



## CONTROLLED RELEASE FORMULATION AND EVALUATION OF IDARUBICIN MICROSPHERE USING BIODEGRADABLE HYDROPHILIC AND HYDROPHOBIC POLYMER MIXTURES

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### ABSTRACT

The purpose of this study was to characterize and optimize the different proportion of polymer ratios, stirring speed on microspheres produced by the solvent evaporation method of idarubicin, an anticancer drug. Proportions of ethyl cellulose and hpmc were subjected to measurement of morphology, mean particle size, particle size distribution, percentage drug entrapment, drug loading and drug release (*in vitro*). By increasing the concentration of ethyl cellulose the mean particle size also increased and the probability for the ratios 10: 1 showed insignificant. This indicates that it will be significant to provide the concentration of ethyl cellulose not more than 8 parts. With change in the speed of rotation, there is an influence on the size of the microspheres that varied with increase in size with decrease in rotation and was in the range between  $134.0 \pm 9.0$  to  $424.7 \pm 11.2$   $\mu\text{m}$  for 1000 rpm and 500 rpm respectively. But the entrapment capacity did not show much significant change in change in speed of rotation falling between 54% and 62%. The release of idarubicin microspheres showed Higuchi's square root model. This model showed that the idarubicin can be attempted to maintain sustained release and reducing the side effects produced by the drug.

**Key words:** Microsphere, Ethyl cellulose, HPMC, Solvent evaporation method, Idarubicin.

### INTRODUCTION

Controlled release dosage form cover a wide range of prolonged action formulation, which provide continuous release of their ingredients at a predetermined time. One such approach is using polymeric microsphere as carriers of drugs. The shell of encapsulating products provides a barrier between reactive components<sup>1</sup>. Microsphere of biodegradable and non-biodegradable polymers has been investigated depending upon final application. Biodegradable polymers have many advantages that they degrade in biological fluids. They can be injected, implanted and inserted into the body, they are non toxic and surgical removal of the polymer skeleton is not required. Therefore microspheres using various kinds of biodegradable polymers are designed to degrade as a result of hydrolysis of the polymer chains into biologically acceptable and progressively smaller compound.

Idarubicin (4-demethoxydaunorubicin) is a synthetic daunorubicin analog that lacks the methoxyl group in position 4 of the aglycone of the parent compound used for the treatment of acute myeloid leukemia (AML) in adults This analog was significantly more effective than daunorubicin or doxorubicin against certain experimental mouse leukemias<sup>2, 3, 4, 5</sup> and was also less cardio toxic than daunorubicin or doxorubicin<sup>6</sup> Phase I studies in leukemia patients using a 3-day schedule recommended a dose of 8 to 12 mg/m<sup>2</sup>/d<sup>7, 8, 9</sup> and the significant antileukemic activity observed in those studies was later confirmed in phase II trials of pediatric and adult patients with AML<sup>10</sup>. They are available in the form of powder for solution and as intravenous injection. Owing to high degree of toxicity and side effects, we have selected this drug for controlled released using biodegradable polymers.

Considering the need for a controlled release of idarubicin, an anticancer drug, we made an attempt to formulate microsphere loaded idarubicin using mixture of biodegradable polymers by varying the speed of rotation and evaluating the effect of speed of rotation on the physical parameters as well on the release pattern.

### MATERIALS AND METHODS

#### Materials

Ethyl cellulose and HPMC 4KM was obtained from E Merck India Ltd (Mumbai, India), Idarubicin was purchased from Pfizer India Ltd (Mumbai, India). Dichloro methane, ethanol was purchased from Himedia Chemicals (Mumbai, India). All other materials used in the dissolution studies were of analytical reagent grade and were used as received.

#### Preparation of microsphere

Idarubicin microspheres were prepared by solvent evaporation method as described elsewhere<sup>[11]</sup>. Briefly, different amount of ethyl cellulose was dissolved in 30 mL of mixture of dichloro methane and ethanol in the ratio 1:1 by using a magnetic stirrer. To this the required proportion of HPMC 4KM was added and continued stirring until a uniform dispersion was ensured. The proportion of ethyl cellulose: HPMC varied to produce a total weight of 150 mg. The required quantity of idarubicin (20mg) was kept constant and was dispersed in the polymer mixture. 200 ml of water containing 0.01% tween 80 was prepared. The polymeric solution was allowed to flow through a hypodermic needle no. 22 drop wise into the aqueous mixture under two different speed of rotation stirred at 500 rpm and 1000 rpm using a mechanical stirrer with 4 blade paddle. Stirring was continued at room temperature for 1 hour, until the solvent mixture evaporated completely. After evaporation of the solvent mixture the microspheres formed were filtered through 0.45  $\mu\text{m}$  membrane filter and dried at room temperature overnight until no weight loss was observed. All the batches were prepared in triplicate.

