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Electrodialytic Extraction in Drug Analysis. I.[1] Description of Apparatus and Study of Extraction Condition

NOBUTAKA TSUNAKAWA[2]

Pharmaceutical Research Laboratory, Research Laboratories, Daiichi Seiyaku Co., Ltd.[2]

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A new method of extraction, *i.e.* electrodialytic extraction was proposed. Electrodialytic extraction apparatus was also devised. Extraction conditions were examined and the following results were obtained.

1. Electrodialytic extraction was more effective than shaking extraction.
2. Possibility of electrodialytic extraction of a test component could be demonstrated by using a liquid sample.
3. Acetic acid (0.5 M) was chosen as the acidic standard carrier solution.
4. A suitable current density was 20 mA/cm^2 when using this apparatus and 0.5 M acetic acid as the carrier solution.
5. It is desirable for the carrier solution to be a weak electrolyte with specific conductivity of about 1×10^{-3} mho/cm.
6. Change in quantity of sample required no essential prolongation of extraction time.
7. The suitable particle size of the sample was about 100 mesh.
8. Cellulose membrane was chosen as the dialysis membrane for extracting low molecular compounds.

The process of drug analysis consists of extraction, separation and determination. There are many studies on separation and determination. However, considering the importance of quantitative extraction of the component from drug preparations, it seems to be strange that there are few studies on the method of extraction. Since the whole analysis primarily depends on the effectiveness of the extraction, it could be said that the studies on the separation and the determination have little meaning unless the components are quantitatively extracted. Thus the investigation to find out an extensive as welll as practical method of extraction has been carried out and the novel technique for complete extraction of ionizable compounds is proposed and named electrodialytic extraction.

Electrodialytic extraction is a new extracting method which has stronger extracting power than the conventional method, and has a capacity to extract many kinds of ionizable compounds with several kinds of carrier solutions such as diluted acetic acid, diluted ammonia, *etc.* In this paper the concept of electrodialytic extraction, the apparatus used and the extraction conditions examined are reported.

Experimental

Apparatus——The electrodialytic extraction apparatus, shown in Fig. 1—3, was used. Fig. 1 is a general view of this apparatus in which a_1 and a_2 are electrode vessels. The structure of the electrodialytic extraction apparatus is indicated in Fig. 2 and is composed of the following parts: membranes, b_1—b_7, sample chamber, c, carrier solution path, d_1—d_5, sample inlet, e, stopper, f, and flow of carrier solution, g. The extracting system is shown in Fig. 3 schematically. In the present study two extracting systems, A or B, were used.

Procedure——The sample was placed in the sample chamber, then the sample chamber filled with a carrier solution and stoppered. The fresh carrier solution was introduced with a constant flow rate pump

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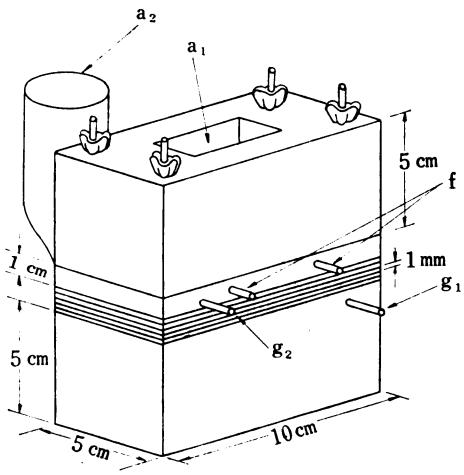

Fig. 1. Electrodialytic Extraction Apparatus

a_1,a_2: electrode vessels
f: stopper of sample chamber
g_1: inlet of carrier solution
g_2: outlet of effluent

