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Composites comprising cholesterol and carboxymethyl cellulose

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Abstract

Whereby cholesterol presents one of the major fatty substances in human body, carboxymethyl cellulose is a water-soluble derivative of cellulose, the most abundant dietary fiber. Whereas on one hand *in vivo* precipitation of cholesterol is the major cause of atherosclerosis, dietary fibers are on the other hand known for their ability to clean the fatty plaque deposited on intestinal pathways, and prevent its build-up in other critical areas within the organism. In this work, a method for the preparation of a composite material comprising cholesterol and carboxymethyl cellulose from 1-hexanol/water biphase mixtures is reported. Specificity of the interaction between the composite components in the given conditions of synthesis inhibits the tendency of solid cholesterol to adopt typical plate- or needle-shaped morphologies. Instead, control of the thixotropic behavior of the constituent polymer phase leads to the formation of bubbling, multi-layered cholesteric films. In view of the major illnesses that involve biological precipitation of cholesterol crystals, these findings may be considered as pointing towards the interactional specificity of potential chemotherapeutic and/or nutritional significance. Scanning electron microscopy, thermal and diffractometric analyses were performed as parts of the characterization of the prepared material.

Keywords: Cholesterol; Carboxymethyl cellulose; Crystallization; Film; SEM; Thixotropy

1. Introduction

Cholesterol is the most abundant steroid in mammalian species. It is also an essential biomolecule for the functioning of the human body, where it is involved in maintaining the flexibility and proper transport balance of cellular membranes, and acts as a precursor for the synthesis of bile acids and steroid hormones. Its abundance in the body is governed by the levels of its dietary intake and internal production. On the other hand, its efficient transport and utilization throughout the body depends on the solubilizing action of lipid bilayers, micelles and vesicles in bile and lipoprotein complexes in blood. When an unbalanced state between these two effects occurs, the conditions for its precipitation *in vivo* become favored. Thus it forms gallstones and atherosclerotic deposits, and eventually endangers the health of individual organisms. Understanding the physicochemical pathways involved in crystallization and deposition of cholesterol, as has been the aim of this work, therefore presents one of the important contemporary challenges with far reaching potential benefits.

It has been long known, for instance, that solvents affect morphology and structure of cholesterol crystals [1,2]. However, in all previous cases of re-crystallization in solution, either plate- or needle-shaped cholesterol crystals (respectively corresponding to monohydrate and anhydrate modifications [3], although this is a rule with exceptions [4]) were obtained. Spiral, helical and tubular morphologies of cholesterol crystals have been also observed, but only as transient microstructures that physiologically evolve into thermodynamically stable cholesterol platelets [5,6]. Beside plates, needles, arcs and spirals as forms of cholesterol crystals [7,8], different cholesterol derivatives have recently been observed to self-assemble into tubular structures [9]. Nevertheless, the typical observations of platelet-shaped cholesterol crystals are consistent with the well-known crystallographic fact according to which the crystals of 3-hydroxy steroids and their hydrates show a tendency towards adopting double layer crystal structure with end-for-end arrangement of approximately parallel molecules [10]. Whereas cholesterol monolayers have never been observed yet [11], the double-layer ordering mechanism explains the formation of typical platelet morphologies during cholesterol precipitation. As a matter of fact, layered structural arrangement of cholesterol precipitates can be considered as a natural mechanism for maximization

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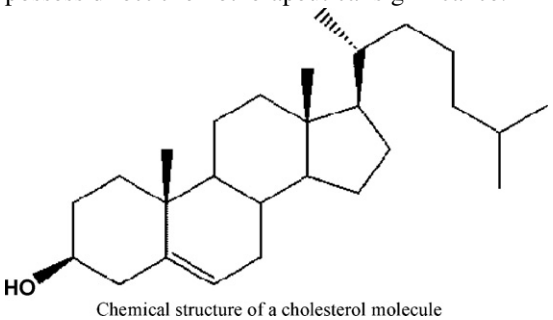
of cholesterol coagulates build-up in arteries, gallbladders and intestinal lumens without constraining the natural flow of life-sustaining fluids. On the other hand, relatively high free surface energy of plate-shaped crystallites (comparing to spherical particle shapes that correspond to minimal surface energy of multi-atomic or multi-molecular aggregates [12]) favors their adhesion on membranes and walls of the respective organs and tissues, decreasing their susceptibility towards efficient dissolution and removal by means of proper cleansing agents.

