

Evaluation and Optimization of Influence of Permeability Property and Concentration of Polymethacrylic Polymers on Microspheres of Metformin HCl

Ikramul Hasan¹, Shovan Paul¹, Sharmin Akhter², Navid Jubaer Ayon¹
and Md. Selim Reza¹

¹Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Dhaka
Dhaka-1000, Bangladesh.

²Department of Pharmacy, University of Asia Pacific, Dhanmondi, Dhaka-1209, Bangladesh

ABSTRACT: Metformin HCl microspheres were prepared with the aim of increasing its bioavailability and decreasing gastrointestinal side effects by means of sustained action. Eudragit RSPO and Eudragit RLPO, polymers of different permeability characteristics were used to prepare different microspheres. Emulsification solvent evaporation technique using acetone as the internal phase and liquid paraffin as the external phase was the method of choice. Six formulations were prepared using two polymers. The effect of drug loading and polymeric property on the surface morphology, entrapment efficiency, particle size and release characteristics of the microspheres were examined. FTIR and DSC studies established compatibility of the drug with the polymers. SEM studies clearly revealed the effect of drug loading and polymeric nature on the surface morphology of the microspheres. Entrapment efficiencies were within 77.09-97.11% and particle size of all the batches were in the acceptable range. Release data were treated with different mathematical kinetic models. The drug release profile showed that Eudragit RSPO and Eudragit RLPO have opposite effect on drug release. On the other hand, increase in drug loading results in increased drug release. Kinetic modeling of *in vitro* dissolution profiles revealed that the drug release mechanism varies from diffusion controlled to anomalous type.

Key words: Emulsification solvent evaporation, Metformin HCl, Microsphere, Eudragit RSPO, Eudragit RLPO.

INTRODUCTION

Microencapsulation is the application of a thin coating to individual core materials that have an arbitrary particle size range between 1 and 1000 μm .^{1,2} In pharmaceutical sciences, microencapsulation is widely used to mask tastes or odors, prolong release, impart stability to drug molecules, improve bioavailability, and as multi-particulate dosage forms to produce controlled or targeted drug delivery.³⁻⁵ Thus, it is therefore a rapidly expanding technology for achieving sustained-release dosage forms.

Microencapsulation can be used to retard the release of a drug in the body. This may permit one to control the release of dose to substitute several doses of non-encapsulated drug and also may decrease toxic side effects for some drugs by preventing high initial concentrations in the blood. There is usually a certain desired release pattern. In some cases, it is zero-order, i.e. the release rate is constant. In this case, the microcapsules deliver a fixed amount of drug per minute or per hour during the period of their effectiveness. This can occur as long as a solid reservoir or dissolving drug is maintained in the microcapsule.

Metformin HCl is an oral antidiabetic drug of the biguanide class. It is the first-line drug of choice for the treatment of type II diabetes, particularly, in

Correspondence to: Md. Selim Reza
Tel: +880-2-8612069, Fax: +880-28615583
E-mail: selim.du@gmail.com

overweight and obese people and those with normal kidney function.⁶⁻⁸ Its use in gestational diabetes has been limited by safety concerns. It is also used in the treatment of polycystic ovary syndrome^{9,10} and has been investigated for other diseases where insulin resistance may be an important factor.¹¹ Metformin HCl works by suppressing glucose production by the liver.

Metformin HCl is a BCS class III drug and like other drugs of this group it shows lower level of absorption. The absolute bioavailability of a Metformin HCl 500 mg tablet given under fasting conditions is approximately 50 to 60%. Studies using single oral doses of Metformin HCl 500 mg to 1500 mg, and 850 mg to 2550 mg, indicate that there is a lack of dose proportionality with increasing doses, which is due to decreased absorption rather than an alteration in elimination.¹²

Among the different approaches used for obtaining sustained-release delivery systems, microspheres' system appears as one of the most efficient systems because of less local gastric interference and most importantly limited risk of dose dumping.

Use of different types of methacrylic resins (Eudragit) for controlled drug delivery appears particularly attractive due to their high chemical stability properties.¹³ Eudragit RLPO and RSPO are pH-independent polymers, inert to the digestive tract, impermeable to water, as well as capable of swelling and release active ingredients by diffusion.¹⁴ As a result microspheres of Metformin HCl using Eudragit RSPO or Eudragit RLPO will give a time dependent controlled release which will ultimately potentiate the pharmacologic efficacy of the drug.¹

MATERIALS AND METHODS

Metformin HCl was collected as a gift sample from Beximco Pharmaceuticals Limited, Bangladesh. All other ingredients were procured from local market.

