Reactive extraction of 2-keto-gluconic acid
Mechanism and influencing factors

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Abstract
The comparative study on reactive extraction of 2-keto-gluconic acid with Amberlite LA-2 in
three solvents with different dielectric constants indicated that the solvent polarity controls the
extraction mechanism. Thus, the reactive extraction in low-polar solvents (n-heptane, n-butyl acetate)
occurs by means of the interfacial formation of an aminic adduct including two extractant molecules. If
solvent with higher polarity is used (dichloromethane), each reactant participates with one molecule to
the interfacial reaction. Due to the opposite phenomena induced by the pH increase, the optimum
extraction pH was 3.

Keywords: 2-keto-gluconic acid, vitamin C, reactive extraction, Amberlite LA-2, n-heptane, n-butyl
acetate, dichloromethane, distribution coefficient, extraction constant.

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 organism. 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
generate 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 (Reischtein 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 and Roche companies 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-keto-gluconic 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-diketo-gluconic acid using mutant strains of Erwinia sp. The
2,5-diketo-gluconic acid is deoxidized into 2-keto-gluconic acid by a mutant strain of
Corynebacterium sp. [3,4]. A similar method uses Bacillus thuringiensis [4].
The one step fermentation for 2-keto-gluconic 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]. Similar to the Reichstein process, the 2-keto-gluconic acid obtained by one or two steps fermentation methods is finally transformed into vitamin C.

Indifferent of the used method, 2-keto-gluconic acid is inevitably involved in the production of vitamin C. The structural differences between these two compounds are due only to the lactonization (Figure 1), thus their physical and chemical characteristics are rather similar. Therefore, the separation and purification of vitamin C require a lot of difficult stages, with high material and energy consumption. 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 acidic medium at low temperature [3]. Due to vitamin C low stability in aqueous solutions, each step requires 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 preliminary objective of our experiments was to establish the optimum conditions for high selective separation of the two compounds by analyzing the individual extraction mechanism, kinetic and the influencing factors. In this paper the results obtained for individual reactive extraction of 2-keto-gluconic acid from aqueous solutions are presented.

**Materials and method**

The experiments have been carried out using an extraction column with vibratory mixing, which offers high interfacial area and the possibility to reach rapidly the equilibrium state. The laboratory equipment has been described in detail in previous papers [6]. 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 5 mm amplitude. The perforated disk 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 25°C. The resulted emulsion was broken in a centrifugal separator at 8000 rpm.

The initial concentration of 2-keto-gluconic acid in aqueous solution was of 1 g/l (5.15x10⁻³ M). The reactive extraction was made with Amberlite LA-2 solved in three solvents with different dielectric constants (Table 1). The extractant concentration in organic phase was between 0 and 120 g/l (0.32 M). The volumetric ratio of aqueous and organic phase was of 1 (20 ml of each phase).

![Figure 1. Chemical structures of vitamin C and 2-keto-gluconic acid.](image)
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Table 1. Dielectric constants of the used solvents at 20°C [7]

<table>
<thead>
<tr>
<th>Solvent</th>
<th>n-Heptane</th>
<th>n-Butyl acetate</th>
<th>Dichloromethane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dielectric constant</td>
<td>1.90</td>
<td>5.01</td>
<td>9.08</td>
</tr>
</tbody>
</table>

The pH-value of initial aqueous solution was varied between 1 and 6. The pH adjustment was made with a solution of 3% sulfuric acid or 3% sodium hydroxide, function on the prescribed pH-value (the initial pH-value of 2-keto-gluconic aqueous solution were 2.8). 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 the extraction experiments.

The extraction process was analyzed by means of the distribution coefficient. For calculating this parameter, the 2-keto-gluconic acid concentrations in the initial solution was measured by high performance liquid chromatography technique (HPLC) with a Shodex RSpak DE-613 column (6 mm diameter, 150 mm length), provided with VIS detector at 430 nm. The mobile phase was an aqueous solution of 2 mM perchloric acid with a flow rate of 0.5 ml/min. For calculating the acid concentration in organic phase the mass balance has been used.

