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Research Paper

Development of highly stabilized curcumin nanoparticles by flash nanoprecipitation and lyophilization

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ABSTRACT

The influence of critical operating parameters on the Flash Nanoprecipitation (FNP) and resulting material properties of curcumin (CUR) nanoparticles has been evaluated using a confined impinging jets-with-dilution mixer (CIJ-D-M). It has been shown that the mixing rate, molecular weight of polymeric stabilizer (i.e., polyethylene glycol-*b*-poly(DL-lactide) di-block copolymer; PEG–PLA) and drug-to-copolymer mass ratio all exert a significant impact on the particle size and stability of the generated nanosuspensions. The attainable mean particle size and span of the nanoparticles through optimization of these process parameters were approximately 70 nm and 0.85 respectively. However, the optimized nanosuspension was only stable for about two hours after preparation. Co-formulation with polyvinylpyrrolidone (PVP) substantially extended the product lifespan to 5 days at ambient conditions and two weeks at 4 °C. Results from zeta potential measurement and X-ray photoelectron spectroscopy (XPS) suggested that the enhanced stability is probably due to the formation of an additional protective barrier by PVP around the particle surface, thereby suppressing the dissociation of PEG–PLA from the particles and preventing CUR leakage from inside. Long-term storage stability (>1 year) could be achieved by lyophilization of the optimized nanosuspension with Kleptose (hydroxypropyl-β-cyclodextrin), which was shown to be the only effective lyoprotectant among all the ones tested for the CUR nanoparticles. At an optimal concentration of Kleptose (1.25% w/v), the redispersibility (S_f/S_i; ratio of the final and initial particle sizes) and encapsulation efficiency of lyophilized CUR nanoparticles were about 1.22% and 94%, respectively.

1. Introduction

Recent advances in nanotechnology have made possible the development of various novel nanoparticulate drug delivery systems for specific biological applications [1,2]. Despite the considerable progress being made in elucidating the particular advantages and mechanisms of such systems in disease treatment, only a few of them managed to make their way to clinical trials or have been approved by US-FDA over the past four decades. In addition to the safety issue, the major challenge in nanoformulation development lies in devising a truly pragmatic and cost-efficient approach or technology capable of achieving the desired product characteristics (particle size in particular) and stability. Although any particle with size below 1 μm (1000 nm) can be classified as a nanoparticle, only the particles whose sizes fall within the range of 50–200 nm have been shown to be effective for targeted drug delivery [3,4]. In addition, when formed or prepared from aqueous solutions, such ultrafine particles often display various forms of physical instability (i.e., aggregation, recrystallization and/or Ostwald ripening) owing to their high surface-to-volume ratio and consequential high surface energy [5]. Circumvention of such stability problems with nanoformulations would require the use of stabilizers such as polymers [6,7], electrolytes [8], lipids [9,10] and surfactants [11,12].

Currently, the technologies available for nanoparticle fabrication can be broadly classified into two main types: top-down and bottom-up. The former utilizes mechanical means to reduce

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Nomenclature

AFM	atomic force microscopy
CIJ-D-M	confined impinging jets-with-dilution mixer
CUR	curcumin
DSC	differential scanning calorimetry
F-68	Pluronic F-68
FNP	flash nanoprecipitation
IBU	ibuprofen
ITZ	itraconazole
LEU	leucine
MIVM	multi-inlet vortex mixer
PEG–PLA	polyethylene glycol-*b*-poly(DL-lactide)
PI	polydispersity index
PS-PEO	polystyrene-*b*-poly(ethylene oxide)
PVP	polyvinylpyrrolidone
PVA	polyvinyl alcohol
Re	Reynolds number
TGA	thermogravimetric analysis
TPGS	d-α tocopheryl polyethylene glycol 1000 succinate
XPS	X-ray photoelectron spectroscopy