Preparation of Metformin HCl microspheres.¹⁶ Microspheres containing highly water-soluble Metformin HCl were prepared by non-

aqueous emulsion solvent evaporation method using two different (poly-trimethylammonioethyl methacrylate) copolymers: Eudragit RLPO and RSPO, having different permeability characteristics. Weighed quantities of Eudragit RLPO or RSPO were completely dissolved in 20 ml acetone. Weighted quantity of Metformin HCl was then added and stirred using a overhead stirrer. The volume ratios of oil₁: oil₂ phases were typically 20:80. The drug polymer mixture was then slowly introduced into 100 ml liquid paraffin previously emulsified with 1% Span 80, while stirring at 1500 rpm held by the mechanical stirrer equipped with a three-blade propeller at room temperature. The whole system was stirred for 3 hrs. The prepared microspheres were kept undisturbed for few minutes to allow settling. The settled microspheres are then washed several times with n-hexane. After serial washing, microspheres were dried in an oven at room temperature not exceeding 25°C.

Formulation design. Total six batches (each of 2g) were prepared, three using eudragit RSPO and the other three using eudragit RLPO. These two groups of microspheres were prepared in three degrees of drug loading (30, 50 and 70%).

Table 1. Formulation protocol of different batches of microspheres.

Batch no. (Batch size- 2.0g)	Drug		Polymer	
	Metformin HCl (g)	Eudragit RLPO (g)	Eudragit RLPO (g)	Eudragit RSPO (g)
A 1 (30% Drug + 70% Polymer)	0.6	1.4	0	0
A 2 (50% Drug + 50% Polymer)	1.0	1.0	0	0
A 3 (70% Drug + 30% Polymer)	1.4	0.6	0	0
B 1 (30% Drug + 70% Polymer)	0.6	0	0	1.4
B 2 (50% Drug + 50% Polymer)	1.0	0	0	1.0
B 3 (70% Drug + 30% Polymer)	1.4	0	0	0.6

***In vitro* characterization of Metformin HCl microspheres:**

Determination of drug entrapment efficiency.

50 mg of microspheres was taken in a mortar and triturated properly until fine powder was formed. 20 mg fine powder was taken in a screw cap test tube. 5 ml buffer was added to the powdered microsphere and was vortexed for 10 minutes. Then the solution was filtered and taken in a 100 ml volumetric flask. The volume of the solution was adjusted to 100 ml with the buffer solution. The absorbance was taken at 233 nm. From the absorbance value, the amount of Metformin HCl entrapped was determined using standard curve. The drug entrapment efficiency was calculated by using the following equation-

$$\text{Entrapment efficiency (\%)} = \frac{\text{Calculated conc. of drug}}{\text{Theoretical conc. of drug}} \times 100$$

Particle size analysis. Particle size analysis was conducted in a laser diffracting particle size analyzer (Partica®). The microcapsules were dispersed in suitable solvent keeping the particle diameter unchanged.

***In vitro* dissolution study of microsphere containing Metformin HCl.** *In vitro* dissolution study was performed in a paddle type (Type II) dissolution apparatus. Weighed amount of microspheres containing 100 mg drug was taken from each batch of formulation for dissolution purpose. Phosphate buffer of pH 6.8 was used as dissolution media, paddle speed was set at 100 rpm and temperature was maintained fixed at 37°C.

The dissolution process was carried out for 10 hours and 5 ml dissolution sample from each dissolution media was withdrawn at a predetermined intervals of 1st hour, 2nd hour, 3rd hour, 4th hour, 5th hour, 6th hour, 7th hour, 8th hour, 9th hour and 10th hour. Each time 5 ml of dissolution sample was compensated by fresh 5 ml phosphate buffer (pH 6.8).

Dissolution samples withdrawn were filtered and taken in test tubes and analyzed spectrophotometrically in a UV-VIS spectrophotometer. The dissolution study for each batch was performed three

times. The average of the percentage of release was calculated for each batch to find percentage of release.

Surface morphologic Study by scanning Electron Microscopy (SEM). Scanning electron microscopic study was employed to study the morphology and surface topography of the microspheres. The microspheres were mounted on the SEM sample stab (aluminum stabs) which were coated with a double-sided sticking tape, sealed and finally coated with gold (200 Å) under reduced pressure (0.001 torr) for 15 minutes using an ion sputtering device. The platinum coated samples were scanned using scanning electron microscope (S-3400N, Hitachi) under varying magnifications and photomicrographs of suitable magnifications were obtained. The microspheres were dried completely before examination.

Compatibility Studies:

Fourier transform infrared spectrophotometry (FTIR). The IR spectrum of the pure drug, pure polymers and optimized microsphere formulation were obtained to prove the chemical integrity and compatibility of the drug in the microsphere. The samples (about 5 mg) were powdered and intimately mixed with 250 mg of pure dry powdered potassium bromide (KBr) and the mixture was pressed into a disc using special mold and hydraulic press. The mixtures were taken in a diffuse reflectance sampler and spectrum was recorded by scanning in the wavelength region of 400 to 4000 cm⁻¹ in a FTIR spectrophotometer.

