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# Determination of Cobalt in Foods Using $\beta$ -Cyclodextrin Epichlorohydrin Polymer Functionalized with 1-(2-Pyridylazo)-2-naphthol

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## ABSTRACT

$\beta$ -Cyclodextrin epichlorohydrin polymer ( $\beta$ -CDEP) is functionalized with 1-(2-pyridylazo)-2-naphthol (PAN) and its application for the determination of cobalt by a direct polymer phase spectrophotometry is proposed. Cobalt(II) ions are included/adsorbed by the synthesized polymer to form cobalt-PAN complex. The maximum absorbance of the complex in  $\beta$ -CDEP was 573 nm. The working range of the calibration graph was 0.04-0.36  $\mu\text{g/mL}$  of cobalt. The interferences from copper, chromium, iron, manganese, nickel, cadmium, zinc, and molybdenum that form colored species with PAN in the polymer phase were investigated. The method was successfully applied to the determination of cobalt in food samples with satisfactory results and recoveries of 95.8-103.7%.

Key words:  $\beta$ -cyclodextrin epichlorohydrin polymer, polymer phase spectrophotometry, cobalt, PAN

## INTRODUCTION

$\beta$ -Cyclodextrin ( $\beta$ -CD) is a very stable oligosaccharide that is composed of seven glucose units. It can form inclusion complexes with several organic compounds by incorporating them into the hydrophobic cavities of  $\beta$ -CD. Szejtli<sup>(1)</sup> has reviewed possible or already realized applications of  $\beta$ -CD in the fields of food, commodity, pharmaceutical industry, chemical engineering, and agriculture.  $\beta$ -Cyclodextrin epichlorohydrin polymer ( $\beta$ -CDEP) that was cross-linked by epoxy chloropropane<sup>(2)</sup> still retains the inclusion property of  $\beta$ -CD.  $\beta$ -CDEP used in present investigation is a transparent, colorless solid that is insoluble in aqueous solutions. Its cavity is fairly hydrophobic and can be applied to include organic compounds that contain hydrophobic aromatic groups to form inclusion complexes. A few applications of  $\beta$ -CDEP have been reported in previous studies<sup>(3-8)</sup>.

Cobalt is an important nutritional element and is a metallic component of vitamin B<sub>12</sub>. Several methods for the determination of cobalt have been reported, which mainly focused on spectrophotometry<sup>(9-12)</sup>, flow-injection<sup>(13,14)</sup>, liquid chromatography<sup>(15,16)</sup>, and capillary electrophoresis (CE)<sup>(17)</sup>. In addition, Bae<sup>(18)</sup>, Filichkina<sup>(19)</sup>, and Uchiyama<sup>(20)</sup> reported voltammetry, laser thermal lens spectrometry, and photoacoustics, respectively. However, not all the above mentioned methods are suitable for routine trace determinations. Spectrophotometry, especially extraction-spectrophotometry, has often shown low sensitivity, selectivity and interference from some cations. HPLC and CE have suffered from more or less time-consuming procedures.

In the present work,  $\beta$ -CDEP is functionalized with 1-

(2-pyridylazo)-2-naphthol (PAN) to obtain the modified polymer ( $\beta$ -CDEP-PAN).  $\beta$ -CDEP-PAN can form a colored complex with cobalt, and the trace cobalt is directly determined by the polymer phase spectrophotometry. This method has two important advantages that sensitivity and selectivity are much higher than those of the conventional spectrophotometric method in solution and that concentration and color development of the metal concerned take place simultaneously. It seems to be a useful technique for the determination of trace amounts of cobalt ions. This paper describes a procedure for polymer phase spectrophotometry and the trace amounts of cobalt in laver (a type of edible algae) are determined with PAN and a 5-mm quartz cell.

## MATERIALS AND METHODS

### I. Reagents and Apparatus

#### (I) Apparatus

A Shimadzu UV-240 spectrophotometer (Shimadzu Ltd., Japan) equipped with 5-mm and 10-mm quartz cells was used to measure absorbance and spectra. To release excess solution of the developed color polymer that was packed in a sample cell, a small hole was made at the bottom of 5-mm quartz cell with an emery wheel before use. A digital pH-meter, model Orion 290A (Orion Research Inc., Boston, MA, U.S.A.), was used to measure pH-values of solutions. A thermostatic rotatory shaker, model Peking SHZ-2 (Beijing Analytical Instrument Company, Beijing, China), was used for the inclusion procedures.

