Chapter 2

Literature Study

Chapter 2 will review the existing literature on drug delivery and drug delivery systems, different routes of administration and natural polymers used as excipients in drug delivery systems. Natural polymers chitosan and its derivative, TMC, will be discussed with regards to their utility in drug delivery formulations. This chapter will also focus on nanoparticles, the reasons for their popularity and the ways they can cause toxicity. In conclusion, hemocompatibility will be defined and a method to improve hemocompatibility explored.

2.1 Drug delivery systems

Therapeutic efficacy of medicine is determined by its solubility in aqueous environments, as well as the rate of dissolution it displays (York, 2002)[1]. These characteristics are dependent on physicochemical properties of the active pharmaceutical ingredient (API) present in the medicine, such as the polymorphic form, crystal size and stability of the API, its pK_a-value and the surface area of the API available for interactions (Ashford, 2002c; York, 2002). However, medicine does not consist of APIs alone. Rather, they consist of APIs in conjunction with certain excipients, forming a drug delivery system (DDS), the main purpose of which is the safe and efficient administration of an API in a way that is convenient for patients (Allen *et al.*, 2005; Ashford, 2002c; Steinberg *et al.*, 1996; York, 2002).

The World Health Organization, or WHO (1999), defined an excipient as a substance other than the API, included in a DDS to perform certain functions (Hamman & Tarirai, 2006; Steinberg *et al.*, 1996; WHO, 1999). Originally, these functions were only to provide the correct weight, consistency and volume for successful API administration, but nowadays excipients play a much larger role in drug delivery (Hamman & Tarirai, 2006; Pifferi *et al.*, 1999). Excipients currently are responsible for increasing therapeutic efficacy of medication, by regulation of the pharmacological properties of APIs, such as improvement of the stability, bioavailability and biodistribution of an API and regulation of the rate of API release from the medicine (Allen & Cullis, 2004; Pifferi *et al.*, 1999; WHO, 1999; York, 2002).

The intended function and site of action of an API dictates which excipients will be used in the DDS, as well as the way the medication will be administered (York, 2002). Excipients typically used include disintegrating agents, lubricants, diluents, suspending- or emulsifying agents, chemical stabilizers and, as excipients play an important role in medication identification, colour and flavour agents (Ashford, 2002c; WHO, 1999).

[1] Harvard referencing style, according to author's guide of the International Journal of Pharmaceutics.

2.2 Routes of administration

There are various possible routes of medication administration, including oral, nasal, ocular, transdermal, rectal and parenteral routes, each with certain applicable dosage forms (Ahuja *et al.*, 1997; York, 2002). Of these, the oral route is most popular, as it is a relatively safe and simple way of administering medicine, with a great variety of applicable dosage forms, namely tablets, capsules, granules, powders, gels, solutions, suspensions, emulsions and syrups (York, 2002). Dosage forms for other routes of administration, apart from those already mentioned, include suppositories, ointments, creams, pastes, lotions, aerosols, implants and inhalations (York, 2002). An API administered in the same dosage form, but via different routes, or via the same route, but in different dosage forms, can display differences in bioavailability (Ashford, 2002a), emphasizing the importance of exploring the different routes and dosage forms suitable for each API and disease to be treated (York, 2002).

2.2.1 The oral route

In the gastrointestinal (GI) tract, general absorption most readily takes place in the small intestine, where the surface area is largest and blood supply is abundant (Guyton & Hall, 2006b; York, 2002). As most APIs are weak acids or weak bases, the small intestine is also the place with the most favourable pH for absorption of APIs in oral medication (York, 2002). However, not all APIs fall in this category and some APIs need to be released at other sites in the GI tract for maximal absorption. To achieve this, DDSs which release their APIs at specific sites in the GI tract can be formulated; sites where the pH values are most suitable for the absorption of the specific API, be it in the stomach (pH 1.0-3.5), the small intestine (pH 7.5-8.0) or the large intestine (pH 7.5-8.0) (Arbit & Kidron, 2009; Guyton & Hall, 2006a; York, 2002). The rate of API release from the DDSs at these specific sites can also be predetermined for controlled or extended therapeutic effects (Collett & Moreton, 2002). Modifying the API release time of a DDS allows better management of therapeutic levels in the body. This means that the effect of the API lasts longer and consequently, medication can be taken fewer times during the course of treatment. Better management of therapeutic levels also reduces the occurrence of side-effects, especially due to high API concentrations in the plasma (Allen & Cullis, 2004; Collett & Moreton, 2002; Sinha & Kumria, 2001).

However, before the API is even released from the DDS there are already factors influencing the medication and altering bioavailability (Arbit & Kidron, 2009; York, 2002), such as enzymes of the GI tract, which degrade some of the unreleased API (Ashford, 2002b). The food content of the GI tract

and the changes in pH it causes, other medication used, as well as the dosage form and the size of the dose taken can affect the release and absorption of the API (Ashford, 2002a; Ashford, 2002b). Once absorbed, the API-containing blood from the GI-tract passes through the liver where the API is further degraded in a process called first-pass metabolism (Ashford, 2002b). The cumulative effect of these factors results in irregular bioavailability displayed by oral medication (Arbit & Kidron, 2009; York, 2002). In addition, the movement of the medication through the GI tract to the site of API release tends to be timely, resulting in slow onset of therapeutic effect (York, 2002).

APIs with large molecular structures and molecular weights, such as protein or peptide APIs (Snyman *et al.*, 2003), are hydrophilic and have poor hydrolytic stability and low bioavailability in the GI tract (Arbit & Kidron, 2009; Sandri *et al.*, 2005). This instability generally makes them unfit for oral administration, necessitating the consideration of other routes of administration, for example, the parenteral route (Casettari *et al.*, 2012; Sandri *et al.*, 2005).

2.2.2 The parenteral route

Although the parenteral route of administration has many advantages over the oral route, such as faster onset of therapeutic effect, better and more consequent bioavailability, the route is invasive, does not encourage patient compliance and is therefore a much less attractive option (Sandri *et al.*, 2005; York, 2002). As Gardner (1987) pointed out, patients not suffering from life-threatening diseases, such as diabetes or cancer, will much rather make use of other routes of administration, than frequently receiving injections (Gardner, 1987). This is still true 25 years later.

Various dosage forms can be administered via the parenteral route, including solutions, suspensions or emulsion injected under the skin, into a muscle or directly into the blood stream (York, 2002). It is predominantly used in cases where fast onset of therapeutic effect is needed, or when an API is unfit for delivery via other routes due to poor stability, metabolic effects or when the patient is unconscious (York, 2002). Depending on the API and the excipients used, the half-life of intravenously injected medicine can range from a few minutes to several days (Crommelin *et al.*, 2002).

In conjunction with the weight, consistency and volume improvement they give, ideal excipients can play a vital role in patient compliance, especially when dealing with oral dosage forms (Hamman & Tarirai, 2006; WHO, 1999).

2.2.3 Other routes

Though not always as popular or convenient, other routes of administration are also needed.

The ocular route is easily accessible, although it is not always very comfortable to use. Because of the high rate of tear clearance from the eye, a large part of the administered medicine is lost before it can be absorbed (Achouri *et al.*, 2013). This route makes use of solutions, ointments and creams for the delivery of APIs (York, 2002).

The nasal route, like the parenteral route can be used for the systemic administration of APIs. The nasal membranes provide a large surface for absorption with a rich supply of blood vessels and absorbed APIs do not undergo first-pass metabolism (Djupesland, 2013; Suman, 2013). The applicable dosage forms, however, are limited to solutions and inhalations (York, 2002).

Transdermal applications, in the form of ointments, creams, lotions, solutions or topical aerosols, are mostly used for local API effects, although systemic effects can also be achieved. The hydrophobicity or hydrophilicity of the applied dosage form will determine how the API is released and thus, what effect it will have (York, 2002).

Although the rectal route is highly inconvenient, it is very useful for the delivery of APIs when the oral route is unavailable, as with unconscious or vomiting patients. The API effect can be local or systemic, but API absorption tends to be irregular. Dosage forms used in this route include suppositories, ointments, creams and solutions (York, 2002).

2.3 New APIs and new excipients

New APIs are discovered almost daily, calling for modification of existing excipients or the discovery of new excipients (Chang & Chang, 2007). New or improved excipients are necessary, not only to overcome the incompatibilities between the existing excipients and the new APIs, but also because the APIs often have physicochemical and pharmacokinetic properties that are not ideal and the APIs or the excipients in the DDS can cause toxicity (Beneke *et al.*, 2009; Chang & Chang, 2007; Pifferi & Restani, 2003). The balance of the excipients in a DDS is extremely important, as too much of it in a formulation can cause toxicity, even if the excipient itself is non-toxic (Allen & Cullis, 2004). The right excipients, in the right balance in a DDS can extend the efficacy period of an API and even reduce the amount of side-effects it causes (Pifferi *et al.*, 1999).

Polymers (natural and synthetic) have proven to be an attractive source of excipients for use in pharmaceutical formulations (Beneke *et al.*, 2009; Guo *et al.*, 1998). Natural polymers are of notable

interest as they are abundant, can easily be chemically modified and usually are biocompatible and biodegradable (Malafaya *et al.*, 2007; Satturwar *et al.*, 2003). These polymers are classified according to their origin, be it plant-, algae-, microbe-, fungus- or animal-derived (Beneke *et al.*, 2009; Pifferi & Restani, 2003; Sinha & Kumria, 2001).

2.3.1 Plant-derived polymers

Plants are a renewable and cost-effective source of polymers. These polymers tend to be non-toxic, biocompatible and biodegradable, making them favourable for use as excipients in DDSs (Beneke *et al.*, 2009; Scholtz *et al.*, 2013; Shirwaikar *et al.*, 2008). Plant-derived polymers currently and potentially used in pharmaceutical formulations include cellulose, hemicellulose and pectin obtained from plant cell walls, starch, inulin found in garlic, onion, artichoke and leeks, rosin from the resin of pine trees and gums produced by plants after injury (Beneke *et al.*, 2009; Carabin & Flamm, 1999; Rana *et al.*, 2011; Satturwar *et al.*, 2003; Sinha & Kumria, 2001; Varshosaz *et al.*, 2006).

