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## Formulation and *In vitro* evaluation of centchroman-loaded biodegradable microspheres

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Centchroman, a non-steroidal contraceptive was formulated as biodegradable microspheres using alginate, chitosan, albumin and poly (lactic-co-glycolic) acid polymers. They were evaluated for physico-chemical characteristics, *in vitro* release profile in phosphate buffered saline (pH 7.4) and stability on storage at different conditions. All the formulations exhibited a prolonged drug release with significant ( $P < 0.05$ ) increase in stability at specified conditions of storage. It was observed that PLGA microsphere was the best biodegradable carrier for Centchroman amongst the others studied.

**C**ENTCHROMAN, a non-steroidal contraceptive, has several advantages over the existing hormonal contraceptives<sup>1</sup>. A number of implantable drug delivery systems have been reported for a variety of contraceptive drugs<sup>2</sup>. The present investigation examines microspheres of centchroman with some long acting, biodegradable carriers namely, alginate,<sup>3,4</sup> chitosan<sup>5,6</sup> ovalbumin,<sup>7,8</sup> and poly (lactic-co-glycolic) acid (PLGA).<sup>9,10,11</sup> All the carriers have been classified as non toxic and are biodegradable in nature and have been extensively used to achieve controlled release of a variety of drugs<sup>12</sup>.

### MATERIALS AND METHODS

Centchroman (Torrent Pharmaceuticals Limited, Ahmedabad); Chitosan (Fisheries Research Institute, Trivandrum); Chicken egg albumin, Span 60 and Tween 80 (Sigma Chemicals Co. USA); PLGA (80:20) (Polysciences, Inc., Warrington, PA). All other reagents were of analytical grade.

#### Preparation of microspheres:

(i) **Calcium Alginate Microspheres**<sup>6</sup>: To 50 ml of 1% w/v solution of sodium alginate in distilled water, drug (50 mg,

drug: polymer = 1:10) was added and dispersed with mechanical agitation for 15 min. This dispersion was added dropwise to an aqueous solution (200 ml) of calcium chloride (2 % w/v) with gentle agitation. Microspheres were formed instantaneously which were allowed to rigidize for 15 min and then were collected by filtration, washed thoroughly with distilled water and vacuum dried.

(ii) **Chitosan microspheres**<sup>6</sup>: Chitosan and the drug (50 mg, drug:polymer=1:10) were dispersed in 25 ml 6% v/v glacial acetic acid. This was added dropwise to 150 ml of 4 % w/v sodium hydroxide solution containing 6 ml of 25 % v/v glutaraldehyde as cross linking agent. The microspheres formed were allowed to rigidize and then collected by filtration, rinsed with a dilute solution of sodium hydroxide followed by thorough washing with distilled water and vacuum dried.

(iii) **Albumin microspheres**<sup>13</sup>: Chicken egg albumin and the drug (100 mg, drug:polymer=1:10) were dispersed in 5 ml of distilled water using high speed stirrer at 2000 rpm. Then arachis oil containing 0.1 % v/v Span 60 was added slowly with continuous stirring. The dispersion was stirred for about 5 min, then 1.0 ml of 25 % v/v glutaraldehyde solution was added slowly and stirring was continued for 2 h. The microspheres formed were collected by filtration and were washed continuously with n-hexane till oil was

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removed completely followed distilled water containing 0.1% tween 80 thrice and finally with distilled water and then dried under vacuum.

In all the above cases, 0.1 % w/w/ methyl paraben was added as the preservative to the final formulation.

**(iv) PLGA microspheres: (Solvent evaporation method)<sup>14</sup>**

The drug (100 mg) and PLGA (drug:polymer=1:5) were dissolved in 6 ml dichloromethane. This solution was added slowly to 100 ml of 1% w/v gelatin solution containing 0.2 % v/v/ of tween 80 under constant stirring (2000 rpm). The stirring was continued for 2.0 - 2.5 h until all solvent was evaporated. The microspheres were collected by filtration on Whatmann #1 paper, washed thrice with distilled water containing 0.1 % Tween 80 and vacuum dried. The microspheres were sieved (# 120) during the final washing process.

