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Cloud Point Extraction of \( \alpha \)-Amino Acids

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Cloud point extraction with a polyethoxylated alcohol (Oxo-C\(n\) E\(x\)) is used to separate five \( \alpha \)-amino acids: alanine, valine, leucine, isoleucine, and phenylalanine (0.75 wt.% in water), and their extraction efficiencies are compared. The variables affecting phase separation and extraction (wt.% surfactant and equilibrium temperature) are optimized using experimental design. The four responses are: percentage of solute extracted (E), residual concentrations of solute (amino acid) and surfactant in the dilute phase, and volume fraction of coacervate at equilibrium. E increases with surfactant concentration and amino acid hydrophobicity in the following order: alanine < valine < leucine < isoleucine < phenylalanine, with respective maximum values: 73, 74, 76, 78.5, and 95%, and decreases with a temperature rise. It also makes sense that aspartic and glutamic acids, much more hydrophilic, are poorly extracted (E \( \sim \) 10%). The trend observed is consistent with water/n-octanol partition coefficient (Log P) of amino acids in pure water. A more detailed study is presented for alanine and phenylalanine. Addition of sodium sulphate or cetylammmonium bromide greatly raises extraction rates.

Keywords \( \alpha \)-amino acids; cloud point; coacervate; extraction; non-ionic surfactant

INTRODUCTION

The extraction and separation of organic compounds have become of great interest as biotechnological or industrial processes (1, 2). In general, substances produced in aqueous media should be separated from impurities or by-products. Data research projects of the EU confirmed antibiotics and other pharmaceuticals are present in sewage and natural waters. In some cases, metabolites were also found in drinking water sources. The removal rate of individual compounds through a waste water treatment is variable, and some standard removal techniques cannot eliminate all the compounds (3). In particular, amino acid separation and determination have become a very important objective in analytical chemistry, since their metabolites are present in a variety of biological, industrial and environmental samples (4). Several methods have been studied as an alternative to the degradation of amino acids (5–10) and various techniques are used for this purpose, such as liquid membranes, ion exchange, chromatography, filtration, evaporation, reverse osmosis, or electrodialysis (3–11). The development of new methods for water treatment is still in progress. Conventional technologies for water disinfection such as chlorination and ozonation, may lead to the formation of harmful substances and by-products (trihalomethanes, for example) (9–12). In the past decades, the interest in the use of aqueous micellar solutions in the field of separation science (13), especially using polyethoxylated alcohols as biodegradable nonionic surfactants, has been growing. The present work concerns the study of cloud point extraction (CPE) as a method of recovery and valorization of several \( \alpha \)-amino acids from aqueous solutions, using the powerful solubilizing property of nonionic surfactant aqueous solutions. In fact, above a cloud point curve, representing a line of lower consolubility contours, \( T_c \), aqueous solutions of most polyethoxylated nonionics (or polyethylene glycols in the presence of electrolyte) form two phases: a surfactant-rich coacervate and a dilute phase (14, 15). In the latter, surfactant concentration is close to its critical micelle concentration (cmc). Therefore, thanks to micellar solubilization, the solute initially present in the solution may be favorably extracted into the surfactant-rich phase after increasing the temperature above \( T_c \). So far, many compounds were extracted using cloud point extraction (16): metal ions (17, 18), organic compounds (19–26), and proteins (27, 28). Amino acid separation and recovery, rather than degradation, may be relevant, e.g., in wastewater of amino acid producing industry. To the best of our knowledge, amino acid extraction has been attempted with reverse micelle system (29) and with aqueous two-phase extraction (PEG-salt system), (30) but not with nonionic surfactant systems.

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MATERIALS AND METHODS

Materials

The surfactant used, a polyethoxylated alcohol: Oxo-C₁₀E₄, having the average formula C₁₀H₂₁(OCH₂CH₂)₄OH, with a cloud point (Tc = 20 °C at 1 wt.% in water) was a gift from SEPPIC (Castres, France). The amino acids: aspartic and glutamic acids, alanine, valine, leucine, isoleucine, and phenylalanine, were supplied by Sigma-Aldrich.