Differential scanning calorimetry (DSC). Differential scanning calorimeter was used to measure the specific heat and enthalpies of transition. Samples, sealed in an aluminum pan in a nitrogen atmosphere went through a thermal transition, the power to the heater was adjusted to maintain the temperature and a signal proportional to the power difference was plotted on the second axis of the recorder known as thermogram. The area under the resulting curve is direct measure of the heat of transition. Thermograms were obtained by using a

differential scanning calorimeter (Shimadzu DSC 60) at a flow rate of 20ml/min and heating rate of 10°C/min over a temperature range of 30 to 300°C.

RESULTS AND DISCUSSION

SEM analysis. SEM study revealed that the microspheres prepared from Eudragit RLPO and eudragit RSPO are generally spherical with few drug particle on the surface. The surfaces of the microspheres prepared by using Eudragit RSPO are smoother than the microspheres prepared using Eudragit RLPO. This may be one of the reasons of higher release retarding capability of Eudragit RSPO than Eudragit RLPO.

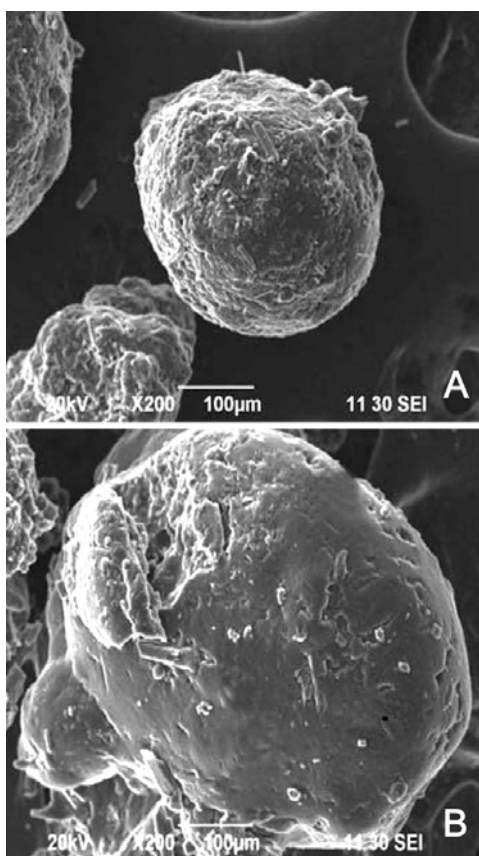


Figure 1. Scanning electron microscopic view of microspheres A. Batch-A2 (Microspheres prepared with Eudragit RLPO) B. Batch- B2 (Microspheres prepared with Eudragit RSPO).

Particle size analysis. Particle size of microspheres of all batches was within the acceptable size ranges in micrometer (44.70µm - 491.91 µm).

Table 2. Particle size distribution of all the batches of microspheres.

Batch ID	Mean size (µm)	Median size (µm)
A1	44.70	28.39
A2	206.44	194.94
A3	491.91	478.18
B1	56.11	49.01
B2	59.95	50.44
B3	82.69	75.95

Drug entrapment efficiency. Eudragit RSPO showed better incorporation efficiency than Eudragit RLPO. On the other hand, drug loading has direct effect on entrapment efficiency. Higher the drug loading higher is the entrapment efficiency.

Table 3. Entrapment efficiency (%) of all the batches of microspheres.

	Sample ID	Drug loading (%)	Entrapment efficiency (%)
Microspheres of Eudragit RLPO	A1	30	77.09
	A2	50	82.91
	A3	70	90.12
Microspheres of Eudragit RSPO	B1	30	88.55
	B2	50	92.4
	B3	70	97.11

***In vitro* dissolution study of microsphere containing Metformin HCl:**

Effect of drug loading and polymeric ratio on release pattern of Metformin HCl microsphere. Microspheres of three different drug loading (30%, 50%, 70%) using Eudragit RSPO and Eudragit RLPO were examined for dissolution pattern. The obtained data were subjected to various kinetic treatments for investigating their release pattern.

From the above release data it is clearly revealed that A1, A2 and A3 which were microspheres of Eudragit RLPO showed lower release retardation than the microspheres of Eudragit RSPO (B1, B2 and B3). After 10 hours almost all the drug of Eudragit RLPO microspheres were released; on the other hand for B1, B2 and B3 only 54.2, 67.3 and 69.78 % drug were released.

