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Selective Separation of Vitamin C by Reactive Extraction

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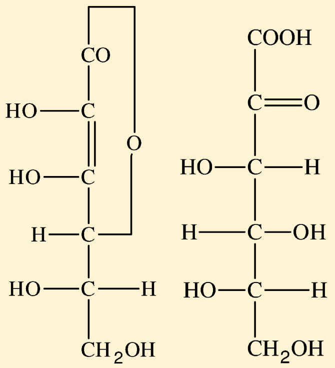
ABSTRACT: Selective separation of vitamin C from the mixture with 2-ketogluconic acid (the main byproduct from the fermentation process) by reactive extraction using Amberlite LA-2 dissolved in dichloromethane has been analyzed. The mechanism of the interfacial reaction pointed out that, indifferent of the pH value, only one molecule of vitamin C or 2-ketogluconic acid and one of extractant react at the interface, the resulting interfacial product being a hydrophobic ammonium salt. The obtained results indicated that it is possible to separate vitamin C by modifying the aqueous phase pH.



INTRODUCTION

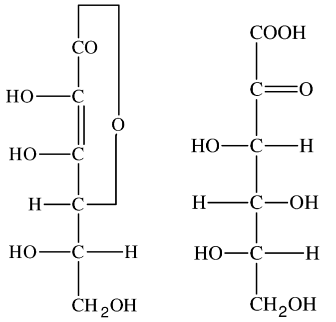
Vitamin C or ascorbic acid is one of the most important vitamins for human nutrition that is supplied by fruits and vegetables, with a large market shared both in the food and drinks industries (more than 50 %) and in the pharmaceutical industry. It is an essential nutrient for humans, nonhuman primates, and a few other mammals as they cannot synthesize this vitamin. As a potent antioxidant, vitamin C has the capacity to eliminate several different reactive oxygen species, acts as a cofactor maintaining the activity of a number of enzymes, and has an important role in stress resistance.[1−5]

Vitamin C has been a key product (110 000 tonnes per year) over several decades (around 70 years) dominated by the Reichstein process with an overall yield of 60 %.[4] This synthesis is a sequence of easy, high yielding steps, and as a result of many technical and chemical modifications, each step gives over 90 % yield. A new process for vitamin C production has been established recently on the industrial level by BASF and Cerestar. It comprises the conversion of sorbitol to sorbose and subsequently to 2-keto-L-gluconic acid by a mixed culture of *Gluconobacter oxidans* and *Bacillus thuringiensis*, with a yield of 85 %. The process was developed in China and is used by all Chinese producers, with an estimated production of 43.000 t/a.[3,4]

2-Keto-L-gluconic acid is produced as a byproduct in the preparation of vitamin C but can also be obtained by microbiological fermentation of sorbose via sorbosone.[5−7] Hydroxy acids, in particular, ketoalduronic acids, have been found to be useful as raw materials. The six-carbon 2-keto-L-gluconic acid can be used, for example, to produce xylitol, either by decarboxylation to L-xylonic acid or by enzymatic or chemical reduction to L-gulonic acid or L-idonic acid.[6−8]

Regardless of the method used to obtain vitamin C, its recovery from the feed solution including 2-ketogluconic acid is nowadays difficult, particularly because the overall precursor concentration is low. At present the downstream process consist of two steps: in the first one, the final solution is prepurified by ion exchange, and in the second step, the purified solution in concentrated under vacuum conditions, the vitamin C being separated by crystallization in acid medium at low temperatures.[3] This process has several drawbacks: because of vitamin C's high solubility in water, the costs of this process due to the energy consumption are very high, and also due to its low stability in aqueous solutions, each step requires an extremely short residence time, thus amplifying the difficulties of the downstream process.

2-Ketogluconic acid and vitamin C (2,3-diketo-L-gulonolactone) differ in their chemical structure essentially only by the lactone structure (Figure 1); therefore they resemble each



Vitamin C **2-ketogluconic acid**

Figure 1. Chemical structure of vitamin C and 2-ketogluconic acid.

