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Regular Article

Spontaneous vesicle formation by γ-aminobutyric acid derived steroidal surfactant: Curcumin loading, cytotoxicity and cellular uptake studies

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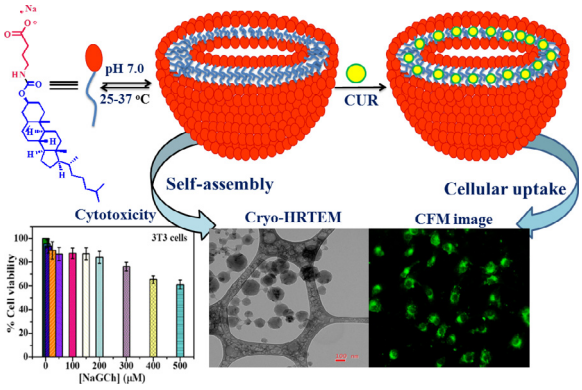
GRAPHICAL ABSTRACT



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ABSTRACT

Cholesterol (Chol) is a ubiquitous steroidal component of cell membrane and is known to modulate the packing of phospholipids within the bilayer. Thus, Chol has been frequently used in the formulation and study of artificial "model membranes" like vesicles and liposomes. In this work, we have developed a novel anionic surfactant by conjugating two biomolecules, cholesterol and γ-aminobutyric acid via a urethane linkage. We have studied its physicochemical behavior in aqueous buffer. The surfactant has been shown to spontaneously form small unilamellar vesicles above a very low critical concentration in aqueous neutral buffer at room temperature. The vesicle phase was characterized by use of fluorescence probe, transmission electron microscopy and dynamic light scattering (DLS) techniques. The vesicle bilayer was found to be much less polar as well as more viscous compared to the bulk water. The vesicle stability with respect to change of temperature, pH, and ageing time was investigated by fluorescence probe and DLS techniques. The loading efficiency of the vesicles for the hydrophobic drug, curcumin, was determined and its release under physiological condition was studied. The in vitro cellular uptake of curcumin-loaded vesicles to human breast cancer cell line (MDA-MB-231) also was investigated. The MTT assay showed that the surfactant was non-cytotoxic up to a relatively high concentration.

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1. Introduction

Cancer registers the second leading cause of death in the USA, accounting for nearly 22.5 % of total deaths [1]. Despite gradual downtrend of its mortality rate (22.97% in 2000 and 22.24% in 2014) [1], cancer still continues to hold its rank over the decades. Curcumin, CUR (see Chart 1 for structure), a polyphenol occurring in the herbal remedy and curry spice turmeric (*Curcuma longa*), has been identified as a potential candidate for treating cancer. Its uses are scripted in the ancient Hindu treatise, the *Ayurveda* [2]. The demand of turmeric as a food coloring and flavouring agent is global. The Food and Agriculture Organization of the United Nations records an annual import of over 2400 metric tons of turmeric into the USA for consumer use. In addition to its stimulant and coloring properties in foodstuffs, CUR exhibits a wide range of pharmacological activities, such as antimicrobial, antioxidant, anti-inflammatory, hepato-protective, anti-Alzheimer's, chemopreventive, and chemotherapeutic properties [3–9]. It is well-established that CUR blocks transcriptional factor activator protein-1 (AP-1), nuclear factor-kappaB (NF-κB) [10], and modulates mitogen-activated protein kinase (MAPK) and phosphoinositide-3-kinase–protein kinase B/Akt (PI3 K-PKB/Akt) signalling pathways that could contribute to anti-proliferation and induction of apoptosis in vitro including K562 myelogenous leukemia cells. Further, CUR is a potent inhibitor of three crucial ATP-binding cassette (ABC) drug transporters, such as MDR1, MRP1 and ABCG2, which play a pivotal role in multidrug resistance (MDR) [11,12]. Studies have also reported that CUR inhibited the activity of p210bcr/abl tyrosine kinase in K562 cells [13]. Due to its diverse properties and myriad applications, over 100 chemical and pharmaceutical companies are producing various curcumin products in different forms—from dairy and drinks to tablets and gels—for daily and medical use. Also, various clinical trials are in different phases or have been done to investigate the therapeutic efficacy of CUR [14].