#### (II) Reagents

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All chemicals used were of analytical reagent grade. Milli-Q water (Millipore Company, Bedford, Mass., U.S.A.) was used throughout the experiment.

A cobalt standard solution (1010 µg/mL) was obtained from Aldrich (Aldrich Chemical Company Inc., Milwaukee, WI, U.S.A.). An aliquot of the cobalt standard solution was diluted to give a final cobalt (II) concentration of 10 µg/mL in the working standard solution.

PAN solution in ethanol was prepared by dissolving an appropriate amount of PAN (Aldrich Chemical Company Inc., Milwaukee, WI, U.S.A.) in 95% ethanol to give a  $1.0 \times 10^{-4}$  mol/L solution.

β-CDEP was synthesized as described earlier<sup>(2)</sup>. Before use, the polymer was ground and sieved first into below 60, 60-80, and over 100 mesh fractions. The 60-80 mesh fraction was washed with Milli-Q water 5-6 times so as to remove the remaining metal ions. Then, the polymer was dried under vacuum at 100°C and kept at room temperature (25°C) in a desiccator.

Buffer solutions used for pH-values in the range of 1.0-12.0 were prepared by mixing sodium citrate and hydrochloric acid solutions for pH-values in the range of 1.0-4.0, by mixing acetic acid and sodium acetate solutions for pH-values in the range of 4.0-6.5, and by mixing sodium hydroxide and potassium dihydrogen phosphate solutions for pH-values in the range of 6.5-12.0, respectively.

The masking solutions used for iron, nickel, and copper were  $4.4 \times 10^{-3}$  mol/L EDTA and the solutions for chromium, manganese, cadmium, zinc, and molybdenum were 0.1 mol/L nitric acid.

## II. Procedure

### (I) Preparation of β-CDEP-PAN

β-CDEP (60-80 mesh) was shaken mechanically with PAN solutions of different pH-values for 60 min at room temperature (25°C). The polymer was filtered off, washed with Milli-Q water, dried under vacuum, and kept out of light in a desiccator. In order to determine the amount of PAN in β-CDEP-PAN, the amount of PAN in the supernatant solution was determined by measuring its absorbance at 468 nm (the maximum absorption of PAN in the solution) with a 10-mm quartz cell against a reference blank solution that did not contain PAN. The inclusive quantities ( $Q$ ) were calculated as follows:

$$Q = \{[G]_o - [G]_{eq}\} V \times m^{-1}$$

Wherein,  $[G]_o$  is the initial concentration of PAN,  $[G]_{eq}$  is the equilibrium concentration of PAN,  $V$  is the volume of solution and  $m$  is the weight of β-CDEP polymer used.

### (II) Determination of the molar ratio of cobalt to PAN in the polymer phase

β-CDEP-PAN (0.1 g) and 10.0 mL of buffer solution (pH 9.0) were added to a 50-mL stoppered conical flask. The mixture was allowed to stand for approximately 15 min

so that β-CDEP-PAN could be swollen sufficiently and then 5.0 mL of cobalt (II) solution (10 µg/mL) was added and made up to 25 mL with Milli-Q water. After the mixture was shaken mechanically for 30 min, 5.0 mL of the colorless supernatant solution was transferred into a 10-mL volumetric flask, then 1.0 mL of buffer solution (pH 9.0) and 1.0 mL of PAN solution were added and made up to the mark with Milli-Q water and mixed well. The absorbance at 578 nm (the absorption maximum of cobalt-PAN complex in the solution) was measured with a 10-mm quartz cell against a reference blank solution that did not contain cobalt-PAN. The molar ratio of cobalt to PAN in the polymer phase was calculated as follows:

$$R = \{[M]_o - [M]_{eq}\} \times V / (5.5 \times m)$$

Wherein,  $R$  denotes the molar ratio of cobalt to PAN in the polymer phase,  $[M]_o$  is the initial concentration of cobalt (µmol/mL),  $[M]_{eq}$  is the equilibrium concentration of cobalt in the supernatant solution (µmol/mL),  $V$  is the volume of solution (mL),  $m$  is the weight (g) of β-CDEP-PAN polymer used, and 5.5 is the content of PAN in the β-CDEP-PAN (µmol/g) used for analysis.

