Chapter 1

Introduction and aim of study

1.1 Introduction

The use of drug delivery systems (DDSs) allows control over the release of active pharmaceutical ingredients (APIs), as well as improving stability, bioavailability and biodistribution of the API (Allen & Cullis, 2004; WHO, 1999). This means increased time between necessary doses and fewer side effects (Pifferi *et al.*, 1999). Various substances are used in DDSs, including natural and synthetic polymers (Beneke *et al.*, 2009; Guo *et al.*, 1998). Natural polymers are of particular interest as they represent a renewable resource, are freely available at low cost and are biocompatible and biodegradable. They also share similarities with the extracellular matrix and display intrinsic cellular interactions (Malafaya *et al.*, 2007). Polymeric DDSs can be used in various dosage forms for the delivery of APIs via various routes of administration, including the oral, parenteral, nasal and transdermal routes (York, 2002). The focus of this study was specifically on the use of polymeric DDSs for intravenous API delivery.

A natural polymer that has become increasingly popular in recent years is chitosan. Chitosan is derived from the deacetylation of chitin, the second most abundant natural polymer, found in the shells of crustaceans (Malafaya *et al.*, 2007). Reasons for chitosan's popularity stem from its unique mucoadhesivity, its high biocompatibility and biodegradability, its low toxicity and low immunogenicity (Chua *et al.*, 2012; Van der Merwe *et al.*, 2004). As such, it is widely applied, among others as an absorption enhancer across mucosal surfaces (Van der Merwe *et al.*, 2004). The problem, however, is that chitosan is insoluble in neutral and basic environments and is consequently unfit for use in biological systems (Casettari *et al.*, 2012). This necessitated the development of chitosan derivatives, including the partially quaternized derivative *N*-trimethyl chitosan chloride (TMC) (Polnok *et al.*, 2004). TMC is soluble over a wide range of pH values and even improves upon the mucoadhesivity and permeation enhancing effects of chitosan (Polnok *et al.*, 2004; Thanou *et al.*, 2000b). The degree of quaternization of TMC, a measure of the polymer's charge density, is proportionate to its ability to enhance absorption (Snyman *et al.*, 2003). The cationic groups in TMC's structure interact with the negative sites on the cell membrane, causing the tight junctions between cells to open reversibly (Hamman *et al.*, 2003; Thanou *et al.*, 2000a). Like chitosan, TMC has a wide variety of possible applications in the pharmaceutical field (Mourya & Inamdar, 2009). It has been explored for gene delivery and the delivery of vaccines, vitamins, insulin and cancer medication (De Britto *et al.*, 2012; Du Plessis *et al.*, 2010; Li *et al.*, 2012; Mourya & Inamdar, 2009). More recently, TMC in the form of nanoparticles have been explored for the

intravenous administration of protein and peptide APIs and even delivery beyond the blood-brain barrier (Amidi *et al.*, 2006; Wang *et al.*, 2010).

Nanoparticles have been a subject of increasing interest over the last couple of decades. They are already used in many products, including certain foods, cosmetics, clothing and medical equipment and more uses are still begin explored (Sonia & Sharma, 2011; Wani *et al.*, 2011). Polymeric nanoparticles have especially been explored for use in the pharmaceutical industry (Sonia & Sharma, 2011). Even though nanoparticles' popularity is undeniable, controversy exists over the exact classification of their size range. Classifications of 1 to 1000 nm (Soppimath *et al.*, 2001) and 1 to 100 nm (President's Council of Advisors on Science and Technology, 2005) have been suggested, but from a physiological point of view, 1 to 500 nm seems to be the most logical classification, as this is the limit for particle uptake by cells (Rejman *et al.*, 2004).

The small size of the nanoparticle is the centre of its pharmaceutical interest. Being small, they can move through the blood capillaries (5-6 µm) with ease and their high surface to volume ratio makes them an attractive carrier for protein and peptide APIs, as they can carry more API relative to their mass than larger particles can (Aggarwal *et al.*, 2009; Hans & Lowman, 2002). It is because of these characteristics that polymeric nanoparticles have attracted attention as a possible delivery system for intravenous API administration. Applications explored for the intravenous use of these particles include administration of cancer medication, gene therapy and the treatment of Alzheimer's disease beyond the blood-brain barrier (Germershaus *et al.*, 2008; Jain *et al.*, 2012; Roney *et al.*, 2005).

