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FORMULATION AND EVALUATION OF MUCOADHESIVE MICROSPHERES OF DABIGATRAN FOR IMPROVING BIOAVAILABILITY

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ABSTRACT

The objective of the current investigation was to formulate mucoadhesive microspheres loaded with dabigatran utilizing Eudragit RL100 as the mucoadhesive polymer and stabilizing the particles with magnesium stearate so that the formulation could control the release of the drug thereby reducing its dosing frequency and improving the bioavailability. The microspheres were prepared using emulsion coacervation method and the micromeritic features were evaluated. The angle of repose for all formulations was found to be within the range from 23.54 ± 0.122 to 25.47 ± 0.0721°. This indicates that good flow property of powder blend. The bulk density and tapped density values were found to be within the range from 0.37 ± 0.01 to 0.61 ± 0.01 g/cm^3 and 0.44 ± 0.005 to 0.73 ± 0.01 g/cm^3 respectively. The values of Hausner's ratio ranged from 1.16 to 1.20 whereas the Carr's Index ranged from 13.46 to 20.29. The yield of all the batches of microspheres was found to be reasonably good ranging from 53.69% to 74.18%. The drug loading was found to be 73.81 ± 0.345% to 74.44 ± 0.313 % while the size of microspheres ranged from 13.28 ± 0.170 to 17.26 ± 0.255 µm. Swelling study was performed on all the formulation for 24 h and swelling index ranged from 2.93 to 4.18. The mucoadhesion time of the microspheres ranged from 2h41min to 6h 45min. All the formulations were able to sustain the release of dabigtran for more than 12h.

Keywords: Gastroretentive, microspheres, mucoadhesion, swelling, Eudragit RL 100

INTRODUCTION

The relative ease of administration as well as the traditional belief makes oral drug delivery as the most widely recommended route of drug administration. The foremost benefit of oral administration is that it allows for self-administration by the patients and therefore is a highly expedient way to introduce substances into the human body.

Mucoadhesives are synthetic or natural polymers which interact with the mucus layer covering the mucosal epithelial surface and mucin molecules constituting a major part of the mucus. They localize the formulation at a particular region of the body thereby improving bioavailability of drugs with low bioavailability. Mucoadhesive drug delivery systems are delivery systems, which utilize the property of certain polymers to adhere to mucus membranes upon hydration **[1]** and hence can be used for targeting a drug to a particular region of the body for extended period of times **[2]**. Mucoadhesive microspheres are the microparticles prepared by using mucoadhesive polymers and having a diameter of 1-1000 μm **[3]**. Mucoadhesive microspheres can be used to adhere to the mucosal layer of nasal cavity, gastrointestinal and urinary tract for localized as well as systemic effect of drugs.

Dabigatran etexilate is an anticoagulant used for the prevention of venous thromboembolic events or stroke in patients with recent elective hip or knee replacement surgery and atrial fibrillation. It is a univalent reversible direct thrombin inhibitor (DTI) that competitively inhibits thrombin. Additionally dabigatran has also been shown to inhibit platelet aggregation, another step in the coagulation pathway **[4]**.

In the recent years, mucoadhesive microspheres as delivery systems have been frequently studied as the tool to achieve sustained release of drug from the dosage form and improvement of patient compatibility and bioavailability of drugs **[5-12]**. The bioavailability of Dabigatran is reported to be 3-7% on oral administration and its protein binding is 35%. This makes it a good candidate for mucoadhesive delivery. The objective of this work was the development and investigation of mucoadhesive microspheres of Dabigatran using Eudragit RL100 as the mucoadhesive polymer in order to improve its bioavailability and reduce the dosing regimen.

MATERIAL AND METHODS

Dabigatran Etexilate was purchased from Yarrow Pharmaceuticals, Mumbai; Eudragit RL100 was obtained from Oxford Fine chemicals, Mumbai; all other reagents and chemicals used in the investigation were of general or analytical grade.

