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Selective Separation of Ascorbic Acid from (bio) Synthesis Media by Extraction and Transport Through Liquid Membrane

Alexandra Cristina Blaga [1], Lenuta Kloetzer [1], Madalina Postaru[1], Anca Irina Galaction [2] and Dan Cascaval [1 +]

1 "Gheorghe Asachi" Technical University of Iasi, Faculty of Chemical Engineering and Environmental Protection, Dept. of Organic, Biochemical and Food Engineering, 73 D. Mangeron, 700050 Iasi, Romania

2 "Gr.T. Popa" University of Medicine and Pharmacy of Iasi, Faculty of Medical Bioengineering, Dept. of Biomedical Science, 9-13 M. Kogalniceanu Street, 700454 Iasi, Romania

Abstract. The separation of ascorbic acid/vitamin C from a mixture with the main by product in fermentation process using pertraction has been investigated. The studies on extraction and transport of vitamin C using a liquid membrane (dichloromethane with Amberlite LA-2 as carrier - facilitated pertraction) indicated the major parameters that affect the separation efficiency: pH gradient between the two aqueous phases and carrier concentration in the liquid membrane. The overall results obtained in this work showed that liquid membrane systems can effectively be used to selectively separate vitamin C from its mixture with the fermentation by-product, 2-ketogluconic acid.

Keywords: Vitamin C, 2-ketogluconic acid, Amberlite LA-2, pertraction

1. Introduction

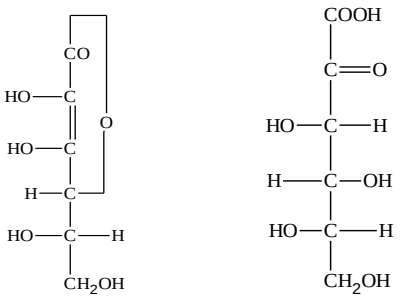
Vitamin C, a key product (110,000 tonnes per year), is over 70 years obtained either by the Reichstein process, with an overall yield of 60% or, more recently, by conversion of sorbitol to sorbose and subsequently to 2-keto-L-gluconic acid by a mixed culture of G. oxydans and Bacillus thuringiensis, with a yield of 85% [1]-[3]. Regardless of the method used to obtain vitamin C, its recovery from the fermentation broth including 2-ketogluconic acid is 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 pre-purified by ion-exchange, in the second step, the purified solution in concentrated under vacuum conditions, the vitamin C being separated by crystallization in acid medium at low temperature. This process has several drawbacks: due to vitamin C high solubility in water, the costs of this process due to the energy consumption is very high, also due to its low stability in aqueous solutions, each steps require extreme short residence time, this amplifying the difficulties of downstream process [1],[2].

2-Ketogluconic acid and vitamin C (2, 3-diketo-L-gulonolactone) differ in their chemical structure essentially only by the lactone structure (Fig. 1); therefore they resemble each other in their chemical reaction properties and have similar physical properties.

Pertraction, defined as the extraction and transport through liquid membranes, is a rather new separation technique and consists in the transfer of a solute between two aqueous phases that are separated by a solvent layer of various sizes. The use of pertraction has many advantages over conventional extraction: needs small quantities of solvent and carrier, owing to their continuous regeneration, and offers the possibility of solute transport against its concentration gradient, as long as the pH-gradient between the two aqueous phases is maintained [4],[5].

+ Corresponding author. Tel.: + 40 - 232 278683; fax: +40 - 232 271311
E-mail address: dancasca@tuiasi.ro.

In this context, on the basis of our previous investigations on the reactive extraction of vitamin C and 2-ketogluconic acid [6]-[8], the aim of this paper is to provide an advantageous process for high selective separation of the two compounds.



Vitamin C **2-ketogluconic acid**

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

2. Materials and Method

The experiments have been carried out using pertraction equipment that allows obtaining and easily maintaining the solvent layer between the two aqueous phases (free liquid membrane) [4], [5]. The pertraction cell has been described in the previous papers and consists on a U-shaped glass pipe having an inner diameter of 45 mm and a total volume of 450 ml, the volume of each compartment being of 150 ml. The aqueous solutions are independently mixed by means of double blade stirrers with 6 mm diameter and 3 mm height, having a rotation speed of 500 rpm. In order to reach high diffusional rates through the solvent layer, the organic phase has been mixed with a similar stirrer, at a similar rotation speed (500 rpm). The area of mass transfer surface, both for extraction and for re-extraction, was of 1.59×10^{-3} m^2. The interfaces between the phases remained flat, and hence the interfacial area constant, for the used rotation speed value.