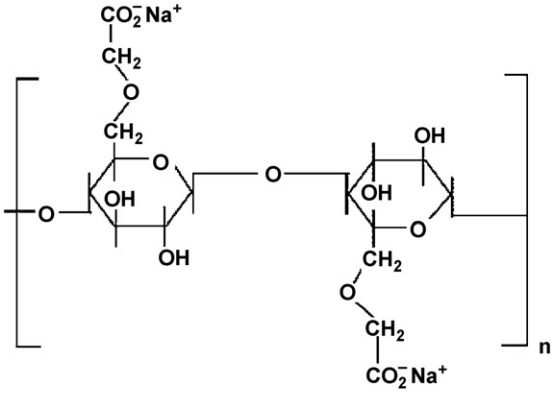
Discovery of the ways to achieve a disruption of the chemical mechanisms that lead to crystallization of cholesterol precipitates in layered forms would present a significant step in the development of chemotherapeutic and/or nutritional methods for fighting against cholesterol coagulates *in vivo*. The only reported case of the formation of spherical cholesterol particles so far involved the use of spray-freezing [13]. However, although such an approach is important in understanding the physical mechanisms of cholesterol dissolution and crystallization, it does not possess direct chemotherapeutical significance.



Chemical structure of a cholesterol molecule

Numerous investigations of the effects of various chemical environments on the crystallization and dissolution of cholesterol are reported in the literature. For example, the effects of solvent type [4], non-solvent phases [14], temperature [1], pH [15], electrolytes [16], the dynamics of solvent systems [17], magnetic field [18], mineral [19] and protein [20] substrates and co-existing phases [21,22] (such as hydroxyapatite deposits, often found interspersed within cholesterol layers in atherosclerotic plaques [23,24]), model bile composition [25–28], various medicinal plants [29] and synthetic biochemical compounds (including phospholipids [30], cholic acid [31] and other sterols [32]) on cholesterol morphology and crystallization mechanisms were previously acknowledged. The majority of these studies suggested a significant sensitivity of cholesterol crystallization processes on the limiting conditions of imposed environments. Such an observation is mostly consistent with the biological nature and multi-functional character of cholesterol molecules. It has also provided a starting point for the actual inquiry into morphological dependencies of cholesterol crystallites upon variously set conditions of its re-crystallization.

Dietary fiber is, on the other hand, well-known for its ability to lower blood cholesterol levels [33,34]. Soluble fiber may, for example, in the small intestine form a gel which binds excessive blood cholesterol delivered by the action of liver, and eventually expel it from the body. As an aqueous-soluble derivative of cellulose, an important cleansing agent against the intestinal plaque, carboxymethyl cellulose (CMC) was chosen as an additional component for the procedures of cholesterol precipitation, attempted at overcoming the inherent and seemingly irresistible tendency of cholesterol crystallites to adopt platelet morphologies. Beside its application as surfactant, thickener, water-binder, disintegrant, lubricant, polymer flocculant, phase and emulsion stabilizer, suspending agent and dirt-attractive, anti-redeposition agent in detergent chemistry, CMC is also used as a chelating agent in precipitation of dietary lipoproteins and the corresponding fabrication of cholesterol-free food products [35]. Viscous CMC has even proven to be a more effective agent for decreasing plasma cholesterol levels through the digestive usage comparing to nonviscous cellulose [36,37]. Similar to cellulose itself, CMC was noticed to decrease liver cholesterol concentrations when introduced in the diet [38]. It has also been used as a hemostatic agent, promoting cellular adhesion and healing from injuries [39,40]. It has lately been used as an enteric polymer, coating pharmaceutical dosage forms [41] and magnetic nanoparticles [42], with a potential application in targeted drug-delivery products. CMC has recently been employed as a pore-sealing additive in cement chemistry, improving strength, fracture toughness, corrosion resistance and durability of the final composite products [43], as well as a biodegradable, low-cost and eco-friendly binding agent in graphitic anodes for lithium ion batteries [44]. It has also been used as an active ingredient in the production of artificial tears [45]. Being a mild acid, CMC may offer an aid in the suppression of electrostatic repulsion present at naturally slightly alkaline pH (∼7.5) of cholesterol solutions, at which *in vivo* deposition of cholesterol usually proceeds [46]. Commercial CMC normally comprises a certain amount of sodium carboxymethyl groups which promote water solubility, and which contribute to an increase of the ionic strength of solution. In accordance with DLVO theory, this effect promotes the contraction of charged layers surrounding individual suspended particles, leading to additional suppression of electrostatic repulsion at large distances.



Molecular structure of sodium carboxymethyl cellulose

2. Experimental

2.1. Materials

The chemicals used in the presented procedures of synthesis were: cholesterol (99+%, Alfa Aesar), carboxymethyl cellulose (M_w = 250,000 g/mol, Polysciences, Inc.), ethanol (anhydrous, 99.5%, Pharmco), and 1-hexanol (J.T. Baker).