Journal of Colloid and Interface Science 428 (2014) 286–294

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

Physicochemical characterization of solid lipid nanoparticles (SLNs) prepared by a novel microemulsion technique

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ARTICLE INFO

Article history:
Received 11 February 2014
Accepted 28 April 2014
Available online 4 May 2014

Keywords:
Solid lipid nanoparticles
Microwave
Tetracycline
Stearic acid
Polydispersity
Encapsulation efficiency
Loading capacity
Stability
In vitro release

ABSTRACT

Hypothesis: Solid lipid nanoparticles (SLNs) produced by conventional microemulsion techniques using thermal heat have specific limitations (e.g. high polydispersity, instability and low encapsulation). Replacing thermal heat with microwave heat may produce SLNs which overcome some of these limitations.

Experiments: Stearic acid-based SLNs prepared with Tween® 20 as the emulsifier were chosen as the optimum formulation to encapsulate and potentially deliver the antibacterial drug tetracycline. All formulations were characterized for their particle size, zeta potential, encapsulation efficiency, loading capacity, thermal and X-ray diffraction analyses. Short-term stability and *in vitro* drug studies were also performed.

Findings: Microwave heating helps to overcome several disadvantages associated with thermal heating (nonuniform, inefficient and slow) and results in improved particle characteristics. There is thus the potential for new opportunities in the development of colloidal carriers. The particle sizes of microwave-produced SLNs were in the desired nanometer range (200–250 nm) with both lower size and lower polydispersity than the conventional SLNs. We take this as an indication of improved stability; however zeta potential measurements were not different, indicating similar stability. True stability testing (visual observation with time) did show that the microwave-induced SLNs were found to be more stable, particularly when refrigerated. The microwave-produced SLNs also demonstrated improved encapsulation efficiency and loading capacity. Thermal and diffraction analysis confirmed a lowered crystallinity of stearic acid with successful incorporation of tetracycline into the SLNs. *In vitro* release studies indicated that, after an initial burst release, SLNs could provide prolonged release of tetracycline. The presence of tetracycline and non-toxicity of carriers towards microbes was confirmed by antimicrobial susceptibility tests.

1. Introduction

High solubility and membrane permeability are two main prerequisites for effective drug absorption. However, many new drug entities and marketed drugs have poor aqueous solubility, low membrane permeability and poor metabolic stability, all of which pose a major challenge to drug development and delivery [1]. The development of colloidal drug carriers, such as micelles, nanoemulsions, liposomes and polymeric nanoparticles has been looked upon as a promising strategy to overcome these issues. However, specific drawbacks including particle aggregation, drug leakage and polymer or solvent toxicity have restricted their use as carrier systems [2]. In recent years, solid lipid nanoparticles (SLNs) have been widely studied as alternative colloidal systems to encapsulate drugs that have limited water solubility.

SLNs are colloidal systems composed of physiological and biocompatible lipids that are solid at room and body temperatures and stabilized with non-toxic emulsifiers [3]. The three main features of SLNs – solid nature, lipid matrix and nano-sized particles – have been theorized to impart biocompatibility, controlled drug release and improved drug dissolution [4]. The solid core of the SLNs, prepared by precipitation of a microemulsion, inhibits coalescence of particles and has been reported to exhibit better stability than liquid microemulsion droplets [5]. The other potential beneficial aspects of SLNs include controlled and sustained release of drugs, avoidance of organic solvents, biodegradability and safety of carrier systems, specific site targeting and the potential for large scale-up and sterilization [6,7]. SLNs have also been extensively

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http://dx.doi.org/10.1016/j.jcis.2014.04.057
0021-9797/

investigated for their oral [8], dermal [9], ocular [10] and parenteral [5] applications as carriers.

A number of procedures have been used in SLN production, with high pressure homogenization and microemulsion techniques being the two most widely employed. Other production procedures include ultrasound-based, solvent-based, phase inversion temperature-based and membrane contractor-based techniques [11]. However, each of these remains with specific disadvantages [12–14] such as the risk of metal contamination when ultrasound is used, high polydispersity, non-uniform and inefficient thermal heating in microemulsion techniques, high energy inputs in homogenization-based techniques, difficulty in solvent removal in solvent-based techniques, particle aggregation and membrane clogging.

The present study investigates a novel microemulsion-based production procedure using microwave energy as the heating source. Microwave-assisted organic and inorganic syntheses have been extensively investigated in recent years [15]. This efficient energy source has been used in the synthesis of compound libraries for generation and optimization of new drug candidates [16]. Microwave technology has also been used in the process of drying pharmaceutical products [17], with particular emphasis on the long-term stability of solid dispersions of drugs [18]. Whilst microwave chemistry is a well-established technique in syntheses, its use in pharmaceutical formulation has not yet reached its full potential, with only a few reported successes [19–21]. To our knowledge, this is the first research that considers the use of microwave heating for formulation of a microemulsion which can be subsequently solidified to generate solid lipid nanoparticles (SLNs).

