In The Name of God

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Preparation and characterization of solid lipid nanoparticles as drug carriers

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Abbas Hemati Azandaryani

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Dedicated To:

Those Who Suffer from Cancer,

My Dear Parents,

&

My Brothers and Sisters

Abstract

Solid lipid nanoparticles (SLN) are colloidal carrier systems providing controlled release profiles for many substances. In this study we prepared aqueous dispersions of lipid nanoparticles using modified pH sensitive derivative of Phosphatidylethanolamine. Firstly 4-(benzylcarbamoyl) butanoic acid was prepared, then the nanoparticles via high sheer homogenization and ultrasonication were produced. To fabricate nanoparticles Tween 80 was used as a surfactant in SLNs, and tripalmitin glyceride and N-glutaryl phosphatidylethanolamine were used as lipid parts. Particle size measurement showed that particles were in nanometric range and polydispersity index proved a narrow particle size. Zeta potential measurement for lyophilized powder showed approximately constant value of -48.0 indicating stability of the product during storage. Atomic force microscopy showed that prepared nanoparticle was in nanometer of 50-600 nm in length and 66.5 nm in height. Differential scanning calorimetry indicated that the majority of SLNs possessed less ordered arrangements of crystals than the corresponding bulk lipids, which was favorable for increasing the drug loading capacity. Drug loading capacity and drug entrapment efficiency (EE %) of the SLNs were calculated to be 25.32% and 94.32% respectively. *In vitro* drug release measurements with static Franz diffusion cells were performed and it is concluded that fabricated SLNs have higher release of the drug (triamcinolone acetonide) in a more acidic condition.

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List of abbreviations

1H-NMR (spectroscopy)	nuclear magnetic resonance (spectroscopy) of protons
AFM	atomic force microscopy
CMC	critical micellar concentration
CMT	critical micellar temperature
DSC	Differential scanning calonmetry
e.g.	exempli gratia (for example)
FT-IR	Fourier transformation infrared spectroscopy
HPH	high pressure homogenization
i.v.	intravenous
LUV	large unilamellar vesicles
MLV	multilamellar liposomes
MVV	multivesicular vesicles
OLV	oligolamellar large vesicles
PE	Phosphatidylethanolamine
PL	Phosphoiipid
MM	mixed micelle
NMR	nuclear magnetic resonance
PCS	photon correlation spectroscopy
PI	polydispersity index
SUV	small unilamellar vesicles
SLN	solid lipid nanoparticles

TA	triamcinolone acetonide
SEM	scanning electron microscopy
ZP	zeta potential

CHAPTER ONE

INTRODUCTION

1. Introduction

1.1. The interest in new drug delivery systems

For many decades treatments of an acute disease or a chronic illness has been mostly accomplished by delivery of drugs to patients using various pharmaceutical dosage forms such as tablets, capsules, pills creams, ointments, liquids as drug carriers. These types of drug delivery systems are known to provide a prompt release of drug. The efficiency of many drugs is often limited by their potential to reach their therapeutic site of action. In most cases; only a small amount of the administered dose of drug reaches the site, while the major drug amount is distributed in the rest of the body depending on the physicochemical and biomedical progenies of the drug.

The availability of new drugs with short biological half-lives (e.g. peptide drugs) and very potent drugs with strong side effects e.g. tumor necrosis factor and their high costs has led to increased interest in the possibility of delivering drugs to their desired site of action (drug targeting). Investigators have therefore attempted to develop new drug delivery systems.

The carrier systems should be able to protect the drug against *in vivo* degradation and prolonging its biological half-life. At the same time the protection of the host against systemic side effects by targeting of the drug to the desired tissue is needed. At the desired tissue the formulation should release the drug in a controlled way in order to maintain a sustained and effective drug level [Lamprecht, 2009]. The drug carrier itself should be stable in the physiological liquid, biodegradable, biocompatible, inert for the drug and the target tissue and able to incorporate sufficient amount of the drug**.** One approach to this

challenge is the use of colloidal drug carriers (Particles < 1 pm) for intravenous (i.v.) drug administration. Colloidal carriers are particles ranging in size From 10 m to 1000 m. They consist of materials in which the active agents (drug or biological active material) is dissolved, entrapped, encapsulated, and / or to which the active agent is adsorbed or attached. Modification of properties of these carriers such as particle size, particle rigidity and surface charge and surface hydrophobicities can lead to the development of suitable carrier systems.