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other in their chemical reaction properties and have similar physical properties (regarding extraction).

The solubility of 2-ketogluconic acid and vitamin C is influenced by the four hydrophilic hydroxyl groups and the acid group, 2-ketogluconic acid being among the strongest monobasic carboxylic acids ($pK_a = 2.66$).[9,10] Both have similar solubility characteristics: they are highly soluble in polar solvents, in particular water, but are only sparingly soluble in organic solvents. Economic separation of the mixture resulting from the fermentation process is therefore difficult.

For these reasons, the selective separation of vitamin C from 2-ketogluconic acid by reactive extraction with Amberlite LA-2 was studied. In this case, 2-ketogluconic acid competes with ascorbic acid in the extraction process. The aim of our experiments is to provide an advantageous process for high selective separation of the two compounds. 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 mixture. It is the object of this study to establish the optimum separation conditions by analyzing the extraction mechanism, kinetics, and the influences of the factors controlling the process.

■ MATERIALS AND METHODS

The experiments have been carried out using an extraction column with vibratory mixing, this laboratory equipment being presented in detail in previous papers.[11,12] All chemicals have been purchased from Sigma Chemical Co, unless otherwise mentioned.

The 2-ketogluconic acid initial concentration in aqueous solution was of $1 \text{ g}\cdot\text{L}^{-1}$, and that of vitamin C was of $7.06 \text{ g}\cdot\text{L}^{-1}$. The reactive extraction was successively carried out with butyl acetate (dielectric constant of 5.01 at 20 °C), dichloromethane (dielectric constant of 9.08 at 20 °C), and *n*-heptane (dielectric constant of 1.92 at 20 °C).[13] The extractant, Amberlite LA-2 (>99 %, Merck), was dissolved in these solvents, its concentration in organic phase varying between (0 and 100) $\text{g}\cdot\text{L}^{-1}$. The pH adjustment of the initial solution has been made with a solution of 5 % sulfuric acid or 5 % sodium hydroxide, the function of the prescribed pH. The pH values were determined using a digital pH meter of Consort C836 type and have been recorded throughout each experiment (no pH changes were recorded during the experiments).

The extraction degree, *E* %, and the selectivity factor, *F*, have been calculated by means of the compound concentrations in the initial solution and in the raffinate. The vitamin C and 2-ketogluconic acid concentrations have been determined using high-performance liquid chromatography (HPLC; Hamilton PRP-X300 column (150 mm × 4.1 mm, 5 μm), 4 mM sulfuric acid solution as the mobile phase, detection being performed by UV absorbance at a wavelength of 210 nm, and the flow rate of $0.5 \text{ mL}\cdot\text{min}^{-1}$).[14]

■ RESULTS AND DISCUSSION

The separation technique of extraction based on reversible chemical complexation (reactive extraction) can be a high-capacity and high-selectivity technology for separating polar organic solutes from dilute solution.[15−20] Based on former results, the reactive extraction of both vitamin C and 2-ketogluconic acid with Amberlite LA-2 can be used to separate the two compounds from their mixture.[11,12] Amberlite LA-2, also called a *N*-lauryltrialkylmethyl amine that has 24 to 28 carbon atoms, is a secondary amine that has been successfully used to separate carboxylic acids from aqueous phases and fermentation broth. The basicity of secondary amines is superior to that of tertiary amines, and the solubility characteristics, together with the ability of secondary amines to react with acids to form the corresponding amine salts, make possible the removal of acidic constituents from aqueous solutions.[17,21−23] Because neither of the compounds is soluble in the organic phase (physical extraction is not possible) and the extractant is not soluble in aqueous phase, chemical reaction occurs at the interface between the aqueous and organic phases, being followed by the interfacial product diffusion into the bulk organic phase.