### (III) Determination of cobalt

β-CDEP-PAN (0.5 g) and 10.0 mL of buffer solution (pH 9.0) were added to a 50-mL stoppered conical flask. The mixture was allowed to stand for approximately 15 min and then 0.5 mL of cobalt(II) solution (10 µg/mL) was added and made up to 25 mL with Milli-Q water. After the mixture was shaken mechanically for 30 min, the colored polymer was transferred into a 5-mm quartz cell using a pipette. The absorbance at 573 nm (the absorption maximum of cobalt-PAN complex species in the polymer phase) was measured against a β-CDEP-PAN blank that did not contain cobalt as a reference.

### (IV) Determination of cobalt in laver

Laver was digested to ash using a general method<sup>(21)</sup>. The ash sample was dissolved with dilute hydrochloric acid and filtered into a 50-mL volumetric flask and made up to the mark with Milli-Q water. A 5-mL aliquot of the samples was transferred into a 50-mL stoppered conical flask, and pH-value of the solution was adjusted to 9.0 with dilute sodium hydroxide. Then 10.0 mL of buffer solution (pH 9.0), 0.5 g of β-CDEP-PAN, and 4 mL of  $4.4 \times 10^{-3}$  mol/L EDTA were added. After 15 min, the mixture was diluted to 25 mL with Milli-Q water and shaken mechanically for 30 min. The colored polymer was filtered and washed with 2 mL of 0.1 mol/L nitric acid three times. The absorbance at 573 nm was determined as described above.

## RESULTS AND DISCUSSION

### I. Effect of pH on the Preparation of β-CDEP-PAN

The results are shown in Figure 1. It can be seen that the inclusive quantities ( $Q$ ) were essentially constant between pH 4.0 and 9.0. It is well known that the cavities of  $\beta$ -CDEP are hydrophobic, therefore neutral, weak polar, and nonpolar molecules are easily included to form their complexes under general conditions. Of course, the size/geometry of the compound, hydrogen bonding between the compound and the secondary hydroxyl groups of  $\beta$ -CDEP/ $\beta$ -CD, van der Waals interaction, and/or hydrophobic interactions are also important factors to the stability of the inclusion complexes. Under the condition of pH 4-9, PAN molecule is neutral and is easily included by  $\beta$ -CDEP. Therefore, pH-values in the range of 4.0-9.0 were selected for the preparation of  $\beta$ -CDEP-PAN.

## II. Conditions for the Measurement of Cobalt (II)

### (I) Absorption spectra

The absorption spectra of PAN and its cobalt complex in the polymer and solution phase are shown in Figure 2. A large increase in absorbance intensity and a blue-shift were observed in the presence of  $\beta$ -CDEP. The maximum absorbance of cobalt-PAN complex was at 573 nm in the polymer phase and at 578 nm in solution phase. The maximum absorbance in the polymer phase moved 5 nm toward the shorter wavelength region than that in solution phase. This result suggests the formation of cobalt-PAN complex and also indicates that the complex was localized in a medium less polar than water, and the state of this complex was highly stabilized. Moreover, the maximum absorbance of PAN was at 468 nm in solution phase and at 472 nm in the polymer phase. Maximum absorbance in the polymer phase moved 4 nm toward the longer wavelength region than that in solution phase. Each spectrum in the polymer phase is similar to that observed in solution phase.

### (II) Effect of shaking time on $\beta$ -CDEP-PAN adsorbing cobalt (II)

In general solid-liquid extractions, in order to adsorb the analytes on the solid adsorbent, the sample solution was often stirred with the adsorbent for a fixed time. In this work, shaking was adopted instead of stirring. No destruction of the polymer particles occurred during shaking, but destruction was often observed when stirred rapidly. The shaking time required for attaining the adsorption equilibrium depended on the volume of the sample solution. Almost all cobalt (II) in a 25-mL sample solution was adsorbed on  $\beta$ -CDEP-PAN within 30 min as shown in Figure 3.