These polymers have a variety of possible uses in pharmaceutical formulations, especially regarding modified release (Beneke *et al.*, 2009). Plant-derived polymers and their derivatives can be used in the production of sustained release tablets (cellulose derivatives), diffusion-controlled DDSs (starch), hydrogels (inulin, cellulose derivatives, gums), microspheres (gums) or films (rosin) (Beneke *et al.*, 2009; Chamarthy & Pinal, 2008; Malafaya *et al.*, 2007; Rana *et al.*, 2011; Satturwar *et al.*, 2003; Satturwar *et al.*, 2004; Shirwaikar *et al.*, 2008; Varshosaz *et al.*, 2006; Vervoort *et al.*, 1998). Inulin and some varieties of gums are predominantly used in colon-specific drug delivery, as these polymers are not affected by the enzymes in the upper GI tract, but are only degraded once they reach the colon (Chaurasia *et al.*, 2006; Rana *et al.*, 2011; Sinha & Kumria, 2001; Vervoort *et al.*, 1998). Gums can also be employed as emulsifiers, disintegrants, thickening agents or stabilizing agents in solid and liquid dosage forms (Rana *et al.*, 2011). Using these polymers as excipients can improve the performance of the DDS (Scholtz *et al.*, 2013), leading to more complete API release and consequently better therapeutic efficacy (Rana *et al.*, 2011).

2.3.2 Algae-derived polymers

Polymers derived from algae are alginates, a component of marine brown algae, and carrageenans, obtained from red seaweeds (Coviello *et al.*, 2007; Malafaya *et al.*, 2007). Alginate is abundantly available and is often used in wound dressings (Malafaya *et al.*, 2007). Because of the gelling properties alginate displays in aqueous solutions, it is used in the production of hydrogel beads and thermo-sensitive microspheres (Abd El-Ghaffar *et al.*, 2012; Coviello *et al.*, 2007; Malafaya *et al.*,

2007; Oddo *et al.*, 2010). Carrageenans, on the other hand, are mostly used in the food industry, but can be used in the production of hydrogel beads (Malafaya *et al.*, 2007).

2.3.3 Animal-derived polymers

One of the most well-known and abundant polymers is the animal-derived polymer chitin, a polysaccharide found in the exoskeletons of crustaceans and the cuticles of insects (Benesch & Tengvall, 2002; Malafaya *et al.*, 2007). Chitin is made up of β(1→4)-glucosamine and *N*-acetyl-D-glucosamine units (Malafaya *et al.*, 2007). When chitin is deacetylated, a biopolymer called chitosan is produced (Benesch & Tengvall, 2002; Malafaya *et al.*, 2007; Jayakumar *et al.*, 2010). This new-formed biopolymer and its derivatives will subsequently be discussed.

2.4 Chitosan

The term "chitosan" is usually used in singular form, yet it describes a large group of polymers differing in the degree of *N*-deacetylation, i.e. the amount of primary amino groups present, and the molecular weight, which can vary from 50 to 2 000 kDa (Casettari *et al.*, 2012; Malafaya *et al.*, 2007). These variables influence the specific properties of each of the chitosan polymers (Felt *et al.*, 1998).

Chitosan (Figure 2.1) is made up of β-(1→4)-2-acetamido-D-glucose and β-(1→4)-2-amino-D-glucose units (Aytekin *et al.*, 2012). Due to unoccupied amino groups in its structure, chitosan is characteristically cationic (Cerda-Cristerna *et al.*, 2011; Felt *et al.*, 1998; Luangtana-anan *et al.*, 2010; Malafaya *et al.*, 2007). These characteristics, along with its biocompatibility, biodegradability and non-toxicity have led to increased interest in chitosan, especially in the pharmaceutical field (Aytekin *et al.*, 2012; Chua *et al.*, 2012; Malafaya *et al.*, 2007; Sieval *et al.*, 1998; Van der Merwe *et al.*, 2004b).

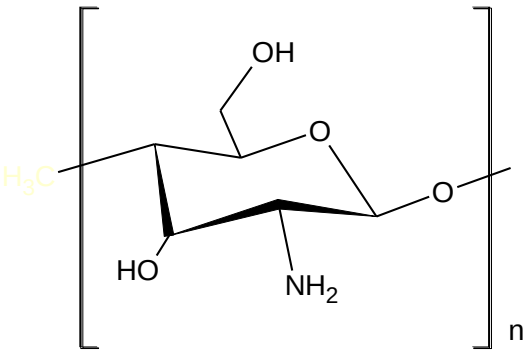

Figure 2.1 – Chemical structure of chitosan (Van der Merwe *et al.*, 2004b)

2.4.1 Applications of chitosan

Chitosan has already been widely applied in a variety of industries, including the food, cosmetic, agricultural and pharmaceutical industries (Sieval *et al.*, 1998; Van der Merwe *et al.*, 2004b). In veterinary medicine, chitosan has been used to improve wound healing, as it has antimicrobial properties (Felt *et al.*, 1998; Ueno *et al.*, 2001). Pharmaceutically, chitosan has been applied to various forms of DDSs, including controlled release drug delivery and delivery via the oral, parenteral, nasal, ophthalmic and transdermal routes. It has also been used to produce tablets, microparticles, granules, beads and liposomes (Felt *et al.*, 1998; Malafaya *et al.*, 2007; Paños *et al.*, 2008; Sieval *et al.*, 1998).

At the root of chitosan's versatility and usefulness lie its unoccupied amino groups (Luangtana-anan *et al.*, 2010). These cationic groups react with negative sites on the epithelial cell membrane, to cause the tight junctions to open (Hamman *et al.*, 2003; Luangtana-anan *et al.*, 2010; Schipper *et al.*, 1997). Through this mechanism, chitosan can enhance absorption across mucosal surfaces, which is highly significant for the transport of large hydrophilic molecules, such as peptide or protein APIs (Casettari *et al.*, 2012; Du Plessis *et al.*, 2010a; Kotzé *et al.*, 1999; Sandri *et al.*, 2005; Thanou *et al.*, 2000a; Van der Merwe *et al.*, 2004b).

However, these reactive primary amino groups are also responsible for chitosan's pK_a value of between 5.5 and 6.5 (Kotzé *et al.*, 1999; Thanou *et al.*, 2000a). This means that the polymer will precipitate from solution above pH 6.5 (Casettari *et al.*, 2012; Malafaya *et al.*, 2007; Sieval *et al.*, 1998). Poor solubility at physiological pH (7.4) strongly impedes chitosan's use in a DDS, especially for oral and parenteral administration (Felt et al., 1998; Sieval et al., 1998; Thanou et al., 2000a). Fortunately, the chemical structure of chitosan can easily be modified to alter certain characteristics (Casettari *et al.*, 2012; Chua *et al.*, 2012; Malafaya *et al.*, 2007; Van der Merwe *et al.*, 2004b).

2.4.2 Chitosan modifications and derivatives

Alterations are made to chitosan's chemical structure to improve upon some of the less favourable characteristics, while still maintaining the favourable qualities. Modifications are usually made by adding alkyl or carboxymethyl groups at the C2 position, or forming chitosan conjugates through covalent bonds (Casettari *et al.*, 2012; Guggi & Bernkop-Schnürch, 2003; Thanou *et al.*, 2001a). Derivatives, such as β-cyclodextrin-linked chitosan and mono-*N*-carboxymethyl chitosan, are generally soluble over a wider pH range than native chitosan, while still being cationic and displaying mucoadhesive properties (Aytekin *et al.*, 2012; Sieval *et al.*, 1998; Tanida *et al.*, 1998; Thanou *et al.*, 2001a). Chitosan derivatives have many possible applications, including the protection of peptide

APIs against degradation in the GI tract when orally administered and use as non-viral vectors for gene delivery (Cerda-Cristerna *et al.*, 2011; Guggi & Bernkop-Schnürch, 2003).

One of the most popular chitosan derivatives is the partially quaternized *N*-trimethyl chitosan chloride, more commonly known as TMC (Polnok *et al.*, 2004).

2.5 *N*-trimethyl chitosan chloride

TMC is synthesized through a reaction called reductive methylation. In this reaction, chitosan is added to methyl iodide in the presence of sodium hydroxide to produce the partially quaternized derivative shown in Figure 2.2 (Sieval *et al.*, 1998; Snyman *et al.*, 2003). The number of steps in the reaction, as well as the duration of these steps, the molecular weight of the chitosan used and the temperature at which the reaction takes place influences the degree of quaternization of the synthesized TMC, as well as its *O*-methylation (Aytekin *et al.*, 2012; Polnok *et al.*, 2004; Snyman *et al.*, 2003). Like chitosan, TMC is mucoadhesive and cationic in character and promotes paracellular absorption (Geisberger *et al.*, 2013; Sandri *et al.*, 2005; Thanou *et al.*, 2000b). In contrast to chitosan, TMC is soluble over a wide range of pH values (Aytekin *et al.*, 2012; Geisberger *et al.*, 2013; Polnok *et al.*, 2004). TMC also has better antibacterial action than chitosan (Sadeghi *et al.*, 2008).

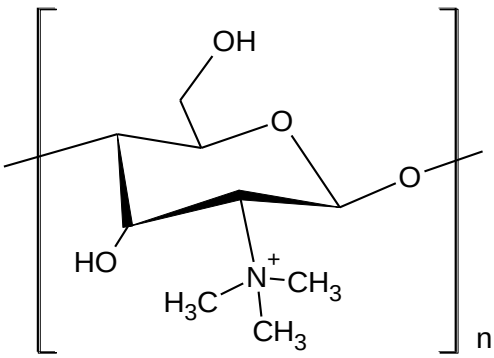

Figure 2.2 – Chemical structure of TMC, indicating the quaternized groups (Van der Merwe *et al.*, 2004b)

2.5.1 Degree of quaternization

TMC promotes absorption in the same manner as chitosan; by reversibly opening the tight junctions between cells (Du Plessis *et al.*, 2010b; Hamman *et al.*, 2003; Sandri *et al.*, 2005; Thanou *et al.*, 2000a). The ability of TMC to open the tight junctions is dependent upon the degree of quaternization (DQ), which is a measure of the polymer's charge density (Hamman *et al.*, 2003;

Thanou *et al.*, 2000a). Generally, a higher DQ is synonymous with more successful opening of tight junctions, as the higher charge density results in more positive TMC molecules to interact with the negative sites on the cell membrane, which in turn, translates as better mucoadhesivity (Hamman *et al.*, 2003; Kotzé *et al.*, 1999; Sandri *et al.*, 2005; Snyman *et al.*, 2003). The increase in the DQ's ability to open tight junctions is not linear, however. Some studies have found that at a DQ above 48% no significant increase in absorption was observed (Du Plessis *et al.*, 2010b; Hamman *et al.*, 2003), while another study suggested the use of TMC with a DQ of 60% for the absorption of peptides (Thanou *et al.*, 2000b). However, TMC with a higher DQ also tend to have higher levels of *O*-methylation at the polymer's 3- and 6-hydroxyl groups (Polnok *et al.*, 2004). A high degree of *O*-methylation has a strongly negative impact on TMC's solubility, thereby decreasing its usefulness as an absorption enhancer (Sieval *et al.*, 1998).

