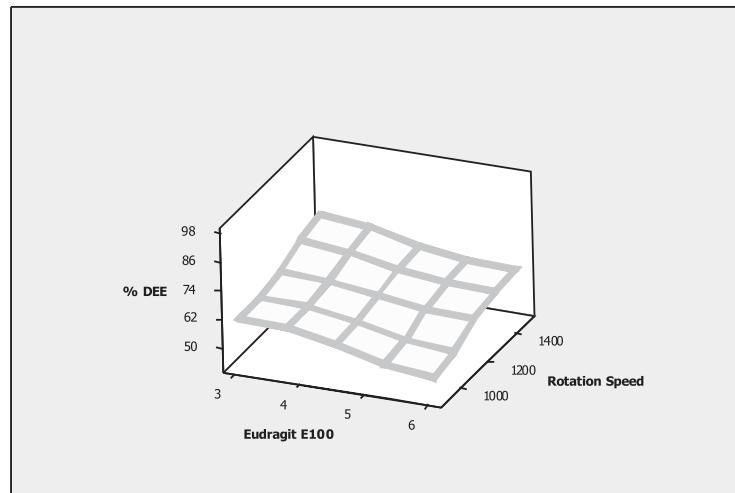
Figure 15. Surface plot showing effect of drug rotation speed and Eudragit E100 on % DEE (drug encapsulation efficiency)
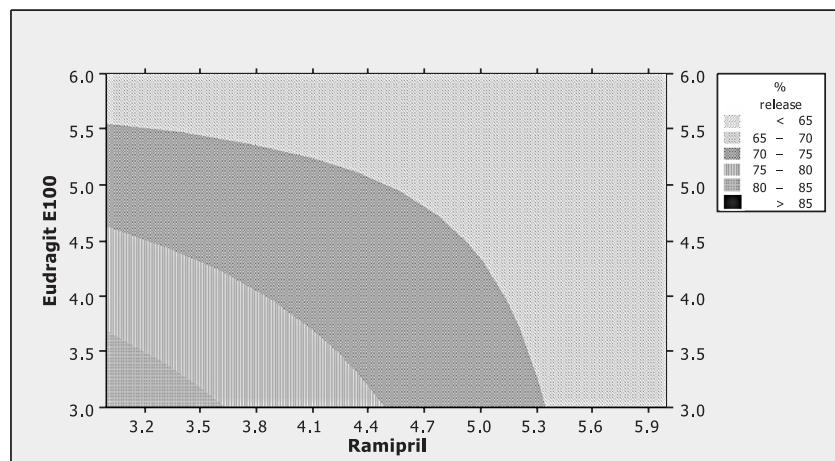