#### Scanning electron microscopy

The shape and surface morphology of the dried microsphere was examined by scanning electron microscope (JOEL, JSM- 6360, and Tokyo, Japan). Prior to examination samples were gold coated under vacuum (JOEL Fine Coat, Ion Sputter, JFC-1100) using a 10.0 - KV accelerating voltage, a 30 mm working distance, a 50  $\mu\text{m}$  objective aperture and a probe current of  $6 \times 10^{-11}$  amps. The object was exposed to two different magnifications to show the nature of the surface of the microspheres. The samples after 10 hours dissolution was also magnified at two different magnification to show the formation of pores and the nature of the release of the drug from the matrix.

#### Encapsulation efficiency

Drug loaded microsphere (100 mg) were powdered and dissolved in methylene chloride and then sonicated for about 30 minutes. The mixture was filtered through 0.45  $\mu\text{m}$  membrane filter (MILLIPORE). The drug content was determined by using Spectrofluorimeter keeping excitation wavelength 470 nm and emission wavelength 580 nm. The entrapment efficiency was calculated using the formula  $EE (\%) = 100 \times (\text{actual drug content} / \text{theoretical drug content})$ . All the batches were prepared in triplicate.

**Table 1: Encapsulation efficiency, percent entrapment and particle size distribution of idarubicin microsphere at various speed of rotation**

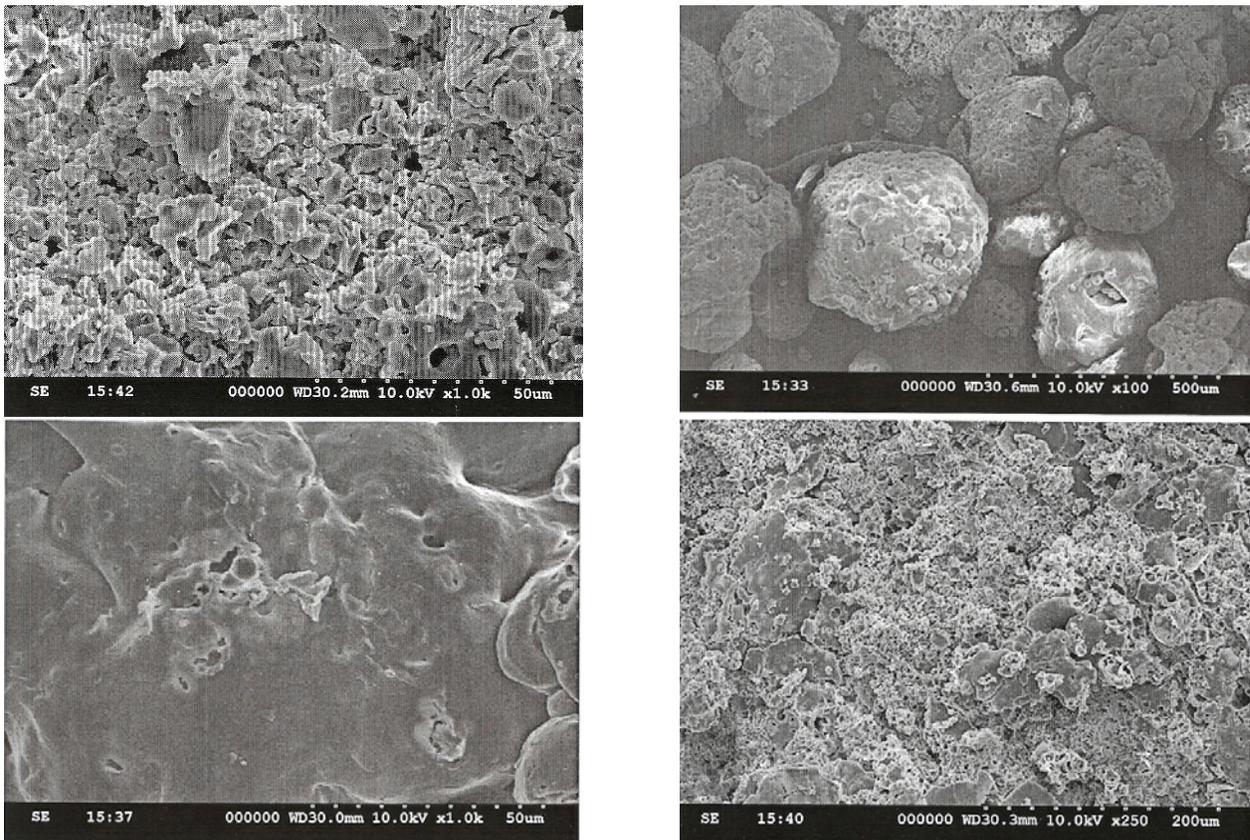
Formulation Ethyl cellulose: HPMC	Percentage yield		Percent entrapment ± SD		Particle size distribution ± SD (µm)	
	500 rpm	1000 rpm	500 rpm	1000 rpm	500 rpm	1000 rpm
2 : 1	59.7± 4.5	54.0 ± 4.6	49 ± 3.6	65 ± 4.5	173.7 ± 12.9	134.0 ± 9.0
4 : 1	53.7± 5.7	59.0 ± 5.6	42 ± 2.5	43 ± 5.7	213.3 ± 14.6	186.7 ± 13.6
6 : 1	52.3±12.7	55.3 ± 9.3	55 ± 3.0	45 ± 5.0	273.0 ± 08.0	228.3 ± 9.1
8 : 1	61.7± 6.0	54.3 ± 4.5	62 ± 2.6	45 ± 5.1	315.3 ± 12.5	288.3 ± 9.5
10 : 1	58.7 ± 7.8	60.3 ± 3.5	44 ± 4.0	46 ± 7.0	424.7 ± 11.2	341.3 ± 10.9

