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Efficient approach to enhance drug solubility by particle engineering of bovine serum albumin

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ABSTRACT

The aim of this study was to investigate the use of bovine serum albumin (BSA) as a solubility enhancer for indometacin (IND) as a model drug. IND-BSA solid dispersions were prepared by both spray drying and freeze drying techniques using IND:BSA solution (20:1 Molar Ratio (MR)) and IND:BSA suspension (100:1 MR). The solid state of IND in solid dispersions was characterised by SEM, DSC and XRD. The aqueous solubility of IND in the presence of increased amounts of BSA was evaluated. Additionally, IND dissolution and release profiles were evaluated. IND in solid dispersions with BSA showed significantly higher solubility in water than that of the physical mixture of both. Enhancement factors of 24,000 and 100,000 were obtained for the solid dispersion formulated in 20:1 MR and 100:1 MR, respectively. Dissolution studies *in-vitro* indicated a significant increase in the dissolution rate of IND from solid dispersions compared to that of the free drug, with almost 95% of the drug dissolved in the first 5 min. Furthermore, an immediate release of IND from BSA solid dispersions was shown. The potential use of albumin as solubility enhancer for poorly soluble drugs, particularly, for immediate release volume-limited dosage forms is reported.

1. Introduction

Poor drug solubility remains one of the major challenges in drug discovery and development process as it leads to erratic absorption and poor oral bioavailability (Bellantone, 2014). Poor solubility is also a key limiting factor in the development of volume-limited formulations such as ocular, intramuscular, pulmonary, and nasal dosage forms. According to Nernst–Brunner/Noyes–Whitney equation, low solubility implies slow dissolution rate, hence, compromising further drug bioavailability. Almost 60–70% of drugs in development are poorly soluble in water with almost 40% being practically insoluble (Bosselmann and Williams, 2012). Over the years, different strategies have been developed to overcome poor solubility issues. This includes micronization and nanonization, particle engineering, amorphisation, solid dispersion, salts formation, the use of surfactant "micellization", cyclodextrins and polymeric complexations (Cavallari et al., 2005; Chen et al., 2011; Del Valle, 2004; Junghanns and Müller, 2008; Owen, 2013; Strickley, 2004). Nevertheless, these strategies are not always able to satisfactorily improve the drug solubility and the combination of more than one strategy may be required. Therefore, the development of innovative solubility enhancing approaches is still needed to keep pace with the dramatically growing number of new drug candidates that are poorly soluble in water.

Known as the most abundant protein in plasma, albumin has been suggested as a non-toxic, biodegradable, highly soluble, and stable pharmaceutical excipient (Evans, 2002; Liu and Chen, 2016). Albumin has several physiological functions such as maintaining plasma osmotic pressure and neutralising free radicals (Evans,

Abbreviations: BSA, bovine serum albumin; IND, indometacin; FD-sol, freeze dried from solution; FD-sus, freeze dried from suspension; SD-sol, spray dried from solution; SD-sus, spray dried from suspension.

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2002; Liu and Chen, 2016). Most importantly, it plays the role of an "*in-vivo* solubilizing agent" allowing the solubilisation of a wide range of biomolecules and drugs in a hydrophilic medium, *i.e.* the plasma (Ghuman et al., 2005; Peters, 1995). The solubility enhancement properties of albumin are mainly due to its remarkable ability to form reversible binding complexes with ligands. This allows the bound molecule to flow in the blood at concentrations higher than that of its initial solubility (Ghuman et al., 2005; Urien et al., 2007). Albumin has two main sites that bind the ligands mainly by hydrophobic and electrostatic interactions. Although the overall charge of albumin is negative at the physiological pH, the two principal binding sites are positively charged which promotes the binding of anionic molecules. Furthermore, albumin has several secondary binding sites increasing the number of bound molecules, *e.g.* up to seven fatty acid molecules (Evans, 2002). Amongst substances showing the highest affinity to albumin, anionic molecules (weak acid) and hydrophobic molecules of medium size (100–600 Da); poorly soluble drugs (Peters, 1995; Urien et al., 2007). Interestingly, albumin molecule possesses numerous accessible free amino and carboxyl groups amenable to form highly soluble salts with acidic or basic drugs, respectively (Owen, 2013; Phelps and Putnam, 1960; Serajuddin, 2007). Furthermore, the buffer capacity of albumin, although weak, may help in enhancing the solubility of drugs that are ionisable in the pH range of albumin solutions (pH 5.2–7) (Curvale, 2009). Despite the exceptional capacity of albumin to physiologically dissolve poorly soluble drugs, no comprehensive study has yet explored the applicability of this unique property *in-vitro*. Though, few and partial data have been transiently mentioned in the literature (Devang et al., 2014; Kim et al., 2011; Li and Yao, 2009). Bovine serum albumin (BSA) is chemically similar to human albumin and is widely used in pharmaceutical industry owing to its abundance, low cost, ease of purification, biocompatibility and biodegradability (Yu et al., 2014).

