RESEARCH PAPER

Accepted 28 June 2000
Revised 19 June 2000
Received 21 February 2000
Indian J. Pharm. Sci., 2000, 62 (5) 380-383

Nanoparticular Antineoplastic Drug Delivery System Using Bovine Serum Albumin

ARPITA BHATTACHARYA AND A. MUKHERJEE*
Division of Pharmaceutical Technology,
Department of Chemical Technology, University College of Science & Technology,
Calcutta University, 92, A.P.C. Road, Calcutta-700009

Targeted drug delivery is an effective means in cancer chemotherapy. Nanoparticulation is one of the useful means for targeted drug delivery. In the present investigation a broad spectrum anticancer drug methotrexate is nanoparticulated in a foreign protein bovine serum albumin. The particle size distribution was measured using a transmission electron microscope. Two sets of nanoparticles were prepared by varying Protein: drug ratio. The pattern of drug release from the nanoparticles were observed in pH 7.4 at 37°. A non-fickian type of release was observed from both sets.

Nanoparticulation is perhaps an useful strategy towards targeted drug delivery[1,2]. Particularly in cancer chemotherapy, a limiting factor is traditional lack of selectivity for antineoplastic drugs towards cancer cells and tissues. Furthermore, the rapidly proliferating cells of bone marrow or gastrointestinal tract can easily be affected by the cytotoxicity of concerned drugs, while on the other hand, emerging resistant cell sublines occuring in normal therapeutic region may demand higher dosage of antineoplastic drug substances[3]. Among various recent strategies adopted, nanoparticulation techniques are already receiving attention due to several advantages[4]. Phagocytable nanoparticles here can provide an effective strategy in drug targeting for concentration of antineoplastic drug substances in liver or bone marrow, additionally a slow releasing formulation will demand less tissue-drug load resulting in lesser toxicity.

This work of a series, is an attempt for development of an upgradable nanoparticulation technology using a broad spectrum antineoplastic drug methotrexate embedded in biodegradable polymer protein. The technology so developed provided coated nanoparticles of relatively narrow particle size range with a slow release profile. An optimized nanoparticulation technique, drug release profile, particle size distribution and release kinetics constitute this study.

* For correspondence

MATERIALS AND METHODS

All solvents used were of HPLC grade and purchased from Spectrochem/SRL, India. Water used was double distilled and filtered through millipore assembly (0.22 μ). Methotrexate was a gift sample from Biochem Industries Ltd., India and BSA was purchased from SRL, India.

Preparation of methotrexate - protein nanoparticles:

Methotrexate (1.5 mg) was dissolved under sonication in 10 ml of ethyl acetate. This solution was emulsified in 30 ml of 0.5% aqueous solution of BSA using Tween 80 (0.4 ml) as an emulsifier. The formed emulsion was stirred for half an hour on a magnetic stirrer; the protein to drug ratio achieved was 100:1. The formed emulsion was then transformed into an optimized microemulsion by dropwise addition of coemulsifier propylene glycol. The total volume of propylene glycol required was 16 ml. The microemulsion was sprayed into acetone using a compressed air driven nebuliser. The nanoparticles formed were separated out by cold (0°) centrifugation at 10,000 rpm for 15 min. They were washed and dried at room temperature under vacuum. Other sets of formulations were run following similar generalised steps including that with polymer to drug ratio of 50:1.

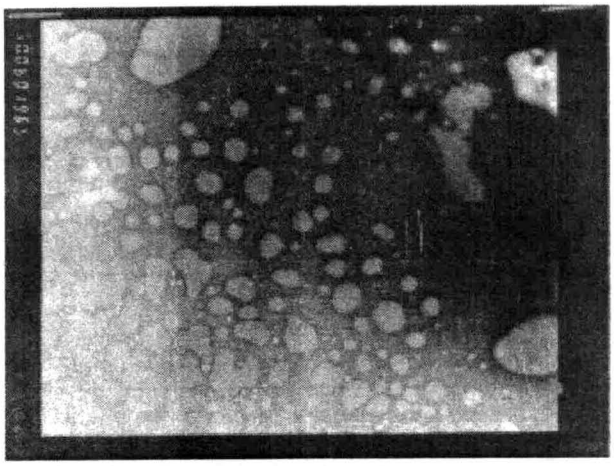

Fig. 1 : Transmission Electron Micrograph of BSA-coated methotrexate nanoparticles at X 50,000 at 75 KV; BSA:Methotrexate ratio 100:1

