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DESIGN AND EVALUATION OF MICROSPHERES OF MIDAZOLAM

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ABSTRACT

The purpose of this research was to formulate and systematically evaluate in vitro performances of Midazolam microspheres. An attempt was made in the present study to deliver the drug in the form of microspheres. Microspheres can increase the site specific delivery system and possible to cross the body fluids like BBB, CSF, etc. Microspheres were prepared by the Emulsion Cross linking method using Eudragit E100 and Eudragit RL100. Microspheres were characterized by SEM, DSC, FTIR, Particle size analysis and evaluated for percentage yield, drug loading, encapsulation efficiency and in vitro drug release. FTIR and DSC studies showed that no chemical interaction occurred between the drug and polymers. The sphericity factor indicated that the prepared microspheres were spherical. Formulation F8 indicated a controlled in vitro drug release. The results indicated that the prepared Midazolam microspheres can be explored for controlled drug release.

KEYWORDS

Midazolam, Microspheres, Eudragit E100, Eudragit RL 100 and Emulsion cross linking method.

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INTRODUCTION

The large numbers of drugs were synthesized by the drug industries every year, like the same everyday many new technological evolve is ever improving the quality of health care. Incorporating an existing drug molecule into a new drug delivery system can significantly improve its performance in terms of efficacy, safety and improved patient compliance¹. Drug Industries nowadays are, therefore, engaged in the development of multiple platform technologies for controlled release, taste-masking, oral fast dispersing dosage forms, newer technology for insoluble drugs, and delivery of drugs through different routes like

intranasal, buccal, pulmonary, transdermal, vaginal, colon, and transmucosal routes etc. Oral controlled release drug delivery is thus a drug delivery system that provides the continuous oral delivery of drug at the predictable and reproducible kinetics for predetermined period throughout the course of gastrointestinal transit. All the pharmaceutical products formulated for systemic delivery via the oral route of administration, irrespective of mode of delivery (immediate, sustained or controlled release) and the design of dosage form (solid, dispersion or liquid) must be developed within intrinsic characteristic of gastrointestinal (GI) physiology^{2,3}. Permeability of the buccal mucosa is 4-4000 times greater than that of the skin. As indicated by a wide range in this reported values, there are considerable differences in permeability between different regions of the oral cavity. In general, based on the degree of keratinization of oral mucosa the permeability of the drug differs.

The scope of the present work is aimed with the development of oral controlled release microsphere delivery systems for the selected drug candidate of Midazolam. Microspheres are solid, approximately spherical particles ranging from 1 to 1000 μ m, they are made up of polymeric materials, which are biodegradable synthetic or natural polymers. The natural polymers include albumin and gelatin, the synthetic polymer include poly lactic acid, poly glycolic acid. Emulsion solvent, phase-separation method and spray drying method are commonly used for the preparation of microspheres⁴. Microspheres are prepared by the evaporating the organic solvent from the dispersed oil droplets containing both polymer and drug. However, the successes of these microspheres formulation are limited based on their duration of residence time at the site of absorption and depends on many factors such as the drug solubility, partition coefficient, polymer composition, molecular weight etc⁵.

MATERIALS AND METHODS

Materials

Midazolam was gifted by Brooks's laboratories Ltd (India). Baddi H.P, Eudragit E100, Eudragit RL100,

Glutaraldehyde, Light liquid paraffin and Citric acid was gifted by Bafana pharmaceuticals. Chennai (India)

Methods

Preparation of Microspheres

Microspheres were prepared by using Eudragit E-100 and Eudragit RL 100 by emulsion cross linking method. The aqueous phase was prepared by using different concentrations of Polymers in phosphate buffer solution (pH 5.5). The drug and citric acid was dissolved in it and the solution was extruded through a glass jacketed syringe in 50 ml of liquid paraffin (heavy and light 1:1 mixture) containing surfactant(tween 80, 0.5% v/v), with continuous stirring on Remi stirrer at 2000 rpm. After 3 h, 1 ml of glutaraldehyde (25% solution, as cross linking agent) was added and stirring was continued for 2 hrs. Microspheres obtained were filtered and washed several times with Petroleum ether to remove oil, and finally washed with water to remove excess of glutaraldehyde⁴. Microspheres were then air dried.