Figure 16. Contour plot showing effect of Eudragit E100 and ramipril on % release of ramipril
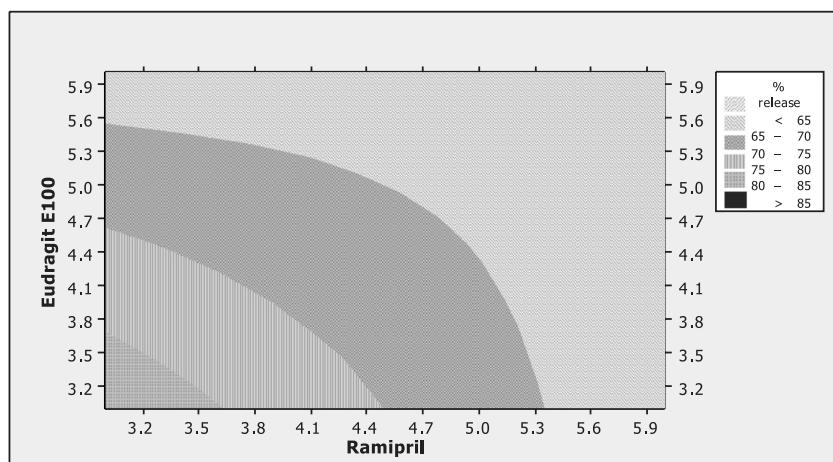
Figure 17. Contour plot showing effect of Eudragit E100 and rotation speed on % release of ramipril
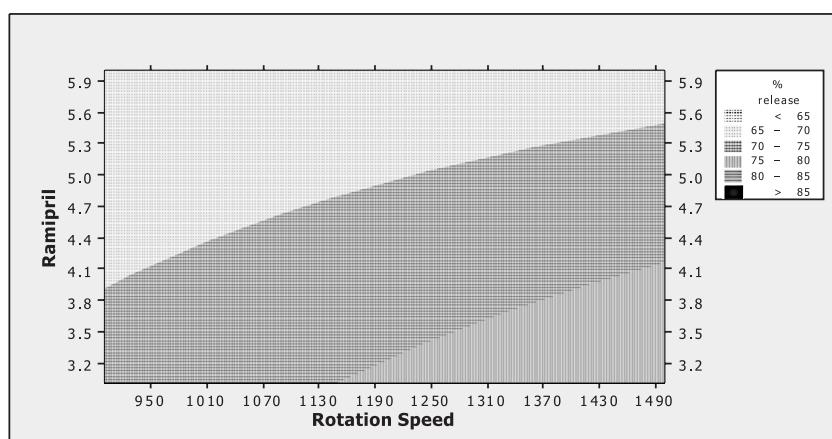
Figure 18. Contour plot showing effect of ramipril concentration and rotation speed on % release of ramipril
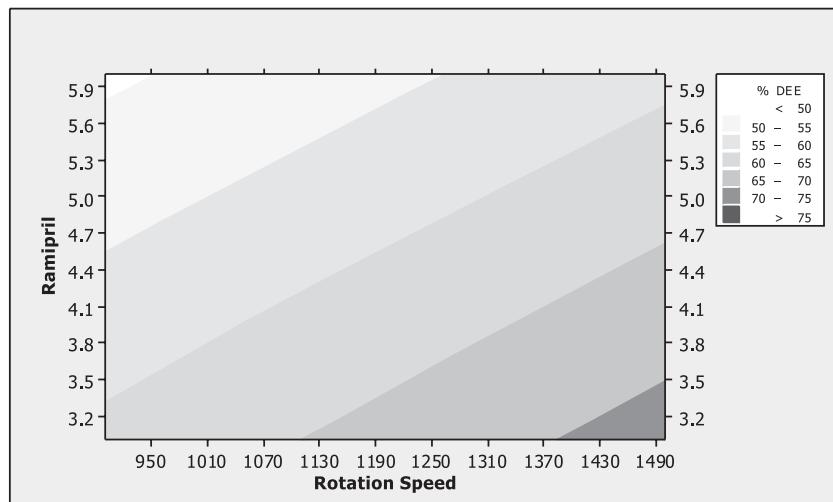

Figure 19. Contour plot showing effect of ramipril concentration and rotation speed on % DEE (drug encapsulation efficiency)
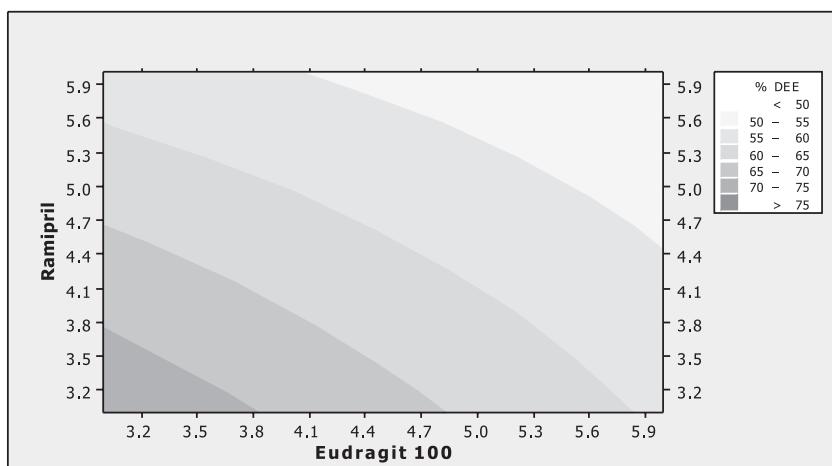

Figure 20. Contour plot showing effect of ramipril and Eudragit E100 on % DEE (drug encapsulation efficiency)
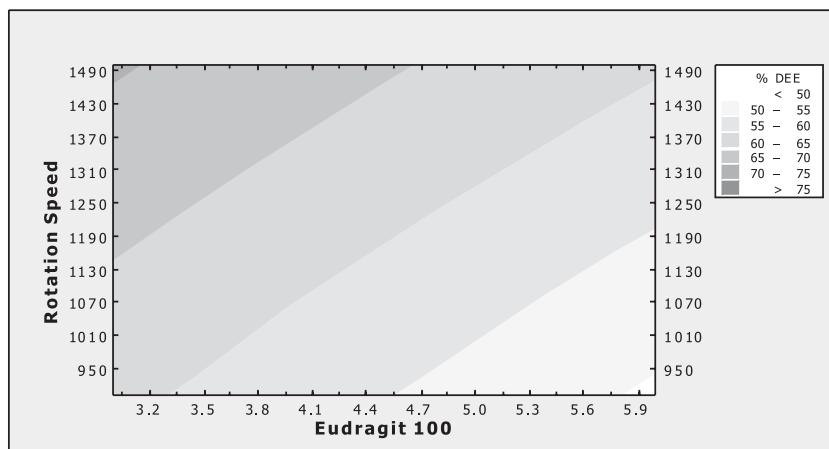
Figure 21. Contour plot showing effect of drug rotation speed and Eudragit E100 on % DEE (drug encapsulation efficiency)

