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Recovery of astaxanthin using colloidal gas aphrons (CGA): A mechanistic study

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Abstract

The aim of this study is to investigate the mechanism responsible for the recovery of astaxanthin using Colloidal Gas Aphrons (CGA), which are surfactant stabilised microbubbles. The latter were produced using different surfactant solutions (Cetyl Trimethyl Ammonium Bromide (CTAB)-cationic, Sodium Dodecyl Sulfate (SDS)-anionic, TWEEN 60-non-ionic and mixtures of TWEEN 60-SPAN 80- non-ionic with varying hydrophobicity) at stirring speed 8000 rpm and stirring time 5 min. Experiments were carried out at varying pH and volumetric ratios of astaxanthin to CGA, and with two different astaxanthin standard suspensions: (i) astaxanthin dispersed in aqueous solutions and (ii) astaxanthin dispersed in ethanolic/aqueous solutions with different compositions of ethanol (20/80 (v/v) and 40/60 (v/v)). When astaxanthin is dispersed in aqueous solutions the separation seems to occur mainly by electrostatic interactions. Therefore the recoveries are higher in the case of the cationic surfactant when astaxanthin particles are strongly negatively charged, as shown by the zeta potential measurements. When ethanol is present, highest recoveries are achieved with CGA produced from the non-ionic surfactant, which indicates that, under these conditions, separation is driven mainly by hydrophobic interactions. In experiments with ethanolic/aqueous suspensions, when the hydrophobicity of the surfactant was increased by increasing volumes of SPAN 80, the CGA produced were less stable; thus higher recoveries of astaxanthin under conditions that favour hydrophobic interactions were not observed.

Keywords: CGA; Astaxanthin; Recovery; Surfactants; Zeta potential

1. Introduction

Astaxanthin (Fig. 1) is a natural antioxidant that belongs to the family of xanthophylls, the oxygenated derivatives of carotenoids [1], and it is mainly used nowadays as a pigmentation source in aquaculture [2]. Furthermore, there is a growing number of research studies concerning its potential benefits to human health [3–5] and due to its biological functions it can be characterized as a value-added product. Currently, the synthetic astaxanthin produced by BASF and ROCHE covers the world market [4,6], however the health conscious lifestyles of the consumers nowadays demand "natural" products, thus much research is conducted on the optimization of the production of astaxanthin from natural sources such as via the fermentation mainly of *Haematococcus pluvialis* and *Phaffia rhodozyma* [7–9], or from utilisation of crustacean by-products [10–12]. Yet natural astaxanthin has received less commercial interest as a potential ingredient for human consumption, partly due to the complexity of downstream processing for the production of pure astaxanthin.

Usually astaxanthin is extracted from cells, using solvent extraction [13]. However, there are some limitations to this technique particularly in large scale applications, due to the toxicity of solvents, their cost, their impact on the environment, and because of the fact that organic solvents may lead to irreversible product degradation. Moreover, solvents need to be removed at the later stage in order to obtain the product in free form. Recently, there is growing interest on the application of surfactants to separation processes [14]. Surfactants possess unique characteristics, such as the tendency to adsorb onto surfaces, to associate in solution to form micelles which dissolve non-polar solutes [15] and finally the fact that most of them can be non-toxic and biodegradable. Moreover, usually these systems have low energy requirements and they can be used to treat degraded materials such as biochemicals [14]. Therefore, surfactant-based separation processes are very promising separation techniques.

One application of surfactant-based separations is the use of Colloidal Gas Aphrons (CGA). These were first described

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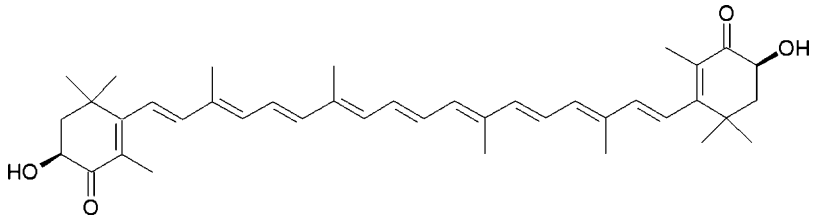

Fig. 1. Molecular structure of astaxanthin (3,3′-dihydroxy-β,β′-carotene-4,4′-dione).

by Sebba (1972) as surfactant-stabilised microbubbles (10–100 μm) generated by intense stirring of a surfactant solution at high speeds (>8000 rpm) [16]. Research studies have been conducted in order to determine the structure of CGA [17] and it is postulated that that they possess a surfactant multilayer structure as can be seen in Fig. 2, and for this reason they have different dispersion characteristics compared to conventional foams. Depending on the surfactant used to produce CGA, e.g. cationic, anionic or non-ionic, the outer surface of the microbubble may be positively, negatively or non-charged respectively, to which oppositely or non-charged molecules will adsorb resulting in their effective separation from the bulk liquid [18], therefore the selectivity of adsorption can be adjusted [19].