The small size, however, is also the centre of its toxic effects. Studies have observed that nano-particles have a greater risk of causing toxicity than larger particles do (Mayer *et al.*, 2009; Wani *et al.*, 2011). Upon entering the blood, nanoparticles are met with numerous blood cells, and interactions with these cells can have deleterious effects (Dobrovolskaia *et al.*, 2008). Most of the particle-blood cell interactions are electrostatic and, as such, polycations like TMC nanoparticles have a great risk for causing toxicity (Cerda-Cristerna et al., 2011; Thanou et al., 2000b). Interaction of particles with red blood cells can cause aggregation or hemolysis (Moreau *et al.*, 2002; Moreau *et al.*, 2000). Interaction with plasma proteins can protect the red blood cells to a certain extent, but it is not all good either (Moreau *et al.*, 2002; Moreau *et al.*, 2000). While most protein interactions prevent hemolysis, interaction with albumin can actually worsen the hemolytic effect of particles (Moreau *et al.*, 2000). Interaction with the complement proteins causes activation of the complement cascade, which leads to inflammation and removal of the particles from circulation via phagocytosis by macrophages (Janeway Jr *et al.*, 2001; Morikis & Lambris, 2005). Plasma proteins

also control coagulation, and interaction with these proteins can activate the coagulation factors, leading to extensive cell aggregation (Cerda-Cristerna *et al.*, 2011).

The extent of particle interactions with the blood components is dependent not only on the properties of the particle (size and charge), but also on the characteristics of the polymer from which the particle is made (Mailänder & Landfester, 2009; Thasneem *et al.*, 2011). Smaller, more positively charged particles tend to be more reactive, binding more proteins and thereby causing more toxicity (Wani et al., 2011). Hydrophobic polymers can easily interact with proteins, providing more opportunity for toxic effects (Huangfu *et al.*, 2009). The hemocompatibility of a particle, that is, its capability to interact safely with blood components, can be improved. One of the popular ways of doing this is by adding poly(ethylene) glycol (PEG) to the nanoparticle formulation (Koziara *et al.*, 2005). The PEG lowers the particle's zeta potential, thereby making it less reactive. It also creates a steric shield around the particle, preventing plasma protein adsorption, minimizing the immunological response, the complement activation and the hemolysis caused (Cerda-Cristerna *et al.*, 2011; Gref *et al.*, 2000).

Although hemocompatibility studies of polymeric nanoparticles are relatively new, the hemocompatibility of quite a few polymers, including lipid-core nanocapsules, hydroxyapatite nanoparticles, ethylcellulose and methylcellulose nanoparticles, pullulan and especially poly lactic-co-glycolic acid (PLGA) and chitosan, have been investigated (Bender *et al.*, 2012; Chandra *et al.*, 2012; Italia *et al.*, 2007; Kulkarni *et al.*, 2005; Ravikumara *et al.*, 2009; Rekha & Sharma, 2009; Shelma & Sharma, 2011; Smitha *et al.*, 2014; Thasneem *et al.*, 2013a; Thasneem *et al.*, 2013b). From these studies we can deduce that hemolysis, activation of the complement system and interactions with plasma proteins are central to the determination of a polymer's hemocompatibility.

Previous studies have shown the non-toxicity of TMC (Amidi *et al.*, 2006; Du Plessis *et al.*, 2010; Thanou *et al.*, 2001), but to our knowledge, no studies determining the hemocompatibility thereof have been conducted and this study therefore aims to help fill the void. Hemocompatibility information is crucial for the utilization of TMC in the form of nanoparticles for intravenous drug delivery.

The aim of this study was to determine the interaction of TMC nanoparticles with specific blood components, specifically looking at the influence of particle concentration, size and the addition of PEG to the formulation.

The objectives were:

- To determine the hemocompatibility of TMC concerning:
  - hemolysis
  - cell aggregation
  - activation of the complement system via the alternative pathway, and
  - interaction with plasma proteins
- To determine the influence of concentration, particle size and the addition of PEG on hemocompatibility.

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