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Gelled oil particles: A new approach to encapsulate a hydrophobic metallophthalocyanine

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ABSTRACT

Chloroaluminum phthalocyanine (ClAlPc) is a promising sensitizer molecule for photodynamic therapy, but its hydrophobicity makes it difficult to formulate. In this study, we have efficiently encapsulated ClAlPc into gelled soybean oil particles dispersed in water. 12-Hydroxystearic acid (HSA) and polyethyleneimine (PEI) were the gelling and stabilizing agents, respectively. The preparation process involved hot emulsification above the gelation temperature (T_{gel}), followed by cooling to room temperature, which gave a colloidal dispersion of gelled particles of oil in aqueous medium. The gelled particles containing ClAlPc had a medium diameter of 280 nm, homogeneous size distribution (polydispersity index ≈0.3) and large positive zeta potential (about +50 mV) and showed a spherical morphology. The gelled oil particle formulations exhibited good physical stability over a 6-month period. ClAlPc interfered with the HSA self-assembly only slightly, and decreased the gelation temperature to a small extent; however it did not affect gelation process of the oil droplets. The amounts of PEI and HSA employed during the preparation allowed us to control particle size and the dispersion stability, a phenomenon that results from complex electrostatic interactions between the positively charged PEI and the negatively charged HSA fibers present on the gelled particles surface. In summary, by using the right ClAlPc, HSA, and PEI proportions, we prepared very stable dispersions of gelled soybean oil particles with excellent ClAlPc encapsulation efficiency. The obtained colloidal formulation of gelled oil particles loaded with ClAlPc shall be very useful for photodynamic therapy protocols.

1. Introduction

Strategies from particulate colloidal dispersion have led to significant advances in drug delivery system research applied to the encapsulation of hydrophobic molecules [1,2]. Such approaches have become essential, because about 40% of the new molecules identified by pharmaceutical companies are practically insoluble in water. The lipophilic character of a molecule limits its bioavailability and absorption, thus preventing the pharmacological and pre-clinical evaluations of hydrophobic drugs [1–4]. In this context, several drug delivery systems based on lipid core have been developed to enhance the low solubility and bioavailability of lipophilic molecules [5,6].

Gelled particles have emerged as a successful system for drug delivery. These gelled particles comprise hydrogels and organogels, and they combine the unique properties of a gel with those of colloidal particles (e.g., very small size) [7–9]. Organogels (Ogg) are semisolid materials in which an organic solvent (e.g., vegetable oil) is entrapped in a three-dimensional fibrous network formed by self-aggregation of the organic gelator. Such gels are normally obtained by directly dissolving the gelator in oil at high temperature, followed by cooling of the solution to a temperature below the gelation transition temperature (T_{gel}). At T_{gel}, the oil flow is no longer observed over long periods [10,11]. Ogg are interesting drug delivery systems for different administration routes [12,13], and their potential as hydrophobic reservoirs has been demonstrated [14–17]. Recently, a new approach based on an original family of lipophilic drug carriers consisting of organogel particles has been successfully developed as a nanocarrier system applied to dye molecules [7,18]. The stabilizing and gelling agents are the main components in the process of Ogg particles production. These

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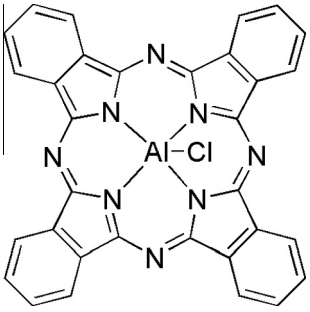

Fig. 1. Chemical structure of chloroaluminum phthalocyanine (ClAlPc).

agents directly influence the particle's physicochemical parameters, such as average diameter and zeta potential, as well as the colloidal dispersion physical stability. Both Ogg particle production and stability depend on the interfacial interactions between the particle and stabilizing agent, reinforced by a possible complexation between the organogelator and stabilizing agent. This complexation occurs mainly through electrostatic interactions with the organic gelator fibers present at the surface of the Ogg particles [7,18].

Phthalocyanines are photosensitizer drugs (PS) belonging to the *second-generation* of sensitizer molecules used in photodynamic therapy (PDT). They replace the less effective *first-generation* PS such as hematoporphyrins [6]. Metallophthalocyanines result from incorporation of diamagnetic metals like zinc, aluminum, or silicon into the phthalocyanine macrocycle. Phthalocyanines containing Al or Zn are considered to be the most photoactive compounds, because their large triplet state quantum yields and long triplet lifetimes give the highest singlet oxygen ($^1O_2$) production [6,19]. Cytotoxic species burst, such as $^1O_2$, occurs when visible light of appropriate wavelength (normally the maximum absorption wavelength of the PS) interacts with a PS in the presence of oxygen, leading to cell death and tumor destruction [20,21]. Chloroaluminum phthalocyanine (ClAlPc) (Fig. 1) is a promising PS because of its strong absorption in the visible spectral region (670–680 nm), exceptional thermal and chemical stability, and excellent photophysical and photochemical properties [19,22–24]. Despite all the potential advantages of ClAlPc for PDT, this metallophthalocyanine is practically insoluble in water, which prevents its direct application in biological fluids. In addition, hydrophobic phthalocyanines dimerize or aggregate, in aqueous medium, which inactivates the PS and diminishes the production of singlet oxygen [24,25] or other reactive oxygen species.

