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Alginate-Based Nanocapsular Antineoplastic Drug Delivery System by Pneumatic Nebulization

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This work is an attempt to develop a generalized upgradable bench top level technology for nanocapsulation of antineoplastic drug substances. Methotrexate was selected as a model drug and calcium alginate was used as a coating material. A nebulizer head was so designed as to atomize a drug-polymer microemulsion directly into calcium chloride cross linker media. Nanocapsules thus formed were compared with those produced by *in situ* gelling technique. Control of various process parameters resulted in the development of superior drug nanocapsules by pneumatic nebulization technique with good yield.

Submicron range (<1 μ) polymer coated nanocapsular drug delivery systems have several potential application including efficient drug delivery, sustained release, drug targeting and better stability[1-3]. Despite the demand and advantages, process developments in this field are relatively slow. This is mainly due to lack of suitable upgradable technology to handle wide range of drug chemicals and a range of useful polymer materials[4]. Preparative techniques available today are either *in situ* polymerization using monomer block materials that can leave undesired chemicals in nanocapsules or *in situ* polymer cross linking that require finer adjustments difficult to perform uniformly in large scale[5].

This work is an attempt of nanocapsulation using a novel pneumatic nebulizer. The process sketch involved compressed air driven nebulization of a microemulsion of drug (in dispersed phase) and polymer (in continuous phase) into a polymer cross linker solution through a designed nebulizer head. The process control parameters are mainly air flow rate, air pressure and fluid flow rate, fluid pressure for optimized delivery of formed nanocapsules. The characteristics of nanocapsules thus formed were evaluated against those produced by known emulsion *in situ* precipitation method[6]. Particle size distribution, drug loading efficiency and particle character were investigated and compared.

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MATERIALS AND METHODS

The model drug used was methotrexate (MXT) which was obtained as a gift from Biochem Pharmaceutical Industries, Ahmedabad. All solvents used were of HPLC grade. Water used was double distilled, filtered (0.22 μ) and degassed. The hydro polymer used for coating was sodium alginate and was purchased from Sigma Chemicals Co., St. Louis, MO.

UV/Vis (Hitachi U-2000) and HPLC (Shimadzu LC-6A) were used as analytical tools for chemical analysis, process development and drug release studies. Transmission electron microscope (TEM, Hitachi-600) was used for particle size distribution and particle nature determination studies. A refrigerated (0°) ultracentrifuge (Sorvall OTD 65 B) was used for harvesting nanocapsules.

Preparation of nanocapsules by emulsion -*in situ* precipitation (Method-I):

Powdered methotrexate (1.5 mg) was dissolved under

sonication (20 KHz) in 10 ml ethyl acetate containing 0.5 ml butylamine. This solution was emulsified in 30 ml of 0.5% aqueous solution of sodium alginate using sorbitan trioleate as emulsifier. Glycerine was used as a co-emulsifier for the preparation of drug-polymer microemulsion. To this microemulsion very slow addition of 10% $CaCl_2$ solution (0.1 ml/min) under continuous stirring (200 rpm) yielded the nanocapsules. The nanocapsules were separated by centrifugation (0°) at 25,000 rpm for 15 min. They were washed and dried at room temperature under vacuum. The polymer:drug ratio used was 100:1.

Preparation of alginate - methotrexate nanocapsules by nebulization technique (Method-II):

Methotrexate (1.5 mg) was taken in the organic phase (10 ml of ethyl acetate containing 0.5 ml butylamine) and emulsified into 30 ml aqueous solution of sodium alginate. The polymer to drug ratio used was 100:1 (w/w). Sorbitan tri-oleate was used as emulsifier. Glycerin was used as a co-emulsifier for preparation of microemulsion. This microemulsion was driven through a specially designed pneumatic nebulizer into cross-linking media of 10% $CaCl_2$ solution. The nanocapsules formed were harvested out by centrifugation (0°) at 25,000 rpm for 15 min, washed, dried and stored.

Nanocapsulation assembly line:

The assembly line for nanocapsulation that uses air - liquid turbulence through a designed nebulizer head was used for nanocapsulation. Microemulsion of drug and polymer solution was used as the liquid phase. A high-pressure compressor is used for air displacement and the microemulsion is feed through an inner tubing such that, regulated flow can create air - liquid interface turbulence at the mouth of the nebulizer (fig.1). This atomised spray of microemulsion emanating from the nebuliser head is directly feed into large volume of cross linker calcium chloride solution. The process parameters were air-pressure, fluid pressure, fluid flow rate and air flow rate. Control of these factors prevented microemulsion droplet coalescing and provided desired air-liquid mist. A fixed spray angle was maintained for tangential entry of microemulsion into cross linker solution.

