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Nanoparticles of poly(styrene-*co*-maleic acid) as colloidal carriers for the anticancer drug epirubicin

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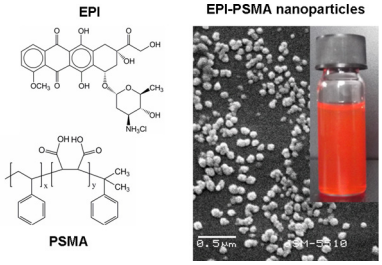
HIGHLIGHTS

- Epirubicin-loaded PSMA nanoparticles were prepared by nanoprecipitation.
- Nanoparticle size could be precisely controlled from 100 to 320 nm.
- Effects of polymer concentration and water/acetone volume ratio were studied.
- Stability of nanoparticles at various pH and drug release were evaluated.

GRAPHICAL ABSTRACT



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ABSTRACT

This article considers the preparation and physicochemical characterization of poly(styrene-*co*-maleic acid) nanoparticles as colloidal carriers for the anticancer drug epirubicin. The nanoparticles were prepared by nanoprecipitation approach in a mixed (water/acetone) solvent system and the drug was loaded by sorption. It was found that the size of nanoparticles could be precisely controlled (from 100 to 320 nm in diameter) by varying the polymer concentration and the acetone/water volume ratio. All nanoparticles possessed negative zeta-potentials at physiological-like conditions. The presence of human serum albumin decreased significantly the absolute value of the zeta-potential, although still remaining negative, of both drug-free and drug-loaded nanoparticles indicating albumin adsorption onto the particle surface. The nanoparticles were stable in phosphate buffered saline within the pH interval from 4.5 to 9.3 (forming precipitates at lower pH and being disintegrated at higher pH). In vitro studies of the epirubicin release indicated a relatively strong association of the drug with the polymer and sustained release of only about 10% of the loaded drug for a period of 7 h. We hope that the results reported in this article could be helpful for improvement of the preparation procedures for poly(styrene-*co*-maleic acid)-based nano-formulations of anticancer drugs with desired physicochemical characteristics, which appear to be important prerequisites for their successful biomedical applications.

1. Introduction

Poly(styrene-*co*-maleic acid) (PSMA) is a synthetic copolymer known to possess amphiphilic properties and complex behavior in aqueous solutions due to its ability to undergo various conformational transitions and to assemble and form associates [1–5]. PSMA is the product of hydrolysis of poly(styrene-*co*-maleic anhydride) (PSMAn) – Fig. 1a. The most distinct chemical features of PSMA (Fig. 1b) are the adjacent two carboxyl groups in the hydrophilic maleic acid monomer combined with the hydrophobic styrene. That chemical structure provides the flexibility for the formation of a comb-like conformation with aggregated

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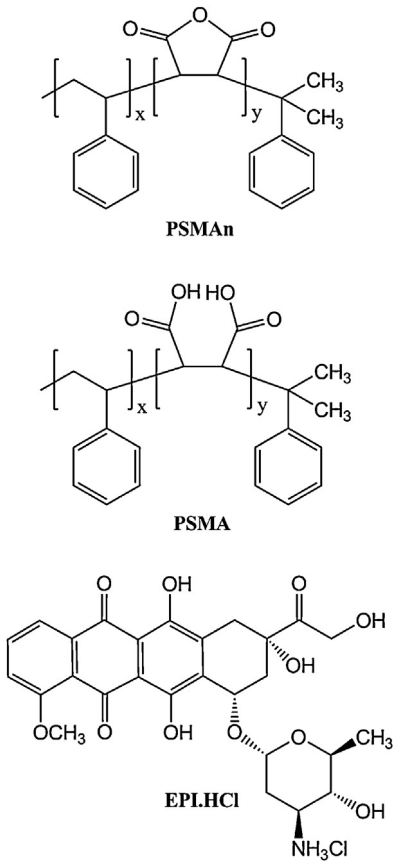

Fig. 1. Chemical structures of PSM-Anhydride, PSM-Acid (PSMA) and epirubicin; $x \sim 12–13$, $y \sim 4–5$.

hydrophobic segments along the chain, which could explain the dispersive properties of these copolymers. It is known that the low molecular weight copolymers could be used as colloidal-dispersing agents to stabilize water-insoluble organic molecules [6]. The colloidal-dispersing properties of PSMA could be utilized also in pharmaceutical technology to prepare colloidal drug formulations. Previous investigations have demonstrated that oligomeric water-soluble PSMA (Mw ~1600–2300) and its derivatives could be used to achieve pronounced improvements in pharmacological properties of various anticancer agents, such as neocarzinostatin [7], doxorubicin [8], pirarubicin [9–11], tanespimycin [12], and zinc protoporphyrin IX [13,14].