EVALUATION OF MICROSPHERES

Particle Size Analysis⁴

Particle size of the microspheres was determined by optical microscopy. The eye piece micrometer was calibrated with the help of a stage micrometer. Average of 100 microspheres was measured randomly. The average particle size was determined by using Edmondson's equation.

$$D = \frac{\sum nd}{\sum n}$$

Where, n = Number of microspheres checked;

D = Mean of the size range

Surface Morphology⁵

The morphology of the microsphere was determined by Scanning electron microscopy. In this study, samples were prepared by lightly sprinkling the powder on a double adhesive tape stuck to an aluminium stub. The stubs were then coated with gold to a thickness of about 300 Å coated under an Argon atmosphere with Gold- Palladium using a Polaron SEM coating unit E5000(Japan). Samples were examined under the Philips SEM 500 Scanning electron microscope.

Percentage yield

The percentage yields of different formulations were determined by weighing the obtained microspheres

after drying. The percentage yield was calculated as follows.

$$\% \text{ yield} = \frac{\text{Amount of Microspheres obtained}}{\text{Theoretical content}} \times 100$$

Drug entrapment efficiency (DEE)⁵

The various formulations of the microspheres were subjected for drug content. In a Glass mortar and pestle 50 mg of microspheres were accurately weighed, crushed and suspended in Methanol to extract the drug from Microspheres. After 24 hours, the resulting solution is then filtered through whatmann filter paper No.44 and then the filtrate was assayed spectrophotometrically at 216 nm for drug content against methanol as blank. Corresponding drug concentrations in the samples were calculated. The percentage drug entrapment was calculated as follows.

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

All the determinations were made in triplicate.

Content uniformity⁶

Randomly samples were taken and weighed. The powdered microspheres equivalent to 100 mg of midazolam and was transferred into a 100ml flask containing suitable aqueous media. The flask was shaken for 24 hours and was kept for 12 hours. The solution is filtered through Whatmann filter paper. 10ml of this filtrate was taken and appropriate dilution was made. The samples were analyzed at 216 nm using UV visible spectrophotometer. The drug content was determined from the standard curve prepared at λ max 216 nm.

Swelling Index⁶

Swelling Index was determined by measuring the extent of swelling of microspheres in phosphate buffer pH 5.5. To ensure the complete equilibrium, exactly weighed 100 mg of microspheres were allowed to swell in buffer for 24 hrs. The degree of swelling was calculated using following formula, $\alpha = (W_s - W_o) / W_o$, where α is degree of swelling, W_o is the weight of microspheres before swelling and W_s is the weight of microspheres after swelling.

In vitro dissolution studies⁷

The release profile of the drug from microspheres clearly indicates that the concentration of polymers slow the release of midazolam from microspheres. At

the end of 12 hours, in vitro drug release from formulations M1 to M9 was found to be 83.1 to 98.9% in the buccal environment. The total cumulative quantity of the drug released at the end of the 12 h dissolution test was below 100% for all dosage forms. This may be in part due to the relatively slow release of entrapped drug from the matrices undergoing testing. Among various formulations, M8 was found to have a good release pattern and controlled release up to 12 hours. Hence, M8 was selected as the optimized formulation and the release was compared with the pure drug of Midazolam.

Thermal Analysis

Differential scanning calorimetry (DSC) was performed on pure drug, drug loaded and blank microspheres. DSC measurement was done on a mettler Toledo DSC 822c.

X-Ray Diffraction (XRD) Studies

The crystallinities of Drug Midazolam and Midazolam loaded microspheres were determined by using an x-ray diffract meter (Brrucker Axs, 08 Advance).

Stability studies⁶

In this study, stability study was done for at conditions like Room temp. (RT) and 40°C and 75% RH. The samples were assayed for drug content at regular intervals for two weeks for the period of six months.

RESULTS AND DISCUSSION

Particle size analysis

Results had shown that as the concentration of Eudragit RL100 and Eudragit E100 polymers increased, the size of the microspheres also increased. Amongst all nine batches of Microsphere, Batch M7 to M9 constantly increased in size while, Batches M1 to M6 shows relatively different in the size of microsphere. Batch M8 Microsphere containing Equal concentration of polymers produced optimum size of Microsphere.

