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Research Papers

Preparation and evaluation of cellulose nanospheres containing 5-fluorouracil

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Summary

Cellulose derivatives have been used to prepare nanospheres (NS) entrapping the drug, 5-fluorouracil (5-FU). Adopting the technique of desolvation for nanosphere formation and drying via a modified procedure, distinct spheres have been obtained, as observed by scanning electron microscopy. Investigation of the in vivo distribution showed a high concentration of the drug in lung tissue.

Introduction

Colloidal drug delivery systems for targeted distribution of anti-cancer drugs are of increased interest for research in elevating the therapeutic efficacy of this class of drugs. Various workers reported the use of microcapsules (Bakan and Anderson, 1976), nanocapsules (Speiser, 1976), macromolecular complexes (Miyao, 1978; Uchman and Grezeskowiak, 1981), erythrocyte carriers (Updike and Wakamiya, 1983) and polymeric beads, as examples of colloidal drug delivery systems. Marty and Oppenheim (1977) prepared ethyl cellulose nanoparticles by a desolvation technique but failed to develop discrete, uniform particles. In the present investigation, nanospheres of methyl cellulose and ethyl cellulose containing 5-FU have been prepared and evaluated for their physical properties and distribution patterns in rat models.

Experimental

Materials and apparatus

5-FU was obtained from Hoffmann La Roche, Switzerland. Ethyl cellulose, A.R. (BDH, U.K.), methyl cellulose, A.R. (Koch-Light, U.K.), Tween 80 (G.C. Chemicals Testing Lab and Allied Industries, India), sodium sulfate, anhydrous, and sodium acetate, A.R. (Glaxo (India) Ltd, Bombay), ethyl acetate, A.R. (I.D.P.L., India), and sodium phosphate, monobasic, A.R. (Reechem Pvt Ltd, India) were used.

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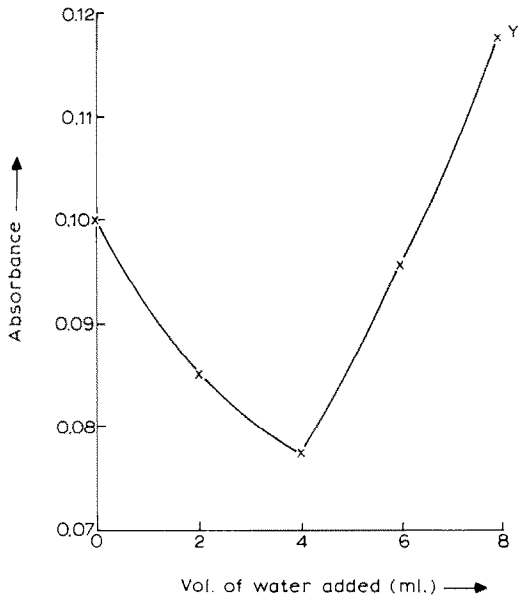

Fig. 1. Absorbance monitoring during desolvation to produce 5-fluorouracil associated ethyl cellulose nanospheres.

A Philips scanning electron microscope (PSEM 501 B), refrigerated centrifuge (Caltan, India) and UV double-beam spectrophotometer (Model CE-594, Cecil Instruments, U.K.) were used.

Preparation of nanospheres

An ethanolic solution (10 ml) containing 5-FU (1.2 mg/ml), ethyl cellulose (1.0% w/v) and Tween 80 (1.0% w/v) was desolvated by stirring at 200 rpm and 30 ± 1°C using distilled water. The extent of desolvation was controlled by absorbance measurements at 550 nm (Fig. 1). The resultant turbid dispersion was again stirred vigorously at 500 rpm for 10 min and dried at 30°C by applying it as a thin film on glass plates. Ethyl cellulose nanospheres were obtained in the form of white, flaky masses.

Methyl cellulose nanospheres were prepared by desolving an aqueous solution (10 ml) containing 5-FU (1.0 mg/ml), methyl cellulose (1.0% w/v) and Tween 80 (0.5% w/v) with sodium sulphate solution (20% w/v) as desolvating agent, at 25 ± 1°C (Fig. 4). The other conditions for desolvation and drying are identical to those used in obtaining ethyl cellulose nanospheres.

