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Novel Complex Coacervates of Albumin and Gelatin a Carriers for 5-Fluorouracil

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Albumin and gelatin have been widely used alone and in combination with other colloids as drug carriers but so far, their combination with each other has not been reported. The present study reports a novel attempt to prepare complex coacervates of albumin and gelatin as carriers for the anticancer drug, 5-Fluorouracil (5-FU). Formaldehyde crosslinked albumin-gelatin complex coacervates, prepared by the complex coacervation-phase separation method, were found to be nearly spherical and had 92.23±2.16% drug entrapment efficiency. These microacervates were evaluated for drug leaching, *in vitro* biodegradability and *in vivo* organ distribution studies. The microcoacervates were found to be more susceptible to pancreatin as compared to pepsin and exhibited diffusion controlled drug leaching in non sink, static conditions. *In vivo* organ distribution studies of the microcoacervates in healthy rats showed a statistically significant distribution of 5-FU in liver and kidneys ($P<0.01$) as compared to the free drug solution, indicating preferential uptake by the organs of the RES. Prolonged 5 FU levels in liver, lungs and kidneys also indicated sustained release of the drug from the microcoacervates.

Natural proteins are widely used as biodegradable, biocompatible drug carriers due to their ready availability, proven safety, cost effectiveness and multiple functional groups available for covalent attachment[1]. Their use as carriers for antineoplastic agents has increased because of their selective uptake in the tumor cells coupled with known lysosomal activity of many cells[2,3]. Of these, albumin[4-9] and gelatin[10-13] have been extensively studied alone and in combination with other colloids like acacia, alginic acid and pectin[14-17] but so far, their combination with each other has not been reported.

The present study reports a novel attempt to prepare complex coacervates of albumin and gelatin as carriers for the widely used anticancer agent 5-fluorouracil (5-FU). Here, albumin, which exhibits a net negative charge, was neutralized with gelatin, which is cationic in acidic medium, at a 1:1 w/w ratio, using Tween 20 for controlling size distribution and formaldehyde as a crosslinking agent.

EXPERIMENTAL

5-FU was gifted by M/s Biochem Pharmaceuticals Ltd., Egg Albumin Powder was procured from M/s. S.D. Fine Chemicals, Gelatin, Pepsin and Pancreatin were procured from M/s Loba Chemie Pvt. Ltd.

All other chemicals and reagents used were of Analytical Grade.

Preparation of Microcoacervates:

5-FU (150 mg) was dissolved in 25 ml of 3% aqueous solution of gelatin with stirring at 55° on a Remi stirrer at 1200 rpm speed. The pH was adjusted between 4 to 4.5 using 10% acetic acid. Albumin solution (25 ml of 3% solution in sodium carbonate buffer at pH 9.8) was added to the gelatin solution with stirring. The coacervates formed were hardened by cooling in ice bath for 30 min and then crosslinked with 10 ml of formaldehyde solution at 50°. They were then successively washed with distilled water and isopropyl alcohol, filtered, dried and stored in a refrigerator till further use[18].

The above procedure was an outcome of optimisation of formulation conditions such as protein concentration, temperature, pH, and Tween concentration. Protein concentration in the range of 1 to 5% was investigated

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while ambient and ice bath temperature was used for formulating the microcoacervates in the pH range of 2 to 6. Tween 20 was used for particle size control in the range of 0.5 to 3%. Each variable parameter was systematically optimised by keeping all other variables constant.

***In vitro* studies:**

Particle size of the microcoacervates was evaluted using an Optik microscope. It was not possible to completely lyze the microcoacervates to determine the drug entrapped within. Hence, drug entrapment efficiency was determined indirectly by analyzing the aqueous supernatant and washings obtained during the preparation of the microcoacervates. The drug was extracted in ethyl acetate: propanol (7:3) and the absorbance was measured at 272 nm on a Hitachi spectrophotometer against a similarly prepared blank[19].