2.5.2 Applications of TMC

Like chitosan, TMC has a wide variety of possible applications, especially in the pharmaceutical field (Mourya & Inamdar, 2009). Its cationic character and mucoadhesive properties have led to TMC being explored for gene delivery (Davies *et al.*, 2008; Geisberger *et al.*, 2013; Mourya & Inamdar, 2009; Sandri *et al.*, 2005). TMC's absorption enhancing effects have also piqued special interest as a delivery system for hydrophilic compounds, both small (like mannitol) and large (like protein or peptide APIs) (Amidi *et al.*, 2006; Kotzé *et al.*, 1997; Sandri *et al.*, 2005; Thanou *et al.*, 2000b). The routes through which TMC may potentially deliver these compounds include buccal, nasal, colonic and oral delivery (Amidi *et al.*, 2006; Mourya & Inamdar, 2009; Sandri *et al.*, 2005; Van der Merwe *et al.*, 2004a). TMC has been explored for the delivery of vaccines through these routes, as they are less invasive than parenteral administration (Keijzer *et al.*, 2011; Sayin *et al.*, 2008). It can also enhance the absorption of nasal and rectal delivery of insulin across the mucosal membranes and serve as a vitamin carrier (De Britto *et al.*, 2012; Du Plessis *et al.*, 2010a). In Van der Merwe's (2004) study, oral delivery of peptide APIs with TMC as carrier was achieved by producing TMC mini-tablets, which was then inserted into a gelatin capsule. The capsule was formulated to release all of the mini-tablets at once, after which the TMC tablets would slowly release the peptide API. By doing so, the API was protected from degradation in the GI tract (Van der Merwe *et al.*, 2004b).

TMC displays antibacterial activity and enhances humoral immunity (Geng *et al.*, 2013; Keijzer *et al.*, 2011). At low concentrations, it also displays radical scavenging activity (Aytekin *et al.*, 2012). These characteristics contribute to TMC's potential role as a delivery system for camptothecin, an anti-

tumor and antimetastatic API, in the treatment of leukemia, melanoma, multiple myeloma and liver cancer (Aytekin *et al.*, 2012; Li *et al.*, 2012; Liu *et al.*, 2010; Zhou *et al.*, 2010).

In recent years, TMC in the form of nanoparticles have been explored. The main interest in this dosage form lies with its cationic surface charge and excellent loading capacity for peptides and proteins (Amidi *et al.*, 2006; Geisberger *et al.*, 2013). Because of this, TMC conjugated with PLGA nanoparticles can potentially be used for the delivery of APIs across the blood-brain barrier (Wang *et al.*, 2010). Amidi et al. (2006) also found that TMC in nanoparticulate form caused less toxicity than a TMC solution. When higher DQ TMC were used to form nanoparticles, the result was smaller particles with increased zeta potential, compared to particles formed with lower DQ TMC (Chen *et al.*, 2007). This means that higher DQ TMC nanoparticles have better interaction with the cell membranes, leading to more successful tight junction opening and absorption enhancement (Hamman *et al.*, 2003; Thanou *et al.*, 2000a).

2.6 Nanoparticles

Nanoparticles have been a subject of increasing interest over the last couple of decades, especially during the last five years. They are already used in many products, including certain foods, cosmetics, clothing, computers, industrial catalysts and medical equipment, and more applications are still being explored (Sonia & Sharma, 2011; Wani *et al.*, 2011). Polymeric nanoparticles have piqued special interest in the pharmaceutical industry (Sonia & Sharma, 2011).

Among other substances, nanoparticles can be produced from natural or synthetic polymers (Hans & Lowman, 2002; Soppimath *et al.*, 2001). There are many techniques for the production of nanoparticles, including emulsion polymerization or coacervation/precipitation, ionic gelation, dispersion polymerization, reverse micellar method and the sieving method (Agnihotri *et al.*, 2004; Roney *et al.*, 2005; Sonia & Sharma, 2011; Soppimath *et al.*, 2001).

There has been much controversy over the exact size range of nanoparticles. Some classify nanoparticles as any particle between the sizes of 10 and 1000 nm (Bender *et al.*, 1996; Soppimath *et al.*, 2001), while others have classified nanoparticles as being approximately 1 to 100 nm in size (President's Council of Advisors on Science and Technology, 2005). Biologically, the nanoparticle range can be specified as 1 to 500 nm, as this is the limit for particle uptake by cells (Rejman *et al.*, 2004).

2.6.1 Characteristics and applications

Pharmaceutically, nanoparticles have shown potential as DDS for the delivery of high molecular weight APIs, such as proteins or peptides, especially in controlled or sustained release formulations (Bertholon *et al.*, 2006; Casettari *et al.*, 2012; Dobrovolskaia *et al.*, 2008; Kumari *et al.*, 2010; Roney *et al.*, 2005; Sadeghi *et al.*, 2008; Soppimath *et al.*, 2001). They can increase the stability, bio-availability and solubility of the API to be delivered, as well as offering a certain extent of protection to orally administered APIs (Kumari *et al.*, 2010; Sadeghi *et al.*, 2008; Soppimath *et al.*, 2001). As nanoparticles have a high surface to volume ratio, they can bind more API relative to their mass, than larger particles can (Aggarwal *et al.*, 2009; Redhead *et al.*, 2001). This means a decrease in the size and frequency of therapeutic dosages, which result in fewer side effects and better patient compliance (Kumari *et al.*, 2010; Schroeder *et al.*, 1998).

Polymeric nanoparticles represent an attractive carrier option for intravenous API administration as they can easily move through the blood capillaries (5-6 µm) and are more stable in biological environments than other colloidal carriers are (Bertholon *et al.*, 2006; Dobrovolskaia *et al.*, 2008; Hans & Lowman, 2002; Roney *et al.*, 2005). The blood-brain barrier is the brain's defence mechanism against pathogens and toxins. As such, it is highly selective and made up of restrictive tight junctions. Nanoparticles made of polymers that improve absorption by opening tight junctions, such as chitosan or TMC, can potentially be used for API delivery beyond this barrier, in the central nervous system, for the treatment of, among others, Alzheimer's disease (Hamman *et al.*, 2003; Roney *et al.*, 2005; Schipper *et al.*, 1997).

The characteristics of nanoparticulate DDS can be altered to serve specific needs, by modifying the particle's size or surface properties (Kumari *et al.*, 2010; Sonia & Sharma, 2011). Modification of these properties allows targeting and thus more effective API delivery (Kumari *et al.*, 2010). Surface properties, such as charge and hydrophobicity dictate how particles will react with cell membranes and blood components after intravenous injection (Aggarwal *et al.*, 2009; Koziara *et al.*, 2005; Kumari *et al.*, 2010; Soppimath *et al.*, 2001). The proteins with which injected nanoparticles interact determine the distribution of the particles throughout the body, the rate of its clearance from the blood and the extent of toxicity it causes (Dobrovolskaia *et al.*, 2009; Lynch & Dawson, 2008; Soppimath *et al.*, 2001).

2.7 Nanoparticle toxicity

When nanoparticles are injected into the bloodstream, they are immediately met by numerous re d blood cells, proteins and immune cells (Dobrovolskaia *et al.*, 2008). Interaction with these components may lead to hemolysis, aggregation, inflammation or other toxic effects, depending on the properties of the injected particle (Cerda-Cristerna *et al.*, 2011; Dobrovolskaia *et al.*, 2009). Characteristics that influence toxicity include size, chemical composition, solubility and surface properties (hydrophobicity and surface charge) (Wani *et al.*, 2011). Smaller, more cationic nanoparticles are more likely to cause toxicity than larger, anionic particles are, as cationic particles can react with negatively charged proteins, eliciting an immune response (Benesch & Tengvall, 2002; Wani *et al.*, 2011). The same counts for hydrophobic particles, readily interacting with the hydrophobic domains on proteins (Huangfu *et al.*, 2009). Because smaller particles have a higher surface to volume ratio, as stated before (Aggarwal *et al.*, 2009; Redhead *et al.*, 2001), they can bind more proteins and in so doing, cause a greater immune response (Dobrovolskaia *et al.*, 2009).

Nanoparticles are foreign to the body, and as such, a biological response is mounted against them by the reticuloendothelial system once they enter the blood stream (Saba, 1970; Soppimath *et al.*, 2001; Thasneem *et al.*, 2011). Intravenously administered nanoparticles up to 200 nm are phagocytised by macrophages in the blood stream, the liver and the spleen (Dobrovolskaia *et al.*, 2008; Schöll *et al.*, 2005; Schroeder *et al.*, 1998; Soppimath *et al.*, 2001). Recognition of the particles by macrophages is dependent on opsonic proteins binding to the surface of the particles, which is in turn dependent on the characteristics of the particles, such as structure, polymer morphology, surface charge, surface hydrophobicity, etc. (Lynch & Dawson, 2008; Mailänder & Landfester, 2009; Saba, 1970; Soppimath *et al.*, 2001; Thasneem *et al.*, 2011). In the liver especially, the nanoparticle-macrophage interactions cause the production of reactive oxygen species (ROS) and certain pro-inflammatory molecules (Forman & Torres, 2001). Excess ROS production can lead to local oxidative stress by creating an imbalance in the biological detoxifying responses, which can lead to inflammation, toxicity and cell damage (Dobrovolskaia *et al.*, 2009; Hoet *et al.*, 2004; Li *et al.*, 2008; Manke *et al.*, 2013).

Because of these toxicity risks, among others, it is crucial to determine a particle's hemocom-patibility profile. Hemocompatibility is a measure of the capability of a particle to interact safely with different blood components. Assessing the hemocompatibility of a particle provides a way to

predict the effects it will have when used in intravenous drug delivery formulations, be it beneficial or deleterious (Dobrovolskaia *et al.*, 2008; Jones & Grainger, 2009).

2.8 Hemocompatibility

When a particle enters the bloodstream, it is met by numerous blood cells. The interactions with these cells determine the particle's hemocompatibility. The nature of the interactions between the particle and the blood components is dependent mainly on the particle's properties, such as surface charge and size, but also on the characteristics of the polymer from which the particle is made (Koziara *et al.*, 2005; Mailänder & Landfester, 2009; Thasneem *et al.*, 2011). Studies have found that toxicity caused by a particle increased with an increase in the molecular weight of the polymer (Cerda-Cristerna *et al.*, 2011; Mao *et al.*, 2005).