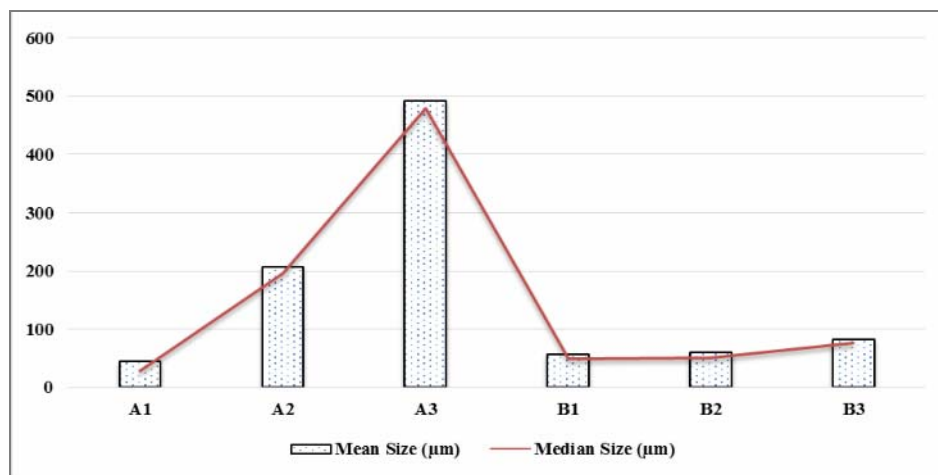


Figure 2. Bar diagram of particle size distribution.

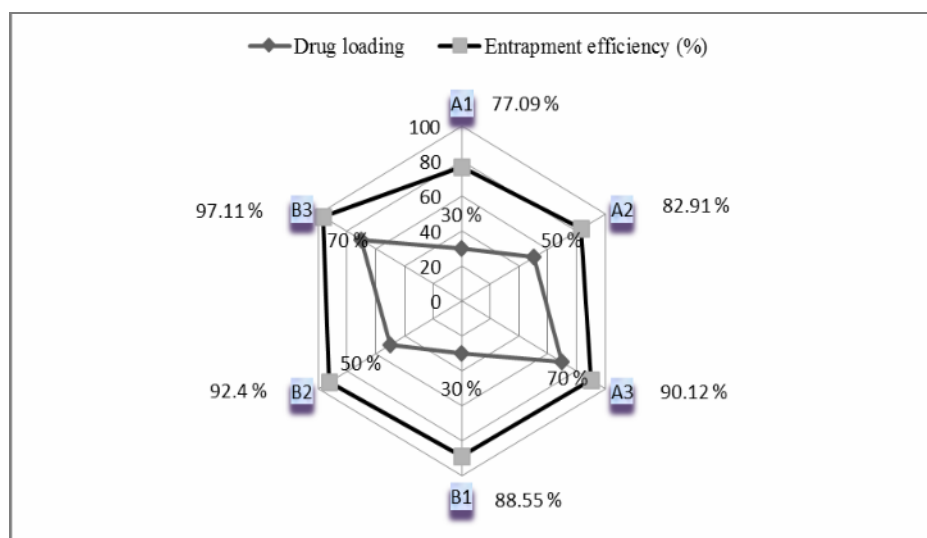


Figure 3. Effect of drug loading and polymeric type on entrapment efficiency.

Table 4. Zero order release profile of A1, A2, A3, B1, B2, and B3.

Time (hrs)	Cumulative % of drug released					
	A1	A2	A3	B1	B2	B3
0	0	0	0	0	0	0
1	50.6	61.6	67.5	12.3	17.5	20.98
2	77.2	80.4	87.8	16.6	23.9	28.5
3	85.3	89.8	92.5	21.5	29.7	37.4
4	88.2	93.3	97.4	26.43	35.4	42.8
5	91.38	95.5	99.5	32.2	40.7	46.4
6	94.5	97.5	99.74	37.8	45.9	53.3
7	97.4	99.3	99.89	43.9	51.5	57.66
8	98.97	99.87	99.9	47.8	54.96	62.21
9	99.38	99.92	99.91	50.4	61.5	65.45
10	99.87	99.93	99.92	54.2	67.3	69.78