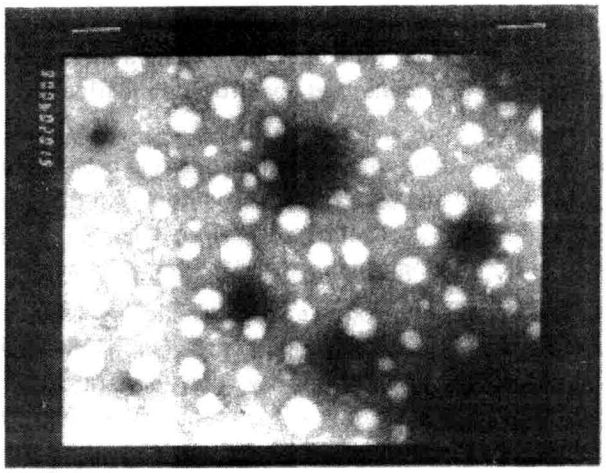

Fig. 2 : Transmission Electron Micrograph of BSA-coated methotrexate nanoparticles at X 80,000 at 75 KV; BSA:Methotrexate ratio 50:1

Drug Entrapment efficiency:

The total drug entrapment efficiency in the nanoparticles were calculated. Ten milligram of each set of nanoparticles 100:1 or 50:1 were dissolved in 1 N NaOH solution and the drug present was measured by serial dilution through UV spectrophotometer. A standard curve of similarly prepared drug solution was used for this reference. The drug entrapment efficiency for 100:1 nanoparticles was 46.8% and that for 50:1 was 55.3%.

Particle Size Distribution:

A drop of the water suspension of the nanoparticles was placed on a carbon grid (C-grid), air dried and the particles were stained with phosphotungstanic acid solution (PTA). Then the C-grid containing the stained nanoparticles were placed in a Transmission Electron Microscope (H 600). Fig. 1 and Fig. 2 show the Transmission Electron Micrographs (TEM) of Protein-coated methotrexate nanoparticles having protein:drug ratio 100:1 and 50:1. Fig. 3 and Fig. 4 represent the particle size distribution of aforementioned nanoparticles. The mean dia of the particles having protein:drug ratio 100:1 was 67.71 nm and that of 50:1 was 68.53 nm.

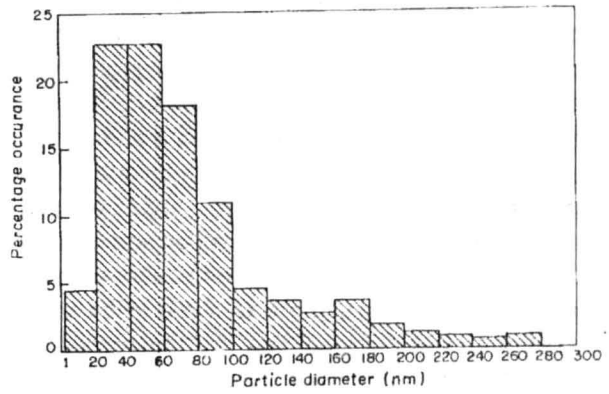

Fig. 3 : Particle size distribution of BSA coated methotrexate nanoparticles
The mean diameter of the nanoparticles having protein:drug ratio of 100:1 is 67.71 nm. Particle size distribution was made from Transmission Electron Micrograph (Fig. 1).