Results and discussion

The reactive extraction of 2-keto-gluconic acid with Amberlite LA-2 occurs by means of the formation of a strong hydrophobic compound at the interface between the aqueous and organic phases. According to the mechanism of the interfacial reaction, the carboxylic group of the acid is involved in this process. The interfacial interactions between the 2-keto-gluconic acid and Amberlite LA-2, Q, could be of hydrogen bonding type with the undissociated carboxylic groups, or of ionic type, if the acid dissociates in the aqueous solution:

\[
\text{CH}_2\text{OH}-(\text{CHOH})_3\text{-CO-COOH} \text{ (aq)} + n \text{ Q} \text{ (o)} \rightarrow \text{CH}_2\text{OH}-(\text{CHOH})_3\text{-CO-COOH.Q}_n \text{ (o)}
\]

In function of the structures of system components and solvent polarity, the acidic or aminic adducts could be formed at the interface [8]. But, as it was observed for reactive extraction with Amberlite LA-2 of other compounds having voluminous molecules and due to the lower initial concentration of 2-keto-gluconic acid, it could be assumed that the formation of acidic adducts is steric hindered [8,9]. Therefore, the interfacial compounds could be of ammonium salt type, formed by neutralization of the carboxylic group with one extractant molecule, or of aminic adducts type, where \( n \geq 2 \). In the second case, the formation of these molecular associations is more pronounced in low-polar solvents and increases the hydrophobicity of the interfacial compound [9].

The pH-value of aqueous phase exhibits a significant influence on reactive extraction degree. Thus, indifferent of the solvent type, Figure 2 indicates that for a certain pH-value of aqueous solution (pH=3) the distribution coefficient reaches a maximum value.

![Figure 2. Influence of pH-value of aqueous solution on distribution coefficient of 2-keto-gluconic acid between water and the three organic solvents (Amberlite LA-2 concentration = 40 g/l).](image-url)
The maximum of distribution coefficient 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 forms [10], the carboxylic group being unable to react with the extractant. 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. But, the pH increase induces the dissociation of the carboxylic group (pKₐ = 2.66 at 25°C [11]) and, consequently, the reduction of the reactive extraction efficiency.

For analyzing the extraction mechanism of 2-keto-gluconic acid, R-COOH, with Amberlite LA-2, Q, in the three considered solvents, it was assumed that n extractant molecules and one acid molecule participate to the formation of the interfacial compound, through ionic and hydrogen bonds. Thus, the reactive extraction can be described by the following interfacial equilibrium:

\[
R\text{-COOH}_{(aq)} + n\text{ Q}_{(o)} \rightleftharpoons R\text{-COOH.Q}_{n(o)}
\]

Therefore, the distribution coefficient, D, is calculated with the relationship:

\[
D = \frac{[R\text{-COOH.Q}_{n(o)}]}{[R\text{-COOH}_{(aq)}]}
\]

where \([R\text{-COOH}_{(aq)}]\) and \([R\text{-COOH.Q}_{n(o)}]\) symbolize the overall concentrations of 2-keto-gluconic acid and extracted compound at the equilibrium state.

According to the interfacial equilibrium, the extraction constant, \(K_{E}\), can be calculated with the following expression:

\[
K_{E} = \frac{[R\text{-COOH.Q}_{n(o)}]}{[R\text{-COOH}_{(aq)}][Q_{(o)}]^n}
\]

\[
\Rightarrow [R\text{-COOH.E}_{n(o)}] = K_{E} \cdot [R\text{-COOH}_{(aq)}][Q_{(o)}]^n
\]

The study on the mechanism of reactive extraction with Amberlite LA-2 was carried out at optimum pH=3, therefore the concentration of undissociated 2-keto-gluconic acid from aqueous phase, \([R\text{-COOH}_{(aq)}]\), is calculated by means of its overall concentration in aqueous phase, \([R\text{-COOH}_{(aq)}]\), and the dissociated acid concentration, \([R\text{-COO}^-_{(aq)}]\). The dissociation constant, \(K_s\), corresponds to the following dissociation equilibrium:

\[
R\text{-COOH} \rightleftharpoons R\text{-COO}^- + H^+
\]

and is determined with the relationship:

\[
K_s = \frac{[R\text{-COO}^-_{(aq)}][H^+]}{[R\text{-COOH}_{(aq)}]}
\]

Thus, the concentration of undissociated 2-keto-gluconic acid is:

\[
[R\text{-COH}_{(aq)}] = [R\text{-COOH}_{(aq)}] - [R\text{-COO}^-_{(aq)}]
\]

\[
[R\text{-COO}^-_{(aq)}] = K_s \frac{[R\text{-COOH}_{(aq)}]}{[H^+]}\]

\[
\Rightarrow [R\text{-COOH}_{(aq)}] = \frac{[R\text{-COOH}_{(aq)}]}{1 + K_s \frac{[R\text{-COOH}_{(aq)}]}{[H^+]}}
\]
Therefore, by combining the eqs. (1), (3) and (7), the following expression for the distribution
coefficent, D, is obtained:

\[
D = K_E \cdot \left[Q_{(o)}\right]^n \cdot \left(1 + \frac{K_s}{[H^+]}\right)
\] (8)

The correlation (8) represents in logarithmic form the equation of a straight line:

\[
\ln D - \ln \left(1 + \frac{K_s}{[H^+]}\right) = \ln K_E + n \cdot \ln [Q_{(o)}]
\] (9)

Because the initial concentration of extractant is higher than the initial concentration
of 2-keto-gluconic acid, \([Q_{(o)}]\) could be assumed to be the initial concentration of Amberlite
LA-2 in organic phase. Consequently, from the slope of the straight line given by eq. (9) it is
possible to determine the number of extractant molecules, n, which participate to the formation
of the interfacial compound, and from its intercept the value of extraction constant, K_E.