Interactions between injected particles and blood components are mostly electrostatic in nature (Cerda-Cristerna *et al.*, 2011; Choksakulnimitr *et al.*, 1995). As such, cationic polymer particles can more easily interact with most blood components, leading to activation of the immune system. They therefore have a greater risk of causing toxicity than anionic particles do (Cerda-Cristerna *et al.*, 2011; Choksakulnimitr *et al.*, 1995; Fischer *et al.*, 2003; Rekha & Sharma, 2011; Soppimath *et al.*, 2001; Wani *et al.*, 2011).

Some common blood component-particle interactions will subsequently be discussed.

2.8.1 Hemolysis

Red blood cells (RBCs) occupy a large cell-volume in the blood. As such, it is likely that injected particles will encounter these cells before any of the immune cells (Dobrovolskaia *et al.*, 2008). Cationic particles can have electrostatic interactions with the negatively charged RBCs, to have one of two undesirable effects: the interaction can cause the RBCs to aggregate (hemagglutination) or it can cause hemolysis (Moreau *et al.*, 2002; Moreau *et al.*, 2000). Hemagglutination is caused by interaction of the particles with the outer RBC surface and is one of the most important aspects of hemocompatibility assessment of a particle intended for parenteral administration (Cerda-Cristerna *et al.*, 2011; Moreau *et al.*, 2002). Hemolysis, on the other hand, is the result of an interaction with the RBC membrane (Moreau *et al.*, 2002). When the electrostatic interaction between the particles and the RBC membranes perturbs the membrane enough to affect permeability, hemolysis occurs, causing the intracellular potassium and hemoglobin to leak out (Dobrovolskaia *et al.*, 2008; Moreau *et al.*, 2000; Shelma & Sharma, 2011). The surface properties of the injected nanoparticles,

especially surface charge, dictate the amount of hemolysis they will cause (Dobrovolskaia *et al.*, 2008). Extensive hemolysis can cause anemia, a life-threatening condition (Dobrovolskaia *et al.*, 2008).

Plasma protein binding to injected nanoparticles prevents the particles from interacting with the RBCs, thereby decreasing the hemagglutination and hemolysis caused (Moreau *et al.*, 2002; Moreau *et al.*, 2000). However, not all protein binding is beneficial, as particle-albumin complexes were found to be even more harmful than the particle on its own (Moreau *et al.*, 2000).

2.8.2 Complement activation

The complement system is comprised of plasma proteins (Janeway Jr *et al.*, 2001). When the first of these proteins are activated, it activates the next protein, which activates the next and so forth, creating a cascade of events that lead to inflammation, removal of foreign particles from the blood stream through phagocytosis and damage to pathogenic cells, thus aiding the immune system (Dobrovolskaia *et al.*, 2008; Janeway Jr *et al.*, 2001; Morikis & Lambris, 2005). The complement system can be activated via three different routes, the classical pathway, the alternative pathway or the lectin pathway, summarized in Figures 2.3, 2.4 and 2.5.

The classical pathway is initiated when the complement C1 protein binds to the surface of a foreign particle or pathogen or when the C1 protein forms a complex with an antibody bound to a foreign particle or pathogen. This serves to mark the particle or pathogen for removal, setting the complement cascade in motion. The C1 complex interacts with the complement C4 protein, cleaving it to C4b, which binds to a C2 protein. This binding allows the C1 complex to cleave the C2 protein to C2b. The C4b and the C2b combine to form C3 convertase of the classical pathway, which cleaves the complement C3 protein into C3a and C3b (Janeway Jr *et al.*, 2001; Walport, 2001).

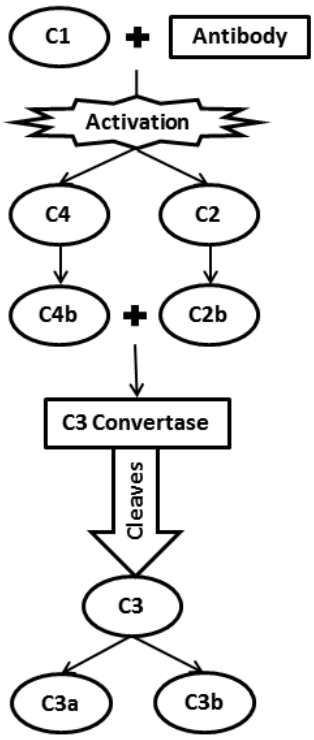

Figure 2.3 – Summary of the classical pathway of the complement cascade, starting when the complement C1 protein interacts with an antibody attached to a pathogen. (Adapted from Janeway Jr et al., 2001)

Unlike the classical pathway, the alternative pathway is not dependent on antibody interaction to initiate the cascade. Instead, the cascade is initiated by spontaneous cleavage of complement protein C3 to form C3b. The formed C3b interacts with factor B, allowing factor D to cleave it into Ba and Bb. In this process, the Bb stays attached to the C3b, thus forming the alternative pathway C3 convertase or C3bBb, cleaving C3 to C3a and C3b as in the classical pathway. As this cascade's start is spontaneous and relatively self-sustaining, a mechanism is needed to protect the body's own cells. The body protects its own cells by expressing proteins, which are not found on the surface of pathogens. These proteins compete with factor B for binding to C3b, as well as cleaving the formed C3b to inactive iC3b, preventing the complement cascade form turning on the body (Andersson *et al.*, 2002; Janeway Jr *et al.*, 2001; Walport, 2001).

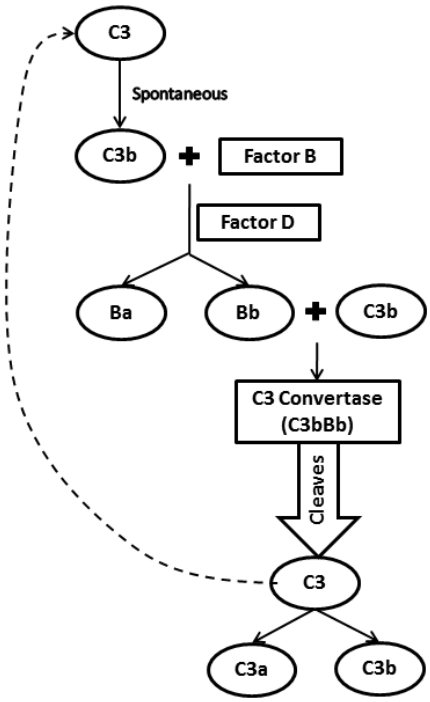

Figure 2.4 – Summary of the alternative pathway of complement activation, displaying its spontaneous start and self-sufficiency. (Adapted from Janeway Jr et al., 2001)

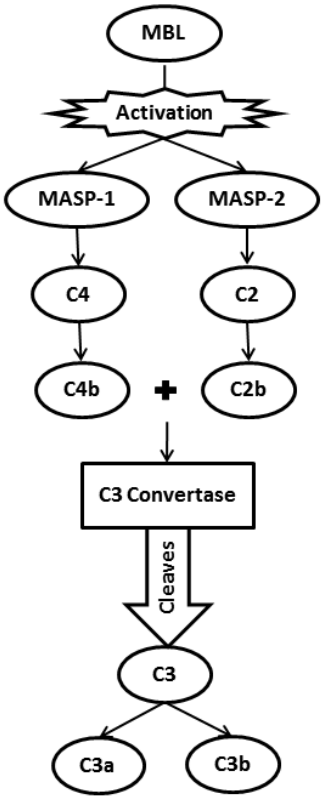

Figure 2.5 – Summary of the lectin pathway of complement activation. MBL = mannan-binding lectin, MASP = MBL-associated serine protease. (Adapted from Janeway Jr et al., 2001)

The lectin pathway is comparable to the classical pathway in its way of producing C3 convertase. To set this cascade in motion, mannan-binding lectin (MBL) interacts with polysaccharide residues (such as mannose) on the surface of a pathogen or foreign particle. This causes activation of the protein complexes MBL-associated serine protease (MASP)-1 and MASP-2, which bind to C4 just as the complement C1 protein does in the classical pathway. The rest of the cascade follows in the same manner as the classical pathway, cleaving C3 into C3a and C3b (Janeway Jr *et al.*, 2001; Walport, 2001).

The C3a and C3b formed in the complement cascade are responsible for the immune responses that follow complement activation. These responses include inflammation, coating of the foreign partide or pathogen to signal phagocytosis by macrophages and directly disrupting pathogenic cell membranes, causing cell death (Morikis & Lambris, 2005).

The greater the extent of complement activation by injected nanoparticles, the faster their removal from circulation and therefore, the less useful they become for the delivery of APIs (Bertholon *et al.*, 2006; Dobrovolskaia *et al.*, 2008). Interaction between the injected particles and the complement proteins is, as with most particle-blood interactions, dependent on the surface properties of the particles, as well as the characteristics of the polymer from which the particles are made (Koziara *et al.*, 2005; Mailänder & Landfester, 2009). Complement proteins interact with OH-groups on the surface or the injected particle (Bertholon *et al.*, 2006). This means that the polymer's structure will have an influence on the extent of complement activation (Bertholon *et al.*, 2006; Dobrovolskaia *et al.*, 2008). If an injected particle has a strong electrostatic interaction with other plasma proteins, before activating the complement cascade, the bound proteins can prevent complement to a certain extent (Andersson *et al.*, 2002; Benesch & Tengvall, 2002; Dobrovolskaia *et al.*, 2008).

As the alternative pathway's activation is spontaneous, it shows potential for use in *in vitro* studies of complement activation.

2.8.3 Plasma protein interaction

Negatively charged, plasma proteins easily form complexes with polycations like TMC nanoparticles (Moreau *et al.*, 2002). Although these electrostatic interactions can have protective effects, as mentioned before, reducing hemolysis and complement activation, the effects are not always beneficial (Andersson *et al.*, 2002; Dobrovolskaia *et al.*, 2009; Moreau *et al.*, 2000).

Interaction with plasma proteins can cause activation of the coagulation cascade (Cerda-Cristerna *et al.*, 2011). Particles formulated for extended release usually have a longer circulation time, which can cause extensive coagulation activation, which can result in the formation of a blood clot or even total occlusion of a blood vessel (Dobrovolskaia *et al.*, 2008).