Surface Morphology

SEM analysis shows that the images of the optimized batch M8 microsphere using equal concentration of Eudragit polymers have uniform and similar morphologies, being a spherical like shape. The Microspheres Produced by emulsion cross linking method, all batches have uniform and similar morphologies.

Drug loading and encapsulation efficiency

From all 9 different batches, Batch M8 of equal composition of Eudragit E 100 and Eudragit RL100 polymers in the drug-polymer ratio of 1:2 shows better drug content and encapsulation efficiency. Encapsulation efficiency ranged from 82.79 to 93.74%. It was found that the encapsulation efficiency increased with increasing amounts of polymers in the microspheres. Formulations M8 and M9 showed a relatively higher encapsulation efficiency, these formulations contained a higher polymer concentration. It can be inferred from the results that there was a proper distribution of midazolam in the microspheres.

The flow property of microspheres was studied by calculating the angle of repose (θ in degrees) and compressibility index (CI, %). The value of θ ranged from 22.77¹ to 25.17¹ indicating that the microspheres had good flow properties. The CI value was found to be in the range of 17.34±0.734 to 20.27±0.47%, which also indicated good flow properties.

In-vitro Dissolution Study

All the formulations of prepared of Midazolam microsphere were subjected to in vitro release studies, these studies were carried out using dissolution media of Phosphate buffer 5.5 pH. The release of midazolam from the Microspheres varied according to the type

and concentration of polymer. The cumulative % releases of batch M1 to M9 were found to be 83.18% to 98.95% in 12 hrs respectively. The total cumulative quantity of the drug released at the end of the 12 h dissolution test was below 100% for all dosage forms. This may be in part due to the relatively slow release of entrapped drug from the matrices undergoing testing. Among various formulations, M8 was found to have a good release pattern and controlled release up to 12 hours. Hence, M8 was selected as the optimized formulation. From the result it was concluded that the increasing polymer concentration of Eudragit RL100. From above observation it was concluded that the polymer concentration increases duration of release increases.

Stability studies

Stability study was carried out for the Formulation M8 by exposing it to different temperatures at Room temp. (RT) and 40°C and 75% RH for six months. The samples were analyzed for content uniformity at regular intervals of two weeks and it was evident that there was no remarkable change in the drug content of the prepared Microspheres. This indicated that the formulation M8 was stable at above mentioned temperatures. Stability studies showed mild changes in the surface characters and not affected by the drug content and encapsulation efficiency.

Table No.1: Composition of Microspheres

S.No	Formulation	M1	M2	M3	M4	M5	M6	M7	M8	M9
1	Midazolam	10	10	10	10	10	10	10	10	10
2	Eudragit E 100	10	20	30				5	10	15
3	Eudragit RL 100				10	20	30	5	10	15
4	Citric acid	3	3	3	3	3	3	3	3	3
5	Distilled Water(ml)	10	10	10	10	10	10	10	10	10
6	Glutaraldehyde (25 % solution)	1	1	1	1	1	1	1	1	1
7	Light Liquid Paraffin(ml)	50	50	50	50	50	50	50	50	50

*All quantities are in mg

Table No.2: Micromeritic Properties of Microspheres

S.No	Batch No	Angle of Repose Mean ($\theta \pm$ SD)	Drug Content	Total Entrapment Efficiency(% \pm SD)	Degree of Swelling (% \pm SD)
1	M1	22.92' \pm 0.10	10.66 \pm 0.854	82.79 \pm 0.525	0.83 \pm 0.52
2	M2	24.3' \pm 0.22	10.52 \pm 0.684	84.77 \pm 0.349	0.78 \pm 0.12
3	M3	22.77' \pm 0.14	10.10 \pm 0.170	86.01 \pm 1.190	0.86 \pm 0.48
4	M4	23.63' \pm 0.12	10.65 \pm 0.219	83.93 \pm 0.064	0.89 \pm 0.58
5	M5	24.22' \pm 0.21	10.28 \pm 0.262	88.09 \pm 1.290	0.95 \pm 0.42
6	M6	24.02' \pm 0.32	10.12 \pm 0.341	83.47 \pm 1.020	1.12 \pm 0.68
7	M7	24.55' \pm 0.27	10.30 \pm 0.332	83.01 \pm 0.696	1.18 \pm 0.46
8	M8	22.84' \pm 0.21	10.21 \pm 0.132	93.74 \pm 0.544	1.22 \pm 0.54
9	M9	25.17' \pm 0.33	10.34 \pm 0.121	87.51 \pm 0.876	1.29 \pm 0.61