An important factor influencing the equilibrium of the system is the pH value, because both vitamin C and 2-ketogluconic acid exist in two forms in the aqueous phase (e.g., ascorbic acid and dehydroascorbic acid, respectively, dissociated and undissociated), of which only one is reactive (ascorbic acid and undissociated form of 2-ketogluconic acid), and the relative amount of each form depends on the pH of the solution. So the reaction step of the extraction is pH-dependent and therefore the overall extraction as well. Table 1 presents the results for the individual separation of the two compounds. Because these studies showed that the most efficient solvent was dichloromethane, the following experiments were developed for this solvent.

Table 1. Extraction Efficiency for the Individual Extraction of Vitamin C and 2-Ketogluconic Acid (Amberlite LA-2 Concentration = 40 $\text{g}\cdot\text{L}^{-1}$)

E, %	vitamin C		2-ketogluconic acid	
solvent	butyl acetate	dichloromethane	butyl acetate	dichloromethane
pH = 1	24.4	45.6	19.2	22.6
pH = 2	14.5	27	23.0	27.7
pH = 3	11.3	23	28.8	35.2
pH = 4	9.8	16.1	18.3	28.5
pH = 5	6.3	12.7	4.7	8.6

The extraction efficiency, quantified by the extraction degree, %, obtained for vitamin C and 2-ketogluconic acid at different pH values and at constant concentrations of Amberlite LA-2, are shown in Figure 2. It is observed that there is a significant difference in the extraction degrees of the two compounds, similar to the individual separation.

Regarding vitamin C, Figure 2 indicates a significant reduction of separation yield by a pH increase. This variation is not the result of the dissociation of acidic group HO from position 3, because both forms of vitamin C, dissociated and undissociated, can react with Amberlite LA-2, as in the similar case of other acidic compound extractions.[11] On the other hand, by increasing the pH value, vitamin C is transformed into its oxidized form, dehydroascorbic acid, thus losing its capacity to react with Amberlite LA-2.[24] But the acidic pH domain required for reactive extraction of vitamin C does not affect the vitamin structure, due to its stability in acidic solutions.[5]

In the case of 2-ketogluconic acid, as illustrated in Figure 2, at a constant extractant concentration, the extraction degree presents a peak with the increased pH. The maximum is the result of the two opposite phenomena that occur by increasing the pH value. At a strong acidic domain, 2-ketogluconic acid exists in aqueous solution in linear or cyclic dimeric form, the

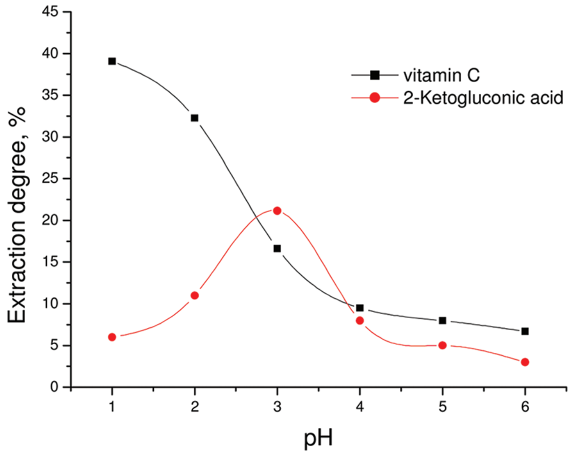

Figure 2. Influence of pH value on reactive extraction efficiency of vitamin C and 2-ketogluconic acid (Amberlite LA-2 concentration in dichloromethane = 40 g·L^{-1}).

carboxylic group being unable to react with the extractant.[12,24] The dimerization of solute becomes less important with the pH increase, thus leading to the increase of concentration in organic phase of the extracted acid. However, the pH increase induces the dissociation of the carboxylic group (pK_a = 2.66 at 25 °C[13]) and, consequently, the reduction of the reactive extraction efficiency. The more pronounced decrease in the extraction degree for 2-ketogluconic acid is due to differences between the acidity of the two compounds. To support this observation, vitamin C has the character of a monobasic acid induced by the enolic group HO− from position 3 (pK_a = 4.2 at 25 °C[24]), while 2-ketogluconic acid is one of the strongest organic acids (pK_a = 2.66 at 25 °C[13]).