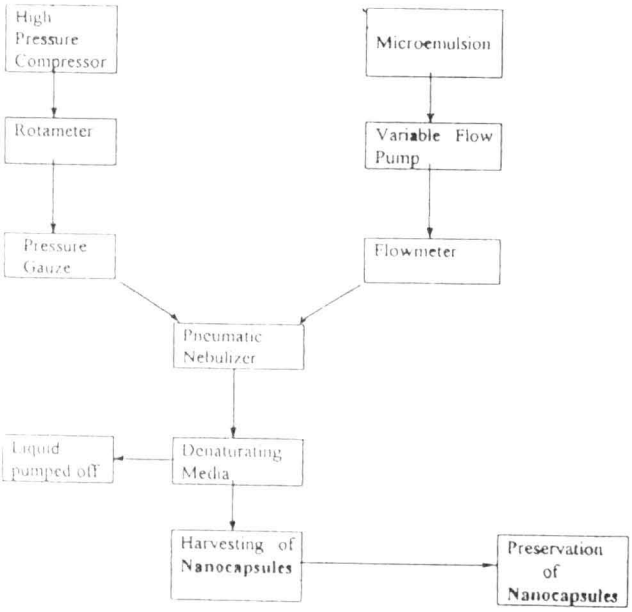


Fig. 1: Nebulizer assembly line sequence.

Design of nebulizer head:

The main objective in designing nebulizer head was to get air-fluid turbulence at the nebulizer mouth without affecting the microemulsion. For that, feed flow area and air annulus area were optimized. To make fine mist from the larger volume of fluid per unit time, higher air jet was required which corresponds to larger air pressure inside the nebulizer. Several trial and error approaches had provided a nebulizer head that can handle various volumes of microemulsions for nanocapsulation. fig. 2 depicts a cross section of the nebulizer head used.

The inner and outer diameter of feed (liquid) flow tube was 1.0 mm and 1.5 mm respectively. Air annulus diameter including feed flow tube was 2.5 mm. Outlet diameter of the

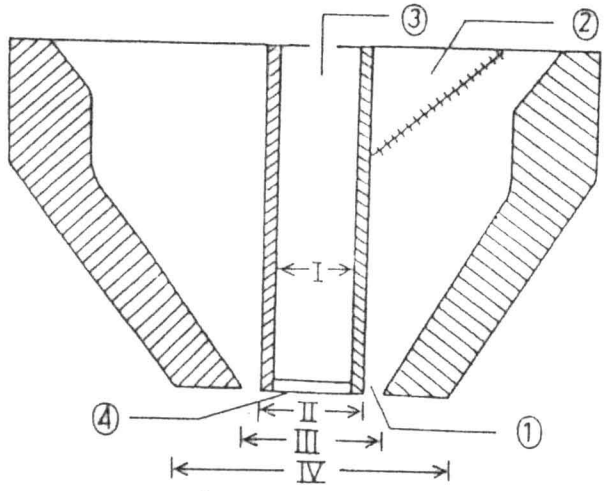


Fig. 2: The cross-section of nebulizer head .

I - 1.0 nm; II - 1.5 nm; III - 2.5 nm; IV - 5.0 nm. 1 – Air annulus, 2- Air flow, 3 – Feed, 4 – Cup lip.

nebulizer was 5.0 mm. Maximum outer diameter of the air-flow bulb was kept 70 mm. The length from the maximum diameter to outlet point was 68 mm. The spray angle maintained was 33.4°.

Particle size distribution studies:

A drop of water suspension of the nanocapsules was placed on carbon-grid (C-grid), air-dried and the particles were stained with phosphotungstanic acid solution (PTA). The C-grid containing PTA stained nanocapsules were placed on transmission electron microscope for TEM micrography. Particle size distributions were measured microscopically (TEM). Transmission electron micrograph of the nanocapsules prepared by following precipitation method and nebulization technique was represented in fig. 3. Particle size distribution was studied in histograms and was presented in fig. 4.

Drug entrapment efficiency:

The total drug load in alginate-MXT nanocapsules was determined by digesting 20 mg of dried nanocapsules in 0.5 ml nitric acid and diluting it to 10 ml with distilled water, pH was adjusted to 7.4 and 20 μl of this sample was directly injected onto the HPLC using Zorbax-ODS column and UV detector set at 257 nm. Mobile phase used was methanol:water, 20:80, containing 0.001% trifluroacetic acid (TFA). Retention time was 16 min at mobile phase flow rate of 0.5 ml/min.