However, the chemistry of CUR is somewhat disappointing. CUR delivery is handicapped by its low water solubility (0.4 μg/mL at pH 7.3) as well as its alkaline degradation [15–18]. A simple way to address the solubility and stability issues is to encapsulate it into nano-aggregates, thus protecting it from degradation and metabolism. During the past two decades, extensive research has been on to developing various CUR nanoformulations. Different nanocarriers, such as polymer nanoparticles [19], polymeric micelles [20], liposomes [21], nano-/micro-emulsions [12,22], nanogels [23] solid lipid nanoparticles [24], polymer conjugates [25], have been formulated for the delivery of CUR to tumor cells. Among various nanodelivery systems, vesicles provide an excellent bilayer microenvironment for CUR solubilization. Vesicles that form spontaneously in aqueous solution and have good long-term stability are even advantageous over liposomes (phospholipid vesicles) as the latter often suffer from instability, poor batch to batch reproducibility, and sterilization difficulties, which limit their application. Kumar et al. have demonstrated enhanced solubilization of CUR in mixed cationic surfactant vesicles [26]. CUR-loaded vesicles formed by nonionic surfactants, such as Tween 80 and F127 in combination with lauric acid and isopropanol showed antimicrobial activity against *Propionibacterium acnes* [27]. Dequalinium-derived cationic vesicles of size 170–200 nm are reported as inhalation formulation with improved stability characteristics and mitochondrial targeting ability for treating acute lung injury [28]. CUR/β-cyclodextrin vesicles are reported to enhance the aqueous solubility of CUR up to 7000 folds, but the vesicles exist in aqueous medium for a month only, showing not-so-good formulation stability [29].

Although there are many reports on liposomal CUR, including two commercially available nanoformulations [30], only a few articles have addressed the chemotherapeutic activity of CUR-loaded vesicles. As a step forward to this direction, in this work we have synthesized a cholesterol-based anionic surfactant, NaGCh (see Chart 1 for structure) and studied its physicochemical behavior in aqueous buffer. In designing NaGCh, two biomolecules, such as cholesterol (Chol) and gamma-amino butyric acid (GABA) were conjugated via a urethane linkage. It is well known that Chol, a typical steroid, is a ubiquitous component of cell membrane and modulates the packing of phospholipids within the bilayer [31]. On the other hand GABA is a four-carbon, non-protein amino acid conserved from bacteria to plants and vertebrates. Although it was discovered in plants long ago [32], but the interest in GABA suddenly moved to animals when it was found that GABA was a chief inhibitory neurotransmitter in the central nervous system. The chemical, physiological, and pharmacological role of GABA have been reviewed in the literature [33,34]. Also, the incorporation of biomolecules like Chol and GABA is expected to render NaGCh cell viable. In a previous paper, we have shown that a structurally similar compound NaChol-Ala, where the amino acid is L-alanine instead of GABA, also formed robust vesicles in aqueous solutions [35]. In the present work, with a wide range of techniques including fluorescence, dynamic light scattering and microscopy, we demonstrate that NaGCh spontaneously forms stable vesicles in neutral buffer at room temperature. These vesicles were investigated for encapsulation and in vitro delivery of CUR to human breast cancer cell line MDA-MB-231. The cytotoxicity and cellular uptake studies were also carried out with the CUR-loaded vesicles.

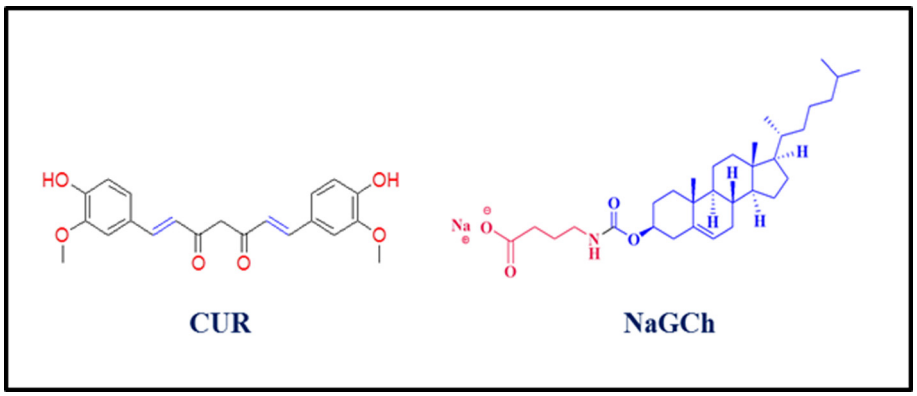

Chart 1. Chemical structure of CUR and NaGCh.