Preformulation study [13]

In order to perform the preformulation evaluation of the drug tests of identification such as physical appearance, melting point and FTIR spectroscopy were carried out. The solubility profile of drug in various solvent systems, incompatibility study by FTIR, partition coefficient was carried out using water and butanol **[14]** and quantitative estimation of drug was also studied. The calibration curve of dabigatran was obtained in riethyl ammonium phosphate aqueous solution (pH 2.5) at 325 nm using UV-Visible spectrophotometer **[15]**.

Formulation of mucoadhesive microspheres [16]

Dabigatran microspheres were prepared according to the oil-in-oil emulsification-coacervation method using Eudragit® RL100 as the polymer. Eudragit RL 100 was dissolved in 15.0 mL of acetone in a 250 mL beaker with stirring at room temperature. Dabigatran (110 mg) and magnesium stearate (0.1 g) were dispersed in the polymer solution. The resulting milky white dispersion was added drop-wise into a beaker containing a mixture of liquid paraffin (50 mL) and span 60 (0.5 g) and homogenized using a paddle stirrer at 500 rpm for 2 h. The formed microspheres were separated by filtration and washed many times with n-hexane to make them completely free from oil. The microspheres were dried at room temperature and stored at 4°C until used. Three batches of the microspheres were prepared for different amounts of the polymer and a control was also prepared using the above method without dabigatran **(Table1)**.

Table 1: Batch Formula for formulation of mucoadhesive microspheres

	F1	F2	F3	F4
Dabigatran (mg)	0	110	110	110
Eudragit RL100 (mg)	2000	2000	3000	4000
Magnesium Stearate (mg)	100	100	100	100
Acetone (mL)	15	15	15	15
Liquid Paraffin (mL)	50	50	50	50
Span 60 (mg)	500	500	500	500

Evaluation of microspheres

Micromeritic features

Angle of repose, Carr's Index, Bulk density, Tapped density and Hausner's ration were determined to assess the flow ability of the prepared granules.

Yield

The formed microspheres were weighed accurately and the yield of the microspheres was determined by comparing the weight of the microspheres against the combined weight of the copolymer and drug using the equation:

$$Yield\ (\%) = \frac{Weight\ of\ microspheres}{Weight\ of\ drug + polymers}\ X\ 100$$

Drug content

The dabigatran content of the microspheres was determined using UV spectrophotometery. 10 mg of microspheres were dispersed in 10 mL of simulated intestinal fluid (SIF, pH 7.2). The dispersion was allowed to stand for 2 h, vortexed for 5 min and then centrifuged at 4,000 rpm for 10 min. The amount of dabigatran contained in each batch of the formulations was determined by the UV at 325 nm. The drug-loading efficiency was then determined by the equation:

Particle Size

The average particle size was calculated using calibrated optical micrometer by dispersing the prepared formulation in distilled water and counting the size of individual particles under an optical microscope.

Morphology

The morphology of dabigatran loaded microspheres was carried out by a scanning electron microscope. Diluted samples were dropped into stubs and allowed for air drying, coated with gold (thin layer) and observed under scanning electron microscope.

***In-vitro* release**

The USP type II paddle apparatus with a paddle speed of 50 rpm was used for studying the release of the drug from the microspheres. The dissolution media used consisted of 900 mL of phosphate buffered saline (pH 6.8) and maintained 37±0.5°C. 5 mL of samples were collected at time points of every hour until 12 h and the media was replenished with the same volume of fresh media. The free dabigatran concentration was estimated using a UV spectrophotometer at a wavelength of 325 nm. The release kinetic was studied by various kinetic models like zero order, first order, Higuchi plot and Korsemeyer-Peppas model. The best fit model was confirmed by the value of correlation coefficient.

Swelling Index

100 mg microspheres were allowed to swell for 24 hr in 6.8 pH phosphate buffer. After 24 hr excess liquid were removed by blotting paper and microspheres were weighed. The degree of swelling was then calculated by the following formula

$$Degree\ of\ Swelling = (M0 - Mt)/M0) * 100$$

Where, S.I = swelling index, M_0 = weight of microsphere at the end and M_t = weight of microsphere at start.

