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# Electrodialytic Extraction in Drug Analysis.III.[1] Some Practical Applications of the Electrodialytic Extraction Method

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Electrodialytic extraction was applied to the following extraction processes:

a) Extraction of dihydrocodeine phosphate from a dihydrocodeine phosphate-carboxymethyl cellulose (CMC) system.

b) Extraction of acrinol from medicated pad.

c) Extraction of thiamine from modified milk powder.

d) Extraction of berberine from powdered phellodendron.

From these expreiments the following results were obtained.

1) In all experiments the superiority of electrodialytic extraction was proved.

2) By using electrodialytic extraction, clear extract was obtained easily from the sample, even from a turbid solution or insoluble materials.

3) The test component which moved in the opposite direction by electric current could be separated by using electrodialytic extraction.

4) It was found that electrodialytic extraction could be used for analysis of food and crude drugs.

In a previous paper[3] it was recognized that electrodialytic extraction was more effective for extraction than shaking extraction, and in the preceding paper[1] of this series it was found that electrodialytic extraction was applicable on a large number of drugs. In this report electrodialytic extraction was applied to some practical preparations.

## Extraction of Dihydrocodeine Phosphate from a Dihydrocodeine Phosphate-Sodium Carboxymethyl Cellulose (CMC) System

CMC, a thickner which is prescribed frequently in syrup preparations, makes the extraction difficult. Consequently, a comparison was made between electrodialytic extraction and other extraction procedures on the recovery of dihydrocodeine from this system.

## Extraction of Acrinol from Medicated Pad

Medicated pads are used in adhesive dressings. Electrodialytic extraction was applied on the extraction of acrinol from medicated pad.

## Extraction of Thiamine from Modified Milk Powder

The determination of thiamine in modified milk powder is described in Standard Method of Analysis for Hygienic Chemists[4] but Ito, *et al.*[5] pointed out that the recovery by the conventional extraction with hydrochloric acid is poor and reported an improved method. In this paper electrodialytic extraction were compared with the standard method and Ito's modification.

## Extraction of Berberine from Powdered Phellodendron

There are no reports on comparison of extraction procedures of berberine. Considering the quantitative assay methods of berberine in powdered phellodendron which are described in

1) This report was presented at the Meeting of Kanto Branch, Pharmaceutical Society of Japan, Tokyo, December 1969. Part II: N. Tsunakawa, *Chem. Pharm. Bull.* (Tokyo), **19**, 2579 (1971).
2) Location: *Narihira 5-6-9, Sumida-ku, Tokyo.*
3) N. Tsunakawa, *Chem. Pharm. Bull.* (Tokyo), **19**, 1164 (1971).
4) "Standard Methods of Analysis for Hygienic Chemists-with commentary," Kanehara shuppan, Tokyo, 1967, p. 137.
5) T. Ito, R. Shibasaki and K. Arase, *Vitamin*, **39**, 109 (1969).

Japanese Pharmacopoea and other extraction procedures,[6-8] reference extraction procedures were decided. These procedures were compared with the electrodialytic extraction.

## Experimental

**I. Extraction of Dihydrocodeine Phosphate from Dihydrocodeine Phosphate——CMC System——**

1. Preparation of Sample: The sample was prepared with the following prescription:

| | |
|---|---|
| Dihydrocodeine phosphate | 27.0 mg |
| Sodium carboxymethyl cellulose | 900.0 mg |
| Purified water | a sufficient quantity |
| | To make 100 ml |

2. Extraction with Chloroform: 1) Sample solution was made alkaline with sodium hydroxide and extracted with chloroform, but this mixture was emulsified and the chloroform layer could not be separable.

2) During the procedure of 1), NaCl was added but the chloroform layer became turbid and the efficient separation of chloroform layer could not be made.

3. Extraction by the CMC Elimination Method: 50 g of NaCl was dissolved in 250 ml of the sample solution and the volume was adjusted to 500 ml by addition of ethanol with stirring. This mixture was centrifuged and 400 ml of the supernatant was taken, the solvent was removed by distillation under reduced pressure on a water bath. The residue was dissolved in 5 ml of methanol and 20 ml of water was added. The solution was titrated with 0.02 N sulfuric aicd (indicator: 3 drops of methyl red–methylene blue test solution).