In this study, we prepared organogel particles containing ClAlPc (ClAlPc Ogg particles) using soybean oil and a low-molecular-weight organic gelator, such as 12-hydroxystearic acid (HSA). We obtained a colloidal suspension of ClAlPc Ogg particles by dispersing the ClAlPc Ogg in an aqueous solution containing the cationic polymer polyethyleneimine (PEI) as stabilizing agent. We characterized the resulting ClAlPc Ogg particles in terms of the average diameter, size distribution, zeta potential, encapsulation efficiency, physical stability, and morphology. In addition, we assessed the thermal behavior of Ogg and ClAlPc Ogg by differential scanning calorimetry. Therefore, the purpose of this study was to develop an innovative class of colloidal particles able to encapsulate a hydrophobic photosensitizer drug for application in PDT.

2. Materials and methods

2.1. Materials

Aluminum phthalocyanine chloride (85% pure), branched polyethyleneimine (PEI, Mn = 600 g/mol), soybean oil, and methanol were purchased from Sigma (Sigma–Aldrich Co., St. Louis, MO, USA). 12-Hydroxystearic acid (HSA, 99%) was obtained from Alfa Aesar GmbH (Karlsruhe, Germany). All the other chemicals were of analytical grade. Ultrapure water was used to prepare the colloidal dispersions.

2.2. Preparation and optimization of ClAlPc Ogg particle formulations

ClAlPc Ogg particles were prepared according to the method described by Kirilov et al. [18], with some modifications. This method involved two steps: organogel preparation and the subsequent formation of the Ogg particle dispersion.

Firstly, the ClAlPc Ogg was prepared by dissolving the gelling agent (HSA) and ClAlPc in soybean oil at 75 °C for 40 min. After complete HSA and ClAlPc dissolution in the oil, the mixture was cooled naturally to room temperature leading to a compact gel formed. Then, 10 ml of an aqueous solution containing the stabilizing agent (PEI) was added to the previously obtained gel. This mixture was heated to 75 °C (above the gel melting temperature) for 40 min, which gave in an oil layer on top of the aqueous solution. Next, the hot mixture was dispersed by ultrasound sonication (Vibra Cell, Bioblock Scientific) for 10 min using a 13-mm probe operating at a power of 400 W and frequency of 20 Hz. The resulting colloidal suspension was cooled to room temperature, to give a dispersion of ClAlPc Ogg particles. All the formulations were stored at 4 ± 2 °C for a period no longer than 6 months.

Table 1
Main components of the ClAlPc Ogg particle formulations and experimental results from physicochemical characterization.

Formulations	PEI (%)[a]	HSA (%)[b]	Mean size (nm)[c]	PdI[c]	ζ Potential (mV)[c]	ClAlPc loading (%)
F1	0.075	6	349.8 (±8.5)	0.41 (±0.04)[e]	+65.5 (±1.9)[f]	10.7 (±0.4)
F2	0.15	6	310.4 (±14.1)	0.47 (±0.01)[e]	+56.7 (±1.2)[f]	22.3 (±0.2)
F3	0.30	6	282.7 (±3.0)[d]	0.34 (±0.03)	+49.3 (±1.8)	60.3 (±1.7)[g]
F4	0.625	6	283.8 (±5.9)[d]	0.37 (±0.02)	+45.7 (±1.8)	63.1 (±2.8)[g]
F5	0.30	3	Unstable formulation (creaming and phase separation)			
F6	0.30	4	323.6 (±9.8)	0.32 (±0.04)	+52.6 (±0.8)	41.6 (±1.3)
F7	0.30	8	249.4 (±7.8)[d]	0.33 (±0.02)	+53.9 (±1.4)	38.4 (±1.7)
F8	0.30	15	Unstable formulation (compact aggregate)			

[a] % w/v.
[b] % w/w, based on oil phase weight.
[c] Mean ± SEM (n = 3).
[d] $p < 0.05$ Compared to *F1*, *F2*, and *F6* formulations.
[e,f] $p < 0.05$ Compared to *F3*, *F4*, *F6*, and *F7* formulations.
[g] $p < 0.05$ Compared to *F1*, *F2*, *F6*, and *F7* formulations.