The main objective of the present study was to compare the SLNs generated by the novel microwave-based microemulsion procedure to those generated by a conventional microemulsion procedure. The research also demonstrates the suitability of the novel microwave induced procedure to encapsulate tetracycline into the SLNs.

Tetracycline was selected as a "choice of drug" since it is a very common antibiotic and the bioassay to test its activity is simple and widely accepted. Tetracycline is a broad spectrum anti-microbial agent that binds to the 30S ribosomal subunit of bacteria, and inhibits protein biosynthesis. Tetracycline and its derivatives find extensive use in the prophylaxis and therapy of human and animal infections. It has been found to exhibit antimicrobial activity against a number of bacteria, mycoplasmas, rickettsia and chlamydia [14]. Tetracycline is a polyketide comprising of a naphthacene ring structure. As a free base, it is an amphoteric compound. The tetracycline molecule comprises of three functional groups: tricarbonylamide, phenolic diketone and dimethylamine imparting three pK_a values, 3.3, 7.7 and 9.7 (Fig. 1) to the tetracycline molecule. Tetracycline free base is very slightly soluble in water (231 mg/L) and its octanol/water partition coefficient is very low ($\log K_{ow} = -1.31$) [22]. Fig. 1 illustrates the chemical structure of tetracycline.

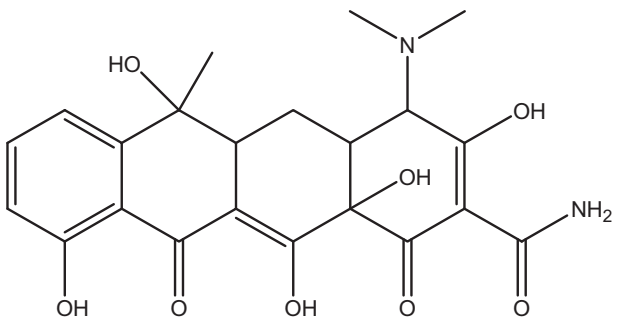

Fig. 1. Chemical structure of tetracycline.

2. Experimental details

2.1. Materials

Unless otherwise stated, all chemicals were used as received without further purification. Imwitor® 900 K (glyceryl monostearate), Dynasan® 114 (glyceryl trimyristate) and Softisan® 142 (hydrogenated coco-glycerides) were generous gifts from Cremer Oleo (Germany). Precirol® ATO 5 (glyceryl palmitostearate) and Compritol® 888 ATO (glyceryl behenate) were supplied by Gattefossé (Germany). Cetyl palmitate was purchased from Merck (Germany). Stearic acid (a fatty acid), Tween® 20 (emulsifier) and tetracycline (⩾98%) were purchased from Sigma–Aldrich (Germany). Ultra-purified water was obtained by reverse osmosis from a MilliQ® Plus purification system (Millipore, Germany) and all other chemicals and reagents were commercially available and of analytical grade.

2.2. Methods

2.2.1. Screening of lipids

The screening of lipids was performed by evaluating the solubility of tetracycline (5–15% (w/w) with respect to lipid mass) in different lipids: Imwitor® 900 K, Dynasan® 114, Compritol® 888 ATO, Precirol® ATO 5, Softisan® 142, cetyl palmitate and stearic acid. The physical mixtures of lipid and tetracycline were heated to 90 °C, and the melts were observed to verify the presence or absence of insoluble drug crystals.

2.2.2. Preparation of solid lipid nanoparticles (SLNs)

SLNs were prepared by two production techniques; a conventional microemulsion method and a novel microwave-based microemulsion method. The composition of both the drug-free (DF) and drug-loaded (DL) SLNs produced by both heating techniques are presented in Table 1. Tetracycline was used at a concentration, referred to as drug loading, of 5%, 10% and 15% (w/w) with respect to stearic acid, and the resultant formulations designated as DL A, DL B and DL C respectively.

2.2.2.1. Conventional microemulsion technique. SLNs were prepared by a conventional microemulsion method according to the procedure described by Gasco with some modification [23]. To summarize; a mixture of water and Tween® 20 was heated to the same temperature (85 °C) as the lipid phase containing stearic acid. Under constant stirring, at 85 °C, the hot aqueous phase was then added to the molten lipid phase in order to produce a hot o/w microemulsion. A dispersion of SLN was then obtained by dispersing the hot o/w microemulsion in cold water (2–4 °C) at a 1:50 ratio (microemulsion: water, v/v). For drug-loaded SLNs, the drug

Table 1
Composition of SLN formulations.

Composition	Formulations (% w/w)			
	DF	DL A	DL B	DL C
Stearic acid	7.5	7.5	7.5	7.5
Tween® 20	12.5	12.5	12.5	12.5
Water	80.0	80.0	80.0	80.0
Tetracycline*	–	5.0	10.0	15.0

DF: drug-free; DL: drug-loaded.
* Drug loading (% w/w) with respect to amount of stearic acid.