Drug leaching studies were performed using the microcoacervate suspension in phosphate buffer stored in a refrigerator. At periodic time intervals, aliquots were withdrawn and analyzed for 5-FU content spectrophotometrically. Biodegradability studies of the microcoacervate suspension in phosphate buffer were conducted in presence of 10 mg each of proteolytic enzymes, pepsin and pancreatin, in refrigerated conditions to protect the enzymatic activity. At periodic intervals, supernatant aliquots were withdrawn and 5-FU was analyzed after extraction of the aqueous phase with ethyl acetate: propanol (7:3) and measuring its absorbance at 272 nm on Hitachi spectrophotometer.

***In vivo* studies:**

The microcoacervate suspension (≅1 mg 5-FU per ml) was injected via the tail vein into healthy Wistar rats of either sex, weighing 200-250 g. Four rats were used for each set of study. The rats were sacrificed at 2,4,6 h and organs like lungs, liver and kidneys were removed, homogenized and homogenates were analyzed for 5-FU content[20]. The homogenates were extracted with ethyl acetate, the extract was evaporated to dryness, the residue was dissolved in distilled water and analyzed for 5-FU content spectrophotometrically at 266 nm against a similarly treated organ blank.

RESULTS AND DISCUSSION

The formulation conditions for preparing the complex coacervates were first optimized. It was observed that at low protein concentration upto 2%, the yield was poor whereas above 3%, irregularly shaped aggregates were obtained. Ice bath temp. was found to be essential for improving the yield and easing the solidification of the microcoacervates, probably by increasing the effectiveness of the coacervation process of the colloidal proteins. Also, variation in the pH from the optimum value of 4-4.5 on either side yielded aggregated fluffy mass. Crosslinking with formaldehyde at 55° was found to improve the stress resistance and flow properties of the microcoacervates.

Above all, a surfactant (Tween 20) was found to play an important role in controlling the particle size of the microcoacervates and improving drug entrapment efficiency. As the Tween concentration increased from 0.5 to 2%, the particle size reduced from 8 to 3 μm, while the drug entrapment efficiency increased upto 92.25%. However, further increase in the Tween concentration produced bigger particles.

Thus, when prepared under the optimized conditions, nearly spherical, microcoacervates having a size range of 2 to 5 μm (d mean = 2.92±2.66 μm), with 92.25±2.16% drug entrapment efficiency were obtained. An aqueous suspension of the microcoacervates stored in a refrigerator upto 2 m exhibited a biphasic diffusion-controlled drug leaching in non-sink, static conditions (18.5±2.19% leaching) with an initial burst effect upto 5 days, probably due to surface associated drug (fig. 1).

In vitro biodegradability was monitored in terms of drug release from the microcoacervates in presence of

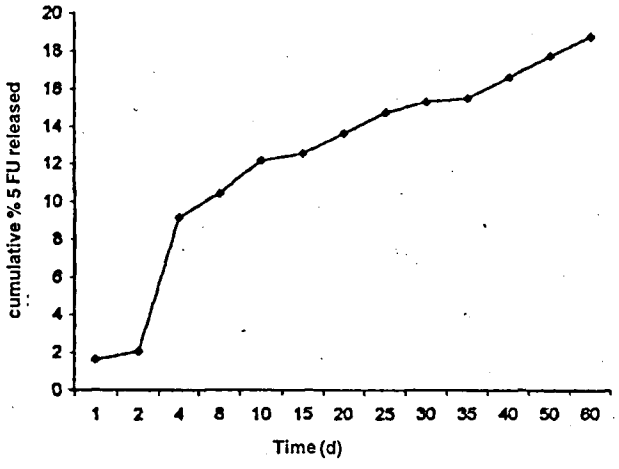

Fig. 1: *In vitro* drug leaching from microcoacervates *In vitro* drug leaching from microcoacervate suspension in phosphate buffer saline stored in a refrigerator upto 2 m.

enzymes, pepsin and pancreatin. It was observed that faster rate of drug release occurred in presence of pancretin (69.30±4.51% in 4 d) as compared to pepsin (27.75±3.25% in 12 d), indicating variable susceptibility of the proteins to proteolytic enzymes. However, 100% release could not be recorded. Instead, drug levels reduced dramatically in the dissolution medium after maximum values were recorded, probably due to enzymatic degradation of 5-FU itself after complete lysis of the carrier proteins. From the plateau type drug release profile (fig. 2), it can be concluded that the peak coincided with the carrier biodegradation giving a sustained release of the drug whereas the gradual decline in drug release may probably be owing to gradual enzymatic breakdown of the released drug itself.