The experiments have been carried out in a pseudosteady-state regime, at the steady-state conditions related to the aqueous phases and unsteady-state mode related to the membrane phase. The aqueous solutions have been separately fed with a volumetric flow of 2.0 l/h. The pertraction has been carried out at 25^{o}C.

The liquid membrane phase consisted of dichloromethane (99%, Aldrich) in which has been dissolved the carrier Amberlite LA-2 (Sigma Chemie GmbH), with concentrations varied between 0 and 100 g/l. The feed phase was an aqueous solution of 7.06g/l vitamin C (99%, Merck) and 1 g/l 2-ketogluconic acid. The pH-value of feed phase varied between 2 and 6. The pH adjustment of the feed phase has been made with a solution of 4% sulfuric acid or 4% sodium hydroxide, function on the prescribed pH-value. The stripping phase consisted of solution of sodium hydroxide with pH = 8 - 12. The pH-values of both aqueous phases have been determined using a digital pH-meter of HI 213 (Hanna Instruments) type and have been recorded throughout each experiment. Any pH change was recorded during the pertraction experiments.

The pertraction has been analyzed by means of initial and final mass flows and permeability factor. The initial mass flow represents the solute mass flow from the feed phase to the liquid membrane, while the final (overall) mass flow the mass flow from the liquid membrane to the stripping phase. The permeability factor has been defined as the ratio between the final mass flow and the initial mass flow of solute. These parameters have been calculated by determining the compounds concentrations in the feed and stripping phases and by using the mass balance for the pertraction system. The concentrations in the aqueous phases have been measured by high performance liquid chromatography technique (HPLC) with a HPLC (Hamilton PRP-X300 column (150 mm × 4.1 mm, 5 μm), 4mM sulfuric acid solution as mobile phase, detection being performed by UV absorbance at a wavelength of 210 nm, and the flow rate of 0.5 mL/min) [9].

3. Results and Discussions

Generally, the facilitated pertraction is strongly influenced by the pH-gradient between the feed and stripping phases (controls the rates of extraction and re-extraction processes), as well as by the carrier concentration inside the liquid membrane [10], [11].

In the case of the separation of vitamin C from 2-ketogluconic acid, the pH-gradient controls the rates of extraction (feed phase - membrane interface) and re-extraction (membrane - stripping phase interface) processes and, implicitly, the rate of transport through the liquid membrane. Thus, the results presented in Figure 2 show major differences between the mass flow variations of the two compounds depending on the pH gradient: with increasing pH of the feed solution, pH_F, there is a continuous decrease for vitamin C mass flow, while for 2-ketogluconic acid the dependence records a maximum of the initial mass flow, corresponding to pH = 3. These variations are due to the fact that 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 can react with Amberlite LA-2 (ascorbic acid and undissociated form of 2-keto-gluconic acid), and the relative amount of each form depends on the pH of the solution. Regarding vitamin C, the 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, at the feed phase interface. 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. 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. The maximum, in 2-ketogluconic acid case, is the result of the two opposite phenomena that occur by increasing the pH-value. At strong acidic domain, 2-keto-gluconic acid exists in the aqueous solution in linear or cyclic dimeric form, the carboxylic group being unable to react with the extractant [12]. The dimerization of solute becomes less important with the pH increase, thus leading to the increase of its concentration in organic phase (liquid membrane). But, the pH increase induces the dissociation of the carboxylic group (pKa = 2.66 at 25 $^{\circ}C$[13]) and, consequently, the reduction of the reactive extraction efficiency, leading to a decrease in the initial mass flow.

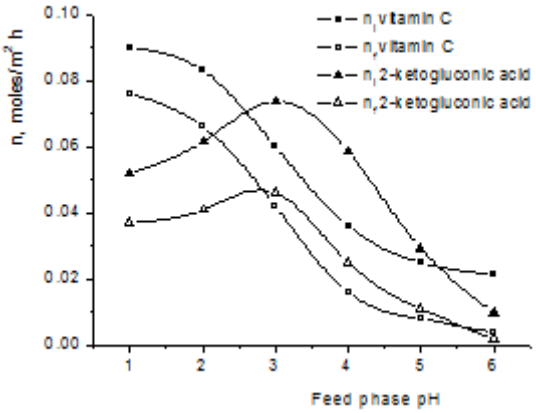


Fig. 2: Influence of pH_F on vitamin C and 2-ketogluconic acid on initial and final mass flows (pH_S = 10, Amberlite LA-2 concentration = 80 g/l)

The variations of final mass flows are similar to those of the initial mass flows, owing to their direct dependence to both compounds concentrations in the organic layer.