All the experiments were conducted in triplicate n=3, Data are shown as mean ± standard deviation

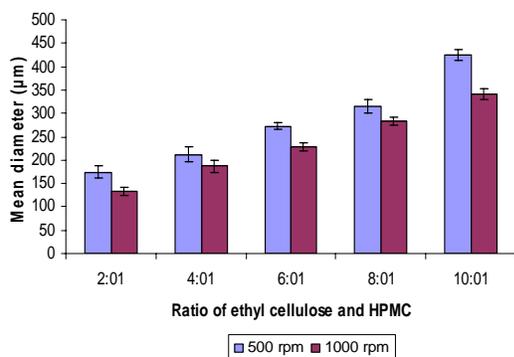
**Table 2: Idarubicin released from microspheres in phosphate buffer pH 7.4 at 500 and 1000 rpm in (%)**

Time (Hrs)	500 rpm					1000 rpm				
	Ethyl cellulose : HPMC									
	2 : 1	4 : 1	6 : 1	8 : 1	10 : 1	2 : 1	4 : 1	6 : 1	8 : 1	10 : 1
0.5	29.01± 3.37	33.81± 4.15	31.4± 4.84	33.50± 3.51	29.33± 1.42	38.0± 3.10	28.58± 2.0	23.86± 3.08	28.68± 1.69	25.16± 2.66
1.0	54.80± 6.70	47.87± 2.03	41.2± 6.62	38.05± 6.21	34.61± 2.59	57.18± 2.31	47.32± 1.72	44.79± 1.88	42.99± 1.85	35.39± 2.65
2.0	73.80± 6.0	59.23± 2.00	58.2± 5.05	51.83± 4.26	43.36± 4.45	71.70± 2.11	61.40± 1.55	60.70± 3.0	57.66± 2.69	49.37± 2.08
4.0	93.45± 2.72	74.33± 2.42	63.9± 5.06	57.42± 5.88	49.64± 4.16	92.61± 2.63	82.95± 1.78	74.67± 2.97	67.67± 2.76	59.31± 3.65
6.0		82.49± 3.05	73.2± 6.14	64.75± 5.16	58.10± 2.76		96.90± 2.04	81.97± 1.59	72.22± 3.16	66.94± 2.02
8.0		94.96± 5.08	81.9± 7.15	74.93± 3.00	65.96± 5.72			94.26± 2.04	81.90± 1.90	76.65± .051
10.0			91.3± 3.53	82.74± 4.41	75.69± 3.00				94.54± 1.13	91.55± 1.63
12.0				93.45± 2.34	91.01± 2.88					