Fourier transforms infra-red spectroscopy (FT-IR) analysis

The Fourier transform infra-red analysis was conducted for the analysis of drug polymer interaction and stability of drug during microencapsulation process. The IR spectra of ramipril, Eudragit E100 and optimized batch F1 floating microspheres were recorded. The IR results are shown in Figures 6 and 7.

Analysis of results by Minitab

The full factorial design was setup to study the effect of various input variables (Eduragit 100, drug complex and stirring speed) on % drug encapsulation efficiency and % release of drug. The polynomial equation for the generalized linear model for 1st order can be depicted as follows:

$$Y = b0 + b_1X1 + b_2X2 + b_3X3 \; b_{12}X1X2$$
$$+ b_{23}X2X3 + b_{13}X1X3 + b_{123}X1X2X3$$

where, Y is the dependent variable, b0 is the arithmetic mean response of the 9 runs, and b1, b2 and b3 are the estimated coefficients for factors X1, X2 and X3. 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 (X1X2, X2X3 and X1X3) are included to investigate nonlinearity.

The polynomial equation for % drug encapsulation efficiency is:

$$Y = 71.23 + 3.73X1 + 4.38X2 - 2.47X3 +$$
$$4.39X1X2 - 0.45X2X3 - 1.25X1X3 +$$
$$15.13X1X2X3$$

The polynomial equation for % drug release is:

$$Y = 60.33 + 5.50X1 + 6.31X2 - 5.15X3 +$$
$$1.98X1X2 + 0.49X2X3 - 0.30X1X3 +$$
$$71.34X1X2X3$$

The interaction plots and main effect plots show relationship of selected variables with the selected observable output variables.

Formulation of suitable dosage form (hard gelatin capsule)

From various evaluation parameters it was found that batch F1 containing low amount of drug as well as polymer at high rotational speed gave optimal drug release in 12 h. So, suitable dosage form, hard gelatin capsule was formulated employing loading dose as well as sustained hollow microcapsules were incorporated as in Table 4 and were subjected to *in vitro* release profile for which the results are shown in Figure. 3

RESULTS AND DISCUSSION

Ramipril is ACE inhibitor which works by reducing load on the heart. In present attempt, the novel approach hollow microspheres were tried for delivery of sustained delivery of ramipril and final delivery in the form of hard gelatin capsule. For formulation of hollow microspheres the full factorial design was employed using three key input variables at two levels i.e., amount of Eudragit (0.75 and 1.50 g), amount of Ramipril (0.75 and 1.5 g) and rotation speed (900 and 1500 rpm). Glyceryl monostearate was used to prevent aggregation of formed microspheres as it may help in formation of

thin layer around the formed membrane of micro-capsules.

The formed microcapsules were evaluated for various parameters such as % drug encapsulation efficiency, particle size and *in vitro* drug release which was found to be highest for batch containing low concentration of drug complex and low concentration of Eudragit E100 at high rotation speed. The 3D surface plot, main effect plots and contour plots also confirm high encapsulation efficiency and high release of the drug. From main effect plot it can be observed that maximum encapsulation is seen at low concentration of polymer and drug complex and high stirring speed. The % release results were also similar to the results for % drug encapsulation efficiency. The optimized batch F1 was selected for further incorporation into the suitable dosage form and hard gelatin capsule was formulated which was also evaluated and showed satisfactory release when compared with batch F1.

The surface characterization was done by using SEM and it shows uniformity in size and shape of microcapsules. Further, the FTIR spectra reveal that there does not exist interaction between components used.

CONCLUSION

The formulation of hollow microspheres for ramipril is a challenging task. The wise selection of formulation ingredients as well as selection of surfactants can provide effective way of preparing them at laboratory level. The full factorial experimental design provides key input variables necessary for providing stable hollow microspheres.

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