Ninhydrin, dimethylsulfoxide (DMSO), sodium acetate, Na₂SO₄, and cetyltrimethylammonium bromide (CTAB) were purchased from Prolabo.

Methods

Cloud Point Measurement and Extraction Experiments

The cloud points were measured with a Mettler FP 900 device, consisting of an oven (FP900), a control unit, and several measuring cells. The cell temperature measurement was performed with a highly accurate Pt100 sensor (probe), integrated in the body of the furnace. In the lower part of the cloud point measuring cell, PF81C, an optical fiber illuminates the three specimens. The light passing through the specimens is converted by three photoelectric cells into electrical signals proportional to the transmitted intensity. The light transmission is measured continuously while the cell temperature increases linearly at the heating rate chosen. The cloud point is the temperature at which the solution becomes cloudy, as a result of the appearance of a second phase.

For the extraction tests, 10 mL of solution containing the surfactant (1–6 wt.%) and the solute (amino acid at 0.75 wt.%) in deionized water were heated in a precise oven for 2 h. The volumes of both phases were then noted. A small amount of the dilute phase was taken using a syringe and analyzed.

Analysis

Surfactant (Oxo-C₁₀E₄) determination in the dilute phase was achieved by reverse phase high performance liquid chromatography (HPLC) under the following conditions: RP18 column (ODS), 95 bar pressure, eluent H₂O/CH₃CN/CH₃OH, 7.5/60/32.5 (vol), flow rate 1 mL/min, light scattering detector (LSD31, EUROSEP Instruments). Three parameters allow optimizing the sensitivity of the detector: the air flow rate, or pressure (1 bar) in the nebulizer, the temperature of the evaporator (55°C), and the gain of the photomultiplier (400 mV).

The amino acids were determined with freshly prepared ninhydrin reagent, consisting of 10 mL of sodium acetate buffer solution at pH = 5.4, 0.8 g of ninhydrin (2,2-dihydroxyindan-1,3-dione), 0.12 g of hydindantin (2,2’-dihydroxy-1H,1’H-2,2’-biindene-1,1’3,3’(2H,2’H)-tetrone), and 30 mL of DMSO (31). For the assay, 1.0 mL of ninhydrin reagent was mixed with 1.0 mL of the bottom phase (dilute phase) of the amino acid extraction test in a capped vial. The vial was shaken by hand, and then transferred to a water bath at 100°C for 30 min to allow complete reaction. After cooling to room temperature, the sample was introduced into a cuvette and the absorbance at 570 nm (Ruhemann purple), measured with a UV–vis spectrophotometer (SAFAS type MC2, photometric accuracy of ± 0.002 absorbance unit), was compared with that of a blank sample (amino acid replaced with deionized water).

RESULTS AND DISCUSSION

Binary and Pseudo-Binary Phase Diagrams

In general, organic solubilizates can interact with the surfactant polar head group or with its hydrophobic chain in the micelle, thus modifying surfactant cloud point according to their chemical nature (19–26, 28, 32–35). As an example, Fig. 1 shows the effect of alanine, phenylalanine, aspartic acid, and glutamic acid on the cloud point curve of Oxo-C₁₀E₄. A significant interaction between the first two, rather “hydrophobic” amino acids and the surfactant induces a cloud point increase, corresponding to an enhanced surfactant solubility in water (28, 32, 33). Consistently, the hydrophilic aspartic and glutamic acids cause a weak depression of the cloud point. Therefore, the cloud point change of the surfactant in the presence of amino acid is related to solute hydrophobicity (Fig. 1 and Table 1). Furthermore, even at very low concentration (0.1 wt.%), the presence of CTAB significantly enhances the cloud point of Oxo-C₁₀E₄ (Fig. 2). In fact, the incorporation of ionic surfactant into the nonionic micelles causes electrostatic repulsion between the micelles, thus hindering coacervate formation and raising the cloud point (33, 34). On the contrary, the cloud point lowering of Oxo-C₁₀E₄ by sodium sulphate (Fig. 2) is due to the salting-out of the surfactant induced by the solvated electrolyte. Indeed, as a well-known structure maker (36), the sulphate ion induces hydrogen bond weakening between surfactant ethylene oxide units and water molecules, making them less available to hydrate micellar aggregates (34, 35). Since Na⁺ does not form complexes with polyethoxylated surfactants, it also salts the surfactant out by dehydration (34).