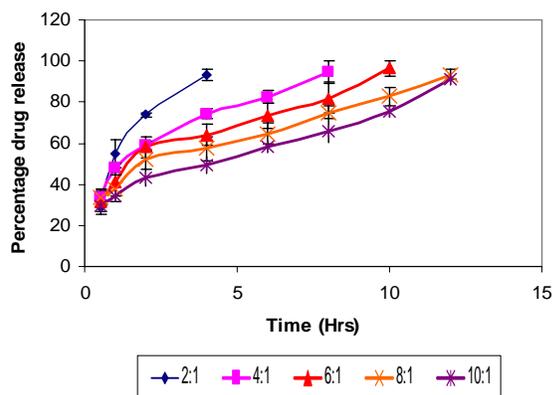
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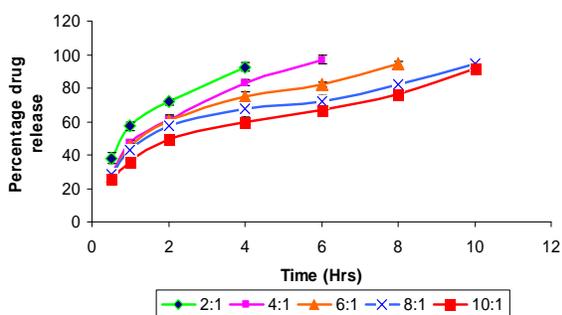
**Figure 1:** Microphotographs showing the shape and surface morphology of the microspheres immediately and after 10 hours dissolution at two different magnifications



**Figure 2:** Effect of speed of rotation on the mean particle size of the microspheres



**Figure 3:** Dissolution profile of idarubicin microsphere at 500 rpm in phosphate buffer pH 7.4



**Figure 4:** Dissolution of idarubicin microsphere in phosphate buffer pH 7.8 at 1000 rpm

#### Particle size distribution

Particle size analysis of the prepared microsphere was measured by using optical microscopy (Olympus CHT - 001, NY) observing around 500 particles. Each batch was analyzed in triplicate.

#### In vitro drug release studies

The in vitro release study of microsphere was carried out using USP XXVI rotating basket method at 50 rpm at  $37 \pm 0.05^\circ\text{C}$ . Dissolution study was performed in 0.2 M phosphate buffer pH 7.4 taking 900 ml for each study. 50 mg of the microsphere was placed in the dissolution medium and 5 ml of the test sample were taken from the medium at predetermined time interval over a period of 10 hours, replacing equivalent volume of fresh buffer. The samples were analysed for the drug content determined by Spectrofluorimeter keeping excitation wavelength 470 nm and emission wavelength 580 nm. The release studies were conducted in triplicate.

## RESULTS

It was shown that microsphere prepared in this study at stirring rates of 500 and 1000 rpm were spherical with smooth surfaces (Figure 1). The effect of the release of the drug from the matrix at different time on dissolution shows that pores are formed for the release. The effect of stirring rate on the particle size of the microsphere is shown in (Table 1, Figure 2). It can be seen that by increasing the rate of stirring from 500 to 1000 rpm, the mean particle size of the microspheres decreased. This is expected because high stirring rate provide the shearing force needed to separate the oil phase into smaller droplets.

By increasing the concentration of ethyl cellulose, the mean particle size of microsphere increased. This observation may be attributed to an increase in the viscosity of the dispersed phase, making the coalescence of emulsified droplet easier<sup>12</sup>.

The entrapment efficiency of idarubicin microsphere prepared in this study was shown to be approximately 65%. There was not much effect on the entrapment efficiency with the variation in the speed of rotation.

The percentage yield of various microspheres was found to fall between 54% and 62%. There was not much effect seen on the percentage yield on the variation in the speed of rotation.