CGA exhibit unique characteristics, including high interfacial area, high stability compared to conventional foams, ability to be pumped and to separate easily from the liquid phase without mechanical aid, reducing in this way the number of operations for product purification/recovery and making them a cost effective separation technique compared to conventional methods such as centrifugation and supercritical fluid extraction. Furthermore, the use of biodegradable surfactants results in environmentally friendly processes while the final product can also be safe for human consumption and in some cases these surfactants can further enhance the bioavailability of lipophilic molecules. For example, the use of TWEEN surfactants can enhance the bioavailability of astaxanthin [20,21], so it would not be necessary to remove the surfactant after the recovery. On the contrary, the presence of surfactant can be beneficial in terms of formulating the final product for human consumption.

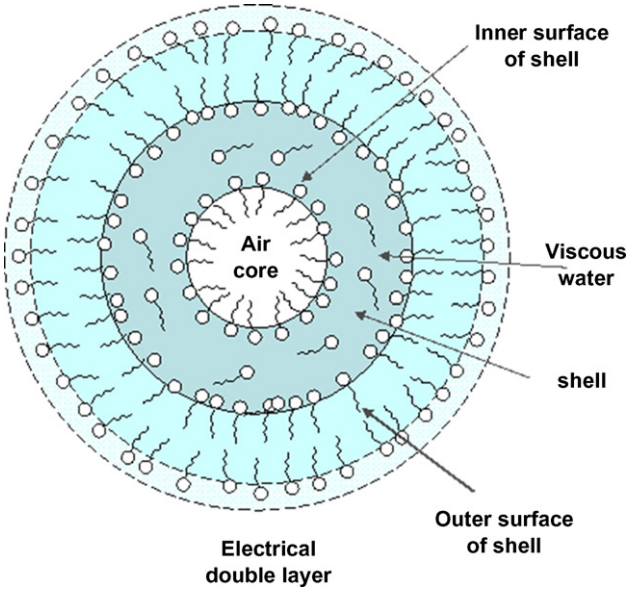

Fig. 2. Proposed structure of CGA by Sebba (1987).

Several applications of CGA have been reported including the flotation of yeast cells [22–27], the removal of toxic wastes from soil [28,29] and waste waters [30] and removal of fine particles [31] among others. More recently, CGA have attracted attention as an alternative method for the recovery of a wide variety of bioproducts from complex systems, including proteins [32–35] polyphenols, such as gallic acid [18] and carotenoids, such as norbixin [36] on laboratory scale.

In the case of the recovery of whey proteins and polyphenols it was shown that CGA act as ion exchangers and the recovery of the product is optimum under conditions that favour electrostatic interactions. However, astaxanthin is a hydrophobic molecule. The purpose of the current study was to investigate the mechanism responsible for the recovery of astaxanthin from standard solutions, using CGA produced from a variety of surfactants under various conditions (pH, presence of ethanol). Additionally, in order to get a further insight into the mechanism, the zeta potential of astaxanthin under various conditions was determined.

2. Experimental

2.1. Materials

All chemicals used were of analytical grade. CTAB (Cetyl Trimethyl Ammonium Bromide), SDS (Sodium Dodecyl Sulphate), TWEEN 60, SPAN 80, synthetic astaxanthin (purity ≥98%), sodium phosphate (Na_2HPO_4) and Tris Base were obtained from Sigma Chemicals (St. Louis, MO). Concentrated hydrochloric acid (HCl), sodium hydroxide (NaOH), ethanol, sodium dihydrogen orthophosphate (NaH_2PO_4) and citric acid were from BDH (Poole, UK). Sodium chloride (NaCl) and potassium chloride (KCl) were from Fisher Scientific (Loughborough, UK).

The laboratory mixer (SL2T) fitted with four bladed impeller ($D = 30$ mm) surrounded by a high shear screen and with a digital readout of the impeller speed was supplied by Silverson (Waterside, Bucks, UK). Zeta potential was measured using a ZetaMaster (Malvern Instruments, UK). The spectrophotometers used were an Ultrospec 1100 pro purchased from Amersham Pharmacia Biotech (Biochrom, Cambridge, UK) and a PerkinElmer lambda 20 UV–vis Spectrophotometer coupled