**Table 1**

<table>
<thead>
<tr>
<th>Wt. % of Oxo-C₁₀E₄</th>
<th>Phenylalanine</th>
<th>Alanine</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.75 %</td>
<td>Oxo-C₁₀E₄</td>
<td>Oxo-C₁₀E₄</td>
</tr>
<tr>
<td>0.75 %</td>
<td>Oxo-C₁₀E₄</td>
<td>Oxo-C₁₀E₄</td>
</tr>
</tbody>
</table>

**Fig. 1.** Effect of amino acids on the cloud point curve of Oxo-C₁₀E₄.
TABLE 1

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Hydropathy index (40)</th>
<th>Water solubility (g/L) (42)</th>
<th>Log P (37)</th>
<th>E (%) at 5 wt. % of Oxo-C_{10}E_{4}</th>
<th>Extraction temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>1.8</td>
<td>166.9</td>
<td>−2.85</td>
<td>73</td>
<td>54</td>
</tr>
<tr>
<td>Valine</td>
<td>4.2</td>
<td>88.5</td>
<td>−2.26</td>
<td>74</td>
<td>55</td>
</tr>
<tr>
<td>Leucine</td>
<td>3.8</td>
<td>23.8</td>
<td>−1.52</td>
<td>76</td>
<td>54</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>4.5</td>
<td>34.2</td>
<td>−1.70</td>
<td>78.5</td>
<td>54</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>2.8</td>
<td>27.9</td>
<td>−1.38</td>
<td>95</td>
<td>54</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>−3.5</td>
<td>8.64</td>
<td>−3.69</td>
<td>11</td>
<td>20</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>−3.5</td>
<td>7.78</td>
<td>−3.89</td>
<td>10</td>
<td>20</td>
</tr>
</tbody>
</table>

at iso-electric pH and 25°C (41).

FIG. 2. Effects of CTAB and sodium sulfate on the cloud point of the system Oxo-C_{10}E_{4}.

**Extraction Efficiency**

**Effect of pH**

The presence of both basic and acidic functional groups in amino acid molecules induces an internal transfer of a hydrogen ion from the -COOH group to the -NH_{2} group to give a zwitterion (Eq. 1), so that according to the pH value, the predominant species is cationic (in acidic medium), zwitterionic (between the two pK_{a} values) or anionic (in alkaline medium). Amino acids show a minimum aqueous solubility at their isoelectric point (pH_{i}), that is, the pH value at which the molecule bears no net electric charge.

\[
\begin{align*}
\text{H}_2\text{N}-\text{CH}-\text{COOH} & \quad \text{In acidic medium} \\
\text{H}_2\text{N}^{+}-\text{CH}-\text{COO}^- & \quad \text{At the isoelectric point} \\
\text{H}_2\text{N}^{+}-\text{CH}-\text{COO}^- & \quad \text{In alkaline medium}
\end{align*}
\]

Now, since ionic species are more soluble in water, they will not be readily solubilized in nonionic micelles. Consequently, only a small amount of ionized solute can be extracted and it makes sense that, as shown in Fig. 3, the maximum extraction ratio (E) is located in the pH region between the two pK_{a} values of the amino acids and practically constant for pH = pH_{i} ± 2 (for alanine, pK_{1} = 2.34, pK_{2} = 9.69, pH_{i} = 6; for phenylalanine pK_{1} = 1.83, pK_{2} = 9.13, pH_{i} = 5.5) (41).

This also entails that pH is the key parameter for surfactant regeneration. Several works have been done on surfactant recovery and recycling after CPE, by a simple pH control (19–22). This requires two steps: back-extraction of acidic or alkaline solutes from coacervate and regeneration of the neutral coacervate.