It can be seen that increase in the speed of rotation shows reduction in the particle size. It can be seen that the particle size does not affect the rate of drug release from the microspheres with low drug loading (42%). Lower drug content create fewer pores within the polymeric network, hence lower rate of drug diffusion is seen

The drug is released from microsphere of different particle sizes (Table 2, Figure 3 and 4) at different rpm. It is shown that drug release is affected by particle size. In case of smaller microspheres produced at 1000 rpm, surface area is increased and a higher number of drug molecules at the surface of microspheres are ready for faster release<sup>13</sup>. It is seen that by increasing the amount of drug loading, a point will be reached when the solid drug particles will begin to form continuous pores or channels within the matrix. At this nature, the release of the drug molecules will diffuse within the pores formed from areas where the drug has previously diffused out from the matrix, because of the matrix becoming more porous and faster release rate of the drug is seen.

## DISCUSSION

In order to develop a controlled release of idarubicin microspheres, combination of different ratios of biodegradable polymers were used and the influence of these combinations were observed for different evaluation aspects. Microspheres were prepared using a gradual increase in ethyl cellulose concentration in combination with a fixed concentration of HPMC 4KM to assess the effect of polymer concentration on the sizes of microspheres. The mean particle size of the microspheres significantly increased with increasing ethyl cellulose ( $p < 0.05$ ) and was in the range  $134.0 \pm 9.0$  to  $424.7 \pm 11.2 \mu\text{m}$  (Table 1, Figure 2). The viscosity of the medium increases at a higher polymer concentration resulting in enhanced interfacial tension. Shearing efficiency is also diminished at high viscosities<sup>14,15</sup>. This resulted in the formation of larger particles.

Encapsulation efficiency was used as a primary criterion in evaluating the quality of the microspheres. The encapsulation efficiency results from the preferential encapsulation of aqueous drops by polymer drops.

The influence of different concentration of polymer is due to its effect on viscosity and solidification rate of the polymer phase. The increased viscosity of the polymer solution as the concentration increases delayed the drug diffusion through the polymeric membrane. Once the polymer solution solidified, the encapsulated drugs do not easily escape from the polymer and thus the encapsulation efficiency remains sufficiently high, approximately 65%<sup>16,17</sup>.

To observe the effect of agitation speed on the size of the resulting microsphere formulation were prepared at two different speed of

rotation. The speed of rotation was found to decrease the mean particle size with increasing the speed, but the increase was not statistically significant. This may be due to the reason that speed of rotation may not be able to break the bulk of the polymer into fine droplets<sup>18</sup>.

*In vitro* release of the prepared microspheres were performed in phosphate buffer pH 7.8 over a period of 12 Hrs. the release of idarubicin increased with increase in ethyl cellulose ( $p < 0.05$ ). The initial burst release from the matrix is attributed to the surface deposited idarubicin as revealed by SEM photomicrograph (Figure 1), while the subsequent release of the drug from the polymer matrix is due to decrease erosion and diffusion of the drug from the matrix. The increased density of the polymer matrix at higher concentration results in an increased diffusional path length. This may decrease the overall drug release from the polymer matrix (Figure 3, 4). Further, smaller microspheres are formed at lower polymer concentration and have a larger surface area exposed to dissolution medium giving rise to faster drug release. The speed of rotation produced different mean particle size, as the speed of rotation increased produces smaller size particle, but the difference in the drug release was not statistically significant (Figure 2).

The data obtained for *in vitro* release was fitted into equations for zero, first and Higuchi release models<sup>19, 20, 21</sup>. Three graph were plotted (i) percentage of drug release Vs time, (ii) log percentage of drug release Vs time, and (iii) percentage of drug release Vs square root of time. The values of "r<sup>2</sup> square" for the three plots were 0.9370, 0.9320 and 0.9932 respectively. The *in vitro* release showed the highest regression coefficient values for Higuchi's model, indicating diffusion to be the predominant mechanism of drug release.

## CONCLUSION

Sustained release idarubicin microspheres were successfully produced using solvent evaporation method by varying the speed of rotation. The *in vitro* release study showed that the release profile could be controlled by the composition of the polymer phase. The intactness of the microspheres showed the integrity of the formulation. The microspheres of different size and drug content are obtained by varying the speed of rotation. Diffusion was found to be the main release mechanism. Thus, further studies on *in vivo* release and correlation with *in vitro* will prove that the attempt for the controlled release of idarubicin will be achieved successfully.

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