

PREPARATION OF ALGINATE NANOPARTICLES BY DESOLVATION TECHNIQUE USING ACETONE AS DESOLVATING AGENT

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ABSTRACT

In order to see functionality and toxicity of nanoparticles in various food and drug applications, it is important to establish procedures to prepare nanoparticles of a controlled size. Desolvation, a thermodynamically driven self-assembly process for polymeric materials. In our study, acetone was added intermittently into 1% alginate solution under stirring at 700 rpm. Amount of acetone added, intermittent timeline of acetone addition, and pH of solution were considered as process parameters to be optimized. Effect of the process parameters on size of the nanoparticles were studied. The results indicated that the size control of alginate nanoparticle was achieved by adding acetone intermittently. The standard deviation of average size of alginate nanoparticles at each preparation condition was minimized by adding acetone intermittently. The intermittent addition in polymeric aqueous solution can be useful for size control for food or drug applications.

Keywords:

INTRODUCTION

Nanoparticles are sub nanosized colloidal structures composed of synthetic or semisynthetic polymers^{1,2}. Nanospheres are solid core spherical particulates which are nanometric in size. They contain drug embedded within the matrix or adsorbed on to the surface. Nanocapsules are vesicular system in which drug is essentially encapsulated with in the central volume surrounded by an embryonic polymeric sheath. In nanocrystals drug is mainly encapsulated in the solution system. Albumin, gelatin, legumin, polysaccharides like alginates or agarose are natural polymers. Natural hydrophilic polymers are studied because of their intrinsic biodegradability and biocompatibility. Natural polymers are classified as proteins and polysaccharides. Proteins are gelatin, albumin, lecithin, legumin and vicillin. Polysaccharides are alginate, dextran, chitosan and pullulan^{3,4}.

Synthetic polymers:- polymers in nanoparticles preparation are those which are used in the preparation of microspheres. Poly lactic acid, methylmethacrylate, polyacrylamide are synthetic polymers^{5,6}.

In order to see functionality and toxicity of nanoparticles in various food and drug applications, it is important to establish procedures to prepare nanoparticles of a controlled size. Amphiphilic macromolecular cross linking, polymerization based methods and polymer precipitation methods are the different methods used for the preparation of nanoparticles. In amphiphilic macromolecular cross linking desolvation technique is mainly used for the preparation of nanoparticles for proteins and polysaccharides^{7,8}. Desolvation, a thermodynamically driven self-assembly process for polymeric materials to prepare nanoparticles. The polymeric molecules form particles of different sizes depending on the preparation conditions such as protein content, pH, ionic strength, concentration of crosslinking agent, agitation speed, amount of desolvating agent etc. The protein or polysaccharide from an aqueous phase can be desolvated by pH change, or change in temperature by adding appropriate amount of counter ions. Cross linking may be affected simultaneously or subsequent to the desolvation step^{9,10}. It contains three steps. Protein dissolution, protein aggregation and protein deaggregation. The appropriate levels of desolvation and resolution, the aggregate size could be maintained and finally these aggregated nanoparticles are cross linked using glutaraldehyde. Sodium sulphate is main desolvating agent. Alcohol, isopropanol, ethanol are added as desolvating agents. The addition can be optimized turbidometrically using nephelometer. Only desolvation can give the final product as nanosphere. Desolvation deaggregates the protein and turns the suspension colloidal and hence milky in appearance. Both lipophilic and hydrophilic drugs can be entrapped in nanoparticles using this technique^{12,13}.

MATERIALS

Alginate is commercially supplied from Sigma Aldrich (St. Louis MO, USA). Analytical Grade high purity acetone is supplied from Fisher Scientifics.

METHODOLOGY

Alginate nanoparticles were prepared using a desolvation method. Alginate powder was added to distilled water. Acetone was added continuously or intermittently into 1% alginate solution at pH 4 under stirring at 700 rpm at room temperature until the solution became just turbid. In continuous addition method acetone was added continuously in the solution with rate addition about 1.0 to 2.0 ml per min and for intermittent method 2ml of acetone was added for every 5 min interval¹¹.

