

Extraction of Water-Soluble Porphyrin and Metalloporphyrins into Acetonitrile by Salting-out

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Abstract: A cationic water soluble porphyrin (5,10,15,20-tetrakis (1-methyl-pyridinium-4-yl)porphyrin, H_2tmpyp^{4+}) and its metalloporphyrins (MP) were easily extracted into acetonitrile separated by addition of sodium chloride (4 mol dm^{-3}) in the presence of sodium perchlorate, where M denotes Zn^{2+} , Cu^{2+} , Co^{3+} , Fe^{3+} , and Mn^{3+} and P^{2-} is porphyrinate ion. The extracted ion-pair complexes were completely dissociated to $[MP(ClO_4)_3]^+$, and $[MP(ClO_4)_2]^{2+}$. The extraction and the dissociation constants were determined by taking into account of the partition constant of sodium perchlorate ($K_D = 1.82 \pm 0.01$). The chemical properties of the separated acetonitrile phase as $E_{T(30)}$ and $D_{II,I}$ were determined and compared with other water miscible solvents (acetone, acetonitrile, 1,4-dioxane, tetrahydrofuran, 1-propanol and 2-propanol). Furthermore, a sensitive and selective method was proposed for the determination of a subnanogram amount of copper(II) in natural water samples by using the present salting-out method and the porphyrins.

Keywords: Salting-out, Acetonitrile, $E_{T(30)}$, Porphyrin, Ion-pair complex, Solvent extraction.

1. Introduction

When water-miscible organic solvent is salted-out from aqueous solution, phase separation occurs due to the decreased solubility of organic solvent in aqueous

solution[1]. The salting-out technique has been used for extraction of metal chelates, ion-pairs, organic compounds, prior to atomic absorption spectrophotometry[2], highperformance liquid chromatography[3,4], porlarography[5], and absorption

spectrophotometry[6]. An advantage using the phase separated solvents could extract charged solutes which are not extracted into normal organic solvents like chloroform. We found that a cationic water-soluble

porphyrin

(5,10,15,20-tetrakis(1-methylpyridinium-4-yl)porphyrin, H_2tmpyp^{4+} (Fig.1)) and its metalloporphyrins were extracted into acetonitrile separated by salting-out in the presence of perchlorate ion, but not extracted into chloroform and 1,2-dichloroethane. Porphyrins and metalloporphyrins are highly sensitive analytical reagents, their molar absorptivities are $(1-5) \times 10^5 \text{ mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}$, and the porphyrins have been widely used for determination of trace amounts of metal ions[7]. Only two papers, however, have been reported for the extraction of the porphyrins and metalloporphyrins using surfactants due to slight solubility of the porphyrins in organic solvents[8,9]

The present paper describes the ion-pair extraction of the cationic water-soluble porphyrins with perchlorate ion into acetonitrile and an analytical method for the determination of a subnanogram amount of copper(II) using the acetonitrile. Furthermore, the values of $E_T(30)$ and $D_{II,I}$ of acetonitrile were measured and compared with those of other water-miscible organic solvents separated by salting-out.

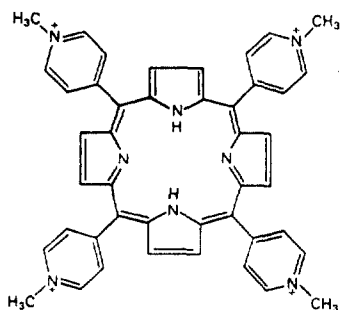


Fig. 1. Structure of 5,10,15,20-tetrakis(1-methylpyridinium-4-yl)porphyrin (H_2tmpyp^{4+}).

2. Experimental

2.1 Procedure. Into a stoppered graduated tube, a 5-cm^3 volume of aqueous sample solution was taken, then 1.169 g (2.00×10^{-2} mol) of sodium chloride was added to the solution. The sodium chloride was dissolved sufficiently and a 5-cm^3 volume of organic solvent was added to the aqueous solution. The mixed solvent was shaken for about 1 min. Two phases were allowed to stand for a few mins. The volumes of the organic and the aqueous phases were measured, and the concentrations of chloride ion and water in the separated-organic phase were determined by argentometry and Karl Fisher titration on a Karl Fisher Moisture Titrator (MKL-200, Kyoto Electronics, Japan), respectively. A $1.000 \times 10^{-2} \text{ mol dm}^{-3}$ perchloric acid aqueous solution containing 3.27 mol dm^{-3} NaCl and 3.47 acetonitrile, which are the same concentrations as found in the aqueous phase after salting-out, was used as a standard hydrogen ion concentration ($-\log[H^+] = 2.000$). All experiments were carried out at 25°C .