From Figure 3 it can be seen that the selectivity factor, calculated as the ratio between the degree of extraction of vitamin C and the 2-ketogluconic acid, decreases strongly with increasing pH, the highest selectivity being obtained at pH = 1. The minimum value for pH = 3 was obtained due to a higher degree of extraction for 2-ketogluconic acid at this pH. These results suggested the possibility of effective separation by selective reactive extraction of vitamin C from the mixture with 2-ketogluconic acid.

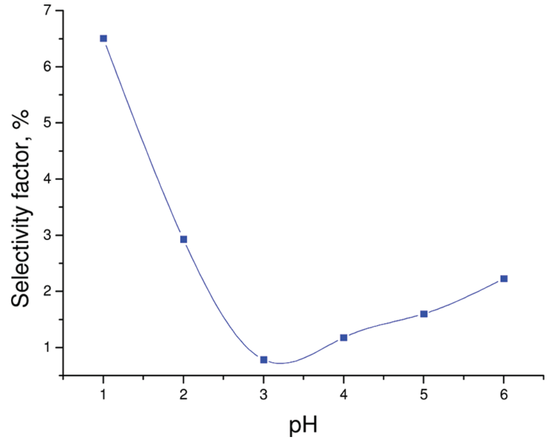

Figure 3. Influence of pH value on the selectivity factor of vitamin C and 2-ketogluconic acid (Amberlite LA-2 concentration in dichloromethane = 40 g·L^{-1}).

Indifferent of the aqueous phase pH, Amberlite LA-2 concentration in the organic phase shows a significant influence on the reactive extraction efficiency and selectivity of vitamin C and 2-ketogluconic acid from the mixture, as shown in Figure 4.

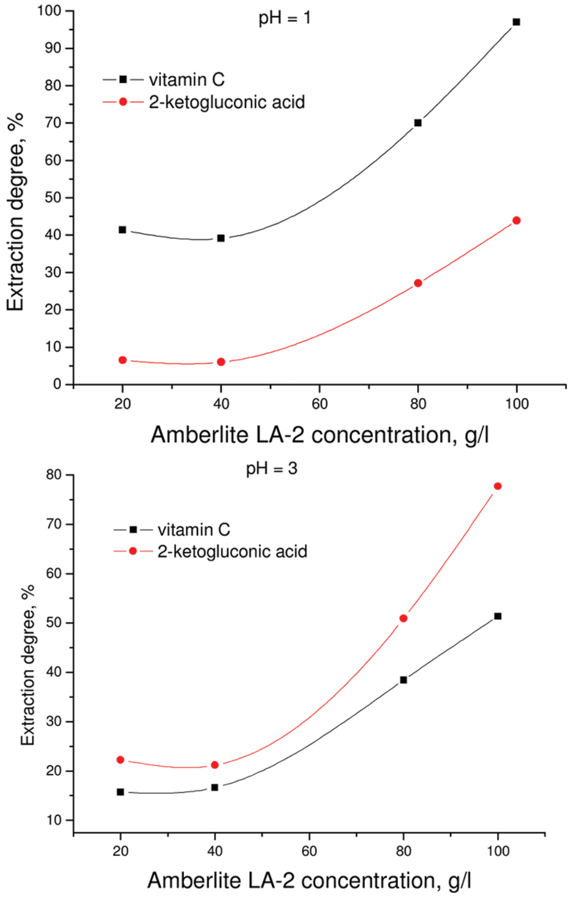

Figure 4. Influence of extractant concentration on the extraction degree of vitamin C and 2-ketogluconic acid (the solvent used was dichloromethane).