As discussed in a previous section, interaction with plasma proteins can assist in recognition of the nanoparticles as foreign by the immune system (Schroeder *et al.*, 1998). The reticuloendothelial system (RES) launches a biological response against the opsonized particles leading to their removal from circulation (Saba, 1970; Soppimath *et al.*, 2001; Thasneem *et al.*, 2011). A by-product of this response, however, is the production of reactive oxygen species and pro-inflammatory molecules (Forman & Torres, 2001). Excessive activation of the RES consequently leads to inflammation, toxicity and cell damage (Dobrovolskaia *et al.*, 2009; Hoet *et al.*, 2004; Li *et al.*, 2008; Manke *et al.*, 2013).

As the interaction between the injected particle and the plasma proteins is dependent on the characteristics of the particle, as well as the polymer it is made of, it is possible to improve the particle's hemocompatibility by altering the surface properties thereof (Koziara *et al.*, 2005; Mailänder & Landfester, 2009; Moreau *et al.*, 2002; Thasneem *et al.*, 2011).

2.9 Methods to improve hemocompatibility

One of the most popular surface modifications made to improve the hemocompatibility of polymeric particles, is the addition of poly(ethylene) glycol, or PEG to the formulation (Koziara *et al.*, 2005). PEG can be chemically attached to the particle surface in a process called PEGylation, or it can be cross-linked to the polymer prior to the synthesis of the particles (Casettari *et al.*, 2012; Kulkarni *et al.*, 2005).

PEG is a polymer whose addition results in a decrease in zeta potential and the extent of reactivity of cations (Cerda-Cristerna *et al.*, 2011; Geisberger *et al.*, 2013; Gref *et al.*, 2000). The decrease in zeta potential is synonymous with a decrease in the surface charge (Casettari *et al.*, 2012). For polycations like TMC nanoparticles, this means less positive charges exposed to interact with the blood components and cause toxicity (Casettari *et al.*, 2012; Sadeghi *et al.*, 2008).

PEG creates a steric shield around the particles, preventing plasma protein adsorption to a certain extent, minimizing the amount of complement activation and the immunological response caused

(Cerda-Cristerna *et al.*, 2011; Chen & Borden, 2011; Dobrovolskaia *et al.*, 2008; Gref *et al.*, 2000). Studies have found that with the addition of PEG, particles had less interaction with cell membranes, thereby inducing less hemolysis (Kim *et al.*, 2005; Mourya & Inamdar, 2009).

As PEG reduces the reactivity and increases the stability of particles (Cerda-Cristerna *et al.*, 2011; Geisberger *et al.*, 2013; Gref *et al.*, 2000), it has presented itself as an almost essential part of intravenous particle formulations.

2.10 Hemocompatibility of polymeric nanoparticles

Although polymers have been a subject of interest for quite some time, the idea of polymeric nanoparticles is relatively new, as are the studies determining the hemocompatibility of these particles, with the first publications only appearing in 2005. Since then the hemocompatibility of a variety of polymeric nanoparticles have been examined. The hemocompatibility of hydroxyapatite, magnetoliposome, PEGylated glyceryl monooleate and gold nanoparticles have been explored, as these particles have possible applications in the delivery of cancer medication (Chandra *et al.*, 2012; Clares *et al.*, 2013; Ganeshkumar *et al.*, 2013; Jain *et al.*, 2012; Venkatesan *et al.*, 2011). Other compounds and polymers' hemocompatibility have also recently been tested. These include pullulan, poly lactic-co-glycolic acid (PLGA) and chitosan.

Pullulan is fungal polysaccharide with the ability to bind to liver cells. This makes it an appealing prospect for gene delivery to the liver, but also a big potential threat, as it would have extended contact with blood components. This necessitated the testing of its hemocompatibility, which included determination of red and white blood cell compatibility, platelet interactions and complement activation. It was determined that the cationic pullulan with the lowest zeta potential had high solubility and displayed the best hemocompatibility (Rekha & Sharma, 2009).

PLGA is a polymer of interest for its cell penetrating abilities, which can potentially be used in nuclear targeting. It has also been investigated for the delivery of the immunosuppressant, cyclosporine. The latter use especially emphasizes the need for hemocompatibility of PLGA particles. Although PLGA nanoparticles have good hemocompatibility, surface modifications were explored to enhance the hemocompatibility and thereby extend the circulation time of the particles. These modifications include addition of glucosamine or the addition of mucin to the PLGA particles. These modifications led to a decrease in plasma protein interactions, thus preventing complement and platelet activation and improving hemocompatibility (Italia *et al.*, 2007; Thasneem *et al.*, 2013a; Thasneem *et al.*, 2013b).

Chitosan has been discussed earlier. Its properties (biocompatibility, biodegradability, etc.) make it an appealing prospective for many pharmaceutical applications (Chua *et al.*, 2012; Sieval *et al.*, 1998). However, its hemocompatibility is poor. Many modifications have been tested, with some success, to improve chitosan's hemocompatibility. These modifications include binding PEG to the surface of chitosan particles, synthesis of a phosphorylcholine-coated glutaraldehyde-cross-linked-chitosan film and developing derivatives, such as *O*-carboxymethyl chitosan or lauroyl sulfated chitosan (Huangfu *et al.*, 2009; Luangtana-anan *et al.*, 2010; Shelma & Sharma, 2011; Smitha *et al.*, 2014). One of the most popular chitosan derivatives, as mentioned before, is TMC (Polnok *et al.*, 2004).

TMC has been shown to be non-toxic by various studies (Amidi *et al.*, 2006; Du Plessis *et al.*, 2010a; Thanou *et al.*, 2001b). It is important to note, however, that, to our knowledge, no hemocompatibility studies have been performed on TMC nanoparticles.

2.11 Conclusion

Polymeric nanoparticles are an attractive option for the delivery of new and existing protein and peptide APIs, as they usually promote bioavailability of the APIs they deliver, as well as being able to bind more per particle mass than larger particles. Chitosan is abundant and biocompatible, but because of solubility problems, it cannot be used in intravenous formulations. This has necessitated the development of the partially quaternized derivative of chitosan, TMC. TMC is biocompatible, biodegradable and non-toxic. Its solubility profile is superior to chitosan's, as well as being a better absorption enhancer across mucosal surfaces, presenting itself as an excipient for use in intravenous drug delivery systems.

All polymers cause toxicity to some extent, however, especially intravenously. When a particle enters the blood, various systems are activated to remove it, decreasing the bioavailability of the API carried by the particle, as well as potentially causing toxicity, e.g. hemolysis, aggregation, inflammation, etc.

This displays the need for hemocompatibility testing before considering a polymer for use in a drug delivery system, especially nanoparticles intended for intravenous use. Although the hemocompatibility of many polymeric nanoparticles have been determined, there remains a void in the knowledge of the hemocompatibility of TMC nanoparticles.

2.12 References

ABD EL-GHAFFAR, M.A., HASHEM, M.S., EL-AWADY, M.K. & RABIE, A.M. 2012. pH-sensitive sodium alginate hydrogels form riboflavin controlled release. *Carbohyd Polym*, 89(2):667-675.

ACHOURI, D., ALHANOUT, K., PICCERELLE, P. & ANDRIEU, V. 2013. Recent advances in ocular drug delivery. *Drug Dev Ind Pharm*, 39(11):1599-1617.

AGGARWAL, P., HALL, J.B., MCLELAND, C.B., DOBROVOLSKAIA, M.A. & MCNEIL, S.E. 2009. Nanoparticle interaction with plasma proteins as it relates to particle biodistribution, biocompatibility and therapeutic efficacy. *Adv Drug Deliver Rev*, 61(6):428-437.

AGNIHOTRI, S.A., MALLIKARJUNA, N.N. & AMINABHAVI, T.M. 2004. Recent advances on chitosan-based micro- and nanoparticles in drug delivery. *J Control Release*, 100(1):5-28.

AHUJA, A., KHAR, R.K. & ALI, J. 1997. Mucoadhesive drug delivery systems. *Drug Dev Ind Pharm*, 23(5):489-515.

ALLEN, T.M. & CULLIS, P.R. 2004. Drug delivery systems: Entering the mainstream. *Science*, 303(5665):1818-1822.

ALLEN, L.V., POPOVICH, N.G. & ANSEL, H.C. 2005. Dosage form design: pharmaceutical and formulation consideration. *In*: *Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems*, 8th ed. Baltimore: Lippincott Williams & Wilkins. 92-141 p.

AMIDI, M., ROMEIJN, S.G., BORCHARD, G., JUNGINGER, H.E., HENNINK, W.E. & JISKOOT, W. 2006. Preparation and characterization of protein-loaded *N*-trimethyl chitosan nanoparticles as nasal delivery system. *J Control Release*, 111(1-2):107-116.

ANDERSSON, J., EKDAHL, K.N., LARSSON, R., NILSSON, U.R. & NILSSON, B. 2002. C3 adsorbed to a polymer surface can from an initiating alternative pathway convertase. *J Immunol*, 168(11):5786-5791.

ARBIT, E. & KIDRON, M. 2009. Oral insulin: the rationale for this approach and current developments. *J Diabetes Sci Technol*, 3(3):562-567.

ASHFORD, M. 2002a. Chapter 15: Introduction to biopharmaceutics. *In*: AULTON, M.E., ed. *Pharmaceutics: The Science of Dosage Form Design*, 2nd ed. Philadelphia: Churchill Livingston. 213-216 p.

ASHFORD, M. 2002b. Chapter 16: The gastrointestinal tract - physiology and drug absorption. *In*: AULTON, M.E., ed. *Pharmaceutics: The Science of Dosage Form Design*, 2nd ed. Philadelphia: Churchill Livingston. 217-233 p.

ASHFORD, M. 2002c. Chapter 17: Bioavailability - physicochemical and dosage form factors. *In*: AULTON, M.E., ed. *Pharmaceutics: The Science of Dosage Form Design*, 2nd ed. Philadelphia: Churchill Livingston. 234-252 p.

AYTEKIN, A.O., MORIMURA, S. & KIDA, K. 2012. Physiological activities of chitosan and *N*-trimethyl chitosan chloride in U937 and 3T3-L1 cells. *Polym Advan Technol*, 23(2):228-235.

BENDER, A.R., VON BRIESEN, H., KREUTER, J., DUNCAN, I.B. & RÜBSAMEN-WAIGMANN, H. 1996. Efficiency of nanoparticles as a carrier system for antiviral agents in human immunodeficiency virus-infected human monocytes/macrophages *in vitro*. *Antimicrob Agents Ch*, 40(6):1467-1471.

BENEKE, C.E., VILJOEN, A.M. & HAMMAN, J.H. 2009. Polymeric plant-derived excipients in drug delivery. *Molecules*, 14(7):2602-2620.

BENESCH, J. & TENGVALL, P. 2002. Blood protein adsorption onto chitosan. *Biomaterials*, 23(12):2561-2568.