***In-vitro* mucoadhesion wash-off test [17]**

Mucoadhesive property of microspheres was determined by *in-vitro* adhesion test. Eggshell membrane was used for this purpose. A 2x1 cm piece of eggshell membrane were taken and fixed on a glass slide (kept at an angle of 45°C). About 100 mg microspheres were spread on rinsed, tissue specimen and hung onto one of the groves of a USP tablet disintegrating test apparatus containing 6.8 pH phosphate buffer. The disintegrating test apparatus was started, the tissue specimen showed regular up and down movements in a beaker. The time required for detaching of microspheres from mucosal surface membrane was recorded by visual inspection.

RESULTS

Dabigatran was yellowish white powder with no odor and taste, soluble in water and methanol while slightly soluble in ethanol having a melting point of 178-180°C and log P value of 3.8. The FTIR spectrum of dabigatran and a physical mixture of dabigatran with Eudragit RL100 suggested no interaction amongst the drug and the polymers. None of the characteristic peaks of dabigatran were found to be affected by the physical mixture **(Figure 1)**.

The calibration curve in UV at 325 nm displayed a regression equation y=0.079x-0.183; R^2 = 0.995 where y is the absorbance and x is the concentration **(Figure 2)**.

Evaluation of microspheres

Micromeritic features

The bulk and tapped density of the formulations ranged from 0.37 ± 0.01 to 0.61 ± 0.01 g/cm^3 and 0.44 ± 0.005 to 0.73 ± 0.01 g/cm^3 respectively. The angle of repose was measured using the fixed funnel method and was found to be ranging from 23.54 ± 0.122 to 25.47 ± 0.072. The values of Hausner's ratio ranged from 1.16 to 1.20 whereas the Carr's Index ranged from 13.46 to 20.29 **(Table 2)**.

Yield and drug loading

The yield of all the batches of microspheres was found to be reasonably good ranging from 53.69% to 74.18% whereas the drug loading ranged from 73.81 to 74.44 % **(Table 3)**.

Particle size and morphology

The particle size was calculated using calibrated eye piece (n=30). The average particles size of the blank particles was 13.28 ± 0.170 μm **(Table 3)**. The SEM photomicrograph revealed that the particles were spherical in shape and had discrete structure **(Figure 3)**.

In vitro drug release

The in vitro release of dabigatran was studied for 12 h and it was found that all the microspheres were able to sustain the release of the drug for more than 12 h with less than 80% drug released at the end of the 12th hour. The release was found to decrease with increase in Eudragit RL100 concentration in the microsphere. The data obtained was fitted to kinetic models to determine the release kinetics of dabigatran from the microspheres **(Figure 4)**.

Korsemeyer-Peppas

The correlation coefficient for each formulation was obtained from the statistical software (Excel) and the best fit was determined by the closeness of the correlation value to one **(Table 4)**.

Swelling index and mucoadhesive property of microspheres

For the evaluation of mucoadhesion eggshell membranes were utilized as the substitute of animal mucosa. The swelling index ranged from 2.93 to 4.18 whereas the time of mucoadhesion was from 2h 41min to 6h 45min **(Table 5)**.