The major obstacle in the use of colloidal carrier systems is rapid clearance of such particles from the blood Stream by the macrophages of the reticuloendothelial system (RES), mainly in the liver and spleen [Barratt, 2003]. The profound involvement of the macrophages in the pathogenesis of diseases such as human immunodeficiency virus (HIV) infection presents a unique opportunity for evaluating cell-specific drug targeting via the use of colloidal carriers. Modification of the properties of the carriers, such as particle size, surface charge and surface hydrophobicity can reduce the RES clearance. The specific targeting of drugs to the desired site can also be achieved using monoclonal antibodies attached to the surface of the drug carriers. Modification of the carrier components can result in the drug release upon exposure of the carrier to the particular environment such as pH changes, temperature changes and the influence of a magnetic chamber [Heiati 1996; Becker *et al.*, 2007].

The following sections deal with fundamental aspects, of colloidal drug carriers as Drug delivery systems as a major group of drug carrier.

1.2. Colloidal drug carriers as Drug delivery systems

High-throughput screening technologies in drug discovery present an efficient way to find new powerful substances. But in recent years it has become evident that the development of new drugs alone is not sufficient to ensure progress in drug therapy. Poor water solubility of drug molecules, insufficient bioavailability, fluctuating plasma levels or high food dependency is the main and common problems. Major efforts have been spent for the development of customized drug carriers to overcome the disappointing *in vivo* fates of the drug. For carriers non-toxicity (acute and chronic), sufficient drug loading capacity, possibility of drug targeting, controlled release characteristics, chemical and physical storage stability (for both drug and carrier) and feasibility of scaling up production with reasonable overall costs are requested [Mehnert and Mäder, 2001; Barratt, 2000]. Colloidal carriers have attracted the main interest because they are promising systems to fulfill the requirements mentioned above. But in the first place, nanosized carriers are treated as hopeful means to increase the solubility and therefore the bioavailability of poorly water-soluble active ingredients belonging to the classes II and IV in the biopharmaceutical classification system (BCS) [Löbenberg and Amidon 2000; Dressman and Reppas, 2000].

The common characteristic of all colloidal carriers is the submicron particle size. Nanometric carriers might differ in materials, composition, drug loading and application spectrum. Corresponding to the broad diversity of colloidal carriers, the possible administration routes vary. Dermal, peroral, parenteral, ocular and pulmonary applications are known for nanocarriers. As upper limit for intravenous administration to avoid embolism in blood vessels no particles above five micrometers and only few particles between one and five micrometers are accepted. Solid particular systems are limited to either the subcutaneous or intramuscular routes of administration; intravenous administration may result in vaso-occlusion [Barratt, 2003].

Although biodistribution studies to organs were performed with radio labeled carriers [Nishikawa *et al.,* 1998], little is known about the detailed fate of the carrier *in vivo*, especially concerning the uptake mechanisms, exchange processes with the physiological environment and degradation rates. Without particle modifying the phagocyte system recognizes circulating colloidal particles in the blood as foreign material and captures them rapidly after intravenous administration [Nishikawa *et al.,* 1998]. While drug delivery keeps difficult to realize as long as carriers are rapidly phagocytized and drug molecules are accumulated in liver and spleen, nowadays first success was achieved for passive drug targeting to solid tumors. Tumor blood vessels present several abnormalities in comparison with normal physiological vessels. In an unspecific way, PEGylated (polyoxyethylene glycolated) particles penetrate the leaky endothelium and deliver drug inside the tumor [Lukyanov *et al*., 2002; Merdan *et al*., 2003]. These small hydrophilic carriers were found to have longer circulating half-lives in the blood than large and hydrophobic particles [Brigger *et al*., 2002; Lukyanov *et al.,* 2003], due to their "water-like" aspect they are more or less invisible for the phagocytes. However, PEG-coated particles are passive systems because their modification in tissue distribution is basically a result of the difference in micro vascular permeability between healthy and altered tissue and of their long circulating properties. Active targeting has not yet been successfully established. Ideas rise up proposing new steps on the way to active targeting. E.g., polysaccharide-decoration of the surface of polymer nanoparticles should serve as anchor to cell surfaces of humans and/or bacteria and virus [Vauthier *et al.,* 2003]. There, oligo- and polysaccharides are universally exposed and often they play a role in biologic activity which is hoped to get influenced by novel carriers.

Focusing on the biofate of lipid-containing drug carriers after peroral application, short chain and medium-chain liquid lipids are known to be easily hydrolyzed and to be