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# Preparation and Evaluation of Gentamicin Biodegradable Polymeric Microspheres

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Gentamicin sulphate is an antibiotic used widely in the treatment of several bacterial infections. It is not absorbed from the GIT and controlled release parenteral formulation of the same will reduce the frequency of infections, better therapeutic efficacy and patient compliance. Chitosan is used as a biodegradable polymer, because the biopolymer is non-toxic, biodegradable, inexpensive and widely used in various medicaments. During the preparation various parameters such as stirring rate, temperature and the concentration of emulsifying agent were varied and found that as the stirring rate, temperature and concentration increase the particle size decrease. The drug content of all the formulations were analysed and ranged between 39.50 – 44.00 mg Chitosan microspheres respectively. The release data was fitted to mathematical models, such as korsmeyer and peppas, and higuchi. The release pattern of all the formulations was observed to be in a biphasic manner characterized by a burst effect followed by a slow release. In vitro drug release studies of the formulations were carried out in a pH 7.4 phosphate buffer at 37°C and cumulative % release of drug at different time interval was determined. The drug release was found to be prolonged releasing up to 80-100% of the drug within 12 hours. The release rate constant of all the formulations ranged from 0.39307 / $hr^{-1}$ to 0.5683 / $hr^{-1}$. The 'n' values of all the formulations were not greater than 0.5 indicating fickian diffusion. From the SEM, it was found that Chitosan microspheres were spherical. Hence the proposed method may be used as an alternative method for making controlled release formulation of gentamicin sulphate for parenteral use.

**Key words**: Microspheres, Gentamicin, Citosan.

## INTRODUCTION

Gentamicin sulphate is an important member of the amino glycoside class of antibiotics, widely used in the treatment of gram positive and gram-negative aerobic bacterial infections. Gentamicin is available as 80 mg/2ml and it is administered at a dose of 1-1 7 mg/kg given $6^{th}$ or $8^{th}$ hourly by IM/IV usually for 7-10 days. Controlled drug delivery system will have the advantage of decrease frequency of administration, to achieve better therapeutic efficiency and to improve patience compliance. This can be achieved by preparing the drug in the form of microspheres for parenteral use, whereby drug release is prolonged.

The use of Chitosan in controlled release is attractive because the biopolymer is non-toxic, biodegradable, inexpensive and widely used with various medicaments [1]. In the present study, water in oil emulsion technique was used in the preparation of microspheres. Water in oil technique has been widely used in the pharmaceutical technology field with different applications, one of which being the preparation of microparticulate drug delivery systems.

The objective of this research work was to formulate gentamicin microspheres using Chitosan for parenteral delivery and to study the various factors affecting drug content, particle size distribution, surface morphology, *and In vitro* release studies.

## MATERIALS AND METHODS

### Materials:

Gentamicin was gift from Karnataka Antibiotic and Pharmaceutical Limited. (KAPL, Bangalore). Chitosan was obtained from Fisheries Research Institute (Trivandrum Kerala). All chemicals and reagents not specified in the text were of analytical grade.

### Methods:

#### A. Preparation of Chitosan Microspheres [2, 3]

Microspheres containing gentamicin were also prepared by water in oil emulsion method. Gentamicin (50mg) was added to a

Chitosan solution (200 mg in 10ml of water). The solution was stirred for 1 hr. at 37°C and once the gentamicin was totally dissolved, the solution was preheated to 80°C and added to 60ml of liquid paraffin previously warmed to the same temperature. This two phase system, plus 1 ml of Tween 85 (1.4% v/v), was stirred under turbulent flow conditions (700 rpm) to form a w/o emulsion, using a mechanical stirrer. After 5 min of continuous stirring, the emulsion was rapidly cooled to 5°C and 100 ml of acetone added in order to dehydrate and flocculate the coacervated particles. Chitosan microspheres were than isolated by filtering the suspension through a sintered glass filter. The procedure was repeated by varying the various parameters such as stirring rate, temperature and the concentration of emulsifying agent as shown in the table 2

**B. Estimation of drug content**:

Fifty milligram of Microspheres were taken and crushed in 25ml of water and left for 24 hr. filtered and filtrate was estimated by using UV spectrophotometer at 356nm. The drug content of all the formulations is given in table 1

**C. Particle Size and Particle Size distribution [4]:**

The particle size and particle size distribution of the prepared formulations were analysed by an optical microscopic method (Allen. 1981, Martin, et. al., 1983). The particle diameters of more than 100 microspheres were measured at random by microscopic observation. The average particle size was determined using the Edmundson (1967) equation.

$$d\ mean = \frac{\sum nd}{\sum n}$$

Where n = No. of microspheres observed, d = Mean size range.