BERTHOLON, I., VAUTHIER, C. & LABARRE, D. 2006. Complement activation by core-shell poly(isobutylcyanoacrylate)-polysaccharide nanoparticles: influences of surface morphology, length, and type of polysaccharide. *Pharm Res*, 23(6):1313-1323.

CARABIN, I.G. & FLAMM, W.G. 1999. Evaluation of safety of inulin and oligofructose as dietary fiber. *Regul Toxicol Pharm*, 30(3):268-282.

CASETTARI, L., VLLASALIU, D., CASTAGNINO, E., STOLNIK, S., HOWDLE, S. & ILLUM, L. 2012. PEGylated chitosan derivatives: synthesis, characterizations and pharmaceutical applications. *Prog Polym Sci*, 37(5):659-685.

CERDA-CRISTERNA, B.I., FLORES, H., POZOS-GUILLÉN, A., PÉREZ, E., SERVIN, C. & GRANDFILS, C. 2011. Hemocompatibility assessment of poly(2-dimethylamino ethylmethacrylate) (PDMAEMA)-based polymers. *J Control Release*, 153(3):269-277.

CHAMARTHY, S.P. & PINAL, R. 2008. Plasticizer concentration and the performance of a diffusion-controlled polymeric drug delivery system. *Colloid Surface A*, 331(1-2):25-30.

CHANDRA, V.S., BASKAR, G., SUGANTHI, R.V., ELAYARAJA, K., JOSHY, M.I.A., BEAULA, W.S., MYTHILI, R., VENKATRAMAN, G. & KALKURA, S.N. 2012. Blood compatibility of iron-doped nanosize hydroxyapatite and its drug release. *ACS Appl Mater Interfaces*, 4(3):1200-1210.

CHANG, D. & CHANG, R.K. 2007. Review of current issues in pharmaceutical excipients. *Pharm Technol*, 31(5):56-66.

CHAURASIA, M., CHOURASIA, M.K., JAIN, N.K., JAIN, A., SONI, V., GUPTA, Y. & JAIN, S.K. 2006. Cross-linked guar gum microspheres: a viable approach for improved delivery of anticancer drugs for the treatment of colorectal cancer. *AAPS PharmSciTech*, 7(3):E1-E9.

CHEN, C.C. & BORDEN, M.A. 2011. The role of poly(ethylene glycol) brush architecture in complement activation on targeted microbubble surfaces. *Biomaterials*, 32(27):6579-6587.

CHEN, F., ZHANG, Z.R. & HUANG, Y. 2007. Evaluation and modification of *N*-trimethyl chitosan chloride nanoparticles as protein carriers. *Int J Pharm*, 336(1):166-173.

CHOKSAKULNIMITR, S., MASUDA, S., TOKUDA, H., TAKAKURA, Y. & HASHIDA, M. 1995. *In vitro* cytotoxicity of macromolecules in different cell culture systems. *J Control Release*, 34(3):233-241.

CHUA, B.Y., KOBAISI, M.A., ZENG, W., MAINWARING, D. & JACKSON, D.C. 2012. Chitosan microparticles and nanoparticles as biocompatible delivery vehicles for peptide and protein-based immunocontraceptive vaccines. *Mol Pharm*, 9(1):81-90.

CLARES, B., BIEDMA-ORTIZ, R.A., SÁEZ-FERNÁNDEZ, E., PRADOS, J.C., MELGUIZO, C., CABEZA, L., ORTIZ, R. & ARIAS, J.L. 2013. Nano-engineering of 5-fluorouracil-loaded magnetoliposomes for combined hyperthermia and chemotherapy against colon cancer. *Eur J Pharm Biopharm*, 85:329-338. DOI: 10.1016/j.ejpb.2013.01.028.

COLLETT, J. & MORETON, C. 2002. Chapter 20: Modified-release peroral dosage forms. *In*: AULTON, M.E., ed. *Pharmaceutics: The Science of Dosage Form Design*, 2nd ed. Philadelphia: Churchill Livingstone. 289-305 p.

COVIELLO, T., MATRICARDI, P., MARIANECCI, C. & ALHAIQUE, F. 2007. Polysaccharide hydrogels for modified release formulations. *J Control Release*, 119:5-24.

CROMMELIN, D., VAN WINDEN, E. & MEKKING, A. 2002. Chapter 35: Delivery of pharmaceutical proteins. *In*: AULTON, M.E., ed. *Pharmaceutics: The Science of Dosage Form Design*, 2nd ed. Philadelphia: Churchill Livingston. 544-553 p.

DAVIES, O.R., HEAD, L., ARMITAGE, D., PEARSON, E.A., DAVIES, M.C., MARLOW, M. & STOLNIK, S. 2008. Surface modification of microspheres with steric stabilizing and cationic polymers for gene delivery. *Langmuir*, 24(14):7138-7146.

DE BRITTO, D., DE MOURA, M.R., AOUADA, F.A., MATTOSO, L.H.C. & ASSIS, O.B.G. 2012. *N,N,N*-trimethyl chitosan nanoparticles as a vitamin carrier system. *Food Hydrocolloid*, 27(2):487-493.

DJUPESLAND, P.G. 2013. Nasal drug delivery devices: characteristics and performance in a clinical perspective - a review. *Drug Deliv Transl Res*, 3(1):42-62.

DOBROVOLSKAIA, M.A., AGGARWAL, P., HALL, J.B. & MCNEIL, S.E. 2008. Preclinical studies to understand nanoparticle interaction with the immune system and its potential effects on nanoparticle biodistribution. *Mol Pharm*, 5(4):487-495.

DOBROVOLSKAIA, M.A., PATRI, A.K., ZHENG, J., CLOGSTON, J.D., AYUB, N., AGGARWAL, P., NEUN, B.W., HALL, J.B. & MCNEIL, S.E. 2009. Interaction of colloidal gold nanoparticles with human blood: effects on particle size and analysis of plasma protein binding profiles. *Nanomed-Nanotechnol*, 5(2):106-117.

DU PLESSIS, L.H., KOTZÉ, A.F. & JUNGINGER, H.E. 2010a. Nasal and rectal delivery of insulin with chitosan and *N*-trimethyl chitosan chloride. *Drug Deliv*, 17(6):399-407.

DU PLESSIS, L.H., LUBBE, J., STRAUSS, T. & KOTZÉ, A.F. 2010b. Enhancement of nasal and intestinal calcitonin delivery by the novel Pheroid™ fatty acid based delivery system, and by *N*-trimethyl chitosan chloride. *Int J Pharm*, 385(1-2):181-186.

FELT, O., BURI, P. & GURNY, R. 1998. Chitosan: a unique polysaccharide for drug delivery. *Drug Dev Ind Pharm*, 24(11):979-993.

FISCHER, D., LI, Y., AHLEMEYER, B., KRIEGLSTEIN, J. & KISSEL, T. 2003. *In vitro* cytotoxicity testing of polycations: influence of polymer structure on cell viability and hemolysis. *Biomaterials*, 24(7):1121-1131.

FORMAN, H.J. & TORRES, M. 2001. Redox signaling in macrophages. *Mol Aspects Med*, 22(4-5):189-216.

GANESHKUMAR, M., SATHISHKUMAR, M., PONRASU, T., DINESH, M.G. & SUGUNA, L. 2013. Spontaneous ultra fast synthesis of gold nanoparticles using *Punica granatum* for cancer targeted drug delivery. *Colloid Surface B*, 106:208-216.

GARDNER, C.R. 1987. Drug delivery - where now? *In*: LLOYD-JONES, J.G. & JOHNSON, P., eds. *Drug Delivery Systems: Fundamentals and Techniques*, Chichester: Ellis Horwood. 11-31 p.

GEISBERGER, G., GYENGE, E.B., MAAKE, C. & PATZKE, G.R. 2013. Trimethyl and carboxymethyl chitosan carriers for bio-active polymer-inorganic nanocomposites. *Carbohyd Polym*, 91(1):58-67.

GENG, X., YANG, R., HUANG, J., ZHANG, X. & WANG, X. 2013. Evaluation antibacterial activity of quaternary-based chitin/chitosan derivatives *in vitro*. *J Food Sci*, 78(1):M90-M97.

GREF, R., LÜCK, M., QUELLEC, P., MARCHAND, M., DELLACHERIE, E., HARNISCH, S., BLUNK, T. & MÜLLER, R.H. 2000. 'Stealth' corona-core nanoparticles surface modified by polyethylene glycol (PEG): influences of the corona (PEG chain length and surface density) and of the core composition on phagocytic uptake and plasma protein adsorption. *Colloid Surface B*, 18(3-4):301-313.

GUGGI, D. & BERNKOP-SCHNÜRCH, A. 2003. *In vitro* evaluation of polymeric excipients protecting calcitonin against degradation by intestinal serine proteases. *Int J Pharm*, 252(1-2):187-196.

GUO, J.H., SKINNER, G.W., HARCUM, W.W. & BARNUM, P.E. 1998. Pharmaceutical applications of naturally occuring water-soluble polymers. *Pharm Sci Technol To*, 1(6):254-261.

GUYTON, A.C. & HALL, J.E. 2006a. Chapter 64: Secretory functions of the alimentary tract. *In*: SCHMITT, W. & GRULIOW, R., eds. *Textbook of Medical Physiology*, 11th ed. Pennsylvania: Elsevier Saunders. 791-807 p.

GUYTON, A.C. & HALL, J.E. 2006b. Chapter 65: Digestion and absorption in the gastrointestinal tract. *In*: SCHMITT, W. & GRULIOW, R., eds. *Textbook of Medical Physiology*, 11th ed. Pennsylvania: Elsevier Saunders. 808-818 p.

HAMMAN, J.H., SCHULTZ, C.M. & KOTZÉ, A.F. 2003. *N*-trimethyl chitosan chloride: optimum degree of quaternization for drug absorption enhancement across epithelial cells. *Drug Dev Ind Pharm*, 29(2):161-172.

HAMMAN, J.H. & TARIRAI, C. 2006. Functional excipients. *Chim Oggi*, 24(5):57-62.

HANS, M.L. & LOWMAN, A.M. 2002. Biodegradable nanoparticles for drug delivery and targeting. *Curr Opin Solid St M*, 6(4):319-327.

HOET, P.H.M., BRÜSKE-HOHLFELD, I. & SALATA, O.V. 2004. Nanoparticles - known and unknown health risks. *J Nanobiotechnology*, 2(art. no. 12):1-15.