Payload of 5-FU in nanospheres

5-FU content in ethyl cellulose nanospheres was determined by dissolving an accurately weighed quantity (50 mg) of the nanospheres in ethyl acetate (1.0 ml). The resulting solution was shaken with distilled water (10 ml). The aqueous layer was isolated and centrifuged at 5000 rpm and subsequently filtered. To an aliquot (1.0 ml) of the clear aqueous filtrate, 0.1 ml of 0.5 M sodium phosphate solution was added and it was again shaken with 8.0 ml of ethyl acetate (Sadee and Beelen, 1980). The organic layer was centrifuged at 5000 rpm for 5 min and the absorbance of the clear solution was measured at 280 nm. Standard blank was prepared in an identical manner without using any drug.

Drug content in methyl cellulose nanospheres was determined by dissolving an accurately weighed quantity (10 mg) of the product in cold water (5°C) and adjusting the volume to 5 ml. The solution (1.0 ml) was subjected to extraction with ethyl acetate (8.0 ml) and 0.1 ml of 0.5 M sodium phosphate solution. The organic layer was centrifuged and analyzed as described for ethyl cellulose nanospheres.

Particle size determination of nanospheres

The scanning electron microscopic observations for determining the overall size and shape of both ethyl cellulose and methyl cellulose nanospheres were performed as described previously (Mukherji et al., 1989).

In vivo study in albino rats A distribution study of 5-FU after release from ethyl cellulose and methyl cellulose nanospheres, in liver, lung and intestine of albino rats and analysis of the drug by HPLC were performed as described (Mukherji et al., 1989).

Results and Discussion

Ethyl cellulose nanospheres

Fig. 2 shows the electron micrograph of ethyl cellulose nanospheres. The particles are spherical and regular, with their size ranging from 190 nm up to a maximum of 1.1 μm with an average

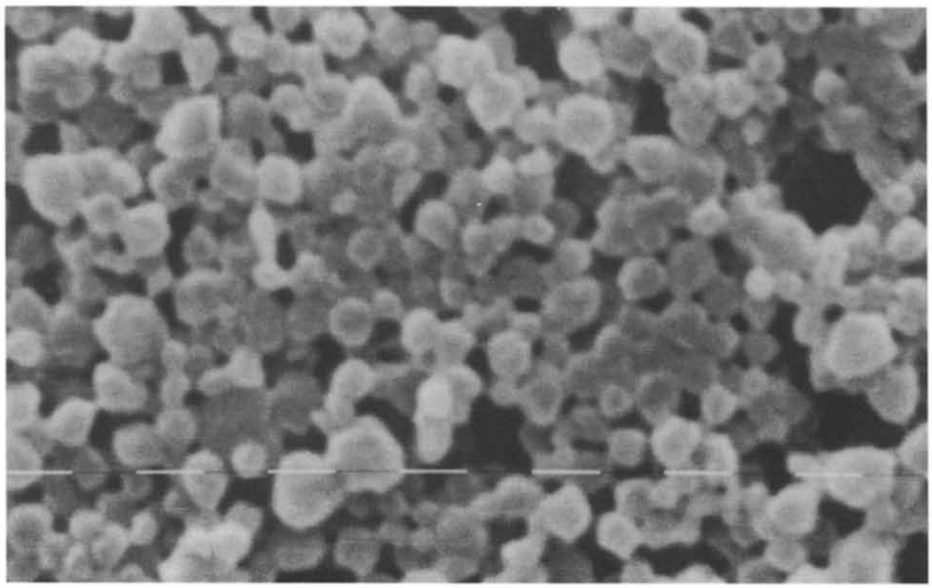

Fig. 2. Ethyl cellulose nanospheres containing 5-FU, as seen through a scanning electron microscope. Thickness of the gold layer was 250 Å. Marker = 1 μm. Magnification, $\times$7000.

diameter of 472 nm. The particle size distribution pattern is shown in Fig. 3.

The method used for the preparation of ethyl cellulose nanospheres was simpler than that adopted for methyl cellulose nanospheres. The vehicle and the desolvating agent employed were selected based on the solubility characteristics of ethyl cellulose. The vehicle used was ethanol due to the free solubility of the carrier in it, and the desolvating agent was distilled water. Hence, the preparation technique has been simplified to the extent that it eliminates intricate purification steps which are sources of considerable drug loss from products. It was found that each mg of ethyl cellulose nanospheres contained 54.05 μg (equivalent to 5.4%) of 5-FU.