**D. *In vitro* release studies[5]**:

Microspheres equivalent to 50mg of drug were weighed and transferred to a flask containing 100 ml of 7.4 pH buffer. The flask was placed on a shaking water bath (37±1°C). Aliquots of samples of 5ml were withdrawn at predetermined time intervals for a period of 12 h. and replaced with equal volumes of fresh fluid. The withdrawn samples were measured for the drug content, spectrophotometrically at 356 nm after suitable dilution. The percentage release from the formulations at various time intervals analysed the release and the curve fitting data is shown in figure 1, figure 2 and table 3.

PHOTOGRAPH NO 1

A TYPICAL PHOTOGRAPH OF SEC 2 FORMULATION

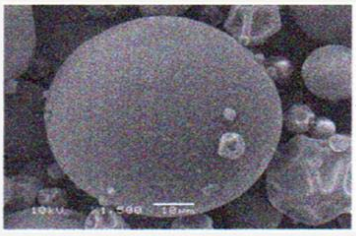


**TABLE 2: EFFECT OF STIRRING RATE, PERCENTAGE OF EMULSIFYING AGENT AND TEMPERATURE CHITOSAN MICROSPHERES**

| F/C | EC % v/v | SR (rpm) | Amt. of water (ml) | Temp | Partide Size (µm) Chitosan |
|---|---|---|---|---|---|
| SEC1 | 1.4% | 300 | 10 | 80°C | 30.35 |
| SEC2 | 1.4% | 500 | 10 | 80°C | 27.50 |
| SEC3 | 1.4% | 700 | 10 | 80°C | 24.70 |
| SEC4 | 1.4% | 300 | 10 | 70°C | 34.50 |
| SEC5 | 1.4% | 500 | 10 | 70°C | 27.70 |
| SEC6 | 1.4% | 700 | 10 | 70°C | 21.80 |
| SEC7 | 2.7% | 300 | 10 | 80°C | 20.35 |
| SEC8 | 2.7% | 500 | 10 | 80°C | 19.38 |
| SEC9 | 2.7% | 700 | 10 | 80°C | 18.60 |
| SEC10 | 2.7% | 300 | 10 | 70°C | 21.10 |
| SEC11 | 2.7% | 500 | 10 | 70°C | 20.2 |
| SEC12/SEC12 | 2.7% | 700 | 10 | 70°C | 19.45 |

FC - Formulation code

EC - Emulsifier Concentration

SR - Stirring rate

TABLE 3: CURVE FITTING DATA OF THE RELEASE RATE PROFILE

| PEPPAS MODEL | SEC1 | SEC2 | SEC3 | SEC4 | SEC5 | SEC6 | SEC7 | SEC8 | SEC9 | SEC10 | SEC11 | SEC12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| K | 0.42278 | 0.39307 | 0.43606 | 0.4258 | 0.40198 | 0.47211 | 0.50542 | 0.43361 | 0.5521 | 0.4736 | 0.5267 | 0.5683 |
| n. | 0.5059 | 0.5605 | 0.4835 | 0.4975 | 0.5084 | 0.424 | 0.4147 | 0.5511 | 0.3877 | 0.5605 | 0.4697 | 0.4343 |
| $r.^2$ | 0.9921 | 0.9765 | 0.9929 | 0.9816 | 0.9917 | 0.9852 | 0.9828 | 0.9653 | 0.9763 | 0.9904 | 0.9983 | 0.9703 |
| HIGUCHI MODEL | | | | | | | | | | | | |
| $r.^2$ | 0.9884 | 0.9780 | 0.9870 | 0.9749 | 0.9892 | 0.9574 | 0.9393 | 0.9653 | 0.8783 | 0.9834 | 0.9920 | 0.9216 |

**Morphological analysis [6, 7]:**

Scanning electron microscopy was carried out on all batches of microspheres by Jeol. JSM – 5600 LV 15KV acceleration voltage, with $1x10^{-9}$ probe current, 1000 - 5000 magnifications. Samples were analysed after they had been gold sputtered (25nm gold film thickness). The SEM of all the formulations shown in Photograph 1.