3. Results and Discussion

3.1 $E_T(30)$ and $D_{II,I}$ values of phase separated solvents. It is expected that the dissociation of sodium chloride and water into the phased separated solvents would alter the chemical properties of the solvents. Thermodynamic quantities which represent a measure of the donor and the acceptor properties of solvents have been proposed by Gutmann and Mayer as donor number and acceptor number[10]. These parameters have succeeded in explaining a lot of chemical reaction in non-aqueous solvents. Furthermore, the electronic transitions of various indicator molecules strongly depend on solvent, so the transition energies in different solvents have been used as an empirical measure of solvent polarity. One of these parameters is the $E_T(30)$ value based on the energy transition of the pyridium phenol

betain[11]. The $E_T(30)$ values have been linearly correlated to the acceptor numbers defined by Mayer[10]. The other parameters is $D_{II,I}$ values calculated by spectral change of $VO(acac)_2$. With an increase of donor number of the solvent, $D_{II,I}$ values increases with the donor number of the aprotic solvent used. However, a protic solvent which can form hydrogen bonds generally yields much higher $D_{II,I}$ alone[12]. Fig.2 and 3 show the $E_T(30)$ and $D_{II,I}$ values measured in the pure solvents, phase-separated organic solvents and the mixed solvents. All these data suggest that the donor and acceptor properties of the phase-separated solvents (except propanol) increase by the salting-out compared to the pure solvents and are smaller than those of the mixed solvents before the salting-out. A large change in the chemical properties of the solvents by salting-out was observed for acetonitrile, 1,4- dioxane and tetrahydrofuran and 2-propanol. Main factor for the change in $E_T(30)$ value is the increased concentration of water in the phase-separated solvent: acceptability of the phase-separated solvent is increased by incorporation of water having a large acceptor number ($AN = 54.8$)[13]. Typical data are

summarized in Table 1 for acetonitrile. The $E_T(30)$ and $D_{II,I}$ values of the acetonitrile phase are larger than those of nitrobenzene[11] and indicate the high donor and acceptor abilities due to a large amount of water dissolved in the solvent. This is a unique characteristic of the solvents separated by salting-out compared to other organic solvents. The high polarity could make an advantage for extraction of ionic compounds which are not extracted into conventional organic solvents that are not miscible with water.

Table 1. Data of phase-separation by salting-out using sodium chloride.

	acetonitrile phase	aqueous phase
volume/cm ³	4.17	6.11
NaCl/mol dm ⁻³	2.49×10^{-2}	3.27
H ₂ O/mol dm ⁻³	4.53	-----
CH ₃ CN/mol dm ⁻³	-----	3.47
$E_T(30)/kJ mol^{-1}$	228	-----
$D_{II,I}/kJ mol^{-1}$	48.0	60.6

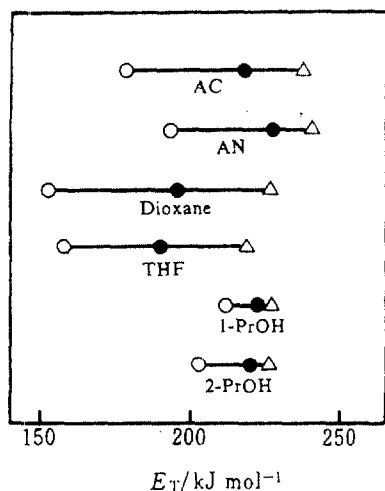


Fig. 2. $E_T(30)$ values of water-miscible pure solvents (○), organic phase after the salting-out (●), and 1:1 mixed-aqueous solution (△).

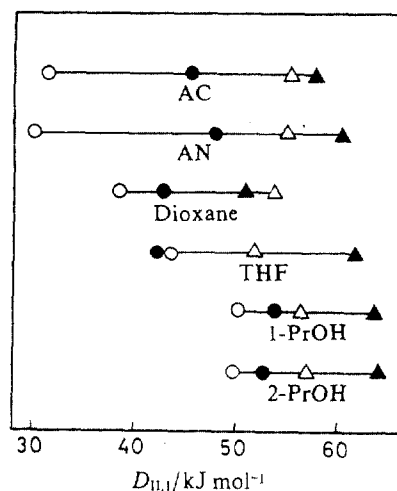


Fig. 3. $D_{II,I}$ values of water-miscible pure solvents (○), organic phase after the salting-out (●), and 1:1 mixed-aqueous solution (△) and aqueous phase (▲).