HUANGFU, P., GONG, M., ZHANG, C., YANG, S., ZHAO, J. & GONG, Y. 2009. Cell outer membrane mimetic modification of a cross-linked chitosan surface to improve its hemocompatibility. *Colloid Surface B*, 71(2):268-274.

ITALIA, J.L., BHATT, D.K., BHARDWAJ, V., TIKOO, K. & KUMAR, M.N.V.R. 2007. PLGA nanoparticles for oral delivery of cyclosporine: nephrotoxicity and pharmacokinetic studies in comparison to Sandimmune Neoral®. *J Control Release*, 119(2):197-206.

JAIN, V., SWARNAKAR, N.K., MISHRA, P.R., VERMA, A., KAUL, A., MISHRA, A.K. & JAIN, N.K. 2012. Paclitaxel loaded PEGylated gleceryl monooleate based nanoparticulate carriers in chemotherapy. *Biomaterials*, 33(29):7206-7220.

JANEWAY JR, C.A., TRAVERS, P., WALPORT, M. & SHLOMCHIK, M.J. 2001. The complement system and innate immunity. *In*: *Immunobiology: The Immune System in Health and Disease*, 5th ed. New York: Garland Science. Available from: http://www.ncbi.nlm.nih.gov/books/NBK27100/ [Date of access: 15 November 2013]

JAYAKUMAR, R., MENON, D., MANZOOR, K., NAIR, S.V. & TAMURA, H. 2010. Biomedical applications of chitin and chitosan based nanomaterials - a short review. *Carbohyd Polym*, 82(2):227-232.

JONES, C.F. & GRAINGER, D.W. 2009. *In vitro* assessments of nanomaterial toxicity. *Adv Drug Deliver Rev*, 61(6):438-456.

KEIJZER, C., SLÜTTER, B., VAN DER ZEE, R., JISKOOT, W., VAN EDEN, W. & BROERE, F. 2011. PLGA, PLGA-TMC and TMC-TPP nanoparticles differentially modulate the outcome of nasal vaccination by inducing tolerance or enhancing humoral immunity. *PLoS ONE*, 6(11):art. no. e26684.

KIM, D., EL-SHALL, H., DENNIS, D. & MOREY, T. 2005. Interaction of PLGA nanoparticles with human blood constituents. *Colloid Surface B*, 40(2):83-91.

KOTZÉ, A.F., LUEβEN, H.L., DE LEEUW, B.J., DE BOER, B.G., VERHOEF, J.C. & JUNGINGER, H.E. 1997. *N*-trimethyl chitosan chloride as a potential absorption enhancer across mucosal surfaces: *in vitro* evaluation in intestinal epithelial cells (Caco-2). *Pharm Res*, 14(9):1197-1202.

KOTZÉ, A.F., THANOU, M.M., LUEBEN, H.L., DE BOER, A.G., VERHOEF, J.C. & JUNGINGER, H.E. 1999. Enhancement of paracellular drug transport with highly quaternized *N*-trimethyl chitosan chloride in neutral environments: *In vitro* evaluation in intestinal epithelial cells (Caco-2). *J Pharm Sci*, 88(2):253-257.

KOZIARA, J.M., OH, J.J., AKERS, W.S., FERRARIS, S.P. & MUMPER, R.J. 2005. Blood compatibility of cetyl alcohol/polysorbate-based nanoparticles. *Pharm Res*, 22(11):1821-1828.

KULKARNI, A.R., HUKKERI, V.I., SUNG, H.W. & LIANG, H.F. 2005. A novel method for the synthesis of the PEG-crosslinked chitosan with a pH independent swelling behavior. *Macromol Biosci*, 5(10):925-928.

KUMARI, A., YADAV, S.K. & YADAV, S.C. 2010. Biodegradable polymeric nanoparticles based drug delivery systems. *Colloid Surface B*, 75(1):1-18.

LI, N., XIA, T. & NEL, A.E. 2008. The role of oxidative stress in ambient particulate matter-induced lung diseases and its implications in the toxicity of engineerd nanoparticles. *Free Radical Bio Med*, 44(9):1689-1699.

LI, Z., LI, X., CAO, Z., XU, Y., LIN, H., ZHAO, Y., WEI, Y. & QIAN, Z. 2012. Camptothecin nanocolloids based on *N,N,N*-trimethyl chitosan: efficient suppression of growth of multiple myeloma in a murine model. *Oncol Rep*, 27(4):1035-1040.

LIU, X. P., ZHOU, S. T., LI, X.Y., CHEN, X.C., ZHAO, X., QIAN, Z.Y., ZHOU, L.N., LI, Z.Y., WANG, Y.M., ZHONG, Q., YI, T., LI, Z.Y., HE, X. & WEI, Y.Q. 2010. Anti-tumor activity of *N*-trimethyl chitosan-encapsulated camptothecin in a mouse melanoma model. *J Exp Clin Canc Res*, 29(1):art. no. 76.

LUANGTANA-ANAN, M., LIMMATVAPIRAT, S., NUNTHANID, J., CHALONGSUK, R. & YAMAMOTO, K. 2010. Polyethylene glycol on stability of chitosan microparticulate carrier for protein. *AAPS Pharm Sci Tech*, 11(3):1376-1382.

LYNCH, I. & DAWSON, K.A. 2008. Protein-nanoparticle interactions. *Nano Today*, 3(1-2):40-47.

MAILÄNDER, V. & LANDFESTER, K. 2009. Interaction of nanoparticles with cells. *Biomacromolecules*, 10(9):2379-2400.

MALAFAYA, P.B., SILVA, G.A. & REIS, R.L. 2007. Natural-origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications. *Adv Drug Deliver Rev*, 59(4-5):207-233.

MANKE, A., WANG, L. & ROJANASAKUL, Y. 2013. Mechanisms of nanoparticle-induced oxidative stress and toxicity. *Biomed Res Int*, 2013(art. no. 942916):1-15.

MAO, S., SHUAI, X., UNGER, F., WITTMAR, M., XIE, X. & KISSEL, T. 2005. Synthesis, characterization and cytotoxicity of poly(ethylene glycol)-graft-trimethyl chitosan block copolymers. *Biomaterials*, 26(32):6343-6356.

MOREAU, E., DOMURADO, M., CHAPON, P., VERT, M. & DOMURADO, D. 2002. Biocompatibility of polycations: *in vitro* agglutination and lysis of red blood cells and *in vivo* toxicity. *J Drug Target*, 10(2):161-173.

MOREAU, É., FERRARI, I., DROCHON, A., CHAPON, P., VERT, M. & DOMURADO, D. 2000. Interactions between red blood cells and a lethal, partly quaternized tertiary polyamine. *J Control Release*, 64(1-3):115-128.

MORIKIS, D. & LAMBRIS, J.D. 2005. Chapter 1: The building blocks of the complement system. *In*: MORIKIS, D. & LAMBRIS, J.D., eds. *Structural Biology of the Complement System, Volume 1*, Florida: CRC Press. 1-18 p.

MOURYA, V.K. & INAMDAR, N.N. 2009. Trimethyl chitosan and its application in drug delivery. *J Mater Sci - Mater M*, 20(5):1057-1079.

ODDO, L., MASCI, G., DI MEO, C., CAPITANI, D., MANNINA, L., LAMANNA, R., DE SANTIS, S., ALHAIQUE, F., COVIELLO, T. & MATRICARDI, P. 2010. Novel thermosensitive calcium alginate microspheres: physico-chemical characterization and delivery properties. *Acta Biomater*, 6(9):3657-3664.

PAÑOS, I., ACOSTA, N. & HERAS, A. 2008. New drug delivery systems based on chitosan. *Curr Drug Discovery Technol*, 5(4):333-341.

PIFFERI, G. & RESTANI, P. 2003. The safety of pharmaceutical excipients. *Farmaco*, 58(8):541-550.

PIFFERI, G., SANTORO, P. & PEDRANI, M. 1999. Quality and functionality of excipients. *Farmaco*, 54(1-2):1-14.

POLNOK, A., BORCHARD, G., VERHOEF, J.C., SARISUTA, N. & JUNGINGER, H.E. 2004. Influence of methylation process on the degree of quaternization of *N*-trimethyl chitosan chloride. *Eur J Pharm Biopharm*, 57(1):77-83.

PRESIDENT'S COUNCIL OF ADVISORS ON SCIENCE AND TECHNOLOGY. 2005. *The National Nanotechnology Initiative at five years: Assessment and recommendations of the Nantional Nanotechnology Advisory Panel*. Washington, D.C.: Executive Office of the President of the United States.

RANA, V., RAI, P., TIWARY, A.K., SINGH, R.S., KENNEDY, J.F. & KNILL, C.J. 2011. Modified gums: approaches and applications in drug delivery. *Carbohyd Polym*, 83(3):1031-1047.

REDHEAD, H.M., DAVIS, S.S. & ILLUM, L. 2001. Drug delivery in poly(lactide-co-glycolide) nanoparticles surface modified with poloxamer 407 and poloxamine 908: *in vitro* characterization and *in vivo* evaluation. *J Control Release*, 70(3):353-363.

REJMAN, J., OBERLE, V., ZUHORN, I.S. & HOEKSTRA, D. 2004. Size-dependent internalization of particles via the pathways of clathrin- and caveolae-mediated endocytosis. *Biochem J*, 377(1):159-169.

REKHA, M.R. & SHARMA, C.P. 2009. Blood compatibility and *in vitro* transfection studies on cationically modified pullulan for liver cell targeted gene delivery. *Biomaterials*, 30(34):6655-6664.

REKHA, M.R. & SHARMA, C.P. 2011. Hemocompatible pullulan–polyethyleneimine conjugates for liver cell gene delivery: *In vitro* evaluation of cellular uptake, intracellular trafficking and transfection efficiency. *Acta Biomater*, 7(1):370-379.

RONEY, C., KULKARNI, P., ARORA, V., ANTICH, P., BONTE, F., WU, A., MALLIKARJUANA, N.N., MANOHAR, S., LIANG, H.F., KULKARNI, A.R., SUNG, H.W., SAIRAM, M. & AMINABHAVI, T.M. 2005. Targeted nanoparticles for drug delivery through the blood-brain barrier for Alzheimer's disease. *J Control Release*, 108(2-3):193-214.

SABA, T.M. 1970. Physiology and physiopathology of the reticuloendothelial system. *Arch Intern Med*, 126(6):1031-1052.

SADEGHI, A.A.M., DORKOOSH, F.A., AVADI, M.R., SAADAT, P., RAFIEE-TEHRANI, M. & JUNGINGER, H.E. 2008. Preparation, characterization and antibacterial activites of chitosan, *N*-trimethyl chitosan (TMC) and *N*-diethylmethyl chitosan (DEMC) nanoparticles loaded with insulin using both the ionotropic gelation and polyelectrolyte complexation methods. *Int J Pharm*, 355(1-2):299-306.

