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Elaboration of ammonio methacrylate copolymer based spongy cationic particles via double emulsion solvent evaporation process

Nadiah Zafar, Ahmad Bitar, Jean Pierre Valour, Hatem Fessi, Abdelhamid Elaissari *

University of Lyon, UMR 5007, LAGEP- CPE, 43 bd 11 Novembre 1918, F-69622 Villeurbanne, France

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ABSTRACT

The aim of present work is to investigate systematic study of the preparation of biodegradable particles via double emulsion solvent evaporation technique. The used formation is based on cationic ammonium methacrylate copolymer Eudragit® RS 100, without the use of any stabilizer. The effect of process parameters like ultra turrax® stirring speed and stirring time, ultrasonication time, polymer amount, and volume of outer aqueous phases on the colloidal properties of particles was investigated. All prepared dispersions were characterized in terms of size, size distribution, and electrokinetic properties, and surface morphology was investigated.

1. Introduction

Increasing interest in the fields of biotechnology, medicine, pharmaceutics, catalysis, ecology, nutrition, etc. leads research to being more focused on encapsulation technologies. Encapsulation of active molecules is frequently hunted for to attain assorted favorable characteristics. These benefits include concentration, shielding and protection of bioactives by creation of a single compartment which separates the active molecules from the surrounding environment [1]. The contemporary microencapsulation still continues to be an imperative formulation strategy since its initiation about 70 years ago. It was commenced with an objective to shield vitamins from oxidation [2]. One of the objectives of advanced drug delivery is to provide drug doses for sustained periods to specific targets in the body [3]. Loading of drugs into microparticles and causing their controlled release upon arrival of microparticles at specific target helps in achieving this objective. Diverse microstructures like liposomes [4], microgels, microemulsions, polymer micelles and colloids are extensively employed as drug carriers. The colloidal carriers present advantages in sustained delivery of DNA vaccines, growth factors and other peptides [5]. Few of the advantages that these carriers provide include enhanced bioavailability, drug protection against degradation and control over the rate of release of entrapped actives [5]. Many newly synthesized molecules have low aqueous solubility and few of these when used in their therapeutic concentrations e.g. Paclitaxel, also show poor solubility in lipid media [6]. Encapsulation of such agents assists in resolving this problem. Additional benefits of encapsulation are safe handling of toxic agents, masking of organoleptic properties like color, taste, odor of molecules and evasion of adverse effects like gastric irritation caused by certain active agents.

Microparticles constituted from biodegradable polymers are increasingly utilized for drug delivery because they can be easily implanted into the body without the need of any surgical removal. The drug release rates from biodegradable polymers can be controlled by factors like biodegradation kinetics of the polymers [7], physicochemical properties of the polymers and drugs [8], thermodynamic compatibility between the polymers and drugs and the shape of the devices [9]. Biodegradable microparticles prepared through various formulatory techniques can be employed for delivery of variety of drug molecules regardless of their molecular weights and water solubility [10,11]. Polymeric microparticles also offer potential for evolution of mucosally administered vaccines. Examples of different biodegradable polymers used in microparticle formation are polyesters, polysaccharides [10], polyanhydrides [12], polyphosphazenes [13] and poly (ortho esters) [14]. Injectable controlled release depots such as drug-loaded biodegradable polymer microparticles can serve as an alternative delivery platform for some of the drugs that (i) possess broad therapeutic window, (ii) need a low daily dose, and (iii) are going to be used for long-term disease treatment [2].

Various methodologies are delved into for encapsulation of different active molecules and the nature of the drug [15] mainly determines the selection of the encapsulation technique [16]. The frequently employed encapsulation techniques include: single emulsion process [17], double emulsion process [18], ionic gelation [19], solvent displacement [20], nanoprecipitation [21,22], supercritical fluid technology, spray drying [23], miniemulsion polymerization [24] and solvent diffusion [25] techniques. In designing any new technique, one must take into consideration some important factors like stability and biological activity of the

* Corresponding author at: University of Lyon, F-69622 Lyon, France.
E-mail address: elaissari@lagep.univ-lyon1.fr (A. Elaissari).

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drugs must not be altered during encapsulation process, encapsulation efficiency should be high, microparticle quality and drug release profile should be reproducible and aggregation or adherence of microparticles should not be exhibited.

Emulsification solvent evaporation method has been widely employed for the preparation of biodegradable microspheres [26,27,28]. This technique has been shown as the most pertinent method to encapsulate hydrophilic drugs (vaccines, vitamins, enzymes, hormones) and proteins within microparticles by many authors [29]. This method offers many attractive prospects for the controlled release of active molecules initially entrapped in the internal droplets. There are two major kinds of double emulsions: water in oil in water (W/O/W) and oil in water in oil (O/W/O) types. Double emulsion consists of three phases and mostly the inner and outer layers are of the same phase [16]. Active molecules may migrate from inner to outer phase of multiple emulsions; thus providing a kind of reservoir suitable for detoxification (overdose treatment) or be used for the removal of toxic agents from waste water. This technique needs only mild conditions like ambient temperatures and constant stirring. Hence, a stable emulsion can be formulated without a compromise on the activity of the drugs.

