

Separation of vitamin C by reactive extraction

1. Mechanism and influencing factors

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Abstract

The separation of vitamin C by reactive extraction with Amberlite LA-2 represents an advantageous alternative to the downstream process applied at industrial scale. The experiments indicated that the separation occurs by means of an interfacial reaction between, the solute and the extractant, the extraction process being controlled by the extractant concentration in organic phase and pH-value of aqueous phase.

The extraction mechanism involves the solubilization of interfacial product by means of solvation or by entrapping into aminic micelles formed in organic phase. Thus, the maximum extraction degree of vitamin C has been reached for a concentration of 160 g l⁻¹ Amberlite LA-2 in organic phase and pH bellow 2. The separation efficiency could be enhanced by addition of a phase modifier, 2-octanol, this effect indicating that the increase of solvent polarity exhibits a favorable effect.

Keywords: reactive extraction, vitamin C, Amberlite LA-2, phase modifier, 2-octanol.

Introduction

Vitamin C, or ascorbic acid, is one of the well-known vitamins, being biosynthesized by microorganisms, plants and most of animals, but not by human organisms. Due to its antioxidant properties, vitamin C plays a vital role in protecting the body. Its deficiency leads to scurvy, a disease characterized by weakness, small hemorrhages throughout the body that cause gums and skin to bleed, and loosening of the teeth [1,2].

Vitamin C is a water-soluble compound, being obtained by extraction from plants, by chemical synthesis, by biosynthesis and by mixed chemical/biochemical methods. The industrial manufacture of vitamin C is carried out by two ways: biosynthesis combined with chemical synthesis (Reischstein process) and two steps fermentation. The annual production is over 70.000 t, China being the most important producer with 43.000 t/year [1-4].

The Reichstein process is used by BASF, Takeda, Roche and implies the biotransformation of sorbitol into sorbose using bacteria cultures (*Acetobacter suboxidans*, *Bacterium xylinum*, *Erwinia sp.*, *Corynebacterium sp.*), followed by the chemical synthesis of di-acetone-ketogluconic acid and, finally, of 2-ketogluconic acid. The last one is chemical rearranged to form vitamin C, using an acid catalyst [1-3].

The production of vitamin C by two steps fermentation method was developed in China since 1987, and it is now applied by Merck, ADM, Genecor International, Eastman, Electrosynthesis Company Inc., MicroGenomics Inc. and BASF. By this method, the glucose is directly transformed into 2,5-diketogluconic acid using mutant strains of *Erwinia sp.* The 2,5-diketogluconic acid is deoxidized into 2-ketogluconic acid by a mutant strain of *Corynebacterium sp.* [3,4]. A similar method uses *Bacillus thuringiensis* [4].

The one step fermentation for 2-ketogluconic acid production from glucose was studied in USA since 1989, the biosynthesis being carried out with the aid of mutant *Erwinia herbicola*, but the conversion yield was inferior to the above methods [4].

Similarly to the Reichstein process, the 2-ketogluconic acid obtained by one or two steps fermentation methods is finally transformed into vitamin C.

Irrespective of the method used for vitamin C production, its separation and purification require a lot of difficult stages, with high material and energy consumption. Because both in Reichstein process and in two steps fermentation the transformation of 2-ketogluconic acid into vitamin C is carried out without acid separation from the broth resulted in the earlier stage, the final solution contains many secondary compounds, among them the 2-keto-gluconic acid being the most important. Furthermore, although each step of vitamin C production has a yield of 90%, the overall yield of glucose conversion into vitamin C is about 60% [3,4].

The separation and purification of vitamin C is carried out in two steps. In the first one, the final solution is pre-purified by ion-exchange. In the second step, the purified solution is concentrated under vacuum conditions, the vitamin C being separated by crystallization in acid medium at low temperature [3]. Due to vitamin C low stability in aqueous solutions, each steps require extreme short residence time, that amplifying the difficulties of downstream process.

For these reasons, the selective separation of vitamin C from 2-keto-gluconic acid by reactive extraction with Amberlite LA-2 was studied. The aim of our experiments is to establish the optimum conditions for high selective separation of the two compounds by analyzing the extraction mechanism, kinetic and the influences of the factors controlling the process. These studies were initially carried out for individual separation of vitamin C and 2-ketogluconic acid from aqueous solutions, and then for their separation from mixtures. In this paper the results obtained for individual reactive extraction of vitamin C are presented.