This fact can be explained by the insolubility of both compounds in dichloromethane and of the extractant in the aqueous phase; therefore the reactive extraction with Amberlite LA-2 occurs through an interfacial reaction, followed by diffusion of the resulting complex in the organic phase. The correlations between the distribution coefficients and dissociation and extraction constants have been previously used for establishing the mechanism of individual reactive extraction of both vitamin C and 2-ketogluconic acid.[11,12] According to previous studies, indifferent of the pH value, only one molecule of vitamin C or 2-ketogluconic acid and one of extractant reacts at the interface. In both cases (vitamin C and 2-ketogluconic acid) the resulting interfacial product is a hydrophobic ammonium salt (a process that is promoted by the acidity of the enolic HO− group from position 3, for vitamin C, and of a carboxylic group for 2-ketogluconic acid).

Figure 4 shows a more important influence of extractant concentration for separation at pH = 1, the degree of extraction for vitamin C reaching a maximum of 98 % in dichloromethane for Amberlite LA-2 concentration of 100 g·L^{-1}. In the same time, there is a significant increase in separation efficiency also for the secondary compound (2-ketogluconic acid), due to increased alkalinity (induced by increasing extractant concentration[25]), which reduces the formation of dimers, similar to the extraction of other acidic compounds.[26] At a pH value of 3, there is a higher degree of extraction for 2-ketogluconic acid compared with vitamin C, because of the reasons outlined above, for the whole domain of Amberlite LA-2 concentration.

According to Figure 5, for pH = 1, the selectivity factor for vitamin C decreases with increasing extractant concentration from 4.26, the value corresponding to a concentration of 20 $g\cdot L^{-1}$, to 2.2 for 100 $g\cdot L^{-1}$ Amberlite LA-2. In contrast, at pH = 3, the selectivity factor for 2-ketogluconic acid weakly increases with increasing concentration of Amberlite LA-2 in organic solvents but is still below 1 throughout the extractant concentration variation. These values prove that the selective separation of vitamin C from the mixture with 2-ketogluconic acid can be realized by reactive extraction with 40 $g\cdot L^{-1}$ Amberlite LA-2 dissolved in dichloromethane, at an aqueous phase pH equal to 1.

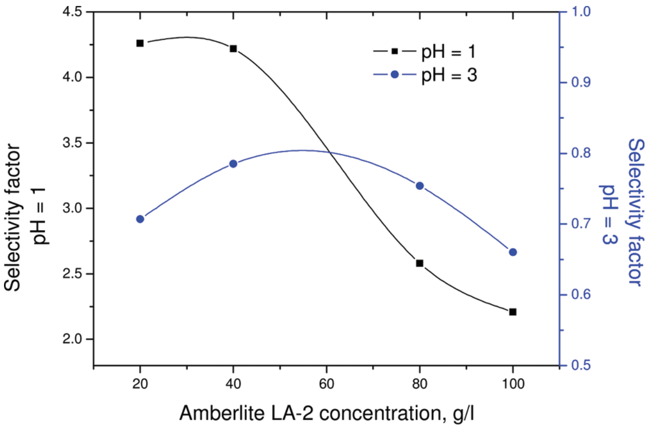

Figure 5. Influence of extractant concentration on the selectivity factor of vitamin C and 2-ketogluconic acid (the solvent used was dichloromethane).

As the solvent polarity is a determinant factor for extraction efficiency, the selective separation studies of vitamin C and 2-ketogluconic acid were realized using three solvents with different polarities: *n*-heptane, *n*-butyl acetate, and dichloromethane. The studies on reactive extraction of 2-ketogluconic acid with Amberlite LA-2 in solvents with different dielectric constants (*n*-butyl acetate, dichloromethane) indicated that the separation occurs by means of an interfacial reaction controlled by the solvent polarity.[11] Thus, if the extracted phase is *n*-butyl acetate, the interfacial product is of aminic adduct type, its structure including two extractant molecules. In the case of the solvent with higher polarity (dichloromethane), the mechanism of reactive extraction involves the formation of a salt by the interfacial reaction between one molecule of each reactant.[11] Table 2 gives the values of extraction constants for the studied extraction systems.