Determining the size of nanoparticles.

Size of alginate nanoparticles was determined by scanning electron microscope. In order to perform the SEM observation, nanoparticle suspension was first diluted with ultrapure water (1/5), and then a drop of the diluted nanoparticle suspension was then directly deposited on a polished aluminum sample holder. Samples were dried in vacuum. The morphology of nanoparticles was observed at 15 kV using a scanning electron microscope (SEM; S-3700 N, Hitachi, Japan).

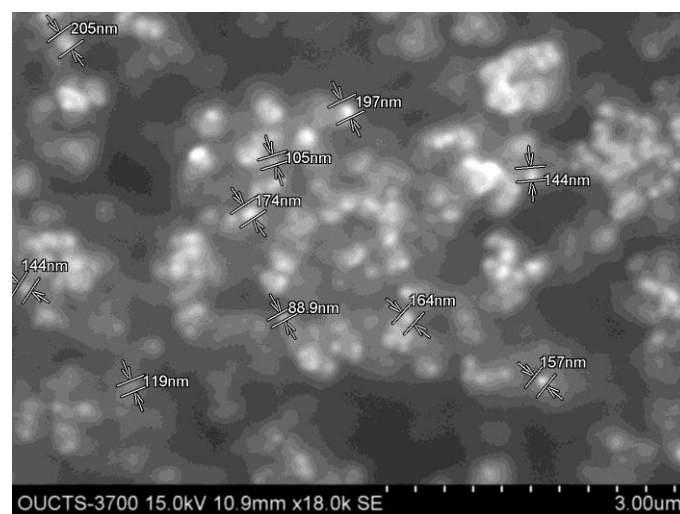


Figure 1: SEM images of Alginate nanoparticles prepared by continuous addition method

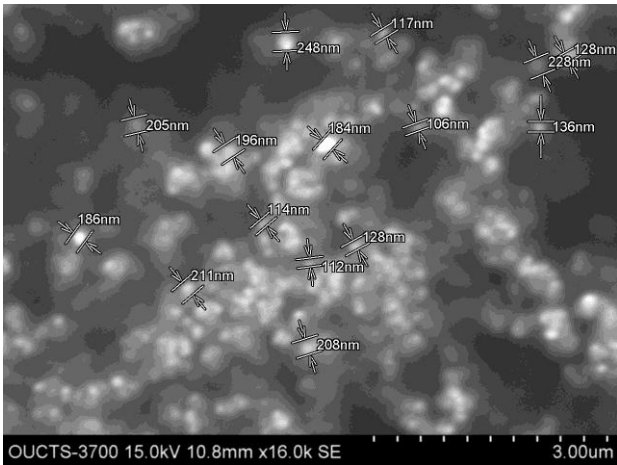


Figure 2: SEM images of Alginate nanoparticles prepared by intermittent addition method

Fourier Transforms infrared Spectroscopy (FT-IR)

The FT-IR spectra acquired were taken for the dried samples. An FT-IR (7000) spectrometer was used for the analysis in the frequency range between 4000 and 400 cm^{-1} (fig no: 3.3). Triturate about 1 mg of the substance with approximately 300 mg of dry, finely powdered potassium bromide IR. These quantities are usually suitable for a disc 13 mm in diameter. Grinded the mixture thoroughly, spreaded it uniformly in a suitable die and compressed under vacuum at a pressure of about 800 Mpa. Commercial dies are available and the manufacturer's instructions should be strictly followed. Mounted the resultant disc in a suitable holder in the spectrophotometer. The IR spectra of the sample were determined from 600-4400 cm^{-1} . Several factors, such as inadequate or excessive grinding, moisture or other impurities in the halide carrier, may give rise to unsatisfactory discs. A disc should be rejected, if visual inspection shows lack of uniformity or if the transmittance at about 2000 cm^{-1} ($5\mu\text{m}$) in the absence of a specific absorption band is less than 75% without compensation.