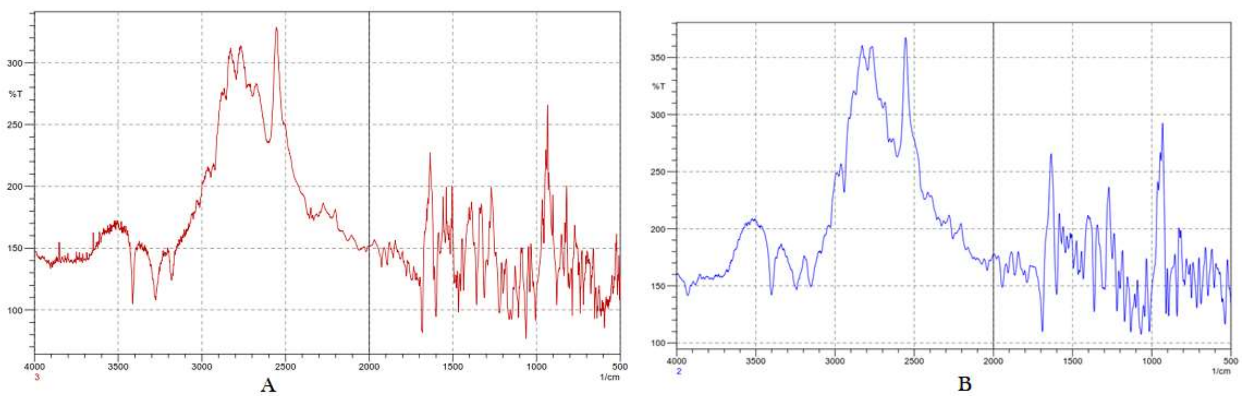

Figure 1: FTIR spectrum (A) Dabigatran (B) physical mixture of dabigatran and Eudragit RL100

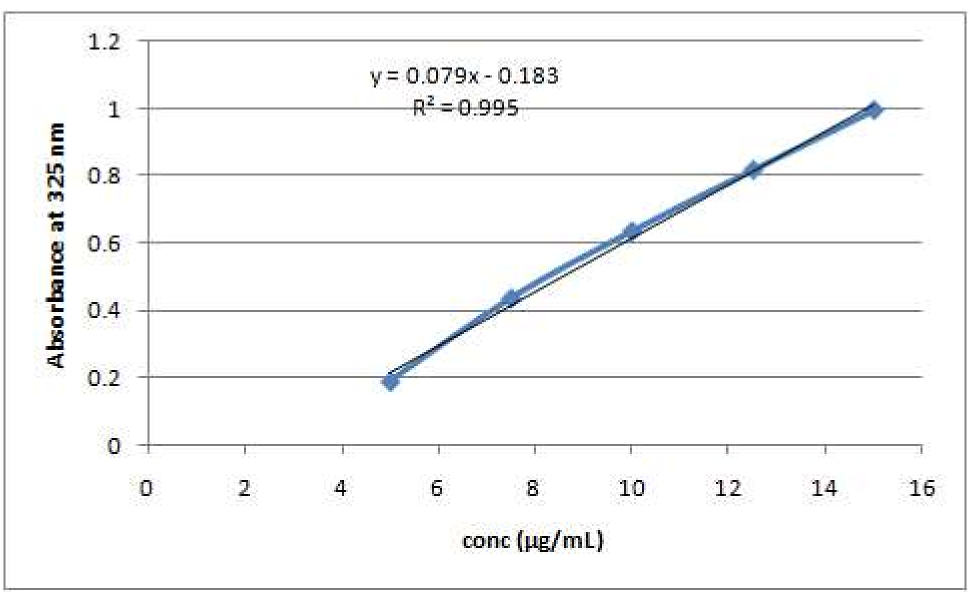

Figure 2: Calibration curve of dabigatran

Table 2: Micromeritic features of the microspheres

Formulation code	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Angle of Repose (°)	Hausner's Ratio	Carr's Index
F1	0.37 ± 0.01	0.44 ± 0.005	23.54 ± 0.122	11.21	1.13
F2	0.45 ± 0.005	0.53 ± 0.005	24.13 ± 0.064	2.57	1.03
F3	0.55 ± 0.005	0.69 ± 0.005	25.24 ± 0.056	15.68	1.19
F4	0.61 ± 0.01	0.73 ± 0.01	25.47 ± 0.072	14.81	1.17

Values are expressed as mean ± SD (n=3)

Table 3: Yield, drug loading and size of microspheres

Formulation Batch	Yield (%)	Drug loading (%)*	Size (µm)**
F1	53.69	-	13.28 ± 0.170
F2	67.55	73.81 ± 0.345	14.28 ± 0.275
F3	74.18	74.53 ± 0.568	16.14 ± 0.167
F4	70.22	74.44 ± 0.313	17.26 ± 0.255

