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Interactions between copper(II) dibrominated salen complex and copolymeric micelles of P-123 and F-127

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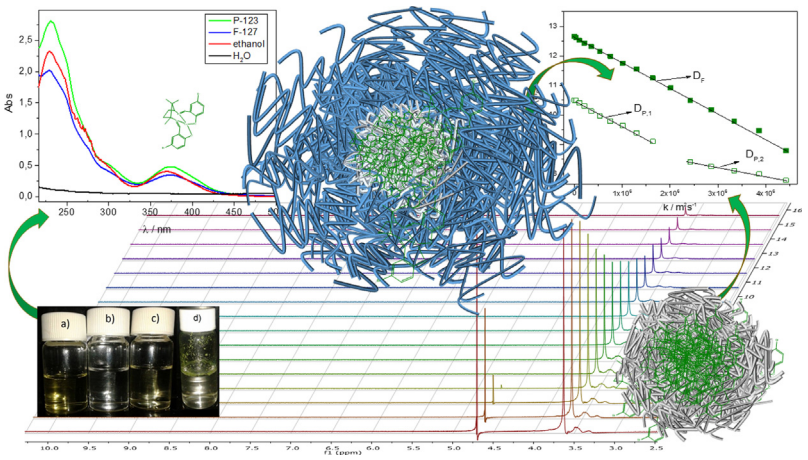
HIGHLIGHTS

- Formulations containing copper(II) salen dibrominated complex and Pluronics F-127 and P-123 micelles have been prepared.
- The encapsulation efficiency of the Cu-salen complex is higher in the Pluronic characterized by a more hydrophobic micelle core.
- Cu-salenBr$_2$/P-123 formulation shows the better spectroscopic stability and storage ability after lyophilization.
- Cu-salenBr$_2$/P-123 formulations shows better IC$_{50}$ than the Cu-SalenBr$_2$ against BALB/c and COS-7 cells.

GRAPHICAL ABSTRACT



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ABSTRACT

Metal-salen complexes have been extensively studied for several applications in nanomedicine as chemotherapic agents substituting, for example, cisplatin based compounds. A recently synthesized copper(II) dibrominated salen complex (Cu-salenBr$_2$) has shown excellent preliminary results against different cell lines; however, its poor water solubility is a major drawback for the use of Cu-salenBr$_2$ in biological fluids. In order to overcome this limitation, Cu-salenBr$_2$ was incorporated in biocompatible micellar systems of Pluronics surfactants F-127 and P-123 by the solid dispersion method, aiming at both the increase in the bioavailability of the complex and the development of a (Cu-salenBr$_2$/copolymer) formulation for intravenous application.

The Cu-salenBr$_2$ formulations were characterized by UV–vis and infrared spectroscopies; this has been complemented by studies on the effect of concentration on the self-diffusion coefficients of micelles. The encapsulation efficiency and the thermal and kinetics stability of Pluronic-Cu-salenBr$_2$ formulations were studied. From these studies, it has been found that P-123-containing formulations are more stable and, consequently, they were chosen for cytotoxicity studies.

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The cytotoxicity of Cu-salenBr$_2$ against fibroblast-like kidney cells (COS-7) and BALB/c mice spleen cells was evaluated, either alone or incorporated in P-123. No significant cytotoxicity was observed for Cu-salenBr$_2$, *per se*, dissolved in the culture medium, however, Cu-salenBr$_2$ solubilized in DMSO/DMEM and Cu-salenBr$_2$/P-123 formulation showed a concentration dependent toxic effect on both COS-7 cell line and mouse spleen cells. The IC_{50} values obtained for the Cu-salenBr$_2$/P-123 formulation were 0.41 and 1.6 μmolL^{-1} for spleen and COS-7 cells, respectively.

1. Introduction

Metal-salen complexes are coordination compounds where the ligand is *N,N'*-bis(salicylidene)ethylenediamine or its derivatives. Their unique properties allow their use in a large number of applications in areas as diverse as food industry, luminescent materials, chemical industry (catalysts) and in pharmaceuticals. In the latter, salen complexes have been extensively studied in cancer treatment, as anti-bacterial and anti-fungal drugs [1,2]. Furthermore, these complexes also show anti-tumoral activity due to their interaction with nucleic acids and proteins, binding and intercalating with DNA, and induce DNA damage [3]. This can be due to the fact that Cu-salen complexes have a slightly distorted square planar structure providing ready binding to the appropriate biological targets [1,4,5].

The biological properties of salen complexes may be improved by changing the structure of the ligand moiety and also through complexation with different types of metals, such as palladium, iron, copper, nickel and zinc [4,6,7]. Meshkini and Yazdanparast, for example, obtained a vanadium salen complex (V-salen) and studied its effects combined with the chemotherapeutic agent taxol, on the proliferative behavior of leukemic cells. This complex has the ability to inhibit the proliferation of cancer cells, by cell death or apoptosis, without significant changes in cell morphology. Additionally, it was verified that such formulation considerably increases cellular death in comparison with the isolated effect of taxol [1].