4. Electrodialytic Extraction: 1 ml of sample was measured accurately and electrodialytic extraction carried out under the following conditions: carrier solution 0.5 M acetic acid, flow rate of carrier solution 0.63 ml/min, current density 20 mA/cm$^2$ and extraction time 15 min. The volume of effluent was adjusted to 10 ml with 0.5 M acetic acid and the absorbance was measured at 285 m$\mu$.

**II. Extraction of Acrinol from Medicated Pad**——1. Preparation of Sample: Absorbent Lint which is described in British Pharmaceutical Codex (1968) was used for the pad. Absorbent Lint was cut into pieces of 1 cm width and 3 cm length, so that each piece could absorb 0.5 ml of 0.05% methanol solution of acrinol, and these were dried in air and stored in a desiccator.

2. Shaking Extraction: A piece of the sample was placed in a centrifuge tube, and 20 ml of 0.5 M acetic acid was added. The extraction was made for 15 minutes with vigorous shaking. After centrifugation, the supernatant liquid was collected by decantation and the extraction of the residue was repeated three times with 20 ml of 0.5 M acetic acid and the absorbance of each solution was measured at 271 m$\mu$.

3. Electrodialytic Extraction: A piece of the sample was placed in the sample chamber of the apparatus[3] and extracted under the following conditions; carrier solution 0.5 M acetic acid, flow rate of carrier solution 1.54 ml/min, current density 20 mA/cm$^2$ and extraction time 60 min. The absorbance fo the effluent was measured at 271 m$\mu$.

**III. Extraction of Thiamine from Modified Milk Powder**——1. Preparation of Sample: 450 g of the modified milk powder[9] was weighed accurately, 18.00 mg of thiamine hydrochloride was added, and after mixing throughly it was used as a sample.

2. Extraction Methods: 1) Extraction Method A: 20 g of the sample was weighed accurately and the sample solution was prepared according to the procedure of Fig. 1. Recovery was obtained by the determination method of 3.

6) K. Yamaguchi, T. Tabata and H. Ito, *Yakugaku Zasshi*, **73**, 1189 (1953).
7) Y. Ichimura and T. Tabata, *Japan Analyst*, **10**, 1097 (1961).
8) A.V. Ananichev, O.B. Stepanenko and P.M. Loshkarev, *Med. Prom. SSSR*, **1**, 37 (1967).
9) "Lebens D" was used and the components of this powder were as follows (in 100 g)

| | | | |
|---|---|---|---|
| Moisture | 2.0 g | $VB_1$ | 0.8 mg |
| Fat | 22.0 g | $VB_2$ | 1.3 mg |
| Protein | 13.3 g | $VB_6$ | 0.3 mg |
| Carbohydrate | 60.5 g | $VB_{12}$ | 0.002 mg |
| Ash | 2.2 g | VE | 7.3 mg |
| Ca | 350 mg | Nicotinic amide | 4.0 mg |
| P | 300 mg | VC | 40.0 mg |
| Fe | 6.0 mg | L-Cystine | 235 mg |
| VA | 2000 IU | Linoleic acid | 3500 mg |
| VD | 600 IU | Folic acid | 0.3 mg |

2) Extraction Method B: 5 g of the sample was weighed accurately and the sample solution was prepared according to the procedure of Fig. 2. Recovery was obtained by the determination method of 3.

3) Electrodialytic Extraction (Extraction Method C): 10 g of the sample was weighed accurately and the sample solution was prepared according to the procedure of Fig.3. Recovery was obtained by the determination method of 3.