SANDRI, G., ROSSI, S., BONFERONI, M.C., FERRARI, F., ZAMBITO, Y., DI COLO, G. & CARAMELLA, C. 2005. Buccal penetration enhancement properties of *N*-trimethyl chitosan: influence of quaternization degree on absorption of a high molecular weight molecule. *Int J Pharm*, 297(1-2):146-155.

SATTURWAR, P.M., FULZELE, S.V. & DORLE, A.K. 2003. Biodegradation and *in vivo* biocompatibility of rosin: a natural film-forming polymer. *AAPS PharmSciTech*, 4(4):1-6.

SATTURWAR, P.M., FULZELE, S.V., PANYAM, J., MANDAOGADE, P.M., MUNDHADA, D.R., GOGTE, B.B., LABHASETWAR, V. & DORLE, A.K. 2004. Evaluation of new rosin derivatives for pharmaceutical coating. *Int J Pharm*, 270(1-2):27-36.

SAYIN, B., SOMAVARAPU, S., LI, X.W., THANOU, M., SESARDIC, D., ALPAR, H.O. & ŞENEL, S. 2008. Mono-*N*-carboxymethyl chitosan (MCC) and *N*-trimethyl chitosan (TMC) nanoparticles for non-invasive vaccine delivery. *Int J Pharm*, 363(1-2):139-148.

SCHIPPER, N.G.M., OLSSON, S., HOOGSTRAATE, J.A., DEBOER, A.G., VÅRUM, K.M. & ARTURSSON, P. 1997. Chitosans as absorption enhancers for poorly absorbable drugs 2: mechanism of absorption enhancement. *Pharm Res*, 14(7):923-929.

SCHÖLL, I., BOLTZ-NITULESCU, G. & JENSEN-JAROLIM, E. 2005. Review of novel particulate antigen delivery systems with special focus on treatment of type I allergy. *J Control Release*, 104(1):1-27.

SCHOLTZ, J.C., VAN DER COLFF, J., STEENEKAMP, J.H., STIEGER, N. & HAMMAN, J.H. 2013. More good news about polymeric plant- and algae-derived biomaterials in drug delivery systems. *Curr Drug Targets*, 14(11):DOI: 10.2174/13894501113149990175.

SCHROEDER, U., SOMMERFELD, P., ULRICH, S. & SABEL, B.A. 1998. Nanoparticle technology for delivery of drugs across the blood-brain barrier. *J Pharm Sci*, 87(11):1305-1307.

SHELMA, R. & SHARMA, C.P. 2011. Development of lauroyl sulfated chitosan for enhancing hemocompatibility of chitosan. *Colloid Surface B*, 84(2):561-570.

SHIRWAIKAR, A., SHIRWAIKAR, A., PRABHU, S.L. & KUMAR, G.A. 2008. Herbal excipients in novel drug delivery systems. *Indian J Pharm Sci*, 70(4):415-422.

SIEVAL, A.B., THANOU, M., KOTZÉ, A.F., VERHOEF, J.C., BRUSSEE, J. & JUNGINGER, H.E. 1998. Preparation and NMR characterization of highly substituted *N*-trimethyl chitosan chloride. *Carbohyd Polym*, 36(2-3):157-165.

SINHA, V.R. & KUMRIA, R. 2001. Polysaccharides in colon-specific drug delivery. *Int J Pharm*, 224(1-2):19-38.

SMITHA, K.T., SREELAKSHMI, M., NISHA, N., JAYAKUMAR, R. & BISWAS, R. 2014. Amidase encapsulated *O*-carboxymethyl chitosan nanoparticles for vaccine delivery. *Int J Biol Macromol*, 63:154-157.

SNYMAN, D., HAMMAN, J.H. & KOTZÉ, A.F. 2003. Evaluation of the mucoadhesive properties of *N*-trimethyl chitosan chloride. *Drug Dev Ind Pharm*, 29(1):61-69.

SONIA, T.A. & SHARMA, C.P. 2011. Chitosan and its derivatives for drug delivery perspective. *Adv Polym Sci*, 243(1):23-54.

SOPPIMATH, K.S., AMINABHAVI, T.M., KULKARNI, A.R. & RUDZINSKI, W.E. 2001. Biodegradable polymeric nanoparticles as drug delivery devices. *J Control Release*, 70(1-2):1-20.

STEINBERG, M., BORZELLECA, J.F., ENTERS, E.K., KINOSHITA, F.K., LOPER, A., MITCHELL, D.B., TAMULINAS, C.B. & WEINER, M.L. 1996. A new approach to the safety assessment of pharmaceutical excipients - The Safety Committee of the International Pharmaceutical Excipients Council. *Regul Toxicol Pharm*, 24(2):149-154.

SUMAN, J.D. 2013. Current understanding of nasal morphology and physiology as a drug delivery target. *Drug Deliv Transl Res*, 3(1):4-15.

TANIDA, F., TOJIMA, T., HAN, S.M., NISHI, N., TOKURA, S., SAKAIRI, N., SEINO, H. & HAMADA, K. 1998. Novel synthesis of a water-soluble cyclodextrin-polymer having a chitosan skeleton. *Polymer*, 39(21):5261-5263.

THANOU, M.M., KOTZÉ, A.F., SCHARRINGHAUSEN, T., LUEβEN, H.L., DE BOER, A.G., VERHOEF, J.C. & JUNGINGER, H.E. 2000a. Effect of degree of quaternization of *N*-trimethyl chitosan chloride for enhanced transport of hydrophilic compounds across intestinal Caco-2 cell monolayers. *J Control Release*, 64(1-3):15-25.

THANOU, M., NIHOT, M.T., JANSEN, M., VERHOEF, J.C. & JUNGINGER, H.E. 2001a. Mono-*N*-carboxymethyl chitosan (MCC), a polyampholytic chitosan derivative, enhances the intestinal absorption of low molecular weight heparin across intestinal epithelia *in vitro* and *in vivo*. *J Pharm Sci*, 90(1):38-46.

THANOU, M., VERHOEF, J.C. & JUNGINGER, H.E. 2001b. Chitosan and its derivatives as intestinal absorption enhancers. *Adv Drug Deliver Rev*, 50(Suppl. 1):S91-S101.

THANOU, M., VERHOEF, J.C., MARBACH, P. & JUNGINGER, H.E. 2000b. Intestinal absorption of octreotide: *N*-trimethyl chitosan chloride (TMC) ameliorates the permeability and absorption properties of the somatostatin analogue *in vitro* and *in vivo*. *J Pharm Sci*, 89(7):951-957.

THASNEEM, Y.M., REKHA, M.R., SAJEESH, S. & SHARMA, C.P. 2013a. Biomimetic mucin modified PLGA nanoparticles for enhanced blood compatibility. *J Colloid Interf Sci*, 409:237-244.

THASNEEM, Y.M., SAJEESH, S. & SHARMA, C.P. 2011. Effect of thiol functionalization on the hemo-compatibility of PLGA nanoparticles. *J Biomed Mater Res A*, 99 A(4):607-617.

THASNEEM, Y.M., SAJEESH, S. & SHARMA, C.P. 2013b. Glucosylated polymeric nanoparticles: a sweetened approach against blood compatibility paradox. *Colloid Surface B*, 108:337-344.

UENO, H., MORI, T. & FUJINAGA, T. 2001. Topical formulations and wound healing applications of chitosan. *Adv Drug Deliver Rev*, 52(2):105-115.

VAN DER MERWE, S.M., VERHOEF, J.C., KOTZÉ, A.F. & JUNGINGER, H.E. 2004a. *N*-Trimethyl chitosan chloride as absorption enhancer in oral peptide drug delivery. Development and characterization of minitablet and granule formulations. *Eur J Pharm Biopharm*, 57(1):85-91.

VAN DER MERWE, S.M., VERHOEF, J.C., VERHEIJDEN, J.H.M., KOTZÉ, A.F. & JUNGINGER, H.E. 2004b. Trimethylated chitosan as polymeric absorption enhancer for improved peroral delivery of peptide drugs. *Eur J Pharm Biopharm*, 58(2):225-235.

VARSHOSAZ, J., TAVAKOLI, N. & ERAM, S.A. 2006. Use of natural gums and cellulose derivatives in production of sustained release metoprolol tablets. *Drug Deliv*, 13(2):113-119.

VENKATESAN, P., PUVVADA, N., DASH, R., KUMAR, B.N.P., SARKAR, D., AZAB, B., PATHAK, A., KUNDU, S.C., FISHER, P.B. & MANDAL, M. 2011. The potential of celecoxib-loaded hydroxyapatite-chitosan nanocomposite for the treatment of colon cancer. *Biomaterials*, 32(15):3794-3806.

VERVOORT, L., ROMBAUT, P., VAN DER MOOTER, G., AUGUSTIJNS, P. & KINGET, R. 1998. Inulin hydrogels. II. *In vitro* degradation study. *Int J Pharm*, 172(1-2):137-145.

WALPORT, M.J. 2001. Advances in immunology: complement (first of two parts). *New Eng J Med*, 344(14):1058-1066.

WANG, Z.H., WANG, Z.Y., SUN, C.S., WANG, C.Y., JIANG, T.Y. & WANG, S.L. 2010. Trimethylated chitosan-conjugated PLGA nanoparticles for the delivery of drugs to the brain. *Biomaterials*, 31(5):908-915.

WANI, M.Y., HASHIM, M.A., NABI, F. & MALIK, M.A. 2011. Nanotoxicity: dimensional and morphological concerns. *Adv Phys Chem*, 2011(art. no. 450912):1-15.

WHO (WORLD HEALTH ORGANIZATION). 1999. *Good manufacturing practices: supplementary guidelines for the manufacture of pharmaceutical excipients*. Berlin.

YORK, P. 2002. Chapter 1: The design of dosage forms. *In*: AULTON, M.E., ed. *Pharmaceutics: The Science of Dosage Form Design*, 2nd ed. Philadelphia: Churchill Livingston. 1-12 p.

ZHOU, L., LI, X., CHEN, X., LI, Z., LIU, X., ZHOU, S., ZHONG, Q., YI, T., WEI, Y., ZHAO, X. & QIAN, Z. 2010. *In vivo* antitumor and antimetastatic activites of camptothecin encapsulated with *N*-trimethyl chitosan in a preclinical mouse model of liver cancer. *Cancer Lett*, 297(1):56-64.