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Synthesis and physicochemical properties of polyacrylamide nanoparticles as photosensitizer carriers

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ABSTRACT

At present, polyacrylamide nanoparticles are attractive to drug delivery. However, some physicochemical characteristics of these nanoparticles still need to be further improved in practice. Polyacrylamide nanoparticles with an average size of 80 nm and a zeta potential of −30 mV were synthesized and used as photosensitizer carriers. The new monobrominated derivatives and parent compounds were the photosensitizers for the photodynamic therapy loaded in the nanocarrier.

The physicochemical characterization of the prepared nanoparticles, drug loading, the ability to generate singlet oxygen and chemical stability were investigated.

The novel tested nanoparticles exhibited a loading percentage of between 80 and 99%, higher generation of singlet oxygen and good stability in comparison with the corresponding starting reagent.

According to these results, the novel polyacrylamide nanoparticles are excellent candidates for drug vehiculization.

1. Introduction

Photodynamic Therapy (PDT) is a minimally invasive therapeutic modality used for the treatment of a variety of cancerous and non-cancerous diseases. This therapeutic alternative utilizes light-sensitive molecules called photosensitizers (PSs), which are drugs activated by light of a specific wavelength. Photodynamic sensitizers produce reactive oxygen species that in biological systems trigger a cascade of biochemical responses that result in cell death (Pereira Rosa et al., 2015; Rodrigues et al., 2013; Yuri Nagata et al., 2012; Weijer et al., 2015).

Nanoparticles (NPs) are innovative biological applications, which have been extensively used due to their advantages of material- and size- dependent physics and chemical interactions with the cellular systems. Nanosized materials have emerged as effective systems for therapeutic applications (Ferreira dos Santos et al., 2015; Maya et al., 2013; Saha et al., 2015).

Depending on the type of nanocarrier and the mode of attachment or loading of PS onto it, the use of NPs in conjunction with PDT may impede the premature release of PS and potential inactivation of the drug by plasma components, thus preventing its nonspecific accumulation and reducing overall photosensitivity. The NPs protect the loaded PSs from degradation, enhance their transport, prevent their aggregation, and represent an emerging technology in the field of PDT that can overcome most of the limitations of classic PS. The properties of PS mainly depend on chemical and physical parameters, such as lipophilicity, type and number of charged groups, charge-to-mass ratio, and type and number of ring and core substituents. Most of the classical PSs have poor solubility under physiological conditions, undesirable pharmacokinetics, and low cellular selectivity.

Although the hydrophobic characteristic of PSs can allow them to penetrate the cell membrane and locate in the photosensitive subcellular compartment, highly hydrophobic PSs could form aggregates in aqueous solution, particularly under physiological milieu, which, in turn, could affect their photophysical and photokilling properties due to their inadequate pharmacokinetics (Paszko et al., 2011; Swarnalatha Lucky et al., 2015).

Positively charged PSs are efficiently taken up by cells and accumulate intracellularly to concentrations higher than in the environment. They are electrostatically attracted by the predominantly negatively charged components of the plasma and

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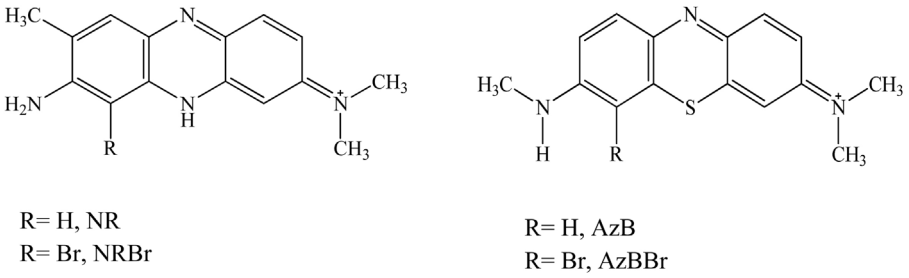

Fig. 1. Chemical structure of PSs.

mitochondrial membranes. The critical force driving such positively charged molecules inside cells and mitochondria is the transmembrane potential.

Many products can behave like PSs and new ones are regularly discovered; however, very few have made it to clinical trial. Each of the currently commercially available PS has specific characteristics, but none of them is an ideal agent (Master et al., 2013; Yano et al., 2011).

There are several vehiculization strategies, such as liposomes, polymeric micelles, polymer nanoparticles, gold nanoparticles, and colloidal silica-based nanoparticles that are well known for improving the therapeutic capability of the PS in aqueous systems. The most commonly used synthetic polymers employed to prepare nanoparticles for drug vehiculization are biodegradable (Li and Huh, 2014). NPs composed of biodegradable materials have the ability to move through capillaries to target cells and evade immune responses which can prematurely eliminate the PS from the body.