Completion of desolvation and nanosphere formation was confirmed by monitoring the absorbance of the mixture to the point marked *Y* (Fig. 1). The drying of ethyl cellulose nanospheres was crucial with regard to particle aggregation. The colloidal spheres exist in a stable, dispersed state at an optimum ratio of ethanol and water when desolvation is terminated. A rise in the proportion of ethanol in the ethanol-water mixture causes the nanospheres to dissolve and to form sheets on drying. In contrast, a decrease in ethanol concentration in the mixture results in the nanospheres aggregating with each other. Thus, the proportion of ethanol and water in the ethanol-water mixture at the end of desolvation should be maintained constant for the stability of

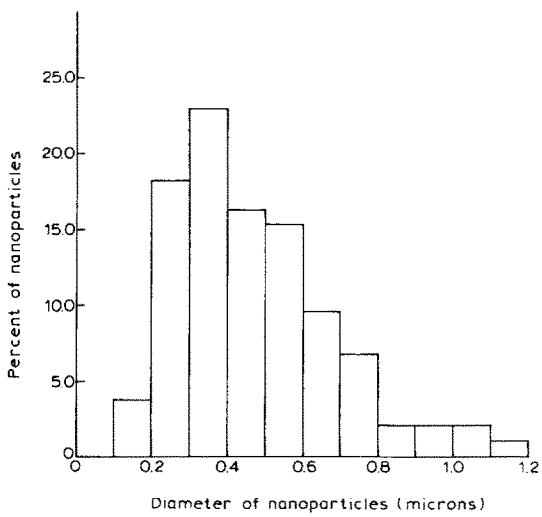

Fig. 3. Particle-size distribution pattern of ethyl cellulose nanospheres containing 5-fluorouracil.

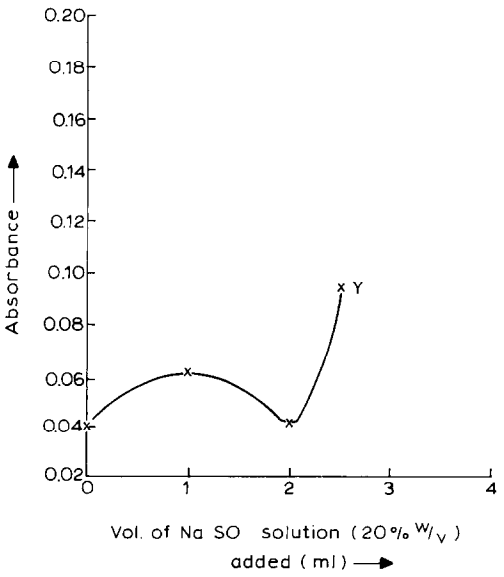

Fig. 4. Absorbance monitoring during desolvation to form methyl cellulose nanospheres.

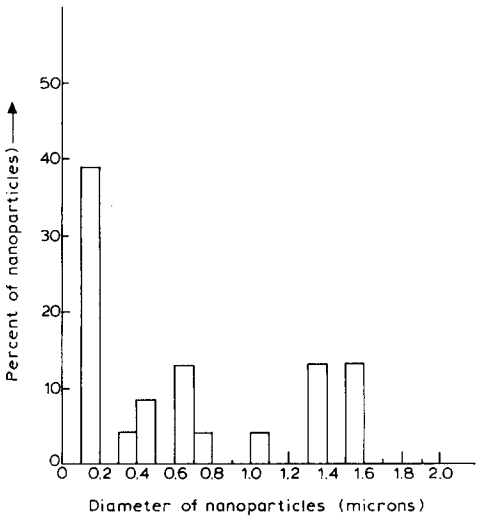

Fig. 6. Particle-size distribution pattern of methyl cellulose nanospheres.

ethyl cellulose nanospheres. In most of the normal drying techniques, the proportions of ethanol and water are disturbed by an increase or decrease of the volume of ethanol as compared to water. This is due to the difference in their volatility which causes faster evaporation of either ethanol or water. The application of the desolvated colloidal