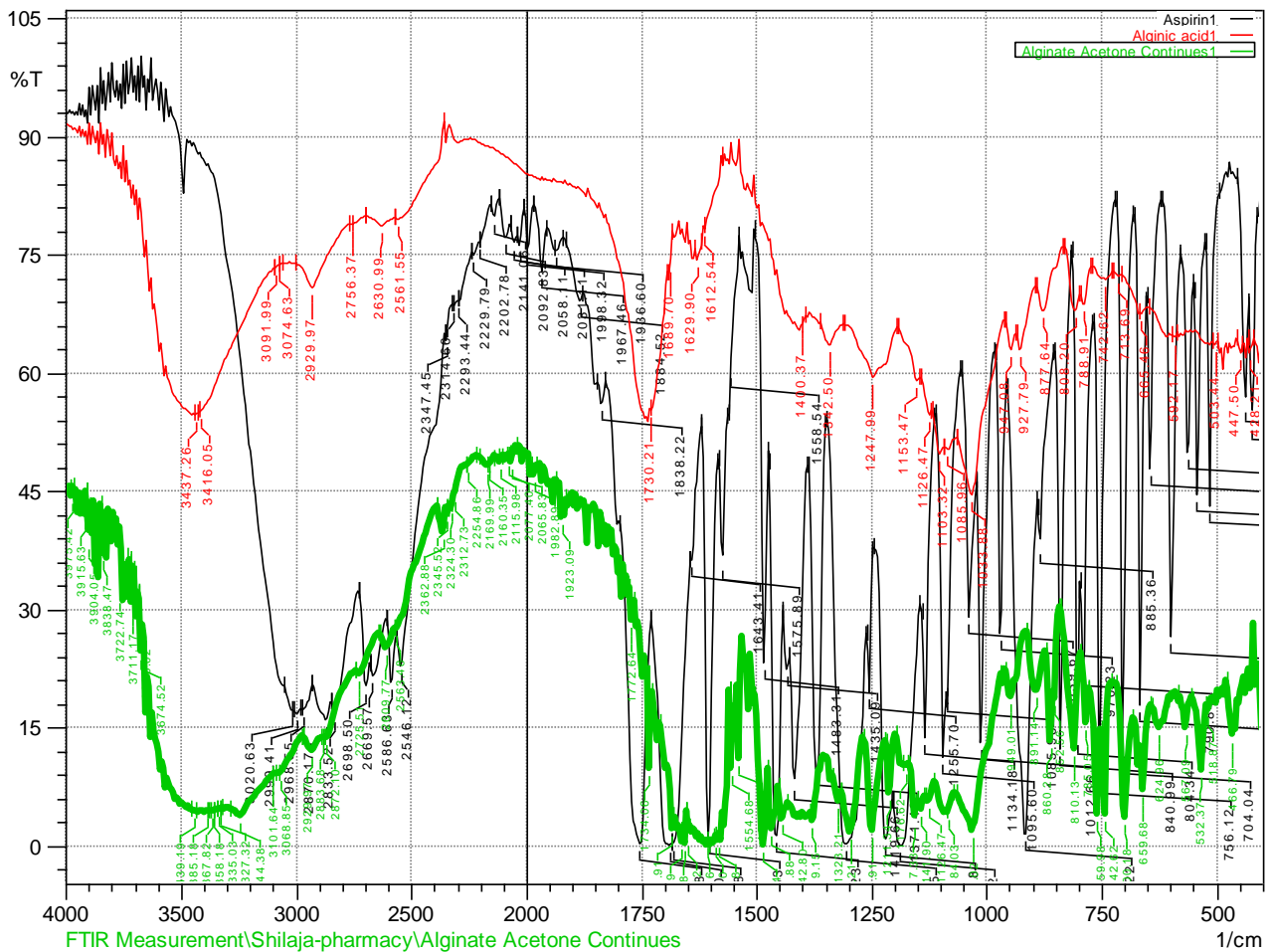


Figure 3: FTIR spectra of aspirin, alginate and formulation (Aspirin loaded alginate nanoformulation)