The increased tumor accumulation of the PSMA-formulated drugs has been explained by the formation of micelles that accumulate in solid tumors via the so-called enhanced permeability and retention (EPR) effect [15–18]. The EPR effect favors the selective accumulation of macromolecular (Mw > 40 kDa) drugs and colloidal delivery systems in the tumor tissue by exploiting the differences between tumor and normal vasculature [19]: the tumor blood vessels are more permeable (with cell-cell gaps as large as 600–800 nm) than normal vasculature and most solid tumors lack effective lymphatic drainage. PSMA-based micellar formulations of anthracyclines (such as doxorubicin and pirarubicin) have been reported to possess high tumor targeting efficiency with little toxicity [8–11]. Anthracyclines are highly active cytostatics with a broad antitumor activity against different cancers and are considered among the most perspective anticancer agents [20–23]. Acute toxicity of anthracyclines affects all rapidly dividing cells (e.g., bone marrow, intestinal epithelial cells), while chronic toxicity affects mainly myocardium and liver. However, the PSMA-formulated doxorubicin has shown a 13-fold increase in tumor accumulation and a 25-fold increase in the plasma level of the drug in a 24-h period after i.v. administration compared with that obtained with free doxorubicin [8]. Further in vivo experiments with PSMA-pirarubicin micelles in mice bearing S-180 tumor have revealed complete tumor eradication in 100% of tested animals with significantly diminished adverse effects [9]. These results appeared to be quite remarkable and inspired our interest in investigation of the physicochemical properties of similar formulations and encouraged us to develop alternative methods for their preparation.

PSMA-anthracycline micelles have been prepared heretofore by association of the drug with the polymer catalyzed by water-soluble carbodiimide (although covalent conjugation was not confirmed), followed by solubilization in alkaline medium and reconstitution of the micelles at pH 7.0 [8,9]. A similar strategy has been utilized for entrapment of other bioactive agents in PSMA micelles [10–14]. The size of the obtained colloidal structures was different in each case: 180 nm for PSMA-pirarubicin [11], 74 nm for PSMA-tanespimycin [12], 176 nm for PSMA-zinc protoporphyrin [13]. However, all these studies have not provided data on how this size could be controlled. On the other hand, it is known that the size of nanoparticle drug carriers appears to be an important parameter that largely determines their in vivo biodistribution and therefore their therapeutic efficacy [24–26]. In view of the fact that no method for preparation of PSMA nanoparticles with controlled size distribution has been heretofore provided, it is our belief that an investigation toward achieving this goal is worthy of presentation.

In this article we report a novel and simple approach for the preparation of drug-free and epirubicin-loaded PSMA colloidal drug carriers by nanoprecipitation, which is intended to allow a fine control of the nanoparticle size distribution. Epirubicin (EPI, Figure 1c), which is considered as a less toxic alternative to the widely popular doxorubicin [27–29], was chosen for our experiments as a model anthracycline drug. As most cytostatics, epirubicin has a low molecular mass (543 Da) and is non-selectively delivered to both tumor and normal tissue, which is the main reason for its undesirable side effects observed in conventional chemotherapeutic treatments. By associating epirubicin with PSMA nanoparticles we intended to prepare a colloidal nano-formulation of the drug. For that purpose, we first prepared drug-free PSMA nanoparticles of desired size from pre-synthesized polymer (PSMA of Mn ~ 2000) by utilizing a nanoprecipitation approach in a mixed (acetone/water) solvent system and then we loaded the drug by simple mixing and sorption. In order to prepare nanoparticles by this method, the polymer itself should be relatively insoluble in water but also to contain hydrophilic segments to ensure the stability of the respective nanoparticles in aqueous medium. For that reason, in contrast to previous reports that have used alternating water-soluble PSMA [8,9], we chose here a non-alternating PSMA copolymer enriched in the hydrophobic monomer (styrene, 75 wt.%) and we took advantage of its diminished water solubility to prepare nanoparticles in phosphate buffered saline by using the nanoprecipitation method. This strategy allowed us to achieve a fine control of the nanoparticle size by two experimental variables – the concentration of polymer and the acetone/water volume ratio. Our study was focused on the physicochemical aspects of the preparation and characterization of nanoparticles, as well as determination of the factors that govern their size distribution, ζ-potential, pH-dependent stability, drug loading and release. We hope that the obtained data could be helpful for further development of novel colloidal formulations of anthracyclines based on PSMA nanoparticles with controlled physicochemical properties, which is a major prerequisite for their successful biomedical application.