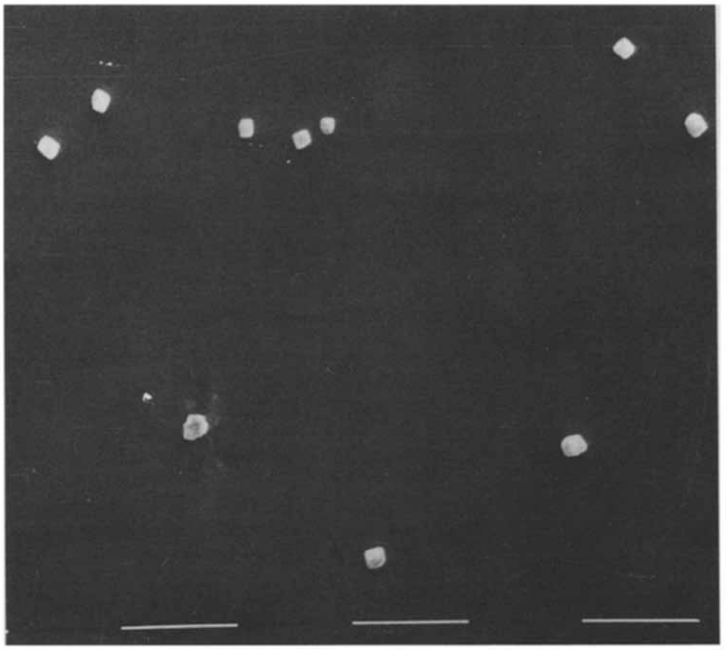

Fig. 5. Scanning electron micrograph showing methyl cellulose nanospheres of 5-fluorouracil. Thickness of the gold layer was 250 Å. Marker = 10 μm. Magnification, $\times$1250.

TABLE 1

Tissue distribution of 5-FU from cellulose nanospheres

	% 5-FU per whole tissue			5-FU (μg) per g of tissue		
	Liver	Lung	Intestine	Liver	Lung	Intestine
EC NS	21.68 ± 0.62	23.44 ± 1.81	14.45 ± 3.51	3.10 ± 0.86	13.01 ± 5.33	2.68 ± 1.03
MC NS	35.43 ± 3.60	21.87 ± 1.61	17.72 ± 4.83	4.27 ± 1.50	12.67 ± 0.22	3.17 ± 1.10

dispersion as a thin film on glass plates and then drying them at 30 °C has not only simplified the procedure but has also ensured the stability of the product. In this technique, even if there is any preferential evaporation of either of the solvents, the particles will not be able to aggregate with one another as they are distributed in the film.

Methyl cellulose nanospheres

Methyl cellulose nanospheres containing 5-FU (Fig. 5) were found to be nearly spherical, with size ranging from 154 nm to 1.54 μm, and an average diameter of 540 nm. The size distribution of the nanospheres, as determined from electron micrograph, is presented graphically in Fig. 6.

The method of preparation of methyl cellulose nanospheres required some changes from that reported by Marty et al. (1978) for gelatin nanospheres. In this experiment, due to the solubility characteristics of methyl cellulose in water, desolvation was carried out at 25 °C to avoid the formation of flocs. On desolvation with sodium sulphate solution methyl cellulose nanospheres were formed together with coarse particles which were removed by controlled filtration.

The 5-FU content of methyl cellulose nanospheres was found to be 11.57 μg per mg of the product (1.157%).

In vivo study

The in vivo study indicated an unequal distribution pattern of 5-FU after release from ethyl cellulose and methyl cellulose nanospheres, 2 h after injection in rats (Table 1). Accumulation of the drug, based on μg/g of tissue, showed a similar distribution in liver, lung and intestine. The ratio of the released drug from ethyl cellulose nanospheres in liver, lung and intestine was found to be 3 : 13 : 2.7, while that from methyl cellulose nanospheres was 4.3 : 12.7 : 3.2. Methyl cellulose nanospheres produced 58% accumulation of the administered dose of 5-FU in the three organs and an effective accumulation of the drug in lung as compared to other organs. The low percentage availability from ethyl cellulose nanospheres may be attributed to the slow release of the drug from ethyl cellulose because of poor solubility of the carrier in aqueous biological fluid.

Summary and Conclusion

The modified method of preparation of cellulose nanospheres reported here produced particles of distinct shape and size as compared to the method reported by Marty and Oppenheim, 1977. Ethyl cellulose and methyl cellulose nanospheres produced preferential deposition of 5-FU in the lung tissue of rats, as compared to liver and intestine.

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