Dvorák et al. have studied the effect of Fe-salen complexes with different axial ligands (triazole, benzotriazole, 5-phenyl-tretrazole) on a large series of different cancer cell lines. These Fe-salen complexes showed half maximal inhibitory concentration (IC_{50}) values between 0.39 and 11.5 μmol L^{-1}, and showed no toxicity in normal cells [4].

Despite the efficacy in cellular inactivation, salen complexes are, in general, insoluble in aqueous media, which is a drawback for their intravenous biomedical applications [2,8]. Thus, the development of drug nano-carrier systems has gained increasing attention. Besides being able to carry anti-cancer agents as drugs or macromolecules (e.g., genes or proteins) these systems allow a greater accumulation of drugs in solid tumor cells when compared to drugs delivered through other matrices. Among nano-carriers, the most common approach involves the encapsulation of hydrophobic drugs into systems, such as dendrimers, liposomes, organometallic compounds (carbon nanotubes) or micelles (e.g., copolymer micelles) [9]. Concerning the latter, P-123 and F-127 Pluronic (Fig. 1a) have been successfully used for different kinds of drugs [10–12].

The micellization process of these amphiphilic species in aqueous media involves the assembly of poly(propylene oxide) – PPO groups to form the hydrophobic core of micelles, responsible for incorporating the hydrophobic drugs, and poly(ethylene oxide) – PEO groups responsible for the stabilization of micelles in biological fluids, thus preventing the adsorption and aggregation by, e.g., proteins [10,11]. Furthermore, they are biocompatible, present a higher circulation time in the blood flow, stability in biological media (since they are hardly recognized by macrophages and proteins) and have a low critical micellar concentration (*cmc*), which makes them resistant to the dilution effects occurring in the blood stream [11–14].

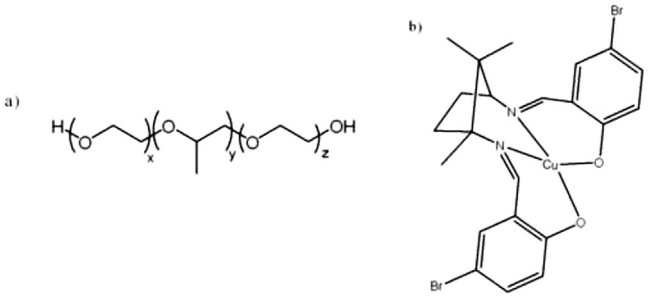

Fig. 1. Molecular structure of (a) F-127 ($x = z = 106$; $y = 70$) and P-123 ($x = z = 20$; $y = 70$) triblock Pluronic^{TM} copolymers and (b) Cu-salenBr$_2$.

It is also worth referring that F-127 and P-123 copolymers can cross the cellular membranes, and thus have an effect on cellular functions as, for instance, mitochondrial respiration, apoptotic signal transduction, activity of drug efflux transporters and genetic expression [10,15]. It has also been reported that these copolymers influence the effects of multidrug resistance (MDR), mainly by inhibiting drug efflux transporters such as breast cancer resistant protein (BCRP), multidrug resistant proteins (MRPs) and P-glycoproteins (P-gp) [12].

In this paper, solubilization studies involving a recently synthesized copper-salen dibrominated complex (Cu-salen-Br$_2$—Fig. 1b) and P-123 and F-127 copolymeric micelles are reported. The Cu-salen compound chosen has shown promising cellular inactivation results presenting IC_{50} of 1.3 and 2.3×10^{-6} mol L^{-1} against breast and colorectal resistant cells, respectively (manuscript in preparation).

2. Experimental section

2.1. Materials

Pluronic^{TM} block copolymers F-127 (M_w = 12600 gmol^{-1}) and P-123 (M_w = 7800 g mol^{-1}) were supplied by Sigma-Aldrich. Organic solvents: methanol, ethanol and acetone were acquired from Fisher Scientific UK. All reagents and solvents were used without further purification. All solutions were prepared using Millipore-Q water. For the NMR studies, D_2O (99.8%) was supplied from Euriso-top.

The copper (II) dibrominated salen complex (Cu-salenBr$_2$) was synthesized according to a procedure described elsewhere [5]. Briefly, (1*R*,3*S*)-1,3-diamino-1,2,2-trimethylcyclopentane (5 mmol, 0.71 g) was dissolved in 20 mL of dry ethanol and treated with 5-bromosalicylaldehyde (10 mmol, 2.01 g) and silica (2.50 g) in an ultrasound bath until the reaction was complete, as monitored by tlc, approximately 30 min. Dichloromethane (50 mL) was added and the silica was filtered off. The solvent was evaporated and the product was isolated by crystallization in ethanol. For the copper complex, $Cu(OAc)_2 \cdot H_2O$ (1.4 mmol) and the ligand (1.2 mmol) were dissolved in methanol (20 mL) and refluxed for a period between 2 h and 4 h. The solvent was evaporated and the residue dissolved in

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