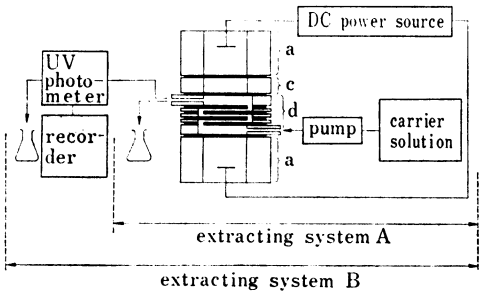

Fig. 3. Extracting System

a: electrode vessel
c: sample chamber
d: multistage separation chamber

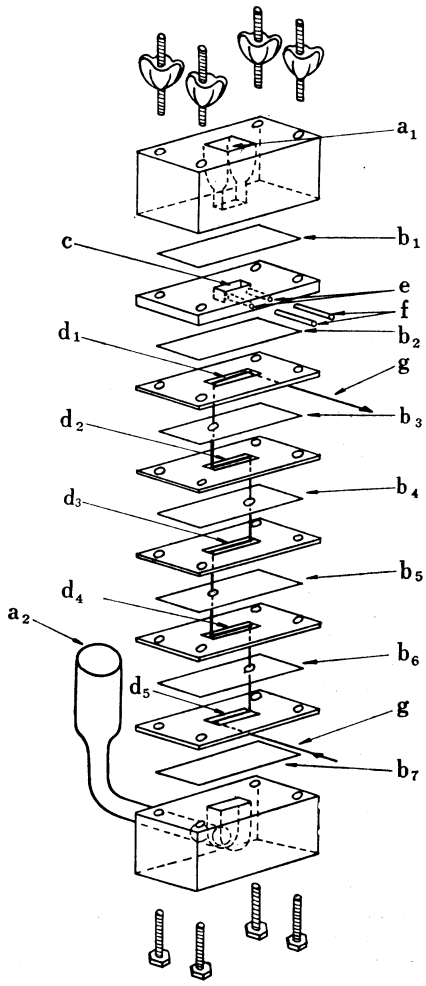

Fig. 2. Disassembled View of Electrodialytic Extraction Apparatus

a_1,a_2: electrode vessels, b_1,b_2,b_3,b_4,b_5,b_6,b_7: membranes, c: sample chamber (5 mm L × 10 mm H × 40 mm W), d_1,d_2,d_3,d_4,d_5: path of carrier solution (5 mm L × 1 mm H × 40 mm W), e: pore of sample chamber, f: stopper of sample chamber, g: flow of carrier solution

through the multistage separation chamber. The test component was removed from the sample with a direct current supplied from a DC power source and sent into the stream of carrier solution through the membrane, and this carrier solution was removed from the separation chamber as the effluent.

Extracting System A——This system is shown in Fig. 3. The same electrodialytic extraction apparatus as that shown in Fig. 1—2 was used. A JLC-2A (Japan Electron Optics Laboratory Co., Ltd.) constant flow rate pump was used, SJ-1055 (Mitsumi Kagaku Co., Ltd.) as the DC power source and dialysis cellulose membrane (Visking Co., Ltd.) for the membrane.

Extracting System B——This system is shown in Fig. 3. The effluent from extracting system A was led into the flow cell of JLC-BC spectrophotometer (Japan Electron Optics Laboratory Co., Ltd.) and the absorbance of this effluent was recorded on a Beckmann log-linear 10 inch recorder.

Sample S——Acrinol base (63.3 mg), 25 g of microcrystalline cellulose and 25 ml of water were mixed uniformly in a mortar. After mixing, this was dried for 1 hour at 60°, pulverized in a mortar, sieved with a 100 mesh sieve and this was taken as sample S (253 μg/100 mg).

Sample L——Acrinol base (25.0 mg) was dissolved in 0.5 M acetic acid and made up to 100 ml.

Comparison of Electrodialytic Extraction Data and Shaking Extraction Data——1) Recovery Test with Electrodialytic Extraction: 100 mg of sample S was placed in the sample chamber of the apparatus, and acrinol base was extracted. The effluent was fractionated every 5 minutes and determined with a spectrophotometer at 271 mμ for calculation of the recovery using the extracting system A. The extraction conditions were as follows; carrier solution 0.5 M acetic acid, flow rate of carrier solution 1.54 ml/min, current density 20 mA/cm² and extraction time 40 min.