Double emulsions are generally prepared via two step emulsification process employing two surfactants e.g. in W/O/W, a low hydrophilic–lipophilic balance (HLB) surfactant is used to stabilize the interface of the W/O internal emulsion and a high HLB one for the stabilization of external interface of oil globules [16,30]. Various drugs and proteins have been encapsulated by this technique e.g. Bovine Serum Albumin [31] (BSA), Plasmid DNA [32], Ciprofloxacin etc.

The objective of this study was to investigate the influence of various process control parameters on the properties of resultant microparticles (e.g. size and zeta potential). The aim was to optimize these parameters for future encapsulation of active molecules via emulsification–diffusion solvent evaporation process. Such studies play an integral role in not only developing better drug delivery systems but also in enhancing human perception of the bioavailability and permeability of the active agents encapsulated in the particles.

2. Materials and methods

2.1. Materials

Ammonio methacrylate copolymer type BNF Eudragit® RS100 (M_w ~ 32,000 g/mol) was purchased from EVONIK (EVONIK, Germany). Dichloromethane (DCM) and NaCl were purchased from Sigma-Aldrich, Germany as used as such. Deionized water was used throughout the work.

2.2. Method

Double emulsion–diffusion solvent evaporation method was selected for the preparation of microparticles. This process involves the formation of double emulsion at a first stage followed by an addition of excess volume of water to facilitate the diffusion of organic solvent into external aqueous phase.

2.2.1. Preparation of double emulsion

1 ml of aq. phase was emulsified in the organic phase (1 g polymer in 5 ml DCM) using ultra sound (Optic ivymen system CY-500) for certain specific amplitude and time. The resultant primary emulsion (w/o) was then transferred to 50 ml of the aq. phase along with stirring using ultra turrax® (T-25 basic IKA®-Werke) at a specific speed and time until the formation of double emulsion. No stabilizer was employed in the formulation. The obtained double emulsion (w/o/w) was diluted with 100 ml of aq. phase and finally subjected to evaporation by using rotavapor (Buchi rotavapor R-124). To ensure that solvent was completely removed, the volume of diluted double emulsion was measured before and after evaporation. Fig. 1 gives a diagrammatic illustration of the process.

2.3. Characterization of the prepared microparticles

The obtained particles were analyzed in three different ways. The hydrodynamic size of the particles and their size distribution were measured after slight dilution. Zeta potential of highly diluted dispersions was investigated as a function of pH. Finally, the morphology of the particles was also carefully examined.

2.3.1. Particles size measurement

The diameter size of the particles and their distribution was studied by using Beckman Coulter LS 13 320 Laser Diffraction Particle Size Analyzer. Prepared dispersions were added dropwisely to the sample cell contained continuous phase (deionized water). The pump speed was adjusted to 31% for proper mixing of the samples. An average diameter of three readings was calculated.

2.3.2. Zeta potential

Zeta potential of each sample was measured at 25 °C by using Zetasizer (Nano-ZS, Malvern®, UK). The microparticles were dispersed in 1 mM NaCl before every measurement. Each measurement was made in triplicate. Zeta potential of the microparticles as a function of pH was measured using auto autotitrator (MPT-2, Malvern®, UK). It was measured at pH range of 3–11 keeping an interval of 1.

2.3.3. Scanning electron microscopy

The shape and appearance of the microparticles was examined by scanning electron microscope (SEM) by using Hitachi S800 FEG microscope at the "Centre Technologique des Microstructures" (CTμ) at the University of Lyon (Villeurbanne, France). A drop of each sample was placed on a flat steel holder (cell) and dried at room temperature. The sample was then coated with platinum under vacuum by cathodic sputtering. An accelerating voltage of 15 kv was used for SEM analysis of the samples.

3. Results and discussion

The objective of this research was to obtain stable cationic particles, which could be adsorbed onto different negatively charged textiles for our future work. Moreover, the first aim was, not to use any stabilizer in the formulation to avoid any complexity in the double emulsion–diffusion solvent evaporation process. The influential factors were investigated in order to fix these parameters to those values, which would give spherical, stable porous particles within a specific size range so that such particles can be easily adsorbed onto different textiles after loading of specific active agents. Without investigating individual parameter it is difficult to know e.g. which polymer amount would give micrometer particles size free from any aggregated particles. To target such objective, double emulsification solvent evaporation process using ammonium methacrylate copolymer (Eudragit® RS100) as polymer. Moreover, the discussion under each parameter is done to provide scientific reasons for the results that are obtained.

3.1. Parameters investigated: water in oil emulsion (W_1/O) stage

Two parameters were first investigated: the polymer amount used in the formulation and the ultrasonication time. All other parameters related to the formulation were kept constant (Table 1).

3.1.1. Polymer amount effect

The amount of polymer Eudragit® RS100 can play an integral role in controlling the particle size and encapsulation efficiency. Various amounts of polymer used were 0.25 g, 0.5 g, 0.75 g, 1.0 g, 1.25 g and 1.5 g. Above 1.5 g of the polymer was not feasible due to high viscosity