In vivo organ distribution pattern of 5-FU from the microcoacervates shows that maximum drug release occurred in 2 h with 20.52±3.36% of the administered dose in liver, 11.15±1.08% in kidneys and 10.47±1.86%

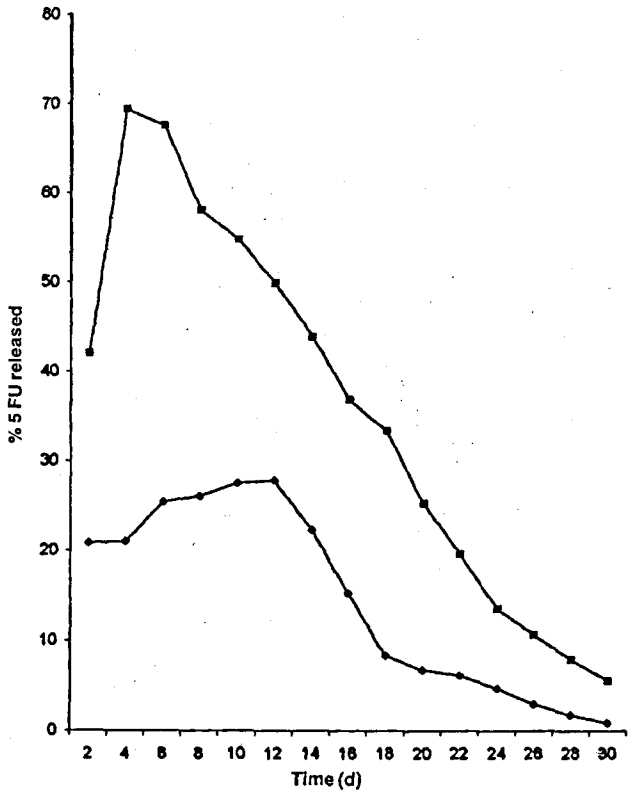

Fig. 2: % 5 FU released from microcoacervates in presence of proteolytic enzymes

***In vitro* 5-FU released from microcoacervate suspension in presence of proteolytic enzymes, Pepsin [-◆-] and Pancreatin [-□-]**

in lungs. Thereafter, the drug levels reduced very slowly in lungs and liver upto 6 h and remained nearly same in kidneys, indicating a sustained release of 5-FU from the microcoacervates (fig. 3a). However, in terms of organ distribution of 5-FU on a weight basis (µg/g organ weight), nearly 46 µg was found in both lungs and kidneys at 2 h whereas that in the liver was 24 µg/g. Notably, these levels

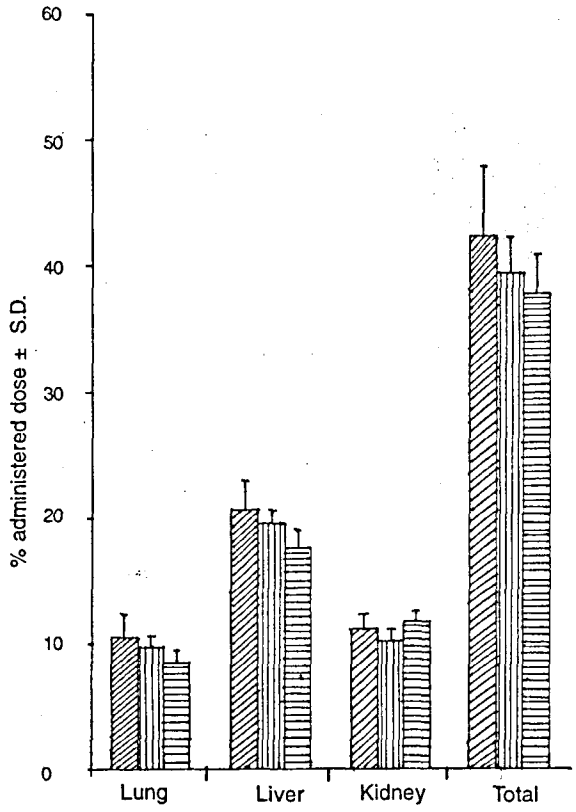

Fig. 3(a): *In vivo* organ distribution of 5-FU from Alb-Gel microcoacervates.