For determining the number of extractant molecules which reacts with 2-keto-gluconic
acid, in correlation with the organic phase polarity, the influence of extractant concentration
on extraction efficiency has been analyzed for n-heptane, n-butyl acetate and
dichloromethane, respectively (Figure 3).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Influence of Amberlite LA-2 concentration on distribution coefficient of 2-keto-gluconic acid
between water and the three organic solvents (pH=3).}
\end{figure}

The experimental results suggest that the influence of extractant concentration
becomes more important with the decrease of the solvent polarity, owing to the formation of
the aminic adducts with higher hydrophobicity.

By means of the experimental data from Figure 3 and by plotting the eq. (9), the
straight lines from Figure 4 have been obtained for three studied solvents.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Graphical representation of the straight lines given by eq. (9) for the three solvents.}
\end{figure}
The values of the straight lines slope depend on the solvent polarity, varying as follows:

- n-heptane \( n = 2.11 \)
- n-butyl acetate \( n = 1.87 \)
- dichloromethane \( n = 0.94 \)

The obtained values indicate the modification of the chemical structure of interfacial compound in function of the solvent polarity. As it was previously presented, the dielectric constant is considered a characteristic of solvent-solute local interactions, inducing the limitation of solute solvation by solvent or extractant, due to the presence of ionizable groups in the solute chemical structure [12]. In the studied case, the reactive extraction of 2-keto-gluconic acid with Amberlite LA-2 in low-polar solvents (n-heptane, n-butyl acetate) occurs by means of the interfacial formation of an aminic adduct with two extractant molecules. If solvent with higher polarity is used (dichloromethane), each reactant participates with one molecule to the interfacial reaction.

The cumulated effect of the pH-value and solvent dielectric constant on distribution coefficient of 2-keto-gluconic acid is plotted in Figure 5.

Moreover, the solvent polarity controls the extraction constant through its influence on the separation mechanism. For the reactive extraction of 2-keto-gluconic acid, in Table 2 are given the values of extraction constants graphically determined for the considered solvents.

**Table 2.** Expressions and values of extraction constants for the studied extraction systems

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Extraction constant</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Heptane</td>
<td>( K_E = \left[ R - COOH.Q_{2(o)} \right] / \left[ R - COOH_{(aq)} \right] \left[ Q_{(o)} \right] \langle E \rangle )</td>
<td>4.48 ((l^2/mole^2))</td>
</tr>
<tr>
<td>n-Butyl acetate</td>
<td>( K_E = \left[ R - COOH.Q_{2(o)} \right] / \left[ R - COOH_{(aq)} \right] \left[ Q_{(o)} \right] \langle E \rangle )</td>
<td>2.72 ((l^2/mole^2))</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>( K_E = \left[ R - COOH.Q_{(o)} \right] / \left[ R - COOH_{(aq)} \right] \left[ Q_{(o)} \right] \langle E \rangle )</td>
<td>1.22 ((l/mole))</td>
</tr>
</tbody>
</table>
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From Table 2 it can be observed that the formation of aminic adducts exhibits a favorable effect on the interfacial equilibrium, inducing its movement to the right side and, implicitly, the solubilization of 2-keto-gluconic acid into the organic phase. This phenomenon counteracts partially the negative effect of the lower polarity of n-heptane and n-butyl acetate.

Conclusions

The studies on reactive extraction of 2-keto-gluconic acid with Amberlite LA-2 in three solvents with different dielectric constants (n-heptane, n-butyl acetate, dichloromethane) indicated that the separation occurs by means of an interfacial reaction controlled by the solvent polarity. Thus, if the extracted phase is n-heptane or 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.

The formation of aminic adducts exhibits a positive effect on the interfacial equilibrium and, consequently, on the solubilization of 2-keto-gluconic acid in the organic phase.

Due to the effects induced by the increase of aqueous phase pH on the acid dimerization and dissociation, respectively, the maximum value of distribution coefficient has been reached at pH=3.

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References