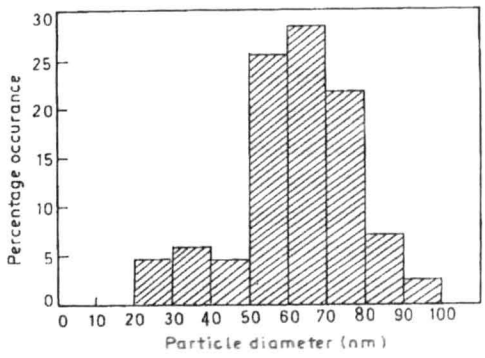

Fig. 4 : Particle size distribution of BSA coated methotrexate nanoparticles
The mean diameter of the nanoparticles having protein:drug ratio of 50:1 is 68.53 nm. Particle size distribution was made from Transmission Electron Micrograph (Fig. 2).

Release Kinetics:

For *in vitro* drug release studies, 10 mg of loaded nanoparticles were taken in a 100 ml round bottom flask and 30 ml of pH 7.4 buffer was added to it. The release studies were carried out at 37° with continuous stirring. The cumulative drug release was measured under sink conditions, at regular time intervals using UV spectrophotometer at 257 nm. For analysis, a standard curve for the drug was first drawn in U.V. spectrophotometer that has provided a fitting curve Y = 0.6508 x - 0.0001, r^2 = 0.9994; Y = Absorbance, X = Concentration.

RESULTS AND DISCUSSION

The principle objective of this work was to develop a suitable technology to nanoparticulate chemotherapeutic drugs in common and available foreign protein for eventual targeting and sustained drug delivery. The preparation of a stable microemulsion seems a primary criterion for success in this work. This and a unique nebulising-denaturing technique followed, provided useable nanoparticles. Selection of emulsifier and coemulsifier seem another critical factor. Among various emulsifiers attempted sorbitan trioleate was found to be very good emulsifier for this purpose. The use of isopropanol as a coemulsifier[5] though gave a good stable microemulsion, was found unsuitable since early partial precipitation of protein materials occurred particularly on storing. This might be due to several factors including formation of some ketonic bodies in isopropanol. However, this problem was solved by using propylene glycol. The method adopted has provided a good yield of nanoparticles. The slow release profile is observed with 97% release over a period of nine days.

The particles found were discrete and majority of them were spherical. The man diameter obtained from the particle size distribution curve of 100:1 Fig. 3 was 67.71 nm. Not much significant change in particle size distribution Fig. 4 was found in the nanoparticles from protein to drug ratio 50:1 where mean diameter was 68.53 nm. It seems the maximum coat attainable has been achieved. The wall thickness of the nanoparticles with different protein:drug ratio (100:1 and 50:1) were thus calculated using relation[6].

$$h = r\,(1-p)\,d^1/3\,(pd2+(1-p)d1)$$

where, h = coating thickness,r = particle radius, p = proportion of the drug in the nanoparticle, d1 = density of the core material, d2 = density of the coating material.

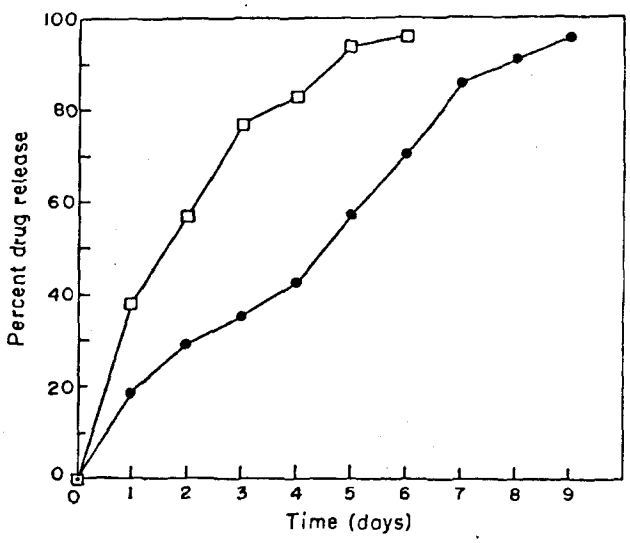

Fig. 5 : *In vitro* cumulative release of methotrexate from nanoparticles

Nanoparticles with protein:drug ratios of 100:1 (□) and 50.1 (•) were stirred at 37° in pH 7.4 buffer, samples drawn at regular time intervals and methotrexate content was measured spectrophotometrically.