3.2 Effect of Salting out Agents. The phase separation depends on the salting-out agents. The effect of salting-out decreases in the following order: $\text{Al}^{3+} > \text{Mg}^{2+} > \text{Ca}^{2+} > \text{Ba}^{2+} \gg \text{Na}^+ > \text{K}^+ > \text{NH}_4^+ > \text{Li}^+$, and $\text{SO}_4^{2-} > \text{Cl}^-$. The main driving force of the salting-out is hydration of these cations and anions. The hydration energies (kJ mol^{-1}) of these ions are 4612 (Al^{3+}), 2198 (Mg^{2+}), 1592 (Ca^{2+}), 1317 (Ba^{2+}), 501.8 (Na^+), 424.5 (K^+), 2198 (Li^+)[14]. Except lithium ion, the salting-out is correlated to the hydration energies of the ions. Small salting-out effect of lithium ion may be due to a large solubility of lithium chloride into acetonitrile. Interestingly, magnesium sulfate led to a salting-in effect for the 1:1 (v/v) water-acetonitrile mixture.

3.3 Partition of Sodium Perchlorate. Before analyzing the extraction of the porphyrin and the metalloporphyrins into acetonitrile using sodium perchlorate, it is necessary to determine the partition constant of sodium perchlorate between acetonitrile and aqueous phases in the presence of sodium chloride. The concentration of perchlorate in acetonitrile was determined by Methylene Blue method. One cm^3 of a Methylene Blue solution ($3.91 \times 10^{-4} \text{ mol dm}^{-3}$), 0.5 cm^3 of sulfuric acid ($5.0 \times 10^{-2} \text{ mol dm}^{-3}$) and a sample solution containing perchlorate ion were taken into a separated funnel, and then 10 cm^3 of 1,2-dichloroethane was added and shaken well for 30 mins. The 1,2-dichloroethane phase was transferred to a test tube with a stopper and 0.5 g anhydrous sodium sulfate was added to the 1,2-dichloroethane phase. The absorbance of the 1,2-dichloroethane solution was measured at 655 nm and the concentration of perchlorate ion was determined.

When sodium perchlorate is distributed into acetonitrile phase as ion-pair and dissociates to Na^+ and ClO_4^- in the acetonitrile, distribution of perchlorate ion is expressed as

$$D_{\text{ClO}_4} = \frac{C_{\text{ClO}_4, \text{org}}}{C_{\text{ClO}_4, \text{aq}}}$$

$$= \frac{[\text{NaClO}_4]_{\text{org}} + [\text{ClO}_4^-]_{\text{org}}}{[\text{ClO}_4^-]_{\text{aq}}} = K_{\text{D}, \text{ClO}_4} (K_{\text{dis}, \text{NaClO}_4}^{-1} [\text{Na}^+]_{\text{org}} + 1) \quad (1)$$

where $K_{\text{D}} = [\text{ClO}_4^-]_{\text{org}} [\text{ClO}_4^-]_{\text{aq}}^{-1}$ and $K_{\text{dis}, \text{NaClO}_4} = [\text{Na}^+]_{\text{org}} [\text{ClO}_4^-]_{\text{org}} [\text{NaClO}_4]_{\text{org}}^{-1}$. Equation (1) can be rearranged to

$$C_{\text{ClO}_4, \text{org}} = K_{\text{D}, \text{ClO}_4} (K_{\text{dis}, \text{NaClO}_4}^{-1} [\text{Na}^+]_{\text{org}} + 1) C_{\text{ClO}_4, \text{aq}} \quad (2)$$