**Effect of Amino Acid Structure**

The extraction percentages obtained for seven α-amino acids (0.75 wt.% in pure water) with Oxo-C_{10}E_{4} aqueous mixtures above their cloud point are illustrated in Fig. 4 and the results corresponding to the 5 wt.% mixtures are reported in Table 1.

FIG. 3. Effect of pH on the extraction extent of amino acids, E (%).
Effect of Surfactant Concentration and Temperature on the Extraction Parameters: Modeling of Extraction Results

The results of amino acid extraction from their 7.5 g/L (0.75 wt.%) aqueous solutions with Oxo-C_{10}E_{4}, according to two variables: wt.% surfactant (X_t), and temperature (T), are expressed by four responses (Y): percentage of extracted solute (E), residual concentrations of solute (X_s), and surfactant (X_{s,w}) in the dilute phase, and coacervate volume fraction at equilibrium (\phi_C) (19–26). Process optimization implies the maximization of E and the minimization of X_{s,w}, X_{s,w}, and \phi_C. For each parameter determined by considering a central composite design (38), the results were analyzed by an empirical fitting. In this method, the experimental values are used to determine the polynomial model constants to be adjusted. The models were checked by plotting computed data against experimental results. The quadratic correlation was chosen to give the slope and the regression coefficient (R^2) the closest to unity.

\[ Y = a_0 + a_1 X_t + a_2 T + a_{12} X_t T + a_{11} X_t^2 + a_{22} T^2 \]  

(2)

Such a correlation allows building the response surface.

For alanine and phenylalanine, the quadratic equations for the properties (E, \(X_{s,w}\), \(X_{t,w}\), and \(\phi_C\)), whose reliability was checked, are as follows:

\[ \text{E}_{\text{alanine}} = 326.22 + 32.96 X_t - 11.68 T - 0.46 X_t T - 0.67 X_t^2 + 0.12 T^2 \]  

(3)

\[ \text{E}_{\text{phenylalanine}} = 226.84 + 3.08 X_t - 5.13 T + 0.21 X_t T - 0.99 X_t^2 + 0.04 T^2 \]  

(4)

\[ X_{s,w}(\text{alanine}) = -149.25 - 1.00 X_t + 6.25 T - 0.06 T^2 \]  

(5)

\[ X_{s,w}(\text{phenylalanine}) = -0.12 + 0.10 X_t + 0.08 T - 0.02 X_t T + 0.06 X_t^2 \]  

(6)

\[ \phi_C(\text{alanine}) = -0.46 + 0.17 X_t + 0.05 T \]  

(7)

\[ \phi_C(\text{phenylalanine}) = 4.12 + 1.32 X_t - 0.16 T - 0.02 X_t T - 0.01 X_t^2 \]  

(8)

\[ \text{X}_{t,w}(\text{alanine}) = 41.78 + 4.40 X_t - 1.28 T - 0.098 X_t T + 0.18 X_t^2 + 0.01 T^2 \]  

(9)

\[ \text{X}_{t,w}(\text{phenylalanine}) = 30.21 + 4.95 X_t - 0.74 T - 0.09 X_t T + 0.09 X_t^2 + 0.01 T^2 \]  

(10)

Extraction Efficiency

Figure 5 represents the three-dimensional isoresponse curves of the studied properties smoothed by the quadratic model (Eqs. 3 and 4). As already shown in Fig. 4, the extent of amino acid extraction (E) increases with \(X_t\), but only weakly beyond 5%. At 5 wt.% Oxo-C_{10}E_{4}, E reaches 94% and 71% for phenylalanine and alanine, respectively. By increasing the hydrophobic character of the amino acid, the presence of a benzene ring in phenylalanine has a positive effect on amino acid solubilization in coacervate micelles. On the other hand, a temperature rise has a slight effect of amino acid extraction. This trend has been observed in other extraction systems (20–26). The most favorable areas for cloud point are thus located in the darker color zones in Fig. 5.