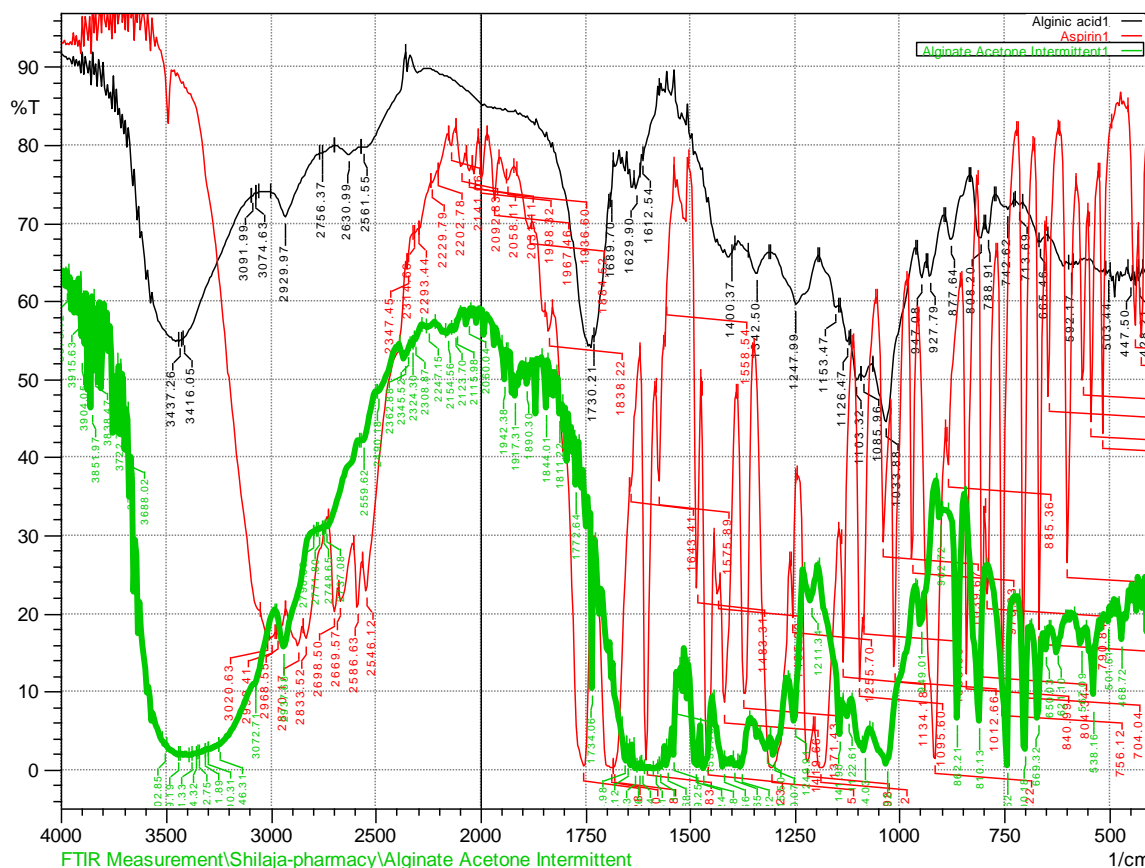


Figure 4: FTIR spectra of aspirin ,alginate and formulation (Aspirin loaded alginate nanoformulation)

CONCLUSIONS

Alginate nanoparticles are prepared by continuous and intermittent addition of acetone as desolvating agent. Effect of the process parameters such as amount of acetone added, intermittent timeline of acetone addition, and pH of solution on the size of the nanoparticles were studied. The results indicated that the size control of alginate nanoparticle was achieved by adding acetone intermittently. The standard deviation of average size of alginate nanoparticles at each preparation condition was minimized by adding acetone intermittently. FTIR spectra indicates there is no polymer drug interaction. Further studies can be performed on drug encapsulation efficiency and *in vitro* release of drug from polymeric nanoparticles.

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