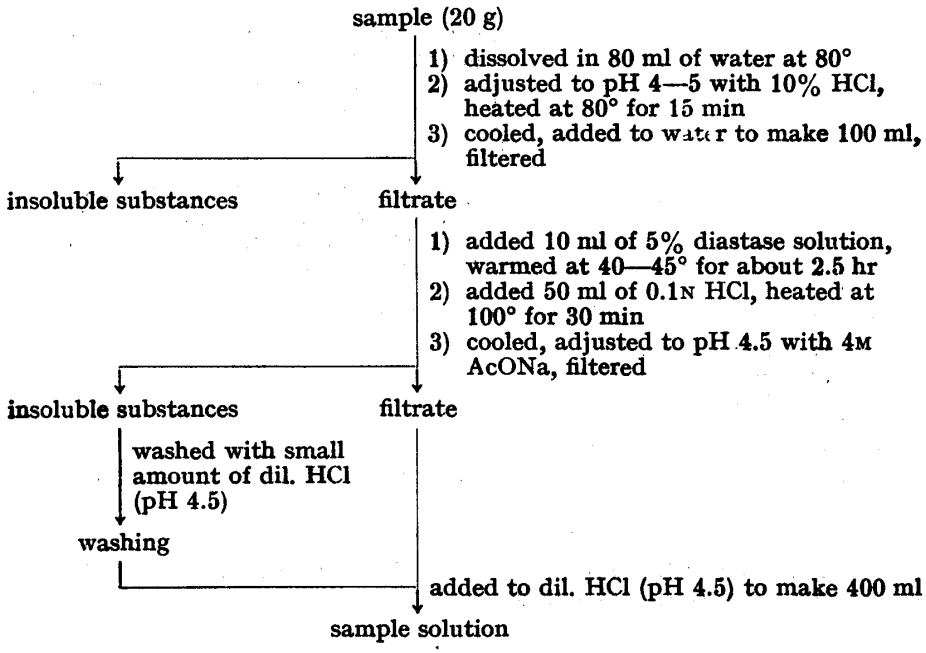


Fig. 1. Preparation Procedure of Sample Solution with Extraction Method A

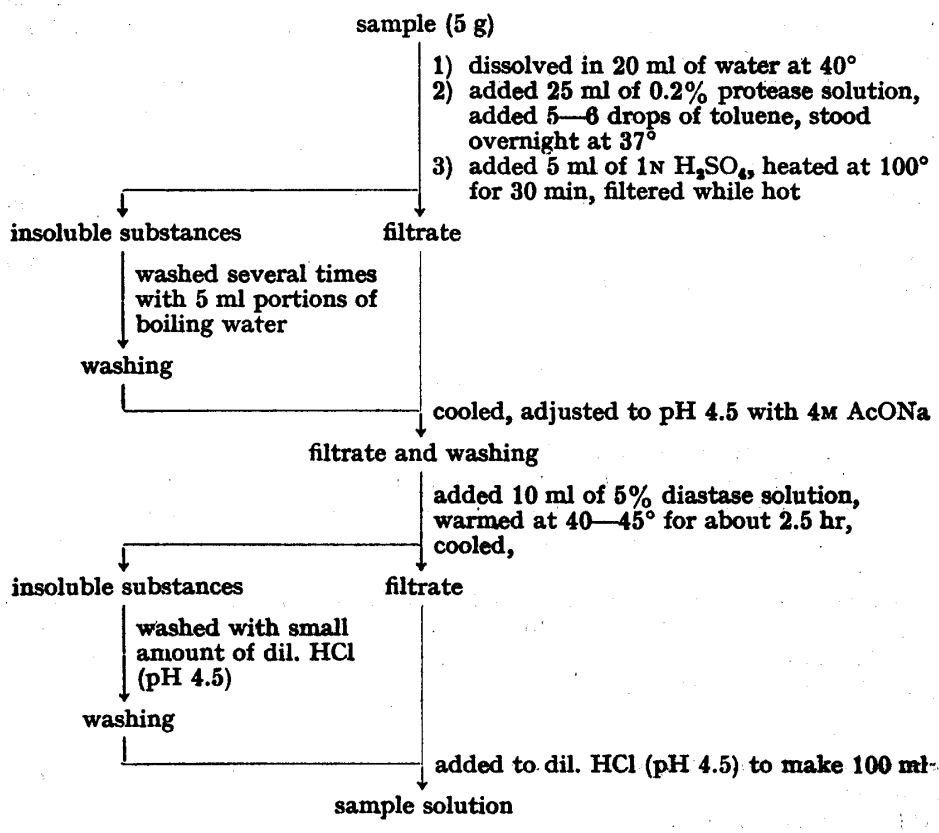


Fig. 2. Preparation Procedure of Sample Solution with Extraction Method B

3. Determination Method: 1) Reagents: a) Diastase Solution: A 5% aqueous solution of commercial diastase[10] was adjusted to pH 4.5 with dilute hydrochloric acid, and this solution was passed through a column of a synthetic zeolite[11] to remove thiamine and used as the diastase solution.

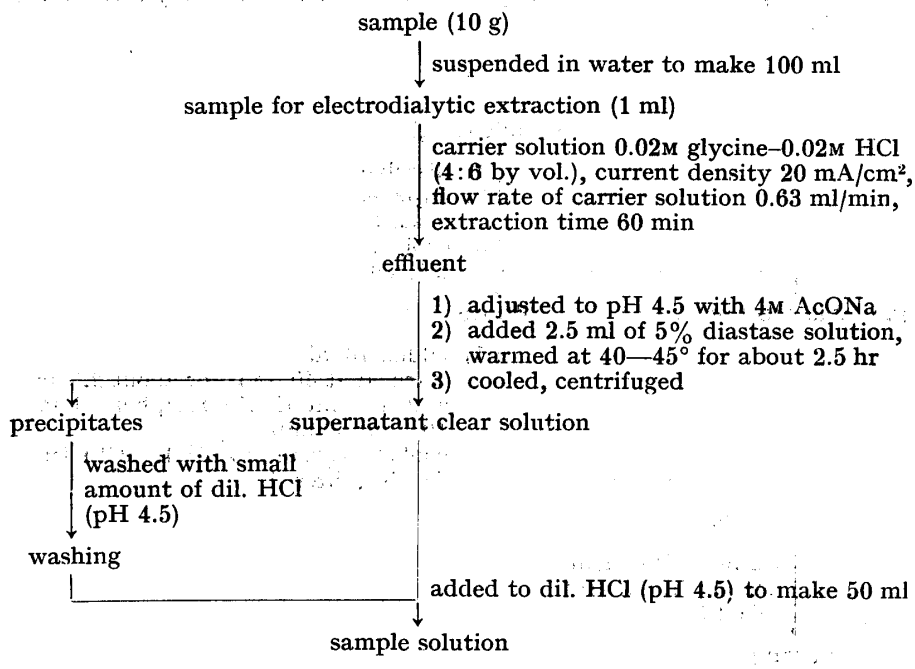


Fig. 3. Preparation Procedure of Sample Solution with Electrodialytic Extraction (Extraction Method C)

b) Protease Solution: Protease[12] was dissolved in Sörensen's phosphate buffer (pH 6.8) to make a 0.2 % solution.

c) Standard Solution of Thiamine : 0.32 g of thiamine hydrochloride (J.P.VII) was weighed accurately after preliminary drying at 80° for 4 hours, dissolved in dilute hydrochloric acid of pH 4.5 and diluted accurately to 100 ml. 1 ml of this solution was taken, dissolved in dilute hydrochloric acid of pH 4.5, diluted accurately to 1000 ml and used as the standard solution.

2) Procedure of Assay: a) Procedure of Adsorption, Washing and Elution: The assay of thiamine was carried out following the standard methods of analysis for hygienic chemists,[4] using an accurately measured volume equivalent to about 2.4 $\mu$g of thiamine hydrochloride.

b) Procedure of Oxidation and Extraction of Thiochrome: Oxidation of effluent with BrCN and extraction of thiochrome with *n*-BuOH were performed in accordance with the Standard Methods of Analysis for Hygienic Chemists.[4]

c) Specific Fluorescence: Intensity of fluorescence was measured with a spectrofluorometer (Hitachi MPF-2) using excitation of 370 m$\mu$ and fluorescence of 425 m$\mu$.