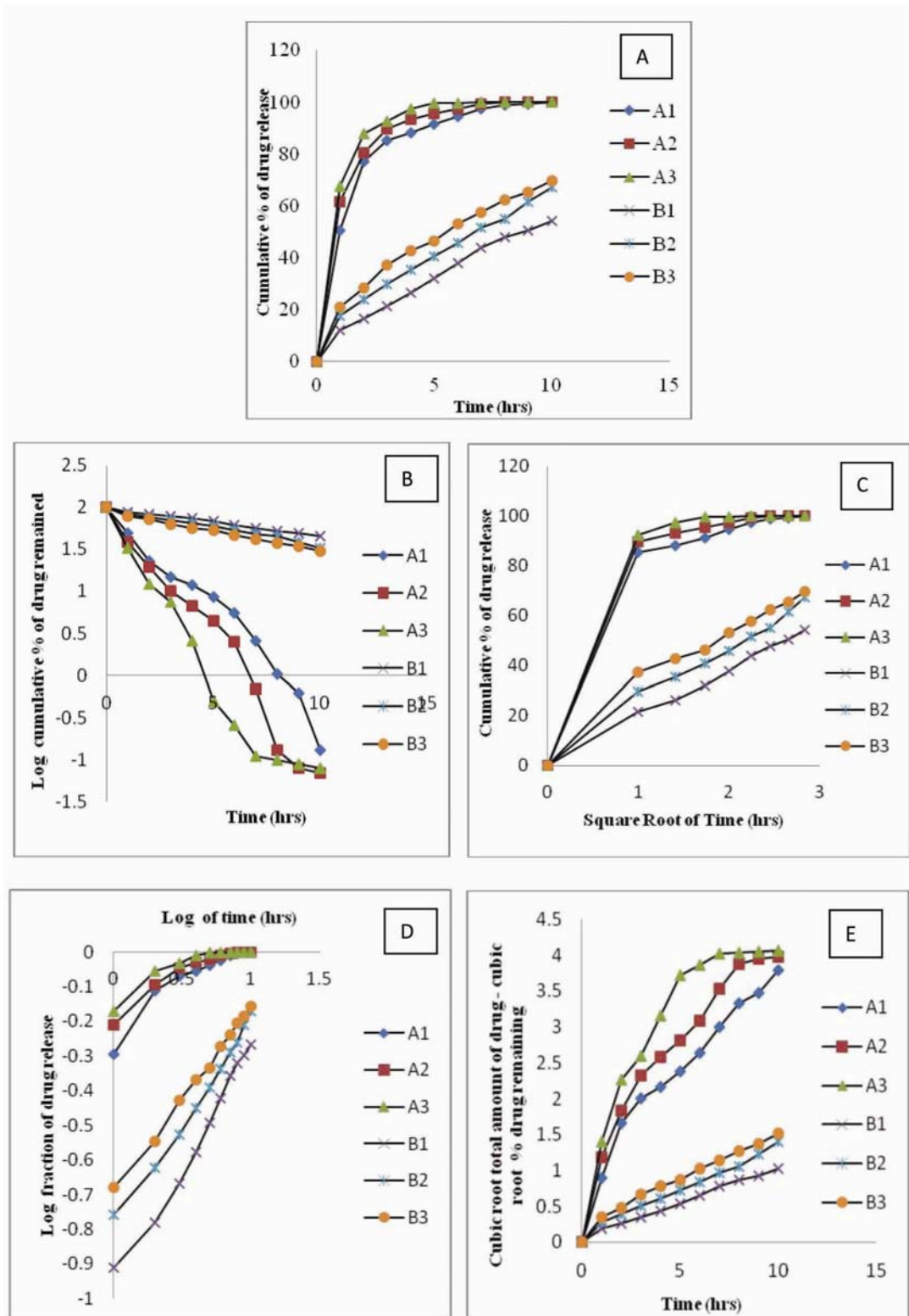


Figure 4. *In vitro* release kinetics of Metformin HCl microspheres; A. Zero order plot, B. First order plot, C. Higuchi plot, D. Korsmeyer plot, E. Hixson Crowell plot

Table 5. Release rate constants and R² values for different release kinetics of six formulations of Metformin HCl microspheres.

Formulations	Zero order		First order		Higuchi		Korsmeyer-Peppas			Hixson- Crowell	
	K ₀	R ²	K ₁	R ²	K _h	R ²	n	K _{kp}	R ²	K _{HC}	R ²
A1	8.146	0,714	-0.57	0.976	31.14	0.901	0.379	0.479	0.941	0.349	0.952
A2	7.368	0.647	-0.73	0.975	28.86	0.858	0.29	0.579	0.941	0.379	0.934
A3	6.579	0.564	-0.77	0.971	26.5	0.791	0.22	0.67	0.93	0.383	0.853
B1	5.179	0.985	-0.07	0.994	17.56	0.973	0.61	0.125	0.986	0.098	0.993
B2	5.984	0.976	-0.09	0.987	20.44	0.985	0.52	0.183	0.988	0.125	0.99
B3	6.242	0.949	-0.10	0.991	21.77	0.997	0.48	0.222	0.995	0.138	0.983

Table 6. The best fitted model and mechanism of drug release from A1, A2, A3, B1, B2 and B3 batches of Metformin HCl microsphere.¹⁷⁻¹⁹

Formulation	Best fitted model	n value	Release mechanism
A1	First order	0.379	Fickian transport
A2	First order	0.29	Fickian transport
A3	First Order	0.22	Fickian transport
B1	Zero order, First order, Korsmeyer-Peppas and Hixson-Crowell	0.61	Anomalous / non- Fickian transport
B2	Zero order, First order, Higuchi, Korsmeyer-Peppas and Hixson-Crowell	0.52	Anomalous / non- Fickian transport
B3	First order, Higuchi, Korsmeyer-Peppas and Hixson-Crowell	0.48	Anomalous / non- Fickian transport

Table 7. Successive fractional dissolution time of six formulations (A1, A2, A3, B1, B2 and B3) Metformin HCl microsphere.