Materials and Method

The experiments have been carried out using an extraction column with vibratory mixing, this laboratory equipment being described in detail in previous papers [5]. The phase mixing was made by mean of a perforated disk with 45 mm diameter and 20% free section. The vibrations had a frequency of 50 s^{-1} and 4 mm amplitude. The mixer position was maintained at the initial contact interface between the aqueous and organic phases. The

extraction time was of 1 minute at a constant temperature of 24°C. The resulted emulsion was evacuated at the base of the column and broken in a centrifugal separator at 4000 rpm.

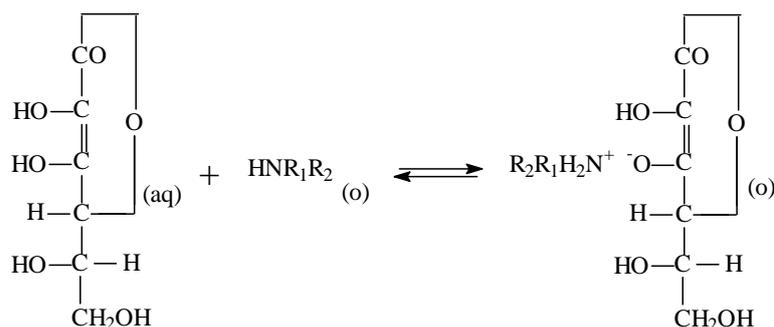
The vitamin C initial concentration in aqueous solution was of 7.6 g l⁻¹. The organic phase was a solution of Amberlite LA-2 in butyl acetate, the extractant concentration varying between 0 and 160 g l⁻¹. In some experiments, 2-octanol was added to organic phase in a volumetric concentration of 15%.

The pH adjustment of the initial vitamin C solution has been made with a solution of 5% sulfuric acid or 5% sodium hydroxide, function on the prescribed pH value (the pH-value of vitamin C solution was of 3.5). The pH values were determined using a digital pH meter of Consort C836 type and have been recorded throughout each experiment. Any pH change was noted during extraction experiments.

The extraction degree has been calculated by means of the vitamin C concentrations in the initial solution and in the raffinate. The vitamin C concentrations have been determined using the iodometric titration [6].

Results and Discussion

The reactive extraction of vitamin C with Amberlite LA-2 occurs by means of the following chemical reaction:



This mechanism of reaction is the result of vitamin C monobasic acid character induced by the enolic group HO from position 3 (pK_a = 4.2 at 25°C [2]). Contrary, the HO group from position 4 exhibits a pronounced basic character (pK_b = 11.6 at 25°C [2]). Moreover, as it was observed for reactive extraction with Amberlite LA-2 of others compounds having voluminous molecules, the formation of acidic or aminic adducts is steric hindered [7,8]. Therefore, the product of the reaction between vitamin C and Amberlite LA-2 includes in his structure only one molecule of each reactant. Because the vitamin C is not soluble in butyl acetate (its physical extraction is not possible) and the extractant is not soluble in aqueous phase, the chemical reaction occurs at the interface between the aqueous and organic phases, being followed by the product diffusion into the bulk of organic phase.

In these circumstances, the extractant concentration in butyl acetate exhibits a significant influence on vitamin C extraction degree, as it can be observed from Figure 1.

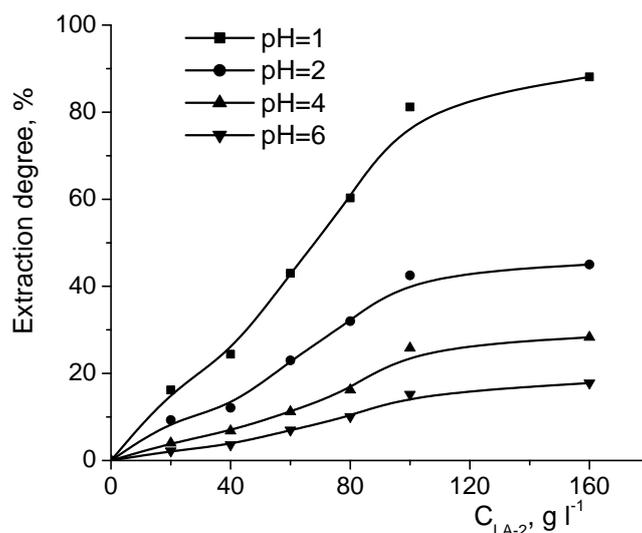


Fig. 1. Influence of extractant concentration in organic phase on vitamin C extraction degree.