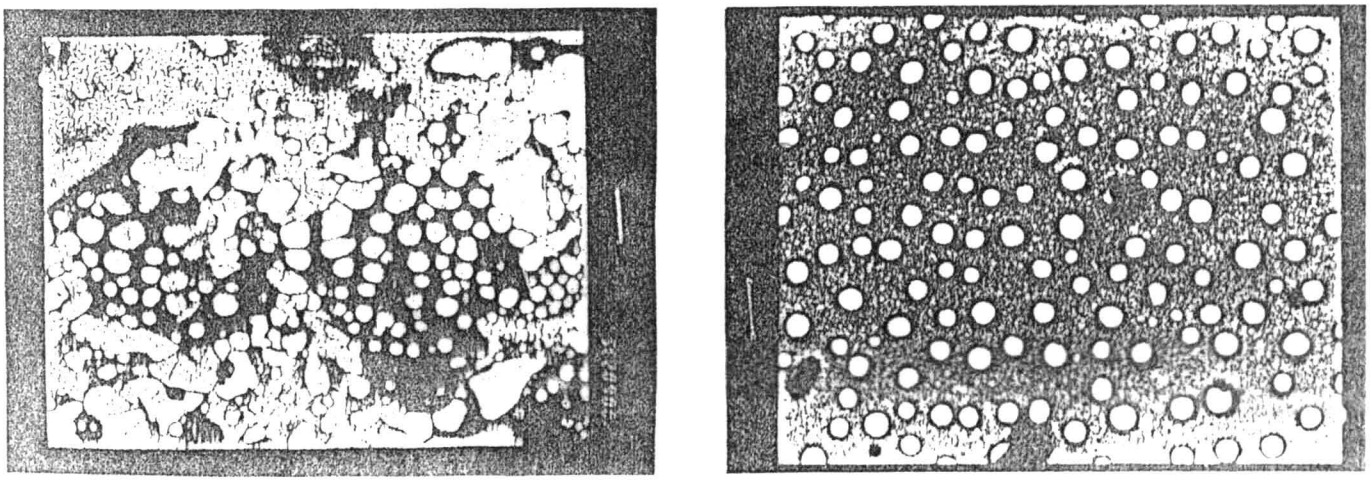
For regular analysis, a std. curve was first prepared with varying conc. of methotrexate vs. HPLC peak area that provided a straight line[7] in same analysis conditions; Y=173.51X+335033.8 [X = concentration, Y = peak area]; r^2 = 0.9985. This standard curve was used for all relevant analysis.

RESULTS AND DISCUSSION

The advantage of pneumatic nebulization technique is that it can handle a larger volume of microemulsion almost continuously. Additionally, process parameters are easily controllable making the technique adaptable for industrial applications.

Submicron size alginate nanocapsules (<1μ) are reported for the first time and is possible by pneumatic nebulization. Being too hygroscopic a gel, nanosize alginate drug carriers were a difficult proposition for conventional techniques. The best attempt so far has been loaded alginate poly-L-lysine nanocapsules (250-850 nm) prepared by *in situ* gelling technique[6]. In pneumatic nebulization system, larger volume of aqueous cross linker solution has significantly reduced problems of residual additives like glycerin or surfactants that were known to pose challenges in proper harvesting of formed nanocapsules. Zwitterionic molecule like methotrexate can be nanocapsulated in this newer technique with reasonably good drug load.

Formation of microemulsion and the rate of addition of cross linker calcium chloride solution has a profound bearing on nanocapsular size distribution and drug loading efficiency



a) b)

Fig. 3: Transmission electron micrograph of alginate–methotrexate nanocapsules.

a) Nanocapsules prepared by precipitation method; polymer:drug ratio 100:1, at X 20,000 at 75 KV. b) Nanocapsules prepared by nebulization technique; polymer:drug ratio 100:1, at X 25,000 at 75 KV.

in method I. Solution turbidity was monitored regularly for microemulsion formation, while slow addition of cross linker solution at a point created a floating gel structure of formed nanocapsules. Choice of surfactant, co-emulsifier, cross linker solution concentration has to be optimized to obtain desirable batch of nanocapsules. Batch variations in this technique are quite large mainly because of several process parameters that are involved and difficult to be controlled. Scale up trials had run into difficulties for nonuniform size distribution and very low drug load. Table 2 provided the particle size range obtained and the total drug load in comparison to these produced by pneumatic nebulization technique.