The plot of $C_{\text{ClO}_4, \text{org}}$ vs. $C_{\text{ClO}_4, \text{aq}}$ gave a straight line with the zero intercept: $K_{\text{dis}, \text{NaClO}_4}^{-1} [\text{Na}^+]_{\text{org}} \ll 1$ and sodium perchlorate dissociates completely in the acetonitrile phase. The partition constant, K_{D} , was found to be 1.82 ± 0.02 from the slope. The value is much larger than the values observed in nitrobenzene ($\log D = -4$ at $0.1 \text{ mol dm}^{-3} \text{ NaClO}_4$)[15] and other solvents ($\log D = -1.2, -2.5, -2.24$ at $0.1 \text{ mol dm}^{-3} \text{ NaClO}_4$ for tributylphosphate, nitromethane, methylisobutylketone and 2-pentanone, respectively)[16,17]. The complete dissociation of sodium perchlorate is ascribed to the large content of water in the acetonitrile phase. The concentrations of sodium chloride were 2.49×10^{-2} and 3.27 mol dm^{-3} in the acetonitrile and the aqueous phases, respectively, and the partition constant of sodium chloride was 7.61×10^{-3} .

3.4 Extraction of $\text{H}_2\text{tmpyp}^{4+}$, $[\text{M}^{\text{II}}(\text{tmpyp})]^{4+}$ and $[\text{M}^{\text{III}}(\text{tmpyp})\text{Cl}]^{4+}$ ($\text{M} = \text{Zn, Cu, Co, Fe}$ and Mn) into Acetonitrile. The above cationic porphyrin and metalloporphyrins were easily extracted into the acetonitrile separated by salting-out in the presence of sodium perchlorate at pH 4.8 (acetate buffer, $10^{-2} \text{ mol dm}^{-3}$). Typical extraction data are given as the function of the concentration of perchlorate in aqueous solution in Fig. 4. $\text{H}_2\text{tmpyp}^{4+}$, $[\text{Cu}(\text{tmpyp})]^{4+}$ was quantitatively extracted into the acetonitrile at 0.3 mol dm^{-3} sodium perchlorate, and

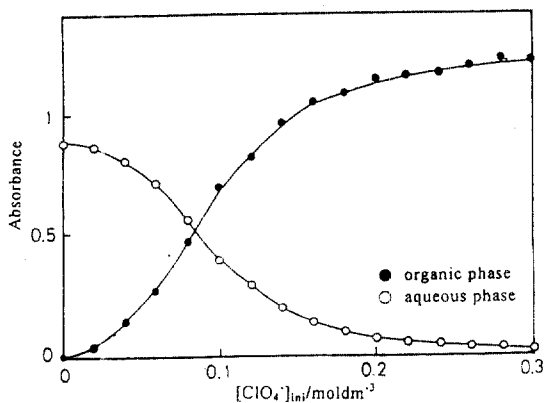


Fig. 4. Change in absorptivities of H_2tmpyp^{4+} in organic and aqueous phases at various concentrations of sodium perchlorate at pH 5.4.

$Mn^{III}(tmpyp)Cl^{4+}$ and $[Co^{III}(tmpyp)Cl]^{4+}$ were extracted at the concentration of perchlorate more than 1.0 mol dm^{-3} . The metalloporphyrins of iron(III), cobalt(III) and manganese(III) are octahedral structure and water molecules are bound to the metalloporphyrins. Thus, the metalloporphyrins of the trivalent metal ions were difficult to be extracted into the acetonitrile phase. On the other hand $[Cu(tmpyp)]^{4+}$ is square planar structure and no water molecule bound to it[18]. In Fig. 5, the log value of distribution ratio of the porphyrin is plotted against the concentration of perchlorate ion in

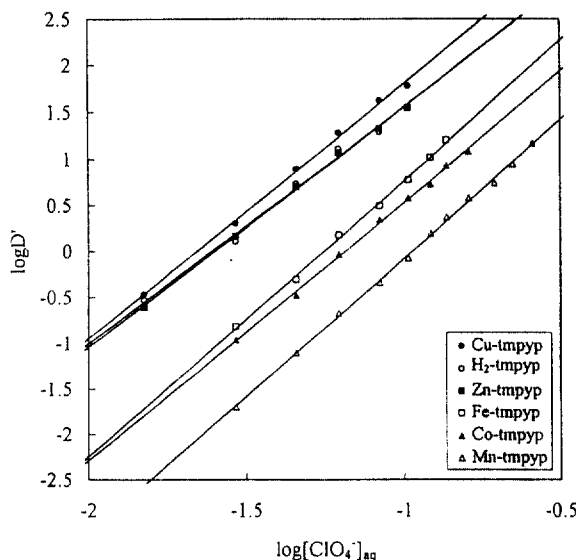
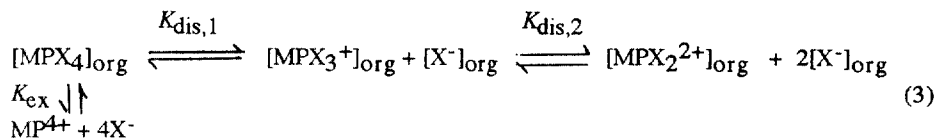