Recovery were calculated by the following equation

$$\text{Recovery } (\%) = \frac{Fa-Fb}{Fa'-Fb'} \times \frac{W_0}{W} \times 100$$

$W$: the amount of sample (g)
$Fa$, $Fb$: the fluorescences of the sample solution and its blank
$Fa'$, $Fb'$: the fluorescences of the standard solution and its blank
$W_0$: weight of sample corresponding to $S$ $\mu$g of thiamine hydrochloride

**IV. Extraction of Berberine from Powdered Phellodendron**[13]——1. Extraction with a Soxhlet Extractor using Methanol: 1 g of powdered, defatted phellodendron was extracted with a Soxhlet extractor for 12 hours. The extract was diluted to exactly 200 ml with methanol. The amount of extracted berberine was determined by measuring the absorbance at 431 m$\mu$.

10) Takadiastase (Sankyo Co., Ltd.) was used.
11) Permutit was used.
12) Pronase P (Kaken Kagaku Co., Ltd.) was used.
13) Powdered phellodendron (Nippon Funmatsu Yakuhin Co., Ltd.) Lot. No. AQY 9635 was used.

2. Shaking Extraction with Ethanol: 0.5 g of powdered, defatted phellodendron was placed in a centrifuge tube, and 25 ml of ethanol was added. The mixture was shaked for 10 minutes. After centrifugation, the supernatant liquid was filtered and the extraction of the residue repeated with 25 ml portions of ethanol until the extract became almost colorless. The recovery of each extraction was obtained by measuring the absorbance at 431 m$\mu$.

3. Shaking Extraction with 0.5M Acetic Acid: 0.5 g of powdered, defatted phellodendron was weighed accurately and placed in a centrifuge tube. 25 ml of 0.5M acetic acid was added, and the mixture was shaked for 10 minutes. After centrifugation, the supernatant liquid was filtered and the extraction of the residue repeated with 25 ml portions of 0.5M acetic acid until the extract became almost colorless. The each recovery was obtained by measuring the absorbance at 420 m$\mu$.

4. Electrodialytic Extraction with 0.5M Acetic Acid: 1 g of powdered, defatted phellodendron was weighed accurately, suspended in water and diluted to exactly 10 ml with water. 1 ml was measured accurately, placed in the sample chamber of the apparatus[3] and extracted under the following conditions; carrier solution 0.5M acetic acid, flow rate of carrier solution 0.72 ml/min and current density 20 mA/cm$^2$. The effluent was fractionated every 10 minutes and the absorbance was measured at 420 m$\mu$.

5. Paper Chromatography of Several Kinds of Extracts: The following 7 kinds of extracts were prepared for paper chromatography.

a) Ether Extract: 10 g of powdered phellodendron was extracted with 100 ml of ether for 1 hour using a Soxhlet extractor.

b) Methanol Extract: The residue of a) was extracted with 100 ml of methanol for 12 hours using a Soxhlet extractor.

c) Aqueous Extract: The methanol extract of b) was evaporated on a water bath to one-tenth of the original volume, then 100 ml of water was added and warmed for a while.

d) Filtrate of c) after Talc Treatment: 2 g of talc was added to the aqueous solution of c), left standing for 20 minutes, and filtered while stirring.

e) Acetic Acid Extract: 1 g of powdered, defatted phellodendron was extracted with 25 ml of 0.5M acetic acid with shaking for 10 minutes.

f) Electrodialytic Extract 1: 100 mg of the defatted phellodendron was extracted with 0.5M acetic acid under the conditions of IV. 4.

g) Electrodialytic Extract 2: 100 mg of the undefatted phellodendron was extracted with 0.5M acetic acid under the conditions of IV. 4.

h) Standard Solution: Berberine hydrochloride was dissolved in 0.5M acetic acid (1000 $\mu$g/ml). The ether extract and the standard solution were used as they were while the other extracts were adjusted to a concentration of berberine hydrochloride (approximately 1000 $\mu$g/ml), and paper chromatography was run for 24 hours on Toyo Roshi No. 51 filter paper on which 1 $\mu$l of each sample was spotted by the ascending method using *n*-BuOH–AcOH–$H_2O$ (4: 1: 3, V/V), and detected by ultraviolet (UV) lamp.