The permeability factor, P, conveys the capacity of solute transfer through the liquid membrane. Permeability factors for both components of the mixture decrease with increasing feed phase pH, as can be seen in Figure 3. Limitations caused by diffusion from the membrane to the stripping phase, more pronounced in the case of vitamin C due to a bulkier cyclic structure, combined with the fact that the initial mass flow of 2-ketogluconic acid is much lower for $pH_F > 5$ explains the higher values for the permeability factor compared to those for vitamin C for $pH_F > 4$. The selectivity factor, F, calculated as the ratio between permeability factors of vitamin C and 2-ketogluconic acid, show a maximum at pH = 3, which can be justified by the large difference between the concentrations of the two compounds in the liquid membrane at this pH value. For 2-ketogluconic acid, the reactive extraction rate is higher than that recorded for vitamin C, so the amount of acid extracted into the solvent layer is much higher than the reextracted one, the final mass flow being smaller than the initial one.

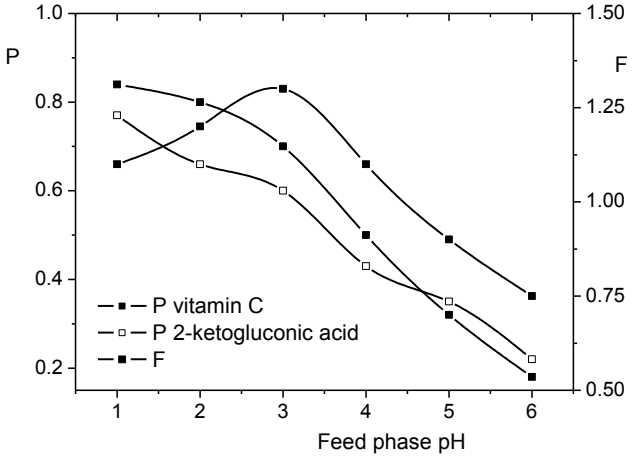


Fig. 3: Influence of pH_F on vitamin C and 2-ketogluconic acid on the permeability and selectivity factors (pH_S = 11, Amberlite LA-2 concentration = 40 g/l)

The concentration of Amberlite LA-2 exhibits a positive influence on both compounds pertraction: the initial and final mass flows are accelerated with the increase of carrier concentration inside the liquid membrane. The increase of the mass flows is the results of a superior concentration of one of the reactants which participates at the interfacial reaction in the extraction process, and the solvation of the interfacial compounds into the organic layer. The highest values of initial mass flows have been recorded for vitamin C.

The same upward trend is recorded for the permeability factors, as evidenced by the results in Figure 4, due to a higher kinetic resistance affecting the overall process of separation.

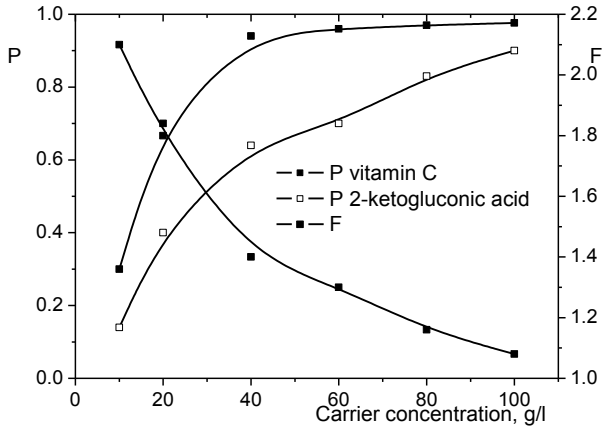


Fig. 4: Influence of carrier concentration on vitamin C and 2-ketogluconic acid on the permeability and selectivity factors (pH_F = 1, pH_S=11, rotation sped = 500 rpm)

For vitamin C, the permeability factor increases up to a concentration of Amberlite LA-2 to 40 g/l, above this value the effect of this parameter becomes negligible. Flattening of the curve is the result of similar efficiency of the extraction and reextraction processes at both interfaces. Since at pH_F = 1, 2-ketogluconic acid is found as a dimer, the permeability factor is lower than the one corresponding to vitamin C. The selectivity factor decreases strongly with increasing carrier concentration, the maximum value (F = 2.1) being reached for amine concentration of 10 g/l. At this concentration value, Amberlite LA-2 preferentially reacts with vitamin C, but the increase of carrier concentration increases the probability of the reaction with 2-ketogluconic.

4. Conclusions

By analysing the experimental data obtained, it was admitted that it is possible the selective separation vitamin C from the mixture with 2-ketogluconic acid by facilitated pertraction with 10 g/l Amberlite LA-2 dissolved in dichloromethane, at feed phase pH equal to 1.

In conclusion, our investigations showed that pertraction could successfully purify vitamin C from the fermentation broth.

5. Acknowledgements

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