Fig. 5. Plot of logarithmic value of distribution ratio of the porphyrin vs. the concentration of perchlorate ion in the aqueous phase.

aqueous phase. The extraction of the porphyrins decrease in the order $[Cu(tmpyp)]^{4+} > H_2tmpyp^{4+} = [Zn(tmpyp)]^{4+} > [Fe^{III}(tmpyp)Cl]^{4+} > [Co^{III}(tmpyp)Cl]^{4+} > [Mn^{III}(tmpyp)Cl]^{4+}$. The slopes of the straight lines are 2.75 ± 0.07 , 2.56 ± 0.15 , 2.59 ± 0.06 , 3.01 ± 0.06 , 2.82 ± 0.05 , and 3.01 ± 0.04 for $[Cu(tmpyp)]^{4+}$, $[H_2tmpyp]^{4+}$, $[Zn(tmpyp)]^{4+}$, $[Fe^{III}(tmpyp)Cl]^{4+}$, $[Co^{III}(tmpyp)Cl]^{4+}$, $[Mn^{III}(tmpyp)Cl]^{4+}$, respectively.



$$\bar{\epsilon} = \frac{Abs_{org}}{C_{MP}} = \frac{\epsilon_1 [MPX_3^+]_{org} + \epsilon_2 [MPX_2^{2+}]_{org}}{[MP^{4+}]_{aq}(V_{aq}V_{org}^{-1}) + [MPX_3^+]_{org} + [MPX_2^{2+}]_{org}} \quad (4)$$

$$= \frac{\epsilon_1 K_{ex} K_{dis,1} K_D [X^-]^3_{aq} + \epsilon_2 K_{ex} K_{dis,1} K_{dis,2} [X^-]^2_{aq}}{K_D^2 (V_{aq}V_{org}^{-1}) + K_{ex} K_{dis,1} K_D [X^-]^3_{aq} + K_{ex} K_{dis,1} K_{dis,2} [X^-]^2_{aq}} \quad (5)$$

The results indicates the binding of two or three perchlorate ions to the porphyrin in the acetonitrile. Metalloporphyrin (MP^{4+}) between the aqueous and acetonitrile phases are given by eq. 3 in the presence of perchlorate ions, where X^- denotes perchlorate ion and $K_{ex} = [MPX_4]_{org}[MP^{4+}]_{aq}^{-1} \times [X^-]_{aq}^{-4}$, $K_{dis,1} = [MPX_3^+]_{org}[X^-]_{org}[MPX_4]_{org}^{-1}$, and $K_{dis,2} = [MPX_2^{2+}]_{org}[X^-]_{org}[MPX_3^+]_{org}^{-1}$. The apparent molar absorptivity ($\bar{\epsilon}$) of the porphyrins in the organic phase is correlated with the above K_{ex} , $K_{dis,1}$ and $K_{dis,2}$ as eqs 4 and 5.

The values of K_{ex} and $K_{dis,1}$ and $K_{dis,2}$ were determined a least-square minimization program with an assumption of $\epsilon_1 = \epsilon_2$. Since the extracted chemical species of $[MPX_4]$ completely dissociates to $[MPX_3^+]$, $K_{dis,1}$ value was not able to be determined. The extraction and dissociation constants determined are summarized in Table 2.

3.5 Effect of pH on Extraction of the Porphyrin and Metalloporphyrins. Copper(II), manganese(III) and iron(III) porphyrins were quantitatively extracted into the acetonitrile at a wide pH range. But free-base porphyrin (H_2tmpyp^{4+}) and $[Zn(tmpyp)]^{4+}$ were not extracted into the acetonitrile at pH lower than 2 due to the protonation of the porphyrin[19] and the dissociation to zinc(II) ion[20], respectively.