2) Recovery Test with the Shaking Extraction: 100 mg of sample S was shaken with 60—100 ml of 0.5 M acetic acid for 5—60 minutes with a shaker and centrifuged. The clear supernatant liquid was then decanted and the absorbance of this solution was determined at 271 mμ, 0.5 M acetic acid being used as the blank.

Comparison of Rates of Extractions from Powder and Solution——Acrinol base was extracted from 100 mg of sample S or 1 ml of sample L using extracting system B. The extraction conditions were as follows; carrier solution 0.5 M acetic acid, flow rate of carrier solution 1.54 ml/min and current density 20 mA/cm². When the pen of the recorder returned to the base line, extraction was stopped and the effluent determined. The recoveries were obtained every 5 minutes by weighing the cut paper from the chart.

Relation between Different Concentrations of Carrier Solutions and Rate of Extraction——Acrinol base was extracted from 1 ml of sample L using extracting system B. The extraction conditions were as follows; flow rate of carrier solution 1.54 ml/min and current density 20 mA/cm². The carrier solutions used are shown in Table V. The recovery for the initial 5 minutes was obtained by weighing the cut paper from the chart every minute.

Effect of Electric Current on Rate of Extraction——Acrinol base was extracted from 100 mg of sample S using extracting system A. The extraction conditions were as follows; carrier solution 0.5 M acetic acid, flow rate of carrier solution 1.54 ml/min and current density 5—25 mA/cm². The effluent was fractionated every 5 minutes and determined.

Relation between Quantities of Acrinol Base and Recoveries by Extraction——Acrinol base was extracted from the 0.5 M acetic acid solutions which contained 50—300 μg/ml of acrinol base, using extracting system A. The extraction conditions were as follows; carrier solution 0.5 M acetic acid, flow rate of carrier solution 1.54 ml/min and current density 20 mA/cm². The effluent was fractionated every 5 minutes and determined.

Effect of Particle Size of Sample on Rate of Extraction——The sample was prepared in the same manner as the sample S and was classified into the six particle sizes of 40—50, 50—60, 60—80, 80—100, 100—200, >200 meshes by sieving. Acrinol base was extracted from these samples, using extracting system A. The extraction conditions were as follows; carrier solution 0.5 M acetic acid, flow rate of carrier solution 1.54 ml/min and current density 20 mA/cm². The recovery for the initial 5 minutes was obtained by the weighing method as mentioned above.

Effect of Pore Size of Membrane on Rate of Extraction——Acrinol base was extracted from sample L, using extracting system B containing various membranes (Millipore's membrane VF, VM, VC). The extraction conditions were as follows; carrier solution 0.5 M acetic acid, flow rate of carrier solution 1.54 ml/min and current density 20 mA/cm². The recovery for the initial 5 minutes was obtained by the weighing method. The recovery was obtained also without applying electric current.

Measurement of Extraction Rate with Nicotinic Acid-Sodium Hydroxide System——1) Apparatus: a) Conductivity Meter: Conductivity Outfit (Yanagimoto Co., Ltd).

b) Electrodialytic Extraction Apparatus: extracting system B.

2) Sample: a) Sample for Measurement of Conductivity: 0—0.1 mole of nicotinic acid was dissolved in 0.1 N sodium hydroxide and made up to 1 liter.

b) Sample for Electrodialytic Extraction: 7.239 g (0.0588 mole), 3.619 g (0.0294 mole) and 0.726 g (0.0059 mole), respectively, of nicotinic acid was dissolved in 0.1 N sodium hydroxide and made up to 1 liter.

3) Procedure: The specific conductivities of sample 2a) were measured. These values were plotted against nicotinic acid concentration and the existence of linear relation between them was confirmed. The initial transport number of nicotinic ion in the sample chamber was calculated from ion equivalent conductivities of Na^+ and OH^-, and this slope of straight line. Also, nicotinic acid was extracted from sample 2b), using extracting system B. The extraction conditions were as follows; carrier solution 0.1N sodium hydroxide, flow rate of carrier solution 1.54 ml/min, current density 10, 20, 30 mA/cm² and extraction time 50 min. The recovery for the initial 5 minutes were determined with a spectrophotometer, and the amounts of electricity carried with nicotinic ion were obtained. The data obtained were compared with the calculated data.