6. UV-Visible Spectra of Acetic Acid Extract and Electrodialytic Extract: Powdered, defatted phellodendron (0.5 g) was placed in a centrifuge tube, 25 ml of 0.5M acetic acid was added. The extraction was made by vigorous shaking for 15 minutes. After centrifugation, the supernatant liquid was filtered with glass filter (No. 3) and used as sample solution ($s_1$). Electrodialytic extraction apparatus which has two multistage separation chambers above and below the sample chamber and with which each effluent can be obtained separately was used. The sample $s_1$ was filled in the sample chamber and extracted under the following conditions; carrier solution 0.5M acetic acid, flow rate of carrier solution 0.72 ml/min, current density 20 mA/cm$^2$ and extraction time 80 min. The effluent of the anode side was combined with the solution remained in the sample chamber. The combined solution ($s_4$) and the effluent of the cathode side ($s_2$) were obtained separately. Each of the solution of $s_1$, $s_2$ and $s_4$ were diluted with 0.5M acetic acid so that they were in the same multiples based on $s_1$, and these solutions were called $S_1$, $S_2$ and $S_4$, respectively. The UV–visible absorption spectra of these solutions were measured from 230 m$\mu$ to 500 m$\mu$ with a recording spectrophotometer ORD-UV-5 (Japan Spectroscopic Co., Ltd.).

## Result and Discussion

### Extraction of Dihydrocodeine Phosphate from a Dihydrocodeine Phosphate-CMC System

It was found that the test component could not be extracted with chloroform because CMC obstructs the separation of the two solvents. The test component was extracted quantitatively by the CMC elimination method shown in Table I, but this method has the two defects. One is the requirement of longer time for centrifugation and evaporation, the other is the requirement of larger volume of solvent. On the other hand, the electrodialytic extraction shown in Table II was very useful because the rate of extraction was very fast and dihydro-

Table I. Recovery of Dihydrocodeine by CMC Elimination Method

| No. | Recovery % | No. | Recovery % |
|---|---|---|---|
| 1 | 100.4 | 3 | 100.7 |
| 2 | 99.3 | $\bar{x}$ | 100.1 |

Table II. Recovery of Dihydrocodeine by Electrodialytic Extraction

| No. | Recovery % | No. | Recovery % |
|---|---|---|---|
| 1 | 100.9 | 7 | 99.1 |
| 2 | 101.9 | 8 | 100.9 |
| 3 | 101.9 | 9 | 97.2 |
| 4 | 100.0 | 10 | 99.1 |
| 5 | 99.1 | $\bar{x}$ | 99.8 |
| 6 | 98.2 | $\sigma$ | 1.48 |

codeine could be determined directly with a spectrophotometer as the CMC was completely eliminated from the effluent.

**Extraction of Acrinol from Medicated Pad**

The recovery was about 90% with the shaking extraction as shown in Table III, while a quantitative recovery was obtained by electrodialytic extraction as shown in Table IV.

Table III. Recovery of Acrinol by Shaking Extraction

| No. | Recovery % | | | | |
|---|---|---|---|---|---|
| | The number of times of extraction | | | | |
| | 1 | 2 | 3 | 4 | Total |
| 1 | 82.04 | 7.64 | 0.15 | 0.09 | 89.92 |
| 2 | 82.96 | 7.96 | 0.14 | 0.09 | 91.15 |
| 3 | 83.44 | 8.00 | 0.15 | 0.09 | 91.68 |
| 4 | 82.28 | 7.32 | 0.15 | 0.10 | 89.85 |
| 5 | 81.52 | 7.44 | 0.14 | 0.09 | 89.19 |
| 6 | 81.38 | 7.62 | 0.15 | 0.09 | 89.24 |
| 7 | 83.42 | 7.52 | 0.14 | 0.09 | 91.17 |
| 8 | 83.61 | 7.44 | 0.15 | 0.09 | 91.29 |
| 9 | 81.34 | 7.38 | 0.15 | 0.09 | 88.96 |
| 10 | 83.32 | 7.46 | 0.15 | 0.09 | 91.02 |
| $\bar{x}$ | | | | | 90.35 |
| $\sigma$ | | | | | 0.97 |