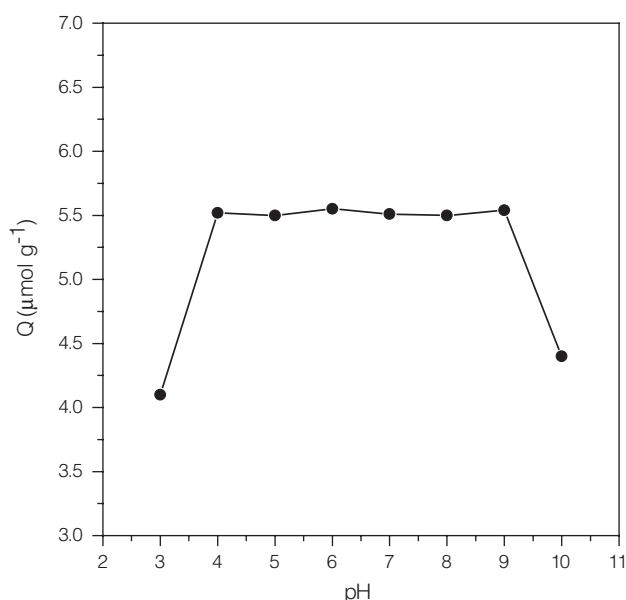
### (III) Effect of pH on $\beta$ -CDEP-PAN adsorbing cobalt (II)

The color of the cobalt complex with  $\beta$ -CDEP-PAN in the polymer phase had the maximum intensity at pH-values between 8.0 and 10.0. The results are the same as those in solution phase. Furthermore,  $\beta$ -CDEP-PAN had the

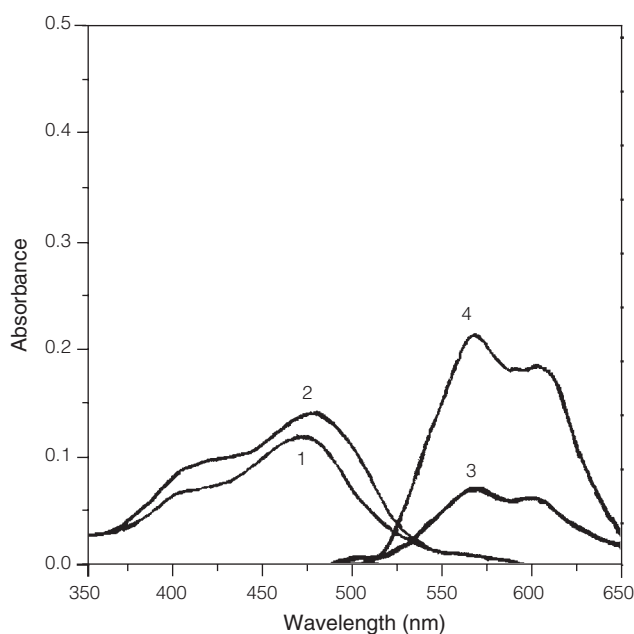
maximum stability at pH-values between 4.0 and 9.0. For the given reasons, the pH-value of 9.0 was selected for analysis.

### (IV) Calibration

A series of standard solutions were prepared in which



**Figure 1.** Effect of pH on the preparation of  $\beta$ -CDEP-PAN. The ionic strength, 0.1 mol/L; PAN,  $1.0 \times 10^{-4}$  mol/L; solution volume, 25 mL; shaking time, 60 min;  $\beta$ -CDEP, 0.5 g.



**Figure 2.** Absorption spectra of PAN and its cobalt complex in  $\beta$ -CDEP.

Solution volume: 25 mL; PAN,  $1.0 \times 10^{-4}$  mol/L; pH, 9.0; Co(II), 2  $\mu\text{g}$ ;  $\beta$ -CDEP, 0.5 g; spectrum 1, PAN in solution phase; 2, PAN in the polymer phase; 3, Co(II)-PAN in solution phase; 4, Co(II)-PAN in  $\beta$ -CDEP phase.