Residual Concentration of Amino Acid (\(X_{s,w}\))

Figure 6 represents the three-dimensional isoresponse curves of the studied property (\(X_{s,w}\)), smoothed by the quadratic model (Eqs. 5 and 6). The concentration of amino acid in the dilute phase, \(X_{s,w}\), decreases as \(X_t\) increases, but varies only slightly with a temperature rise. Hence, the first contact between surfactant and effluent solutions allows 4- and
FIG. 5. Three-dimensional isosurface curves smoothed by a quadratic model, \( E = f(X_t, T) \), calculated by the quadratic model (Eqs. 2 and 3).

FIG. 6. Three-dimensional isosurface curves smoothed by a quadratic model, \( X_{s,w} = f(X_t, T) \), calculated by the quadratic model (Eqs. 4 and 5).

14-fold alanine and phenylalanine concentration reductions, respectively (Table 2).

**Residual Concentration of Surfactant \((X_{s,w})\)**

The behavior of \( X_{s,w} \) vs. \( X_t \) and \( T \) is shown in Fig. 7 (smoothed by the quadratic model Eqs. 7 and 8). The residual concentration of surfactant is low at high temperature and low surfactant concentration. These results are in good agreement with previous studies with other polyethoxylated alcohols (21–26). \( X_{s,w} \) is a very important parameter. A high loss of surfactant in the dilute phase can compromise process reliability. Indeed, the presence of another contaminant in the dilute phase is sufficient to make the process useless. Although Oxo-C\(_{9-15}\)E\(_{2-10}\) compounds are readily biodegradable and do not give rise to bioaccumulation, they are considered as toxic to fish (43), so that it would be detrimental to release and squander them in the dilute phase.

**Volume Fraction of Coacervate**

In order to increase the concentration factor of solute, a minimal volume fraction of coacervate \((\phi_c)\) should be obtained. In fact, according to Fig. 8, and as predicted by Fig. 1, the smoothed value of \( \phi_c \) using Eqs. (9) and (10) is low at high temperature and low surfactant concentration. Now, higher surfactant concentrations lead to higher \( E \) and lower \( X_{s,w} \) values. So, the optimization of the process needs to compromise over the four studied parameters \( E, X_{s,w}, X_{t,w}, \) and \( \phi_c \) (20–26). On the basis of this finding, optimal values of \( \phi_c \) (i.e., 0.2) were obtained using 4 wt.% Dowfax 20B10 and 4 wt.% Oxo-C\(_{10}\)E\(_4\) at 50°C and 40°C, respectively.

**Effect of Salt on the Rate of Extraction \((E)\)**

In general, electrolyte addition induces coacervate volume fraction reduction due to the cloud point lowering of the surfactant solution (22). The presence of \( \text{Na}_2\text{SO}_4 \) decreases the
TABLE 2

Some experimental results of the extraction parameters (E, $X_{s,w}$, $X_{t,w}$, $\phi_C$ and $X_{s,0}/X_{s,w}$)

<table>
<thead>
<tr>
<th>[X_T (wt.%), T(°C)]</th>
<th>E (%)</th>
<th>$X_{s,w}$ (mg/L)</th>
<th>$X_{t,w}$ (wt.%)</th>
<th>$\phi_C$</th>
<th>$X_{s,0}/X_{s,w}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[2, 42]</td>
<td>68.332</td>
<td>R²=0.984</td>
<td>13.006</td>
<td>0.264</td>
<td>3.158</td>
</tr>
<tr>
<td>[2, 45]</td>
<td>66.624</td>
<td>2.503</td>
<td>12.562</td>
<td>0.214</td>
<td>2.996</td>
</tr>
<tr>
<td>[2, 48]</td>
<td>63.012</td>
<td>2.774</td>
<td>11.769</td>
<td>0.114</td>
<td>2.704</td>
</tr>
<tr>
<td>[3, 45]</td>
<td>72.563</td>
<td>2.058</td>
<td>13.236</td>
<td>0.302</td>
<td>3.645</td>
</tr>
<tr>
<td>[3, 48]</td>
<td>69.823</td>
<td>2.263</td>
<td>11.893</td>
<td>0.224</td>
<td>3.314</td>
</tr>
<tr>
<td>[4, 51]</td>
<td>71.095</td>
<td>2.025</td>
<td>12.456</td>
<td>0.101</td>
<td>3.703</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[2, 48]</td>
<td>81.624</td>
<td>R²=0.979</td>
<td>11.145</td>
<td>0.241</td>
<td>5.442</td>
</tr>
<tr>
<td>[2, 50]</td>
<td>80.542</td>
<td>1.378</td>
<td>10.896</td>
<td>0.125</td>
<td>5.139</td>
</tr>
<tr>
<td>[2, 52]</td>
<td>78.893</td>
<td>1.583</td>
<td>10.025</td>
<td>0.035</td>
<td>4.738</td>
</tr>
<tr>
<td>[3, 50]</td>
<td>88.013</td>
<td>0.899</td>
<td>13.112</td>
<td>0.312</td>
<td>8.342</td>
</tr>
<tr>
<td>[3, 52]</td>
<td>87.236</td>
<td>0.957</td>
<td>11.679</td>
<td>0.100</td>
<td>7.835</td>
</tr>
<tr>
<td>[4, 54]</td>
<td>93.123</td>
<td>0.515</td>
<td>11.892</td>
<td>0.012</td>
<td>14.541</td>
</tr>
</tbody>
</table>