## RESULT AND DISCUSSION

Since Gentamicin Sulphate is not absorbed from GIT microspheres of gentamicin Sulphate were prepared. Drug content of all the formulation ranged 39.50 – 44.00 mg (Table1)

The mean particle size of microspheres was found to be in the range of 18.85 µm to 33.90 µm. It can be observed that (Table 2) as the stirring rate increases from 300-700 rpm the particle size decreases, as the temperature increases form 70° C – 80° C the particle size decreases , as the emulsifier concentration increases from 1.4% - 2.7% v/ v there was decrease in particle size.

From the SEM, it was found that microspheres were spherical. As it can be seen from photograph 1 there were no important differences in the shape of the microspheres, those made with different parameter, all the microspheres having smooth surface (a typical photograph is shown ) and might have had more drug close to the surface which was released at once.

The release pattern of all the formulations was observed to be in a biphasic manner characterized by a burst effect followed by a slow release. The burst effect corresponds to the release of the drug located on or near the surface of the microspheres or release of poorly entrapped drug. The slow release period may be due to the medium being diffused into the polymer matrix, whereby degradation occurs and the drug diffusing out of the microspheres.

To obtain the values of the release rate constant and in order to understand the release mechanism, the release data was fitted to mathematical models, such as Korsmeyer and Peppas, and Higuchi.

Kosmeyer and Peppas release model: [4]

In order to determine the model which will represent a best fit for the prepared formulation, the dissolution data as analysed using the Peppas and Korsenmeyer equation.

$M_t/M_\infty = Kt^n$

where Mt is the amount of drug released at tim 't' and M∞ is the amount of drug released at time t=∞. Thus, Mt/M∞ is the fraction of dose released at time t, k is kinetic constant and n is the diffusional exponent

Higuchi reease Model [4]

To study the Higuchi release kinetics the release data are fitted to the following equation