**Size distribution analysis:**

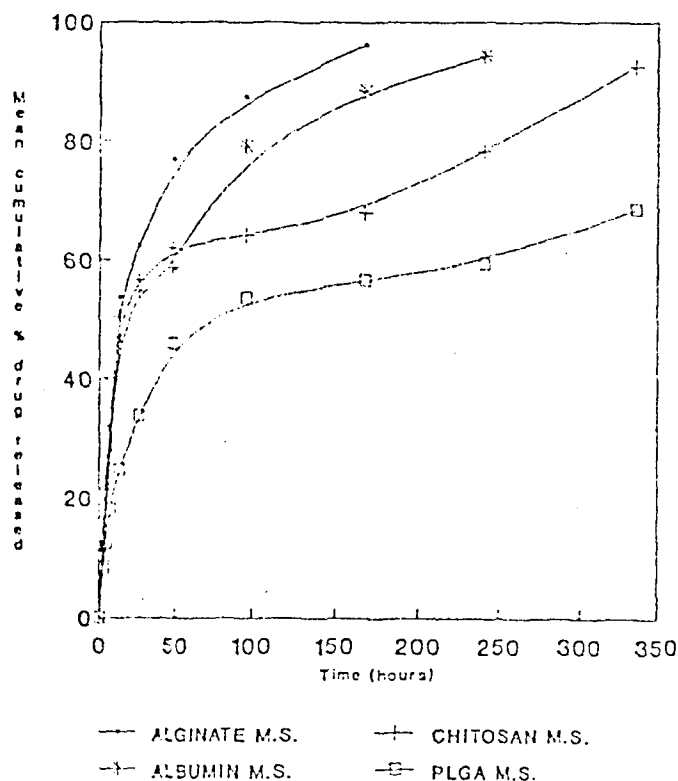
The size distribution for alginate and chitosan microspheres was carried out using a screw gauge (diameter of 100 microspheres/batch were measured individually and average was taken for three batches) and for albumin and PLGA microspheres by light microscopy.

**Drug content :** (For alginate, chitosan and albumin microspheres) Drug from a 100 mg of the microspheres was extracted (by digesting for 36 h) with 10 ml of phosphate buffered saline (PBS, pH 7.4) containing 60% methanol. This solution was filtered and drug content was determined spectrophotometrically at 276 nm after diluting suitably with PBS containing 60 % methanol.

(For PLGA microspheres) 100 mg of microspheres were dissolved in 8 ml dichloromethane and methanol (about 10 ml) was added to completely precipitate the polymer. This was centrifuged at 4000 rpm for 10 min. A sample of clear supernatant was diluted suitably with PBS containing 60 % methanol and drug content was measured spectrophotometrically at 276 nm. The experiment was conducted in triplicates.

**In vitro drug release studies<sup>9</sup>**

100 mg of microspheres were placed in vials containing



**Fig.1 : In Vitro drug release from centchroman loaded microspheres**

20 ml of PBS (pH 7.4) and kept on a shaker water-bath set at 37° and 60 oscillations/min Clear samples of 0.8 ml were withdrawn at predetermined time intervals, mixed thoroughly with methanol (1.2 ml so as to get a final concentration of 60 % methanol in PBS) and drug released was determined spectrometrically at 276 nm against appropriate blank (placebo microspheres treated in the same manner). The experiment was conducted in triplicates.

**Stability studies:<sup>15</sup>**

Microspheres containing 10 mg of drug were stored (in amber colored vials with rubber closure) at different conditions viz., room temperature, 37°, sunlight exposure (stored in plain botosilicate glass vials exposed to day light) and 82.5 % relative humidity (stored in vials without closure in a dessicator containing 26.2 ml concentrated sulphuric acid diluted to 200 ml with water for 28 days. The samples were withdrawn weekly and analyzed for the drug content. The experiment was done in duplicates.

All the results were analyzed statistically using GraphPAD software (version 1.13).

**Table 1: Average diameter and entrapment efficiency of microspheres**

Formulation	D:P	Average diameter* in microns ( $\pm$ S.D.)	% entrapment of drug ( $\pm$ S.D.)
Alginate m.s.	1:10	1325.00 $\pm$ 22.00	78.56 $\pm$ 4.93
Chitosan m.s.	1:10	1178.00 $\pm$ 18.00	79.80 $\pm$ 3.19
Albumin m.s.	1:10	50.10 $\pm$ 7.44	82.61 $\pm$ 3.41
PLGA m.s.	1:5	15.28 $\pm$ 2.14	89.83 $\pm$ 3.04

m.s. = microsphere

D:P = Drug:Polymer

Results are average of three different batches (three trials/batch).