The extraction yield strongly increases with the Amberlite LA-2 concentration up to 100 g/l, over this value the increase becoming slowly. The favorable effect of extractant concentration increase is amplified by pH-value decrease (for 160 g/l Amberlite LA-2 and pH=1, the reactive extraction yields reached the value of 88 - 90%). These results indicate that for high efficiency of extraction is required high Amberlite LA-2 concentration, over the stoichiometric values needed for reacting with vitamin C. This behavior of extraction system is due to the one of the following reasons:

a. owing to the low acidity of enolic HO from position 3, the interfacial reaction occurs slowly, the accumulation of one reactant at the interface (Amberlite LA-2) increasing the rate of chemical reaction, consequently increasing the amount of extracted vitamin C for a certain duration of extraction process.

b. high molecular weight amines, like Amberlite LA-2 (lauryl-trialkyl-methylamine), can form molecular aggregates (20 - 40 monomers) of micelles type, especially in low-polar solvents, like butyl acetate [8]. The formation of these aminic aggregates could induce the increase of interfacial product solubility in organic phase, by solvation or by entrapping it into the micelles (due to the presence of HO groups in the structure of vitamin C, the solubility of the compound formed by interfacial reaction between vitamin C and extractant in butyl acetate is rather low).

For establishing the mechanism responsible for the extractant concentration influence on extraction efficiency, the effects of extraction duration and of 2-octanol addition on vitamin C extraction degree have been analyzed. From Figure 2 it can be seen that the duration doesn't exhibit an important influence on extraction yield, the significant increase of extraction yield being recording only for duration up to 15 s. This variation suggests that the rate of interfacial reaction is rather high and the limiting step of extraction process is not of kinetic type.

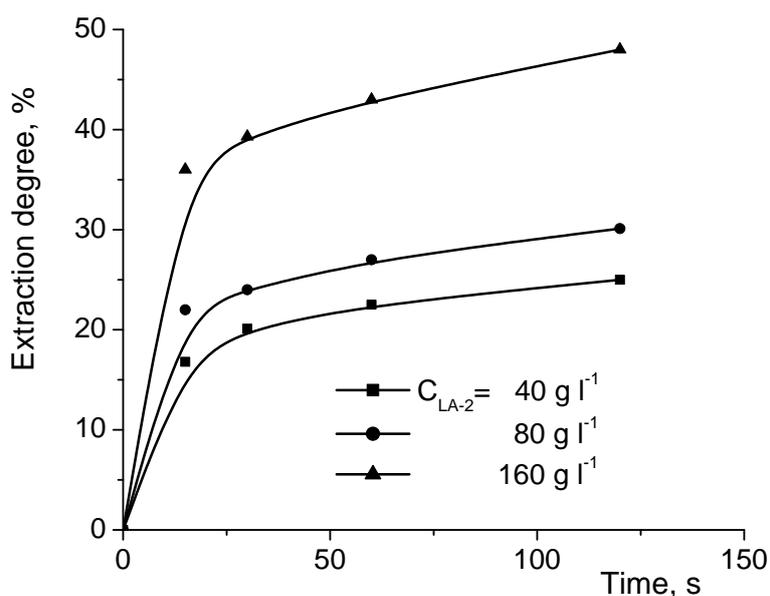


Fig. 2. Influence of extraction duration on vitamin C extraction degree (pH = 2).

The addition of 2-octanol in organic phase leads to the increase of extraction degree (for 15% vol. 2-octanol, the extraction yield was higher with 6 to 23%, function of pH-value) (Figure 3). But, the physical extraction of vitamin C didn't become possible even in presence of 2-octanol.

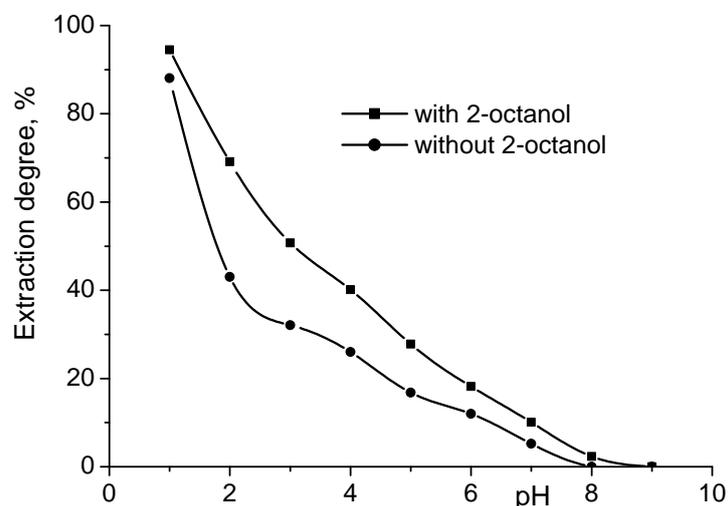


Fig. 3. Influence of 2-octanol addition on vitamin C extraction degree ($C_{LA-2} = 160 \text{ g l}^{-1}$).