*All the readings were in triplicate measurements

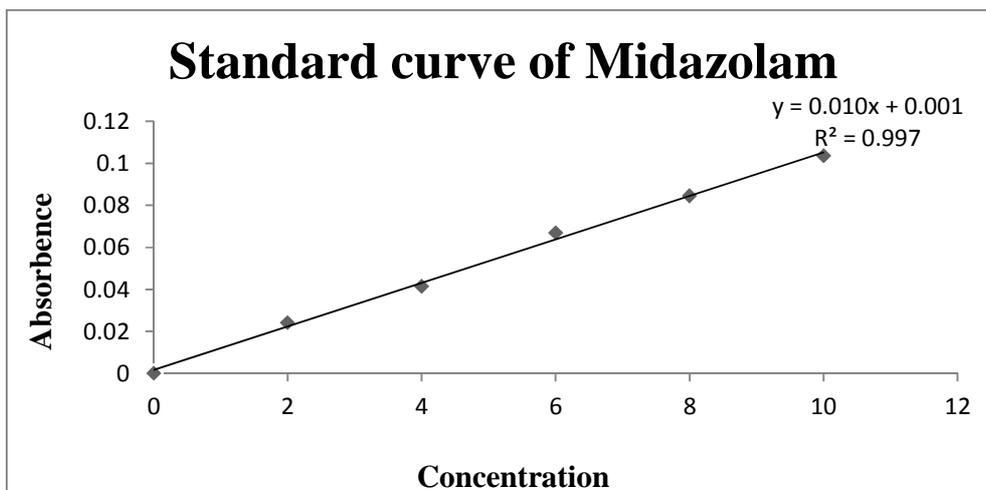


Figure No.1: Standard curve of Midazolam

FTIR studies

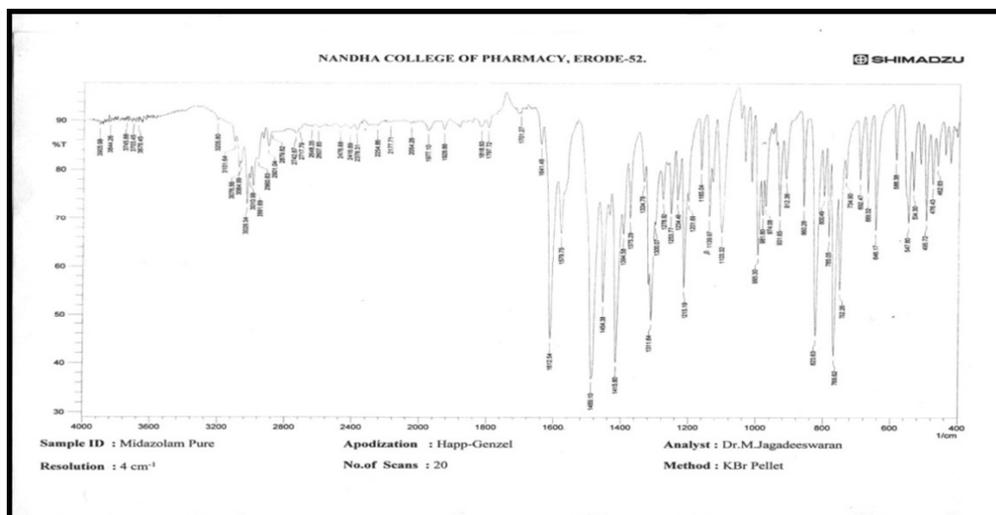


Figure No.2: IR spectrum of midazolam (pure)