Recent accelerated biodegradation studies demonstrated the biodegradability of the polyacrylamide nanomaterials (Kolya and Tripathy, 2014). Polyacrylamide (PAA) nanoparticles are promising vehicles for photodynamic therapy. The PAA nanoparticle matrix, generally a porous hydrogel protects the embedded active form of PS from enzymatic or environmental degradation and permits singlet oxygen and other kinds of reactive oxygen species (ROS) diffusion through the pores. In view of the above-mentioned facts, we developed a nanocarrier strategy using biocompatible polymers to incorporate NR, NRBr, AzB and AzBBr.

The four PSs shown in Fig. 1 have very similar structures. Neutral Red (NR) is a phenazine-based dye, which is very useful as a biological probe, and has been widely utilized for various purposes in many biological systems, such as the staining of cellular particles and the intracellular pH indicator. In addition, NR has been used as a PS in PDT and good results have been obtained (Fischer et al., 2005; Singh et al., 1999).

The new phenazine cationic dye, monobrominated Neutral Red (NRBr), was synthesized and characterized in our laboratory. This compound proved to have the potential to be used as a PS because it presents less aggregation in different solvents in comparison with the parent compound. The improved photoproperties exhibited by NRBr were accompanied by significant increases in the photoantimicrobial action compared to NR (Urrutia et al., 2015). This newly synthesized phenazine dye has shown a slight discoloration in the studied experimental conditions. In this context, photosensitizer-loaded nanocarriers can have the capability to reduce this effect modifying the physicochemical properties (Urrutia et al., 2015).

The phenothiazine, Azure B (AzB), is a positively charged compound used as a phototherapeutic agent due to its appropriate biological, chemical, photochemical and photophysical properties; therefore, AzB and the new derivatives, monobrominated Azure B (AzBBr), which were synthesized and characterized in our work group, are candidate agents to produce photocytotoxicity in biological media (Fig. 1) (Montes de Oca et al., 2013). The introduction of bromine atom into the chromophoric system caused an increase in the lipophilicity. Moreover, the *in vitro* photodynamic activity against LM-2 murine mammary carcinoma cells demonstrated that the photocitoxicity of AzBBr remained unchanged or decreased. The lower efficiency to inactivate cultured tumor cells may be attributed to the aggregation effects of phenothiazinium derivatives (Montes de Oca et al., 2013).

The photodynamic inactivation in cell suspensions of *Candida albicans* observed for AzB and AzBBr derivatives can be associated with a different distribution of agents in the cell, mainly due to the number and location of the positive charge on the PS. Although studies of photodynamic action mechanism indicated that photoinactivation of *C. albicans* cells induced by phenothiazinium derivatives produce singlet molecular oxygen, a contribution of other reactive oxygen species cannot be discarded in the photoinactivation (Alvarez et al., 2014). Besides, the entrapment of these PSs could offer therapeutic advantages in comparison to free PS active agents since it could prevent different adverse effects.

2. Experimental section

2.1. Materials

NR (3-amino-7-dimethylamino-2-methyl phenazine hydrochloride) and AZB (3-(Dimethylamino)-7-(methylamino)phenothiazine-5-ium chloride) were purchased from Sigma Chemical Co, and its purity was confirmed by RP-HPLC (> 98%). NRBr and AzBBr were synthesized in our laboratory according to the procedure previously described (Montes de Oca et al., 2013; Urrutia and Ortiz, 2013).

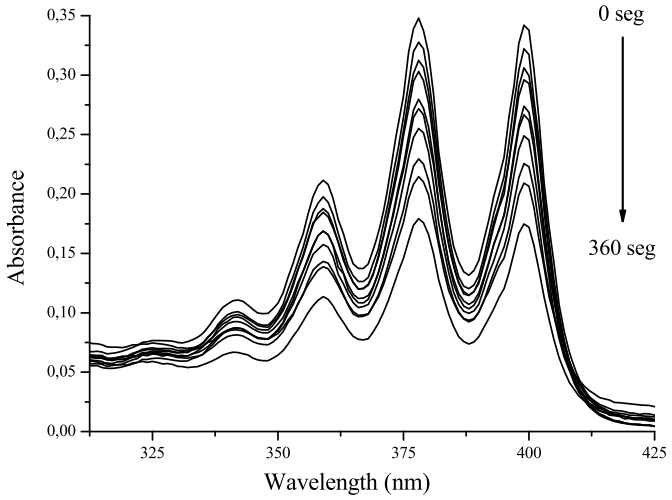

Fig. 2. UV–vis absorbance spectra of ABDA in solution with AzB irradiated at different times.