the cobalt concentrations are accurately known. Under the optimum conditions developed (pH, 9.0;  $\beta$ -CDEP-PAN, 0.5 g; shaking time, 30 min), cobalt was adsorbed onto  $\beta$ -CDEP-PAN to form  $\beta$ -CDEP-PAN-cobalt complex and the absorbances of the colored complex were directly measured at 573 nm by the polymer phase spectrophotometry. The calibration curve was obtained by plotting of absorbances (573 nm) against cobalt concentration. Good linearity was obtained for up to 0.36  $\mu\text{g/mL}$  cobalt. The regression curve might be expressed by the equation:  $A_{(573\text{nm})} = 0.0852 X + 0.0067$  ( $R = 0.9986$ ,  $N = 6$ ), where X is the weight ( $\mu\text{g}$ ) of cobalt in the sample solutions of 25 mL. The relative standard deviation (RSD) for nine replicate determinations was 1.5% for 1  $\mu\text{g}$  of cobalt in a 25-mL sample solution. The detection limit of the method was 0.04  $\mu\text{g}$ , which was calculated as three times of the standard deviation of the blank (3s criterion).

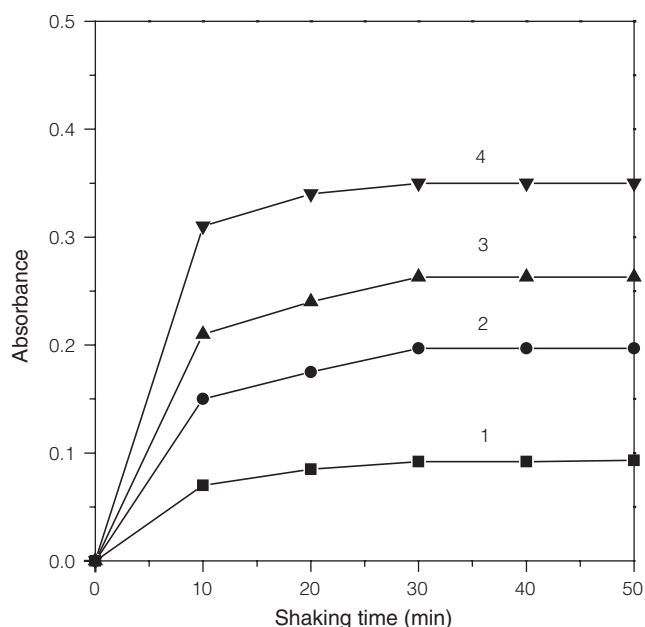
The recoveries of cobalt were in the ranges of 95.8–103.7% for 1  $\mu\text{g}$  cobalt in 25-mL of sample solutions.

#### (V) Sensitivity

The sensitivity of the proposed method had been compared with that of the conventional extraction-spectrophotometric method<sup>(22)</sup>. The sensitivity for triplicate determinations was 43 times as high as that of the previous method with a 25-mL sample solution that contains 1  $\mu\text{g}$  of cobalt (II).

#### (VI) Molar ratio of cobalt to PAN in the polymer phase

In the polymer phase, the molar ratio of cobalt to PAN



**Figure 3.** Effect of shaking time on  $\beta$ -CDEP-PAN adsorbing cobalt (II).

Solution volume, 25 mL; pH, 9.0;  $\beta$ -CDEP-PAN (60–80 mesh), 0.5 g; Co(II): 1, 1  $\mu\text{g}$ ; 2, 2  $\mu\text{g}$ ; 3, 3  $\mu\text{g}$ ; 4, 4  $\mu\text{g}$ .

in the inclusive complex was measured depending on the fact that the addition of cobalt(II) will result in formation of  $\beta$ -CDEP-PAN-cobalt complex. Initially in the sample solution containing no cobalt(II) ion, no complex will be present and the absorbance of the polymer is due solely to  $\beta$ -CDEP-PAN which contains 5.5  $\mu\text{mol}$  of PAN per gram. As cobalt ions were added, a part of the cobalt(II) would be consumed due to formation of the complex in the polymer phase. By the determination of the excess cobalt(II) in the supernatant solution, the molar ratio of cobalt to PAN in the polymer phase could be found to be 1:2 which is identical with that observed in solution phase. From this result, we may suppose that not the whole molecule of PAN, but only one group of the molecule such as a pyridine ring is resided in the cavity of  $\beta$ -CD.