$X_{s,0}=7.5g/L$ (initial concentration of amino acid).

cloud point (T_c) of Oxo-C_{10}E_4 (Fig. 2) and increases the values of (T−T_c). Therefore, in the presence of salts, at a given temperature, smaller coacervate volumes with high surfactant concentration were obtained (results not shown). Inducing a decrease of amino acid aqueous solubility by a salting-out phenomenon, the presence of the electrolyte increases the extraction extent (E) of amino acid (Fig. 9). According to Saito and Shinoda (39), the addition of electrolyte to nonionic surfactant solutions increases their hydrocarbon solubilization capacity, by lowering surfactant cmc, so that, at a given surfactant concentration, more micelles are present.

Effect of Cetyltrimethylammonium Bromide (CTAB) on the Extraction Yield (E): Extraction with Mixed Micelles

When nonionic and ionic surfactants co-exist in an environment, both surfactant species can interact and provide additional beneficial properties to the system. In most cases, mixed micelles form, that can lead to synergic effects (44). Figure 10 shows the synergic effect of the Oxo-C_{10}E_4/CTAB system toward CPE of alanine and phenylalanine, that is, the extraction extent (E) is highly improved with the mixed micelle system compared with that obtained using neutral micelle system (at 0 wt.% of CTAB). In pure aqueous solution
the dipolar ion (zwitterionic) form \( (^-OOC-RCH-NH_3^+) \) of the amino acid predominates. Thus, the cationic surfactant (CTAB) reinforces micellar solubilization thanks to Coulombic interactions.

**CONCLUSIONS**

Coacervate extraction (CPE) was used to separate amino acids from water. The best compromise between the parameters governing the extraction effectiveness (surfactant concentration and temperature) was found using a suitable experimental design and three-dimensional empirical curve fitting. In pure water, extractions at temperatures ranging between 43°C and 55°C yielded extraction extents between 10 and 95%, depending on amino acid structure. CPE of amino acids is governed by their hydrophobicity. These phenomena can have an application in separation of hydrophobic and hydrophilic amino acids. Low surfactant concentration (≤ 4 wt.%) should be used to have a smaller volume fraction of coacervate. Na\(_2\)SO\(_4\), as a salting-out electrolyte, and CTAB, as a mixed micelle forming surfactant, increased the extraction extent of amino acids. The extraction of the solute was high within the pH range situated between the two pKa values of amino acids. Thus, pH is a key parameter for surfactant recovery and recycling in CPE of amino acids.
**NOMENCLATURE**

Symbols

- $\text{cmc}$: Critical micelle concentration
- $E$: Extraction efficiency (%)
- $\log P$: log octanol/water partition coefficient
- $T_e$: Clouding temperature or cloud point (°C)
- $X_{aw}$: Residual concentration of amino acid (g/L)
- $X_i$: Residual concentration of surfactant (g/L)
- $\phi_c$: Volume fraction of coacervate

**REFERENCES**


15. Refer to the references for detailed information on each study.
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