As it was indicated in literature, if the carboxylic acids are extracted from high concentrated solutions with amines in non- or low-polar solvents, or if the solubility of the interfacial reaction product in the same solvents is low, a third phase can form in the extraction systems. This phase contains high amount of acidic adducts, formed in the first

case, or of interfacial reaction product, in the second one [8-10]. For increasing the solubility of these compounds and for eliminating the third phase, in the organic phase are added so-called “phase modifiers”, usually high molecular weight alcohols (n- and i-octanol, -decanol). The main favorable effect of phase modifiers is due to the increase of solvent polarity.

In this case, owing to the superior polarity of 2-octanol (the dielectric constant of 2-octanol is 10.3, and of butyl acetate 5.01 at 20°C [11]), its addition exhibits a favorable effect both on third phase elimination and on interfacial compound solubilization in solvent phase.

The increase of extraction efficiency due to the increase of solvent polarity cumulated with high rate of the interfacial reaction underlines that the solubilization and diffusion of interfacial product into organic phase bulk represents the limiting step of the separation process. Consequently, the increase of amine concentration in butyl acetate induces the increase of interfacial product hydrophobicity, either by solvation or by entrapping it into the micelles.

Another important factor is the pH-value of aqueous solution. Figures 1 and 4 indicate the significant reduction of vitamin C extraction yield with the pH increase.

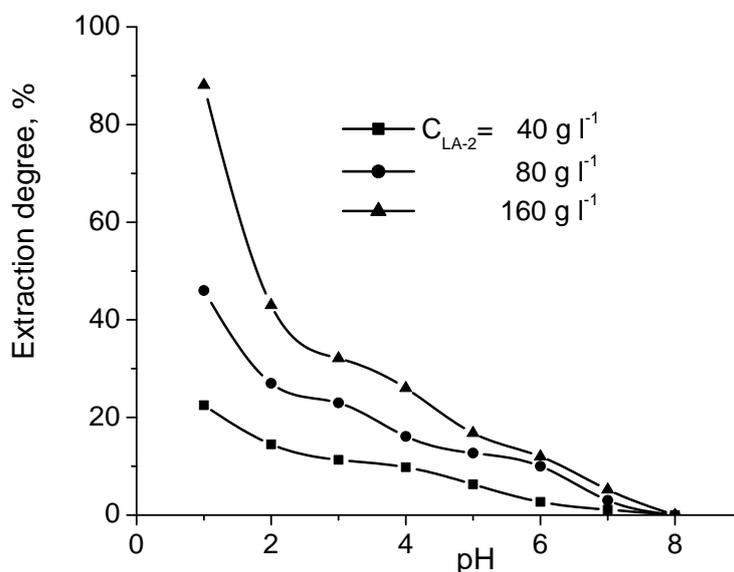


Fig. 4. Influence of the pH-value of aqueous solution on vitamin C extraction degree.

This variation is not the result of the dissociation of acidic group HO from position 3, because both forms of vitamin C, dissociated and non-dissociated, can react with Amberlite LA-2, as in the similar case of others acidic compounds extraction [7-10]. On the other hand, by increasing pH-value, vitamin C is transformed into its oxidized form, dehydroascorbic acid, thus losing its capacity to react with Amberlite LA-2 [2]. But the acidic pH domain required for reactive extraction of vitamin C doesn't affect the vitamin structure, due to its stability in acidic solutions.

Conclusions

The studies on reactive extraction of vitamin C with Amberlite LA-2 dissolved in butyl acetate indicated that the separation occurs by means of an interfacial reaction of first order between the two components. The extraction process is controlled by the extractant concentration in organic phase and pH-value of aqueous phase. For Amberlite LA-2 concentration of 160 g/l and acidic pH-domain, the extraction degree of vitamin C reached 90%, as the result of the solubilization of interfacial product by solvation or by entrapping into aminic micelles formed in organic phase.

The separation efficiency was enhanced by addition of a phase modifier, namely 2-octanol, the extraction yield being increased with about 6 - 23%. This variation indicated that the increase of solvent polarity exhibits a favorable effect on interfacial formed compound solubilization into organic phase.

These experiments will be continue and developed for kinetic studies and for mathematical quantification of the main parameters influences, in the purpose of extraction process modeling, optimization and scaling-up.

Notations

C_{A0} - initial concentration of vitamin C in aqueous solution, $g\ l^{-1}$

C_{LA-2} - initial concentration of Amberlite LA-2 in organic phase, $g\ l^{-1}$

Subscript

aq - aqueous phase

o - organic phase

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