The aim of this study is to comprehensively investigate the potential use of BSA as a solubility enhancer of IND (a model drug). IND is a member of acid class of non-steroidal anti-inflammatory drug that is described as poorly soluble and highly permeable (Class II) drug (El-Badry et al., 2009). It is has been reported that IND binds with high affinity to albumin molecule (Bogdan et al., 2008) and that its chemical structure is susceptible to form salt bridges with albumin amino groups (Trivedi et al., 1999). Prepared by two different particle engineering techniques (freeze drying and spray drying), IND-BSA solid dispersions are characterised by different scanning electron microscopy (SEM), differential scanning calorimetry (DSC), and powder X-ray diffraction (XRD). IND aqueous solubility in the presence of increased amounts of BSA is evaluated. In addition, IND dissolution rates and release profiles are evaluated *in-vitro* under physiological conditions

2. Materials and methods

2.1. Materials

BSA lyophilised powder was purchased from sigma-Aldrich (UK). IND was purchased from Acros organics (USA). Acetonitrile, sodium acetate, glacial acetic acid, orthophosphoric acid and all other materials and reagents used in the analytical methods were of analytical grade of purity and were all purchased from sigma-Aldrich (UK).

2.2. Solid dispersions preparation

2.2.1. Freeze dried solid dispersion

Initially, 0.12 g IND was dissolved in 5 mL ethanol and gradually added to 100 mL BSA solution (1% w/v), a clear IND:BSA hydro-alcoholic solution (20:1 IND:BSA MR) was formed. Alternatively, 0.5 g IND was dissolved in 15 mL ethanol and gradually mixed with 100 mL BSA solution (1% w/v), this resulted in the formation of a BSA colloidal suspension (100:1 IND:BSA MR). The resulting solution and suspension were immediately frozen in liquid nitrogen and freeze-dried for 72 h using (VirTis BenchTop Pro, UK).

2.2.2. Spray-dried solid dispersion

For the spray dried formulations, resulting BSA-IND solution and suspension were spray-dried using a lab-scale nozzle-type spray dryer (Buchi, Switzerland) operated at feeding rate of 1 mL/min and an inlet and outlet temperatures of 120 °C and 50–60 °C, respectively.

The resulting solid dispersions were named as shown in Table 1:

IND and BSA powders, at similar molar ratios to the above, were mixed to produce dry physical mixtures (IND:BSA-PM) for comparison purposes.

2.3. Differential scanning calorimetry (DSC)

DSC analysis of IND, BSA, IND:BSA-PMs and the solid dispersions were performed using differential scanning calorimeter (Mettler Toledo, DSC822e, UK). Samples (2–5 mg) were weighed and crimp-sealed in aluminium pans with the led pierced to ensure constant pressure. Analysis was carried out under nitrogen gas flow (20 mL/min) over a temperature range of 25 °C–300 °C and at a heating rate of 10 °C/min. The obtained thermograms were analysed using STAReSW 10.00 software.

2.4. X-ray diffractometry

IND, BSA, IND:BSA-PMs and the solid dispersions were also characterised by X-ray diffraction through Bruker diffractometer (Bruker AXS D8 Advance; Bruker Corporation, Billerica, MA, USA). The diffractometer operated at room temperature and at 40 kV. The scanning diffraction angle (2θ) ranged from 2° to 45° with a step size of 0.1°. The data were collected and analysed using DIFFRAC plus XRD commander software (Bruker Corporation).

2.5. Scanning electron microscopy

Scanning electron microscopy (SEM) images of IND and BSA as raw powders, and the solid dispersion powders were obtained using Zeiss Evo50 electron microscope (Oxford instrument, UK). Prior to imaging, the samples surfaces were sputter-coated with gold. The electron microscope was operated at an accelerating voltage of 30 kV under low-vacuum mode.

2.6. Aqueous solubility studies

The aqueous solubility of IND was carried out by adding excess amounts of IND (50 mg) to 1 mL BSA solutions of serial concentrations of (0, 0.3, 0.6, 0.9, 1.8 and 3.6 mM). In order to evaluate the solubility of IND formulated with BSA in the solid dispersions, calculated amounts of IND:BSA solid dispersions were added to 1 mL distilled water to obtain a range of BSA concentrations similar

Table 1
Composition of IND:BSA solid dispersions.

Formulation Code	IND:BSA molar ratio	Preparation Method
FD-sol	20:1	freeze dried from solution
SD-sol	20:1	spray dried from solution
FD-sus	100:1	freeze dried from suspension
SD-sus	100:1	spray dried from suspension