Table IV. Recovery of Acrinol by Electrodialytic Extraction

| No. | Recovery % | No. | Recovery % |
|---|---|---|---|
| 1 | 100.3 | 7 | 99.9 |
| 2 | 99.7 | 8 | 101.1 |
| 3 | 99.8 | 9 | 98.7 |
| 4 | 101.0 | 10 | 100.4 |
| 5 | 100.2 | $\bar{x}$ | 100.1 |
| 6 | 99.6 | $\sigma$ | 0.66 |

**Extraction of Thiamine from Modified Milk Powder**

The results were shown in Table V. Ito, *et al.*[7] reported that extraction method B gave a higher recovery than A. In this work quantitative recoveries were demonstrated with the method B and C and a lower recovery was obtained by the method A. The extraction method B gave the largest variance of results. The time required for each extraction process was as shown in Table V. The extraction was completed in the shortest time in the case of C (about

Table V. Recovery of Thiamine by Extraction Methods A, B and Electrodialytic Extraction

| No. | Recovery % | | |
|---|---|---|---|
| | Extraction method A | Extraction method B | Electrodialytic extraction |
| 1 | 66.9 | 97.8 | 99.5 |
| 2 | 63.6 | 101.8 | 97.8 |
| 3 | 62.6 | 93.6 | 103.2 |
| 4 | 60.4 | 100.5 | 97.5 |
| 5 | 65.0 | 91.4 | 103.3 |
| 6 | 62.9 | | 98.1 |
| $\bar{x}$ | 63.6 | 97.0 | 99.9 |
| $\sigma$ | 2.02 | 3.97 | 2.45 |
| Time from extraction to adsorption and elution | about 7 hr | about 7 hr+ stand overnight | about 6 hr (about 2 hr) |

Table VI. Recovery of Berberine by Four Kinds of Extraction Methods

1) Soxhlet Extraction

| Extraction time (hr) | Recovery with respect to weight of sample (%) |
|---|---|
| 12 | 3.09 |

2) Shaking Extraction with Ethanol

| Recovery with respect to weight of sample (%) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Number of times of extraction | | | | | | | | | | |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Total |
| 1.33 | 0.28 | 0.14 | 0.09 | 0.07 | 0.06 | 0.05 | 0.05 | 0.03 | 0.02 | 2.12 |

3) Shaking Extraction with 0.5M Acetic Acid

| Recovery with respect to weight of sample (%) | | | | | | | |
|---|---|---|---|---|---|---|---|
| Number of times of extraction | | | | | | | |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | Total |
| 2.00 | 0.51 | 0.16 | 0.07 | 0.03 | 0.02 | 0.01 | 2.80 |

4) Electrodialytic Extraction

| No. | Recovery with respect to weight of sample (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Extraction time (min) | | | | | | | |
| | 10 | 20 | 30 | 40 | 50 | 60 | 70 | Total |
| 1 | 2.23 | 0.56 | 0.06 | 0.03 | 0.02 | 0.01 | — | 2.91 |
| 2 | 2.42 | 0.49 | 0.08 | 0.03 | 0.02 | 0.01 | 0.01 | 3.06 |
| 3 | 2.44 | 0.46 | 0.07 | 0.03 | 0.01 | 0.01 | 0.01 | 3.03 |
| $\bar{x}$ | | | | | | | | 3.00 |

6 hours). In case of the method C, it is possible to omit the diastase treatment, adsorption and elution (about 2 hours) because the sample solution was colorless and clear; there was no difference in the value of thiamine whether diastase treatment was included or not; Fb and Fb' were approximately the same and the fluorescence of nicotinic amide was negligible.