particle size down to the nanoscale (e.g., milling and high pressure homogenization) [13,14] while the latter relies on the attainment of a sufficiently high supersaturation level to trigger 'rebirth' of ultrafine particles from a solution phase [15]. Although the top-down approach has been well-established and validated for large-scale utilization in industry, it is generally ineffective for achieving particle size as small as 50 nm for organic drug materials, and the final nano product tends to display a wide particle size distribution. On the other hand, the bottom-up techniques, notably the solvent displacement or antisolvent precipitation process [16], can readily generate nanoparticles of the desired particle size or size range through proper control of the particle nucleation and growth processes in solutions. Of particular merit in this regard is the recently emerging technology, Flash Nanoprecipitation (FNP). This technology relies on rapid mixing of the solvent and antisolvent on a millisecond timescale with the aid of suitably designed high-efficiency mixers to attain a homogeneous and highly supersaturated solution, which is critical for inducing instantaneous precipitation of uniformly sized nanoparticles [17]. Smart mixers that have been specifically developed for the FNP process include the confined impinging jets mixer (CIJM) devised by Johnson and Prudhomme [17] and the multi-inlet vortex mixer (MIVM) developed by Liu et al. [18]. The particular designs and geometric characteristics of these mixers, which are central to their ability to achieve homogenous mixing of the solution and antisolvent streams prior to particle nucleation, can critically impact their mixing efficiency and hence the physical properties (e.g., particle size and morphology) of the resulting nanoparticles [19]. In order to further raise the solute supersaturation for the precipitation process and to minimize Ostwald ripening, the CIJM has subsequently been modified with the introduction of a second dilution (quenching) step [20]. This new CIJ mixer, commonly known as confined impinging jets-with-dilution mixer (CIJ-D-M), has the flexibility to be operated manually without strict reliance on syringe pumps for solvent delivery, which makes it a particularly convenient tool for mass screening of nanoformulation components and repeated testing of nanoformulations. In addition to a high-efficiency mixer, FNP requires the use of a primary stabilizer, typically an amphiphilic diblock copolymer [e.g., (ethylene glycol)-*b*-poly(DL-lactide); PEG–PLA], which serves to halt further growth of nanoparticles and maintain their stability once they are formed.

We have previously reported an FNP method employing an MIVM to prepare highly stabilized curcumin (CUR) nanoparticles for testing in Alzheimer's disease mouse model [21]. Two stabilizers, an amphiphilic diblock copolymer (i.e., PEG–PLA 2–8 k) plus a co-stabilizer (i.e., PVP), were found necessary to maintain the stability of the CUR nanoparticles during subsequent processing treatments (i.e., dialysis and freeze drying). Further work comparing the relative performance of a CIJ-D-M and an MIVM also indicated that while good particle size control could be achieved with either mixer, the stability of the preparation in the presence of copolymer stabilizer alone could not last for more than a few hours even with some process optimization [22]. It should be reiterated that our choice of CUR in this previous work was guided by it having a log*P* value (~3) typical of most drugs and its wide array of proven pharmacological activities (e.g., anti-oxidative, anti-inflammatory, cholesterol-lowering, anti-amyloid, and anti-cancer activities), which may be beneficial to the treatment of Alzheimer's disease and cancers [23,24].

Most of the successful cases with the FNP technology employed highly lipophilic compounds or drugs ($\log P \geqslant 6$), and the primary copolymer stabilizers used served well to maintain the stability of the resulting products [8,25]. Apparently, the extremely high lipophilicity or poor water solubility of such compounds favors their tight binding to the hydrophobic cores of the particles, which accounts for their superior nanoparticle stability. For drugs in the typical log*P* range of 3–5, such binding in the nanoparticle cores is expected to be weaker, which possibly explains why our CUR nanoparticles are relatively unstable when formulated with the copolymer stabilizer alone [22].

As an alternative formulation approach to generating stable curcumin nanoparticles by FNP using the CIJ-D-M, Margulis and co-workers employed a coarse oil-in-water emulsion instead of a simple solution of curcumin to co-mix with water in the initial mixing step inside the mixer [26]. A volatile, partially water-miscible organic solvent of curcumin, cyclopentanone, formed the oil phase of the emulsion, and non-polymeric, food grade surfactants (soybean phosphatidylcholine and glycyrrhizinate salts) were used to stabilize the nanoparticles. It is believed that the high shear forces created by impingement of the coarse emulsion against water provides an effective means of reducing the emulsion's droplet size while the accompanying turbulent mixing facilitates rapid diffusion of the organic solvent to the external aqueous phase, resulting in instantaneous supersaturation inside the droplets. Subsequent dilution with water further augments the supersaturation and expedites the diffusion of cyclopentanone into water, leading to the formation of small monodisperse particles. The organic solvent and water in the final liquid mixture are then rapidly removed by spray drying to yield a dry powder. The nanoparticles produced thus were stable and redispersible in water without any significant increase in particle size after storage for several weeks. The authors also showed that the use of a preformed curcumin emulsion in the initial co-mixing step with water was essential for the formation of stable nanoparticles since its substitution by a solution formulation containing the same ingredients at the same concentrations resulted in immediate formation of visually discernable coarse particles. In addition, "rapid mixing"

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