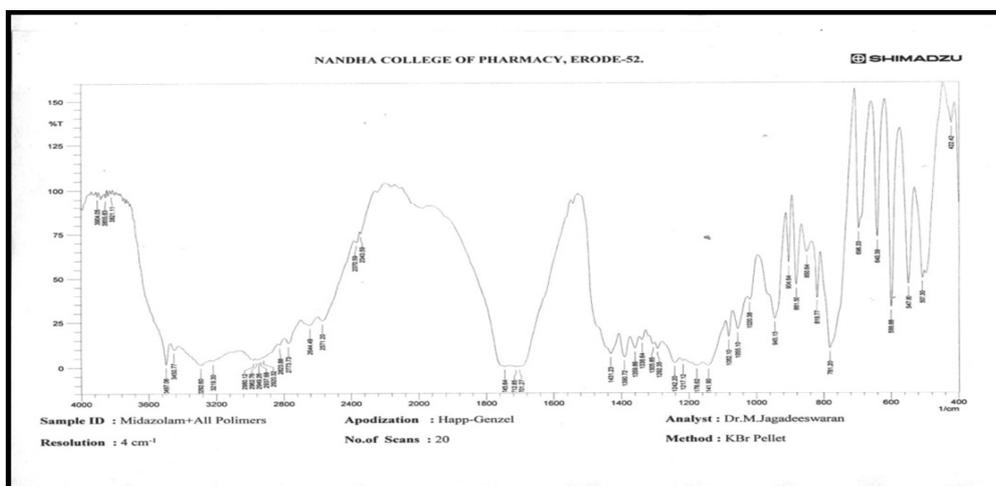


Figure No.3: IR spectrum of midazolam+ all polymers

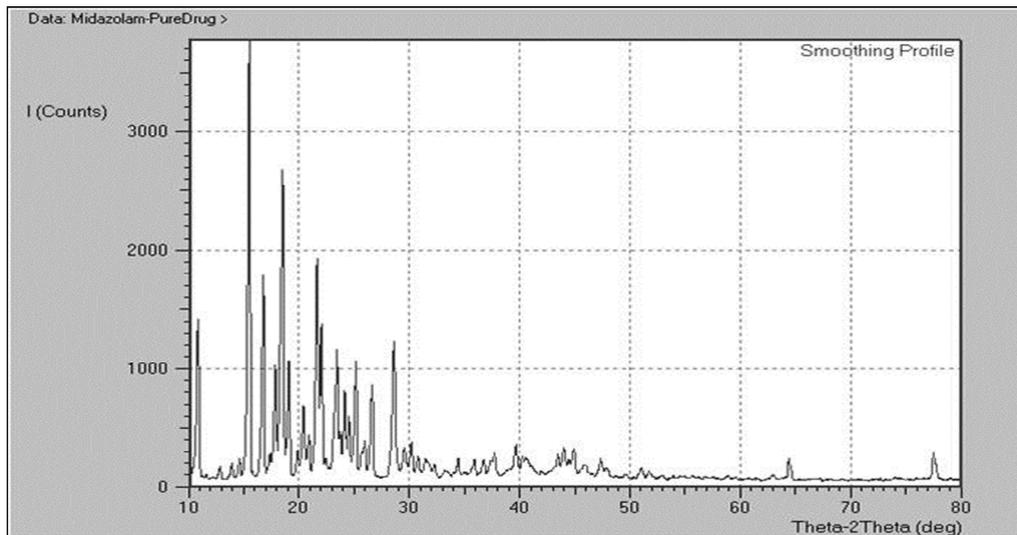


Figure No.4: XRD Spectra of Midazolam-Pure Drug

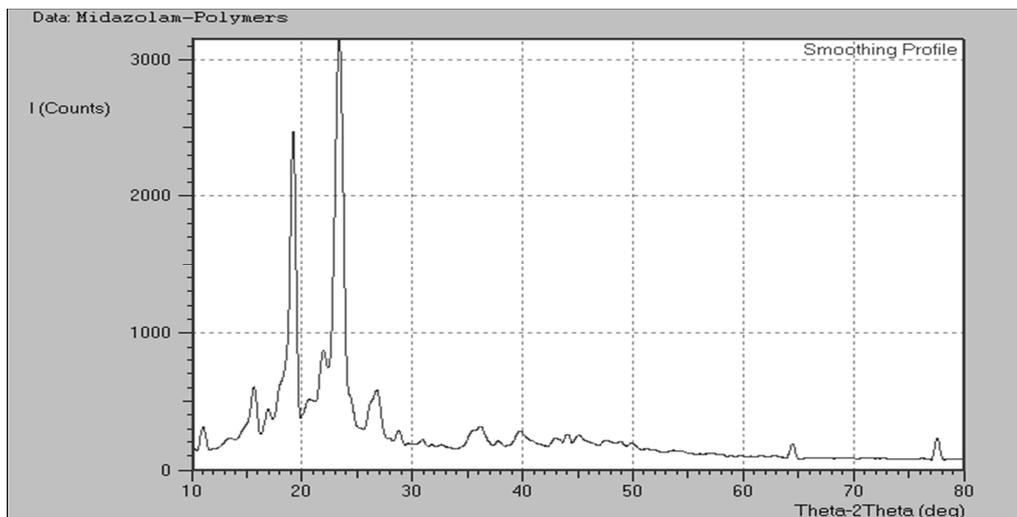


Figure No.5: XRD Spectra of Drug Midazolam and Polymers

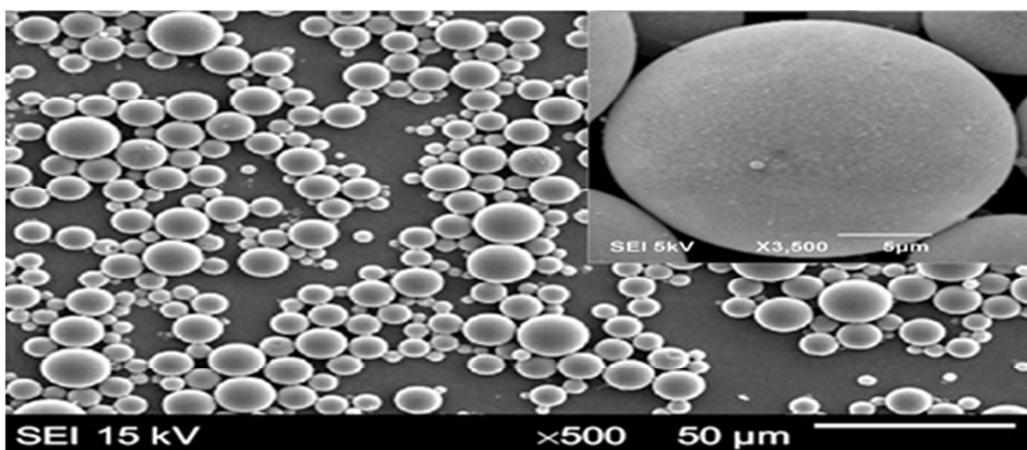


Figure No.6: SEM Photograph of Formulation M8

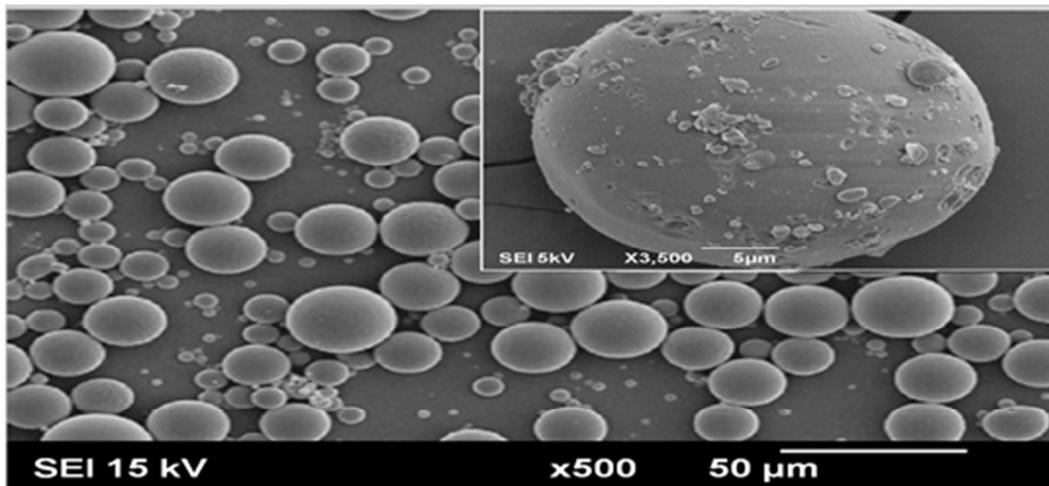


Figure No.7: SEM Photograph of Formulation M9

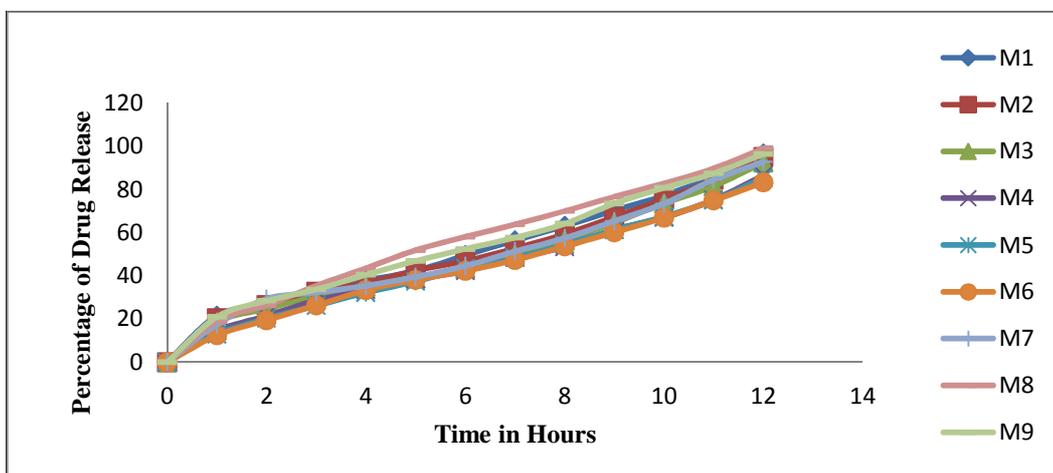


Figure No.8: Drug Release Profile of Batch M1-M9

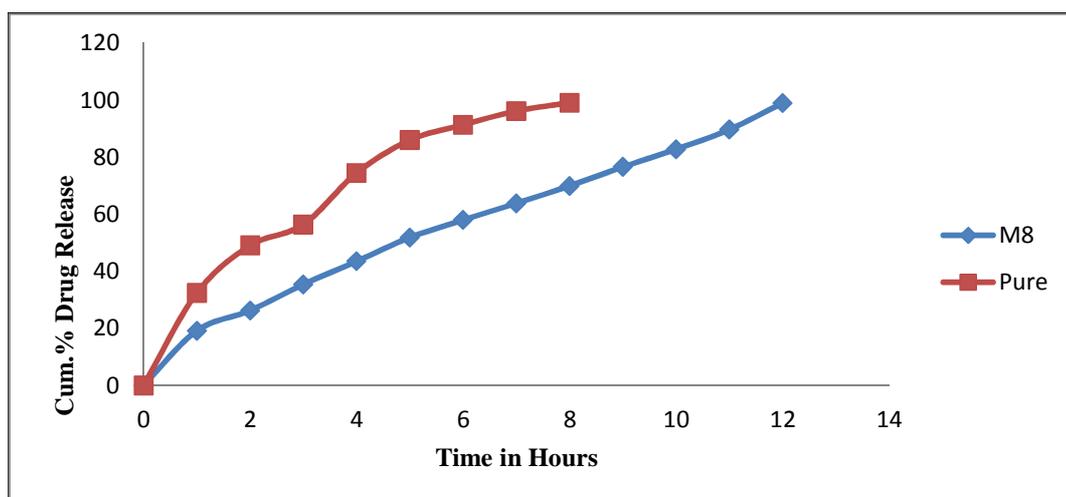


Figure No.9: Comparison of Optimized Batch M8 with Pure drug

CONCLUSION

The emulsion cross linking technique for the entrapment of Midazolam in Eudragit E 100 and Eudragit RL 100 produced a high yield of discrete microsphere with minimal agglomeration, reproducible drug loading efficiency and release profiles from batch to batch. The prepared microspheres showing good micromeritic properties with controlled drug release. Midazolam having pH depended solubility, so that citric acid was incorporated in the formulation. Both the polymers were swellable at mild acidic condition i.e. at the pH of 5.5, thereby the polymers were swell and release the solubilized drug midazolam in a controlled manner, probably as a consequence of prolonged residence at the absorption site, midazolam may ultimately show improvement of bioavailability than conventional oral dosage form. Hence, it can be concluded from the results of present experimental work, that the Midazolam Microspheres are easy to administer, minimize the dose, reduce the side effects and improves the patient compliance and also midazolam might be a right and suitable candidate for controlled drug delivery as Microspheres.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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