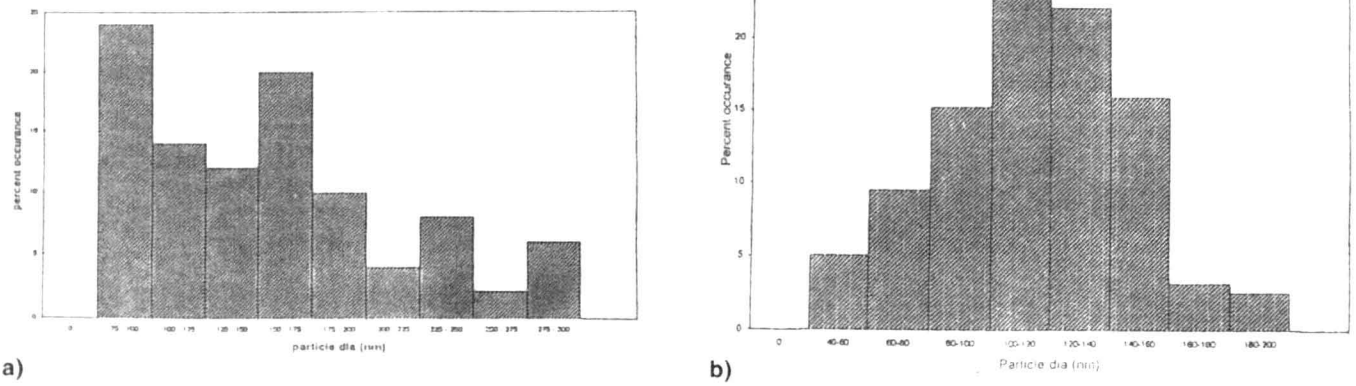
Design of nebulizer head was optimized by several trial and error steps with help from local manufacturers. Material of construction was hard glass though stainless steel head of same dimension was also used for experimentations without any alteration of product nature. Ultrasonic nebulizers and several commercially available nebulizer heads were tried without success; they either released the organic layer in calcium chloride media or produced larger coalesced particles.

Process parameters in this technique were air pressure, airflow rate and microemulsion flow rate. Nanocapsules were formed instantly on contact of the mist with the calcium chloride solution. This has also prevented coalescing of nanocapsules formed and in TEM micrograph discrete nanocapsules were observable. Concentration of calcium chloride and adequate curing time were important. Nanocapsules can be harvested by ultracentrifugation or by successive wash with dry acetone. Yield of nanocapsules can be maximized with a defined spray angle and tangential contact of spray into the cross linker solution. Process parameters were easily controllable and the process can run continuously. The process parameters used are enlisted in Table 1.

The particles observed in TEM micrograph were smooth, spherical and uniformly coated. Fig. 3 shows representative TEM pictures of nanocapsules produced by method I and II respectively. The coating thickness obtained were calculated from arithmetic mean diameter, following the method of described by Luu[8]. Particle mean diameters[9]

TABLE 1: NEBULIZATION CONDITIONS OPTIMIZED FOR METHOTREXATE NANOCAPSULES.

Process parameters	Alginate - Methotrexate nanocapsules (100:1)
Air flow rate	125 ml/sec
Fluid flow rate	180 ml/h
Air pressure	4 atms
Fluid pressure	4 atms
Average nanocapsule diameter	124±21.8 nm
% drug load	33.6 %



a) b)

Fig. 4: Particle size distribution of alginate coated methotrexate nanocapsules.

a) Particle size distribution of nanocapsules with alginate:methotrexate ratio 100:1 prepared by precipitation method from the transmission electron micrograph (fig. 3a). b) Particle size distribution of nanocapsules with alginate:methotrexate ratio 100:1 prepared by nebulization method from the transmission electron micrograph (fig. 3b).

TABLE 2: SIZE DISTRIBUTION AND DRUG LOAD OF NANOCAPSULES PRODUCED IN DIFFERENT TECHNIQUES.

Mean diameter and percent drug load	Nanocapsules from precipitation method	Nanocapsules from nebulization method
Arithmetic mean diameter	165±51.8 nm	124 ± 21.8 nm
Geometric mean diameter	154±43.6 nm	123±19.6 nm
Harmonic mean diameter	145±41.3 nm	122±16.9 nm
Percent drug load	15.7%	33.6%

were calculated from corresponding TEM micrographs and are tabulated in Table 2. Nanocapsules produced by pneumatic nebulization technique were smaller in size and better size distribution when compared with those produced by *in situ* precipitation method.

The percentage drug entrapment efficiency was calculated from the initial amount of drug taken for nanocapsulation and the drug content estimated on dissolution of nanocapsules. % drug load=[{drug taken (mg)–drug loaded (mg)}/drug taken (mg)]x100. Percent drug load of alginate - MXT nanocapsules obtained following method I and method II were tabulated in Table 2. Average of four experiments each following method I and II were used for % drug load calculations.

In summary, submicron sized alginate nanocapsules were produced by a novel pneumatic nebulization technique. Nanocapsules thus produced were of good particle size distribution and carry reasonably good drug load. Antineoplastic drug methotrexate was used as a model drug and nanocapsules produced were compared to those produced by *in situ* gelling technique.

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