Batch no.	T _{25%} (min)	T _{50%} (min)	T _{80%} (min)	MDT (min)
A1	10.79058	67.1922	232.217	114.9921
A2	3.18306	35.92476	185.8234	90.17196
A3	0.62526	15.4776	136.3651	68.05824
B1	186.5672	580.1222	1251.973	684.147
B2	109.3218	414.5822	1023.644	537.8663
B3	76.72848	322.2606	852.7386	440.8225

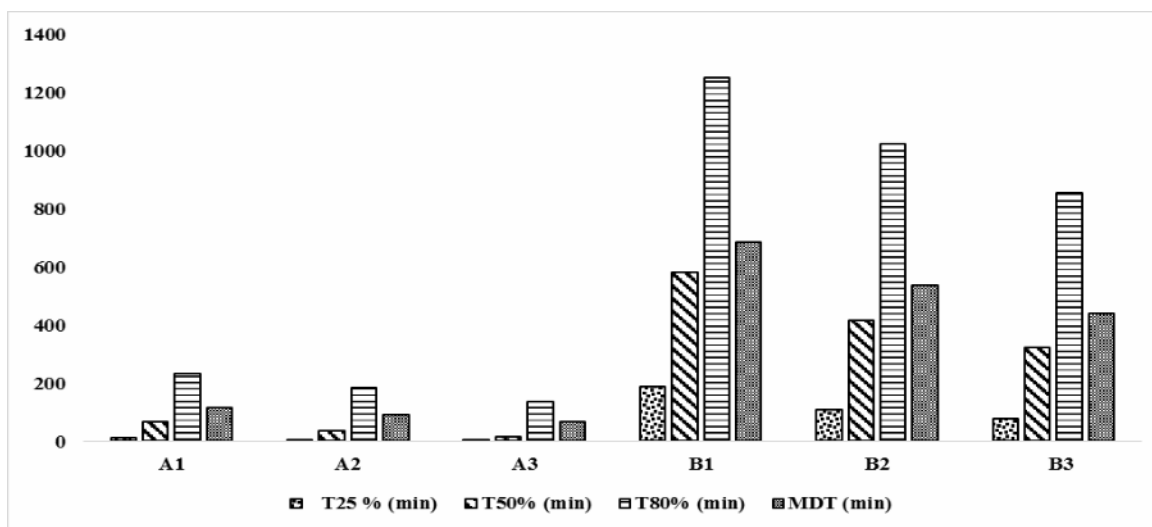


Figure 5. Bar diagram representing successive dissolution time of A1, A2, A3, B1, B2 and B3.

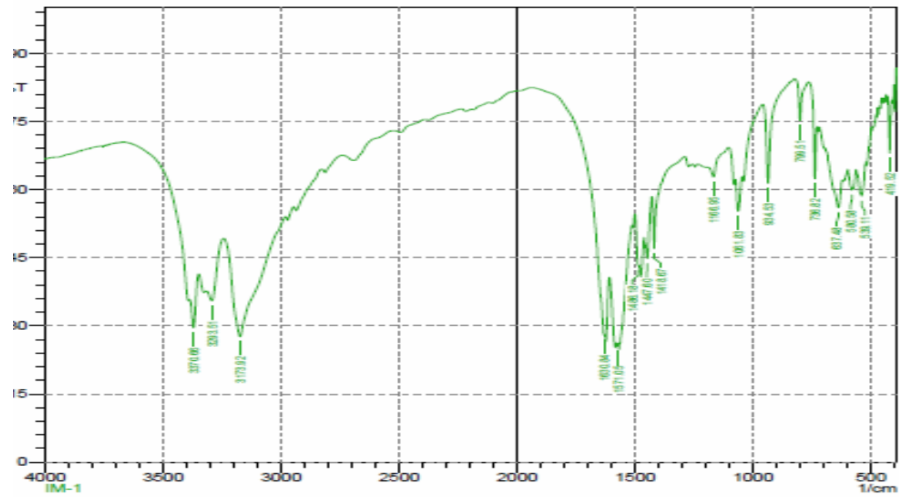


Figure 6. FTIR of pure metformin HCl.