Extraction of iron(III) and cobalt(III) porphyrins

decrease gradually at higher pH due to the formation of hydroxo- and dihydroxometalloporphyrins like $[Co^{III}(tmpyp)OH]^{4+}$ and $[Co^{III}(tmpyp)(OH)_2]^{3+}$. The high extraction of $[Cu(tmpyp)]^{4+}$ and the low extraction of H_2tmpyp^{4+} at pH 1 suggests the separation of $[Cu(tmpyp)]^{4+}$ from the free base porphyrin and led the following sensitive determination of copper(II) based on the present phase-separation by salting-out.

3.6 Determination of Copper(II) by Extraction to Acetonitrile. (a) *Calibration Curve.* Above results imply the quantitative extraction of copper(II) into acetonitrile separated by salting-out of the sodium chloride in the presence of sodium perchlorate. Furthermore the porphyrin not bound to copper(II) is hardly extracted to the acetonitrile after the salting-out at pH lower than 1. The blank absorption is 0.008 at 421 nm and the molar absorptivity is $3.1 \times 10^5 \text{ mol dm}^{-3} \text{ cm}^{-1}$. The high sensitivity and low blank absorbance were developed to the determination of subnanogram amount of copper(II) by solvent extraction of $[Cu(tmpyp)]^{4+}$ into the acetonitrile based on salting-out as follows.

(b) *Formation rate of $[Cu(tmpyp)]^{4+}$.* Rate of metalloporphyrin is very slow compared to the metal complex formation with open-chain ligands[7]. Thus some catalysts have been used to enhance the rate of metalloporphyrin formation. It is well known that the copper(II) porphyrin formation is accelerated by the presence of hydroxylamine[19,21]. Copper(II) porphyrin completely formed and was extracted into the acetonitrile in a few mins. in the presence of $1.5 \times 10^{-3} \text{ mol dm}^{-3}$ hydroxylamine sulfate. The absorbance was independent of the concentration of hydroxylamine sulfate and the extracted copper(II) porphyrin was stable.

(c) *Effect of Diverse Ions.* Metal ions and anions usually encountered in natural water samples did not interfere with the determination of copper(II) by the present method. Interestingly, the serious interference of palladium(II) as reported before[21] was not

Table 2. Distribution constants of free base- and metallo-porphyrins of H_2tmpyp^{4+} .

	$K_{ex}K_{dis,1}$	$K_{dis,2}$
	$\text{mol}^{-3} \text{ dm}^9$	mol dm^{-3}
H_2tmpyp^{4+}	$(7.2 \pm 1.3) \times 10^4$	$(2.9 \pm 1.4) \times 10^{-2}$
$[Zn(tmpyp)]^{4+}$	$(6.4 \pm 0.9) \times 10^4$	$(3.1 \pm 1.1) \times 10^{-2}$
$[Cu(tmpyp)]^{4+}$	$(1.35 \pm 0.13) \times 10^5$	$(8.0 \pm 4.9) \times 10^{-3}$
$[Co(tmpypCl)]^{4+}$	$(4.8 \pm 0.6) \times 10^3$	$(5.1 \pm 2.2) \times 10^{-2}$
$[Fe(tmpypCl)]^{4+}$	$(1.23 \pm 0.05) \times 10^4$	—
$[Mn(tmpypCl)]^{4+}$	$(1.42 \pm 0.07) \times 10^3$	—

observed in the present study because of the palladium being masked by sodium chloride used as a salting-out agent.

(d) *Recommended Procedure for the Determination of Copper(II) in Natural Waters.* A 2.5 cm³ of sample solution was taken into a test tube and followed by addition of 0.5 cm³ of H₂tmpyp⁴⁺, 0.5 cm³ of acetate buffer (0.5 mol dm⁻³, pH 4.5 - 5.3), and 0.5 cm³ of hydroxylammonium sulfate (1.5 x 10⁻² mol dm⁻³). The solution was allowed to stand for a few mins. to complete the formation of [Cu(tmpyp)]⁴⁺ and then 0.5 cm³ of sodium perchlorate (4.00 mol dm⁻³), 0.5 dm⁻³ of sulfuric acid (5 mol dm⁻³), and 1.169 g of sodium chloride were added. A 5 cm³ of acetonitrile was added to the solution and the absorbance of the separated acetonitrile phase was measured at 421 nm against a pure water. The present method was applied to the determination of copper in hot spring water samples. The copper was found to be 2 ppb and 5 ppb for two hot spring

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