**FIGURE 1**

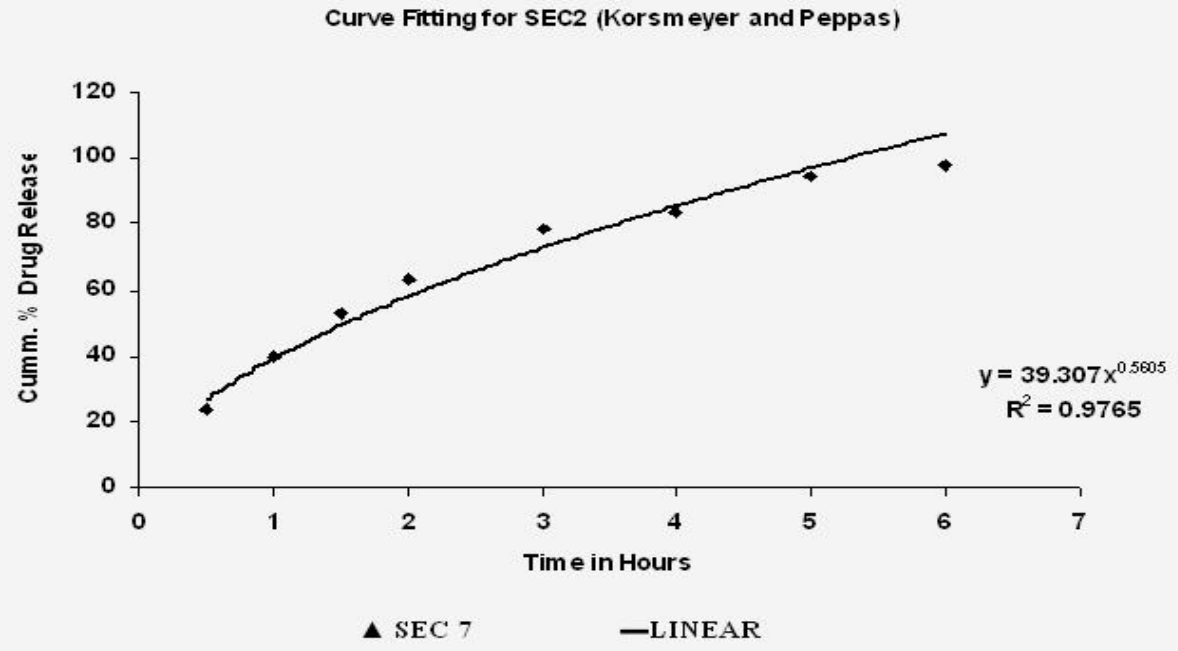
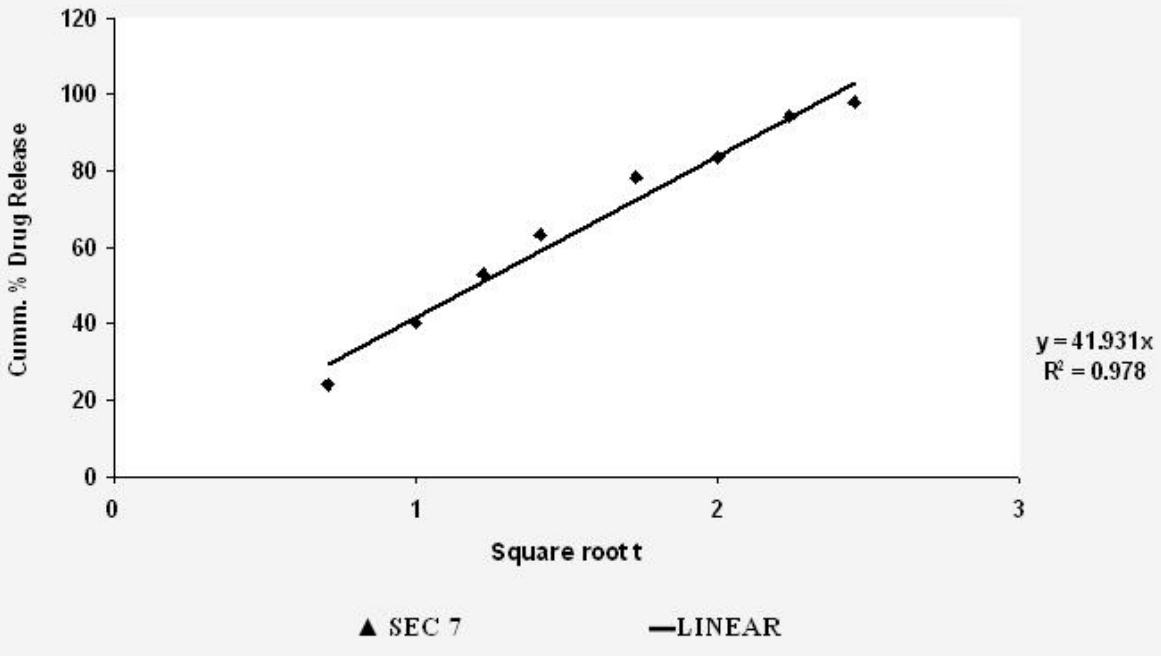



**FIGURE 2**



$F = K. t^{1/2}$

Where 'F' is the amount of drug release, 'K' is the release rate constant and 't' is the release time.

The linear regression analysis is summarized in Table 3 the co-efficient of correlation ($r^2$) was about0.9653 to 0.9983. The release rate constant of all the formulation ranged from 0.39307/$hr^{-1}$ to 0.56803/$hr^{-1}$. The n value of all the formulation were not greater then 0.5 indicating a fickian diffusion. This is conformed by Higuchi plots. Among all the formulation SEC 2 were considered to be best.

## CONCLUSION

The current study aimed to formulate an ideal polymeric microsphere by using gentamicin sulphate as drug with chitosan as polymer was found to successful in terms of Morphological and its physicochemical properties. The release rate constant of all the formulations ranged from 0.39307 / $hr^{-1}$ to 0.5683 / $hr^{-1}$. The 'n' values of all the formulations were not greater than 0.5 indicating fickian diffusion. SEM was observed the Chitosan microspheres were spherical. This method may be used as an alternative method for making controlled release formulation of gentamicin sulphate for parenteral use.

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