The densities of the dried methotrexate and protein were measured by liquid displacement method. Wall thickness of the nanoparticles with protein:drug ratio 100:1 was found to be 11.2 nm and that of 50:1 was 11.1 nm.

The cumulative drug release profile against time from the nanoparticles having different protein:drug ratios were represented in Fig. 3. This shows the per cent drug released based on total drug entrapment against time. The total drug entrapment for 50:1 is higher (55.3%) than that of 100:1 (46.8%) which is expected as the ratio of drug:protein taken initially is higher for the former case.

For 100:1 formulation, the total drug release took 6 days with a maximal release observed of 96.3% where as in case of 50:1 formulation max. release observed was 97% and it took 9 days. The percent loss in entrapped drug material following time release studies is perhaps due to a degree of methotrexate protein binding equlibrium existing *in vitro*. As the wall thicknesses observed in both the formulations were more or less similar subject to a similar release profile it is the drug content that has determined the delayed release.

The statistical fitting of drug release kinetics were studied in emperical release kinetics equations using

TABLE 1 : METHOTREXATE RELEASE PROFILE PARAMETERS IN DIFFERENT PROTEIN: DRUG RATIOS

Formulation	M_1	M_2
Protein:Drug	100:1	50:1
k	0.386±0.002	0.1706±0.011
n	0.553±0.008	0.176±0.008
r^2	0.9987	0.978
t_{50} (days)	1.8	4.6

k, kinetic constant;[7] n, release component;[7] r^2, correlation coefficient; t_{50}, time taken for 50% drug release.

sigma plot (Curve fit algorithm written in Sigma Plot - 1.02, Jandel Scientific, USA).

The release profile for both the formulations can easily fit in the equation[7].

M_t/M_α k t^n

The initial portion of the release curve (M_t/M_α <0.6) was used for this calculation. M_t/M_α is the fraction of drug released up to time t; k is kinetic constant, n is release exponent, related to the release mechanism. The values of n and k were estimated by linear regression of ln (M_t/M_∞) on ln (t) and are tabulated in Table 1. The values of n is 0.43 for Fickian diffusion, 0.43<n>0.85 for non-Fickian transport and 1.0 for case II transport[8]. As in Table 1 n values for both the formulations are between 0.43 and 0.85 indicating a non-Fickian transport controlled both by diffusion and relaxation mechanism.

Nanoparticulation techniques developed is an effective means for study of protein based nanoparticulation. Increasing polymer load does not necessarily provide a better sustained release nanoparticles. Formulation M_2 has provided a good means for slow release nanoparticular antineoplastic drug delivery. Study of process parameters, optimization and biopharmaceutical investigation are need of the hour and the work is in progress in this direction.

ACKNOWLEDGEMENTS

One of the authors (A.B.) Thank CSIR for a grant of Senior Research Fellowship.

REFERENCES

1. Gupta, P.K. and Hung, C.T., **J. Microencaps,** 1990, 7, 85.
2. Aynie, I., Vauthier, C., Chacun, H., Fattal, E. and Couvreur, P., **Pubmed,** 1999, cross. **Antisence Nuclic Acid Drug Dev.,** 1999, 9, 301.
3. Allemann, E., Gurny, R. and Doelker, E., **Eur. J. Pharm. Biopharm.,** 1993, 39, 173.
4. Kramer, P.A. and Burnstein, T., **Life Sci.,** 1976, 19, 515.
5. Bhattacharya, A. and Mukherjee, A., **Indian J. Pharm. Sci.,** 1999, 61, 389.
6. Luu, S.N., Carlier, P.F., Delort, P and Laforit, D., **J. Pharm. Sci.,** 1973, 62, 452.
7. Ritger, P.L. and Peppas, N.A., **J. Control. Rel.,** 1987, 5, 37.
8. Takka, S., Ocak, O.H. and Acarturk, F., **Eur. J. Pharm. Sci.,** 1998, 6, 241.