Table 2. Values of Extraction Constants for the Studied Extraction Systems

	vitamin C	2-ketogluconic acid
n-butyl acetate	4.95 ($L\cdot mol^{-1}$)	2.72 ($L^2\cdot mol^{-2}$)
dichloromethane	14.01 ($L\cdot mol^{-1}$)	1.22 ($L\cdot mol^{-1}$)

The study on vitamin C individual separation by reactive extraction with Amberlite LA-2 indicated that the process occurs by means of an interfacial reaction of first order related to the both extraction system components, indifferent of solvent polarity and pH value. The separation process can be favorably influenced using dichloromethane at pH = 1, the extraction degree reaching a level of 95 % in strongly acidic media. The increase of extractant concentration in rgw organic phase over the stoichiometric need for interfacial reaction with vitamin C exhibits a favorable effect, thus suggesting the solvation of the interfacial compounds with the increase of its hydrophobicity.[12]

The experimental results presented in Figure 6 indicate higher degrees of extraction when using dichloromethane, similar to individual extraction of these compounds, due to a higher extraction efficiency when using solvents with a high dielectric constant for compounds which dissociate in the aqueous phase.[27,28] Considering the fact that there has been a lot of focus recently on the introduction of greener solvents in the pharmaceutical industry, we analyzed the use of *n*-heptane, classified as a green solvent, for the reactive extraction studies.[29] The results showed the smallest extraction degree for this solvent compared with *n*-butyl acetate and dichloromethane.

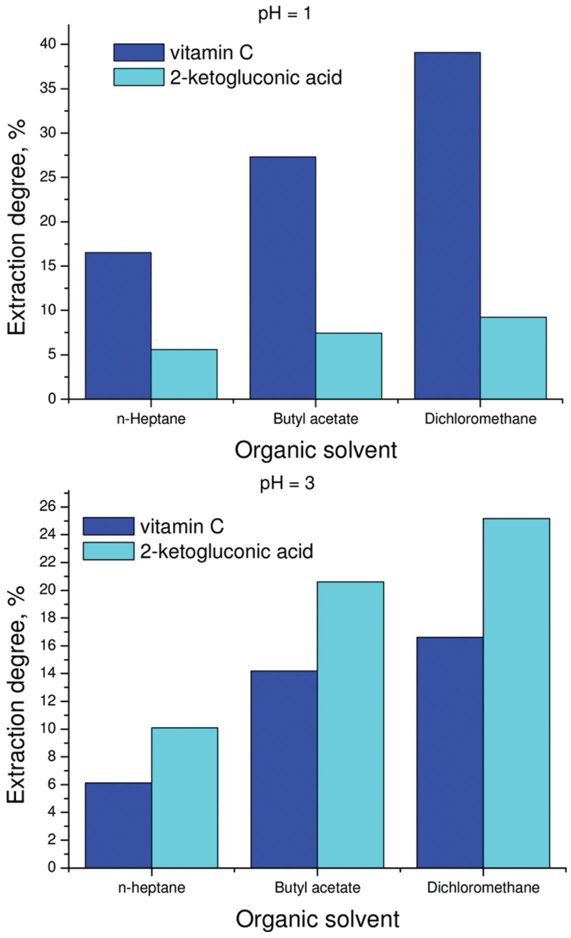

Figure 6. Influence of organic solvent on the extraction degree of vitamin C and 2-ketogluconic acid.

The cumulated favorable effects of the increase both of extractant concentration over the stoichiometric need and of solvent polarity indicate that the solubilization of the interfacial compound is achieved by its solvation by extractant molecules.

CONCLUSIONS

By analyzing the experimental data obtained, it was admitted that the selective separation of vitamin C from the mixture with 2-ketogluconic acid is possible by reactive extraction with 40 g·L^{-1} Amberlite LA-2 dissolved in dichloromethane, at an aqueous phase pH equal to 1.

In conclusion, our investigations showed that the new development of the complex separation process could successfully purify vitamin C from the fermentation broth.

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