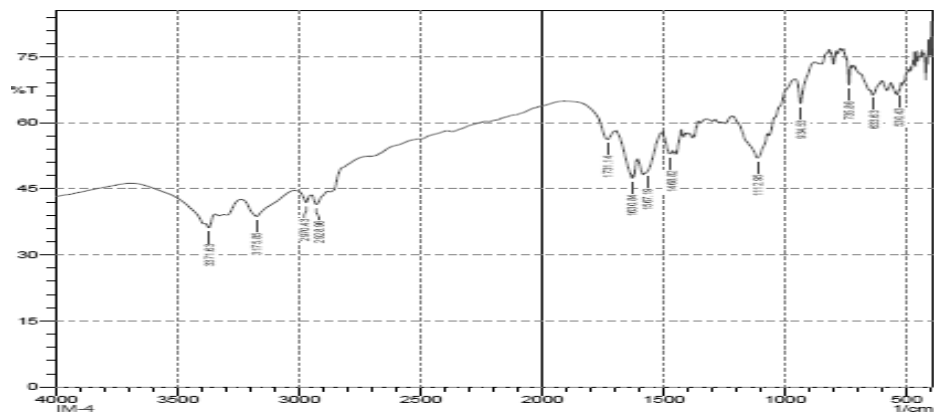


Figure 7. FTIR of A2 (Metformin HCl + Eudragit RLPO).

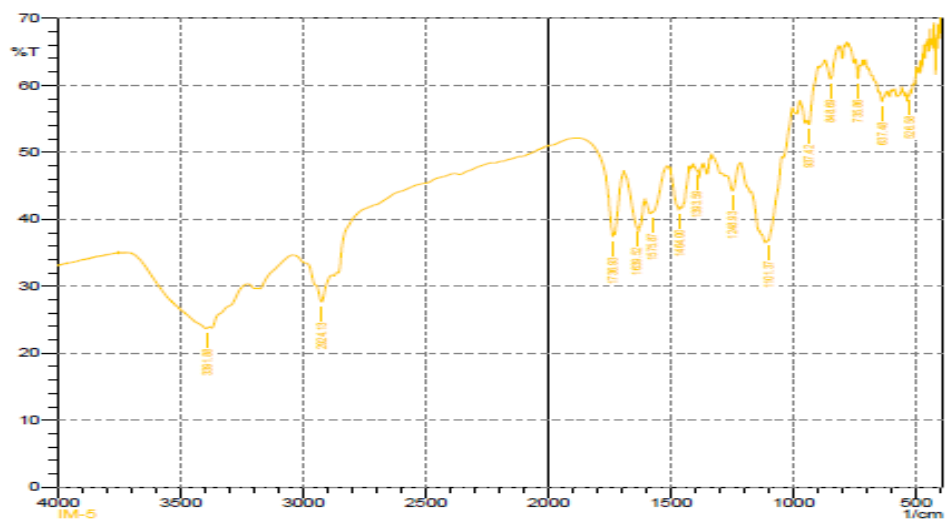


Figure 8. FTIR of B2 (Metformin HCl + Eudragit RSPO).