***In vivo* organ distribution studies in groups of 4 rats from albumin-gelatin micocoacervates in terms of per cent of administered dose in various organs at 2h [▨], 4 h [▥] and 6h [▤].**

remained nearly constant in all three organs upto 6 h, indicating a sustained drug release from the organ-embedded microcoacervates (fig. 3b). A statistically significant ($P<0.01$) difference was found in total distribution of 5 FU to all three organs from the microcoacervates as compared to free drug solution (Table 1). A significant increase in drug distribution to liver and kidneys ($P<0.01$) was also observed, indicating higher uptake of the microcoacervates by the organs of the reticuloendothelial system (RES), mainly by size

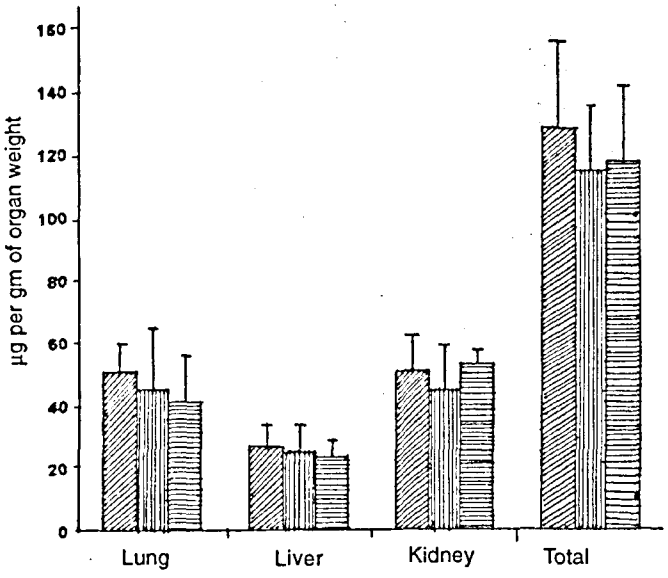

Fig. 3(b): *In vivo* organ distribution of 5-FU from Alb-Gel microcoacervates

***In vivo* organ distribution studies in groups of 4 rats from albumin-gelatin micocoacervates in terms of µg 5-FU per g organ weight in various organs at 2 h [▨], 4 h [▩] and 6h [▤].**

TABLE 1: PERCENT 5-FU LEVELS IN VARIOUS ORGANS

Organs	Percent Administered 5 FU solution	Dose ± S.D. Alb-Gelcoacervates
Lung	7.98±0.41	10.47±1.86
Liver	14.48±2.78	20.52±2.36
Kidney	6.40±0.36	11.15±1.08
Total	28.85±3.55	42.14±5.52

Peak % FU levels in various organs attained after administration of free 5 FU solution and albumin-gelatin micocoacervates

dependent, passive targeting mechanism. The preferential distribution in the liver followed by kidneys and lungs can be attributed to phagocytic and size dependent distribution of administered foreign carriers by the organs of the RES. It has been proved that intravenously administered particles larger than 7 µm are trapped by mechanical filtration by the lungs whereas particles between 2 to 7 µm are phagocytosed by the organs of the RES[21]. As the size of the prepared microcoacervates is in the range of 2.92±2.66 µm, they have been significantly deposited in the liver and kidneys, while the distribution to the lungs remained significantly unchanged. This can be highly beneficial in the treatment of solid tumours where 5-FU is the drug of choice, as it will ensure organ targeting accompanied by sustained release at the site of disposition.

Thus, the present investigation has resulted in preparing a novel complex coacervates of albumin and gelatin with high drug entrapment efficiency of 92.35% and improved enzymatic resistance indicated by an *in vivo* sustained release effect. This can perhaps be used for the purpose of targeting drugs to the RES with an added advantage of sustained delivery of the drug to the target organ(s).

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