#### (VII) Effect of foreign ions

Cobalt (II) reacts with PAN to form a red complex and then the cobalt (II)-PAN complex is oxidized by oxygen to obtain a more stable green cobalt (III)-PAN complex in solution phase. This complex, once formed, does not decompose in the presence of EDTA, even in a strongly acidic medium. The reaction between cobalt (II) and PAN in the polymer phase is different from that in solution phase that the cobalt (II)-PAN complex is more stable in the polymer phase. In the present study, metal ions did not interfere except for copper, chromium, iron, manganese, nickel, cadmium, zinc, and molybdenum (as shown in Table 1). Iron reacts with PAN to form a colored complex that has a large absorbance at 620 nm. The complex is much more stable even in a strongly acidic medium, but it could be masked with 4 mL of  $4.4 \times 10^{-3}$  mol/L EDTA solution. In the same condition, EDTA could also eliminate the interferences of copper and nickel. The interferences of manganese, cadmium, zinc, chromium, and molybdenum could easily be eliminated because acids can decompose these metal-PAN complexes. After the colored  $\beta$ -CDEP-PAN complexes were macerated in 0.1 mol/L nitric acid and shaken mechanically for about 2 min, these complexes were decomposed and the interferences of the metals were

**Table 1.** Effect of foreign ions on the determination of 2  $\mu\text{g}$  cobalt (solution volume, 25 mL) by the  $\beta$ -CDEP-PAN complex method

Foreign ions	Weight-ratio of Co to foreign ion	Co found ( $\mu\text{g}$ )	Relative error (%)
$\text{Cu}^{2+}$	1:125	2.01	+0.5
$\text{Cr}^{3+}$	1:25	2.08	+4.0
	1:100	2.05	+2.5
$\text{Fe}^{2+}$	1:25	2.09	+4.5
$\text{Fe}^{3+}$	1:10	1.95	-2.5
	1:100	1.89	-5.5
$\text{Mn}^{2+}$	1:50	2.07	+3.5
$\text{Ni}^{2+}$	1:50	2.04	+2.0
$\text{Cd}^{2+}$	1:100	2.06	+3.0
$\text{Zn}^{2+}$	1:10	2.01	+0.5
	1:100	2.03	+1.5
$\text{Mo}^{2+}$	1:20	2.03	+1.5



avoided. In such conditions, no release of PAN from the polymer was observed as a consequence of the treatment with nitric acid.

#### (VIII) Determination of cobalt in laver

The proposed method was applied to the determination of cobalt in laver samples. The results are given in Table 2. The cobalt contents in four different laver samples collected from different regions are in the range of 0.44–0.53  $\mu\text{g/g}$ . The recoveries of cobalt added are in the range of 95.8–103.7%. In order to further validate the proposed method, cobalt contents in the samples were determined simultaneously by atomic absorption spectroscopy (AAS) (Instrument parameters: lamp current, 7 mA; flame, air- $\text{C}_2\text{H}_2$ ; wavelength, 240.7 nm; slit, 0.2 nm) and the results are also shown in Table 2. As demonstrated, the results obtained by the proposed method are in good agreement with those obtained by AAS. Therefore, the developed method is very sensitive and selective.

### CONCLUSIONS

The results demonstrate the application of  $\beta$ -cyclodextrin epichlorohydrin polymer phase spectrophotometry to the determination of micro amounts of cobalt in food samples. The sensitivity and selectivity of the method are much higher than those of the corresponding spectrophotometric method in solution phase.

### ACKNOWLEDGMENTS

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**Table 2.** Trace amounts of cobalt in laver samples

Samples	Co found ( $\mu\text{g/g}$ ) <sup>a</sup>		Co added <sup>b</sup> ( $\mu\text{g}$ )	Recovery (%)
	Present method	AAS		
Laver 1	0.50 $\pm$ 0.02	0.51 $\pm$ 0.02	1.0	95.8
Laver 2	0.44 $\pm$ 0.01	0.45 $\pm$ 0.01	1.0	102.3
Laver 3	0.53 $\pm$ 0.02	0.55 $\pm$ 0.02	1.0	97.7
Laver 4	0.47 $\pm$ 0.01	0.45 $\pm$ 0.01	1.0	103.7

<sup>a</sup>Average  $\pm$  standard deviation of three determinations.

<sup>b</sup>In 25 mL of sample solutions.

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