**Extraction of Berberine from Powdered Phellodendron**

The recoveries using 4 kinds of extraction methods were as shown in Table VI. Electrodialytic extraction and Soxhlet extraction gave better recoveries than the other methods. But Soxhlet extraction required longer time than the electrodialytic extraction. Paper chromatograms of 7 kinds of extracts and the standard solution are shown in Fig. 4. Three kinds of spots were found in this experiment, *i.e.* $B_1$ which can be identified with UV lamp in the extracts of b,c,d, and e; $B_2$ which is a yellow spot of berberine; and $B_3$ which can be observed faintly with UV lamp. Electrodialytic extracts showed the same pattern as that of the standard. It was found that the defatting process could be omitted in electrodialytic extraction. UV-visible spectra of acetic acid extract and electrodialytic extract were as shown in Fig. 5. The substance which is extracted at the anode side by electrodialytic extraction showed the same spectrum as that of berberine, while the substance which is extracted at the cathode side showed a different spectrum from that of berberine and gave the same spot of $B_1$ on paper chromatography. In this experiment, if the spectrum of $S_2$ is added to that of $S_4$ the combined spectrum coincides with that of $S_1$. It was found from this that $B_1$ was separated from berberine ($B_2$) by electrodialytic extraction.

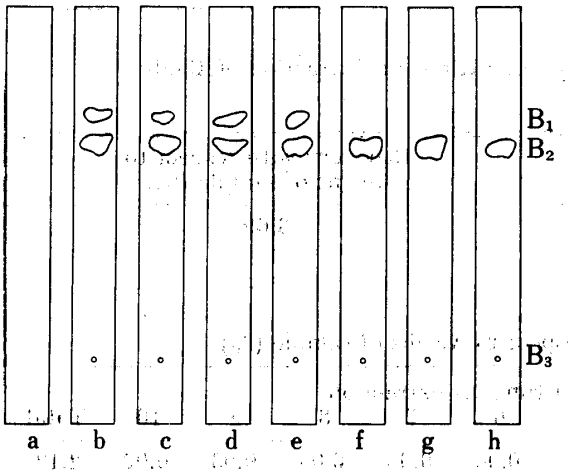


Fig. 4. Paper Chromatograms of Several Kinds of Extracts of Powdered Phellodendron

extracts used:
a) ether extract
b) methanol extract
c) aqueous extract
d) filtrate of c) after treating with talc
e) acetic acid extract
f) electrodialytic extract 1
g) electrodialytic extract 2
h) standard solution
filter paper: Toyo Roshi No. 51
solvent: *n*-BuOH–AcOH–$H_2O$ (4:1:3)
detection: UV-lamp

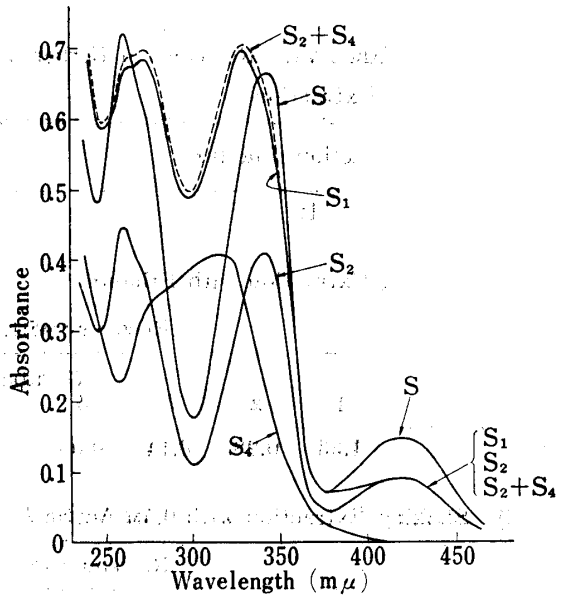


Fig. 5. UV-Visible Spectra of Acetic Acid Extract and Electrodialytic Extracts

S : standard solution of berberine hydrochloride
$S_1$: acetic acid extract
$S_2$: cathode side extract of $S_1$
$S_4$: solution of combined anode side extract and residual solution in the sample chamber

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