Results are represented as mean ± standard deviation; *n =3; **n=30

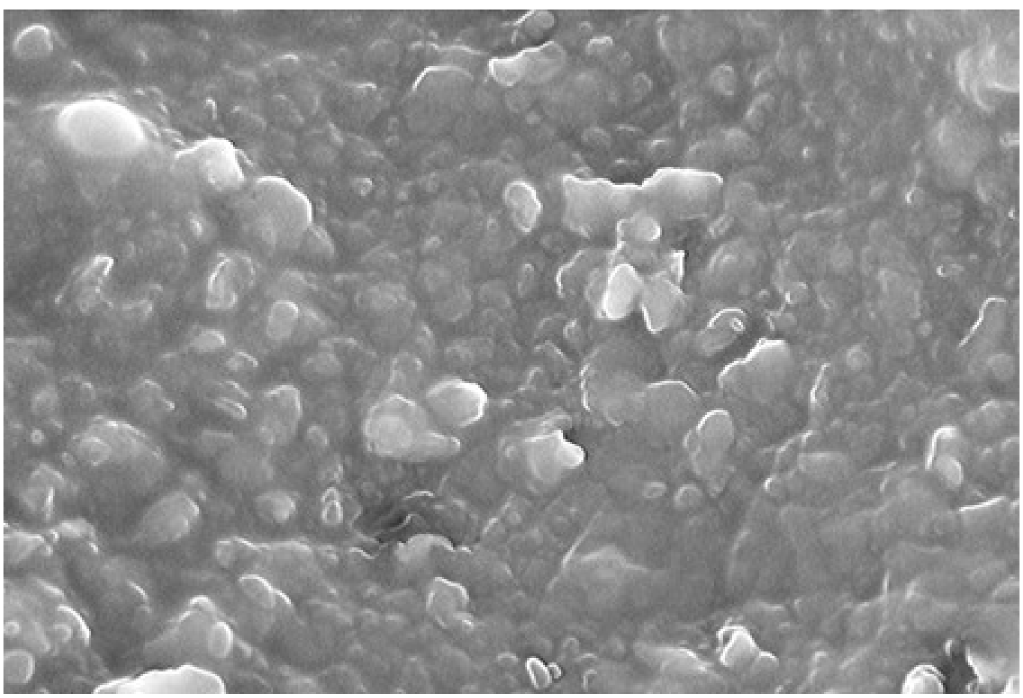

Figure 3: SEM image of F1

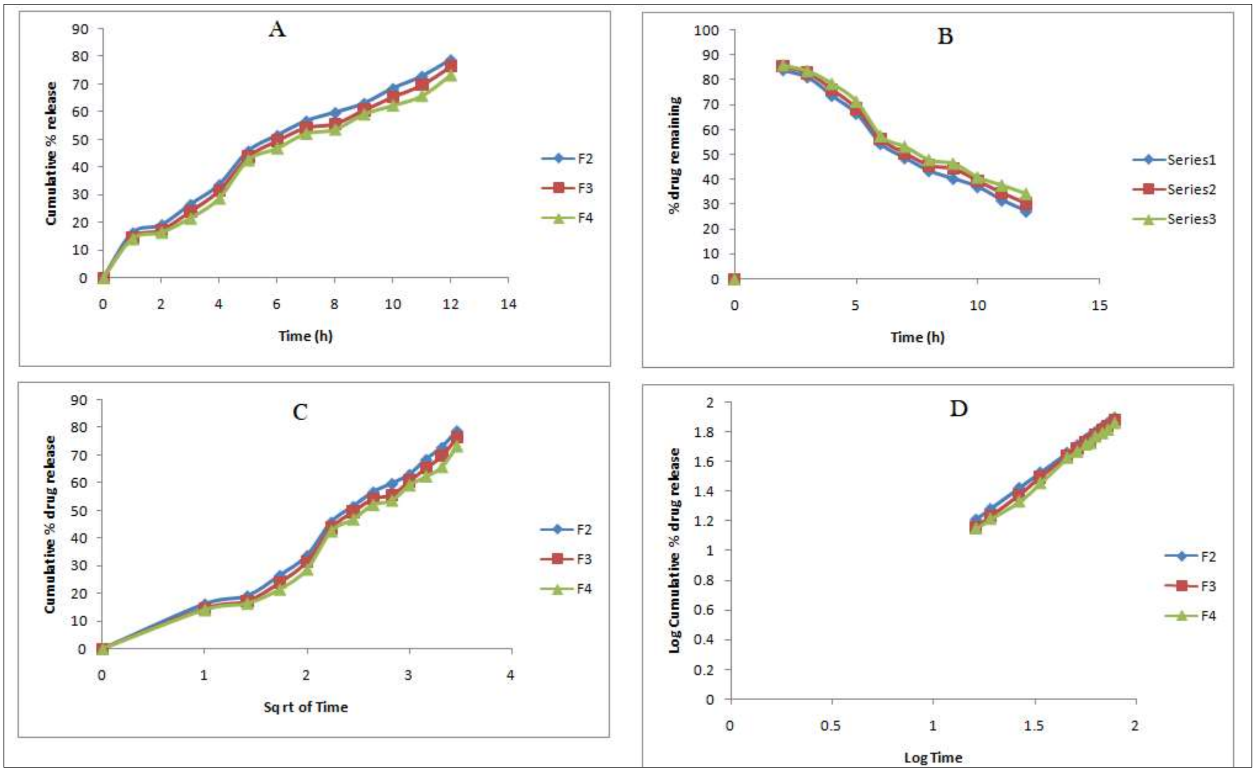

Figure 4: Release profile of dabigtran (A) zero order (B) First order (C) Higuchi (D)

Table 4: Correlation coefficients of various mathematical models

Formulation batch	Zero order	First order	Higuchi	Korsemeyer-Peppas
F2	0.97	0.095	0.969	1
F3	0.971	0.075	0.963	0.999
F4	0.969	0.057	0.957	0.997

Table 5: Swelling Index and Mucoadhesion of the microspheres

Formulation Batch	Swelling Index	Mucoadhesion time (h)
F1	2.93	2.41
F2	3.75	2.53
F3	3.96	3.57
F4	4.18	6.45

DISCUSSION

The bulk and tapped density play a vital role in pharmaceuticals as it reflects processability of the blend. It also reflects flowability of the blend using various calculative ratios. Angle of repose is a measure of the ability to powder to flow through the hopper of the tablet punching machine. Angle of repose of less than 30° is considered to be good for the flow of the powder. All the results of powder characterization indicate that the formulation blends exhibited good ability to flow and compress in to tablets.

The highest yield was exhibited by the formulation **F3**. The good yield of the microspheres indicates that the formulation process and variables employed in preparing the microspheres are efficient. As such the drug loading percentage was almost similar for all the formulations indicating that the concentration of Eudragit RL100 did not had much effect on drug loading. The highest loading was however found in **F3**. The high drug loading efficiency in all the formulations was also suggestive of the high efficiency of the process parameters used for microsphere formulation.

The size of microspheres increased with the increasing concentration of Eudragit RL100. All the particles were found to be within the micrometer range suggesting a desired efficiency of the method of formulation.

The best fit model was found to be Korsemeyer-Peppas model suggesting that the sustained release of the drug from the microspheres was due to the degradation of the matrix over time.

The mucoadhesion increased with the higher concentrations of Eudragit RL100 in the formulations. The formulation **F4** had the highest degree of swelling, highest mucoadhesion time and released the lowest amount of drug at the end of 12th hour suggesting that it could be able to achieve the highest sustained release. This makes formulation **F4** the most desirable formulation to reduce the dosing frequency of dabigatran.

CONCLUSION

The results obtained from the study indicate that use of Eudragit RL100 as the mucoadhesive polymer could help in achieving sustained release over a longer duration and help in reducing the dose as well as frequency of administration of the medicaments. Further *in vivo* release studies are needed to support for the conclusion of the present investigation.

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