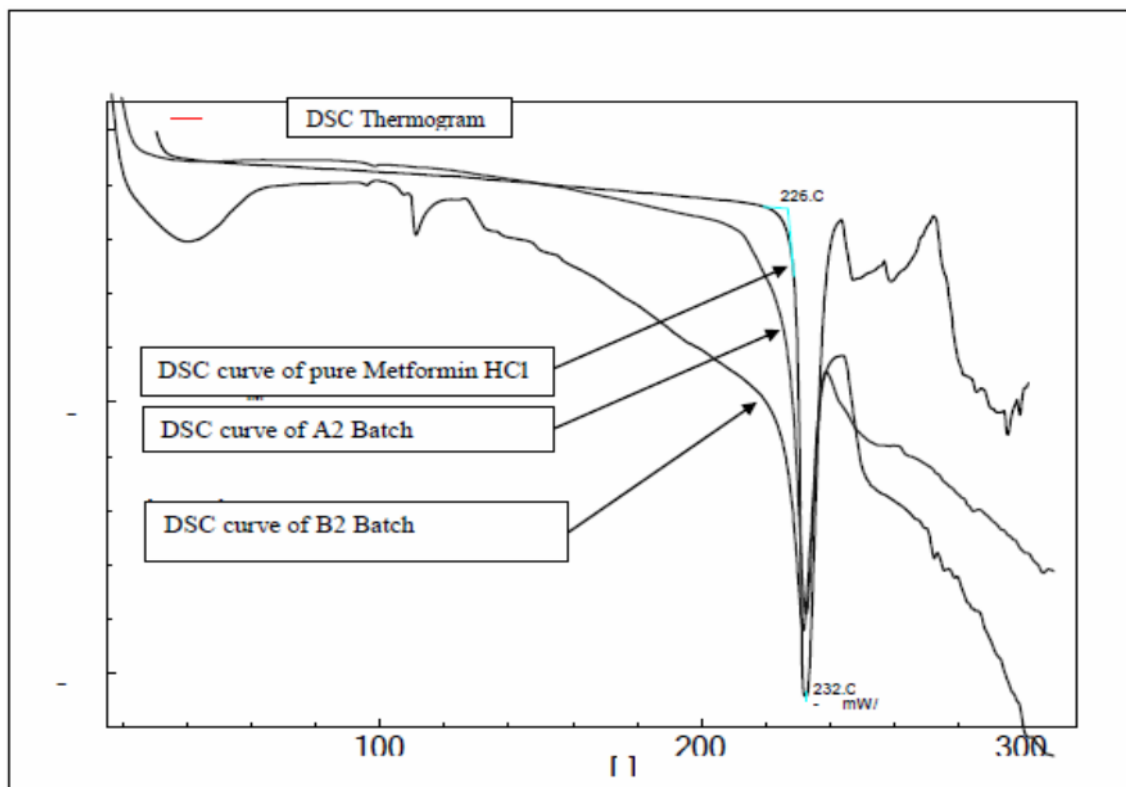


Figure 9. DSC thermogram of pure Metformin HCl and two batches of microspheres.

Successive fractional dissolution time.

Successive fractional dissolution times of six formulations (A1, A2, A3, B1, B2 and B3) of Metformin HCl microsphere are discussed below. $T_{25\%}$, $T_{50\%}$, $T_{80\%}$ and MDT values were determined to characterize the drug release rate from the microspheres and the retaining efficiency of the polymers. A higher value of MDT indicates a higher drug retaining ability of the polymer and *vice-versa*.

Drug compatibility studies:

Fourier Transform Infrared Spectroscopy (FTIR) Study. FTIR study was conducted for the following three samples:

1. Pure drug (Metformin HCl)
2. Sample A2 (Metformin HCl microsphere containing Eudragit RLPO)
3. Sample B2 (Metformin HCl microsphere containing Eudragit RSPO)

A lot of peaks are visible in this spectra but the most prominent peaks are-

- 1) Primary amine N-H stretching at 3370.66 and bending at 1630.84
- 2) Imino compound N-H stretching at 3293.51 and bending at 1571.05
- 3) Secondary amine N-H stretching at 3173.92 and bending at 1571.0

Figure 7 represents spectra of batch A2, a batch of microspheres prepared by using Eudragit RLPO. Secondary amine N-H bending and stretching both are present here. N-H stretching of imino compound and N-H bending of primary amine are absent here, but most importantly N-H bending of imino compound and N-H stretching of primary amine are present at 1578.19 and 3371.63, respectively, which are valid indication of the presence of these two groups.

Figure 8 represents spectra of batch B2, a batch prepared by using Eudragit RSPO. Secondary amine N-H bending is present at 1575.87 though it's

stretching peak is absent. N-H stretching of imino compound and N-H bending of primary amine are present here at 3291.88 and 1639.52, respectively, which are valid indication of the presence of these two groups, in other words indication of minimum interaction.

So, it can be concluded that there is minimum interaction between Metformin HCl and Eudragit RSPO and eudragit RLPO, in other word they are compatible to each other.

Differential Scanning Calorimetric (DSC) study. DSC studies were conducted for the following samples-

- 1) Metformin HCl
- 2) Microspheres of batch A2 (Metformin HCl microspheres containing eudragit RLPO)
- 3) Microspheres of batch B2 (Metformin HCl microspheres containing eudragit RSPO)

No drastic changes occurred to the melting point of the microspheres in comparison with pure Metformin HCl. This means that there is no interaction in between drug and the polymers.

CONCLUSION

Microspheres of Metformin HCl were prepared successfully by emulsification-solvent evaporation technique; in fact it may be the premium technique for encapsulation of Metformin HCl. The prepared microspheres are of good quality as all the microspheres possess spherical shape and the particle size of all the batches are within the acceptable range (1-1000 μm).

Various variables like - drug loading and polymeric type all have direct effect on different characteristics of microsphere.

Microspheres prepared by using Eudragit RSPO showed highest entrapment efficiency and highest retardation of drug release when compared to the microspheres of Eudragit RLPO. Drug loading also influenced entrapment efficiency as well as release rate. The entrapment efficiency and drug release both increase with increase in drug loading.

The other parameters like surface morphology or particle size are also influenced by drug loading and polymeric properties. This however, reveals a newer approach to formulate Metformin HCl microspheres having optimized *in vitro* characteristics

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