\* n = 100 microspheres/batch (three batches)

**Table 2 : Degradation rate constant (K) values of Centchroman in different microspheres at different storage conditions for 28 days**

Formulation	Degradation rate constant K (day <sup>-1</sup> ) under Storage conditions of			
	R.T.	37°	82.5 % R. H.	L.E.
Centchroman*	8.44 x 10 <sup>-4</sup>	4.06 x 10 <sup>-3</sup>	10.5 x 10 <sup>-3</sup>	2.22 x 10 <sup>-2</sup>
Alginate m.s.	7.67 x 10 <sup>-4</sup>	3.76 x 10 <sup>-3</sup>	9.59 x 10 <sup>-3</sup>	1.32 x 10 <sup>-2</sup>
Chitosan m.s.	7.61 x 10 <sup>-4</sup>	3.68 x 10 <sup>-3</sup>	8.67 x 10 <sup>-3</sup>	2.08 x 10 <sup>-2</sup>
Albumin m.s.	7.53 x 10 <sup>-4</sup>	2.76 x 10 <sup>-3</sup>	7.06 x 10 <sup>-3</sup>	1.73 x 10 <sup>-2</sup>
PLGA m.s.	6.91 x 10 <sup>-4</sup>	2.45 x 10 <sup>-3</sup>	6.60 x 10 <sup>-3</sup>	1.51 x 10 <sup>-2</sup>

\*Free drug

Values are mean of duplicates

$K = 2.303/t \times \log$  (Initial amount of drug/amount of drug after time 't')

## RESULTS AND DISCUSSION

Centchroman was encapsulated into biodegradable carriers. The microspheres prepared were spherical in shape. The photomicrographs of albumin and PLGA microspheres revealed that they had relatively smooth surface. The size distribution analysis of microspheres (Table 1) showed that PLGA microspheres had the least diameter with maximum drug loading (89.83  $\pm$  3.04) whereas albumin microspheres exhibited lesser degree of entrapment (82.61 $\pm$ 3.41). Chitosan was better than the alginate both in size distribution characteristics and entrapment efficiency. The *in vitro* release profiles of the

microspheres revealed a biphasic release pattern in all cases (Fig. I).

The drug release rate was particularly faster in case of alginate and albumin microspheres and hence they could not sustain the rate of drug release for a longer time (not more than 7 days) unlike chitosan (upto 2 weeks) and PLGA microspheres (for periods more than 2 weeks). The cumulative % drug released from PLGA microspheres were 8.04 $\pm$ 0.98, 12.36 $\pm$ 1.26, 18.18 $\pm$ 1.28, 24.62 $\pm$ 2.14, 33.76 $\pm$ 2.31, 45.92 $\pm$ 2.42, 53.60 $\pm$ 3.22, 56.64 $\pm$ 3.18, 59.42 $\pm$ 3.54 and 68.56 $\pm$ 3.83 at 2, 4, 6, 12, 24, 48, 96, 168, 240, 336 hours respectively. The initial burst may be

attributed to the drug remaining adsorbed onto the surface of the microspheres and the faster drug release rate may be due to the rapid erosion of the polymer. In all cases, prolonged drug release was achieved by diffusion followed by slow erosion of the polymeric coating<sup>3,6,8,9</sup>.

Stability studies indicated that all the microspheres could retard the drug degradation process as expressed by the drug content.<sup>16</sup> This was evident by the significantly ( $P < 0.05$ ) lowered degradation rate constant ( $K$ ) values of the microspheres than the free drug (Table 2).

PLGA appears to be the superior biodegradable microcarrier for preparing centchroman microspheres. The method for preparation was reliable and reproducible, giving fine microspheres with high drug content. Sustained *in vitro* drug release profile was observed along with improved stability. Further studies are required to employ centchroman microspheres as an implantable contraceptive formulation.

#### ACKNOWLEDGEMENTS

The authors are thankful to Torrent Pharmaceuticals Ltd. for the gift sample of Centchroman.

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