Electrodialytic Extraction of Phenobarbital, using (Tris-hydroxymethyl)aminomethane-Sodium Chloride as Carrier Solution——1) Apparatus: Extracting system B.

2) Carrier Solution: (Tris-hydroxymethyl)aminomethane(0.05 M)-sodium chloride (0.005—0.015 M) was used.

3) Sample: 100 mg of phenobarbital was dissolved in the carrier solution and made up to 100 ml.

4) Procedure: Phenobarbital was extracted from 1 ml of each sample and the extraction conditions were as follows; carrier solution the same as above mentioned 2), flow rate of carrier solution 1.54 ml/min and current density 20 mA/cm². The recovery for the initial 30 minutes were obtained by weighing the cut paper every 5 minutes. The specific conductivity of each carrier solution was measured.

Electrodialytic Extraction of Sulfadiazine using Sodium Hydroxide as Carrier Solution——1) Apparatus: Extracting system B was used.

2) Carrier Solution: Sodium hydroxide (0.005—0.100 M) was used.

3) Sample: 50 mg of sulfadiazine was dissolved in the carrier solution and made up to 100 ml.

4) Procedure: Sulfadiazine was extracted flom 1 ml of each sample and the extraction conditions were as follows; carrier solution the same as above mentioned 2), flow rate of carrier solution 1.54 ml/min and current density 20, 40 mA/cm^2. The recovery for the initial 40 minutes was obtained by weighing the cut paper every 5 or 10 minutes. The specific conductivities of the carrier solutions were measured.

Result and Discussion

As a model composition, acrinol–microcrystalline cellulose was chosen as it was indicated by preliminary experiments that among the usual drugs, binders and excipient this was the most difficult combination to extract a drug from its excipient. The advantage of this new elctrodialytic extraction method was examined by using these model compositions and the extraction conditions generally applicable were selected by using the same samples.

Table I. Comparison of Electrodialytic Extraction Data and Shaking Extraction Data

Extraction time (min)	Recovery with shaking extraction (%) Vol. of solvent (ml) 60	70	80	90	100	Recovery with electrodialytic extraction (%) 1.54 ml/min
5	87.6	87.5	86.8	85.1	85.9	77.0
10	85.9	86.8	87.6	87.6	87.8	89.1
15	84.5	85.1	87.8	87.6	88.0	94.7
20	85.6	84.2	84.2	87.8	87.8	97.6
30	85.9	86.3	88.5	87.6	89.7	100.6
40	85.9	87.6	87.6	87.6	87.8	100.7
60	89.4	87.5	89.4	87.5	86.4	

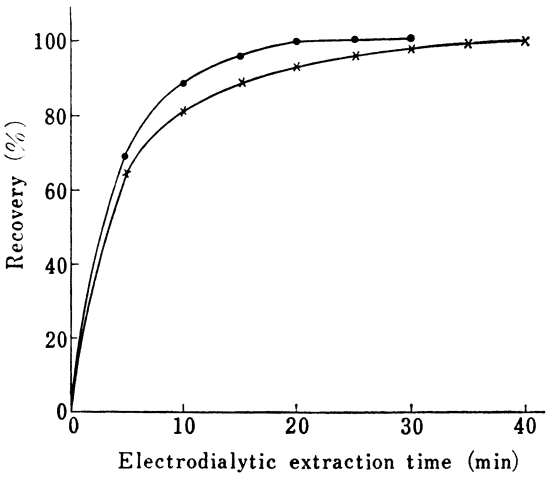

Fig. 4. Comparison of Rate of Electrorodialytic Extraction from Powder and Solution

—×—: powder —●—: solution

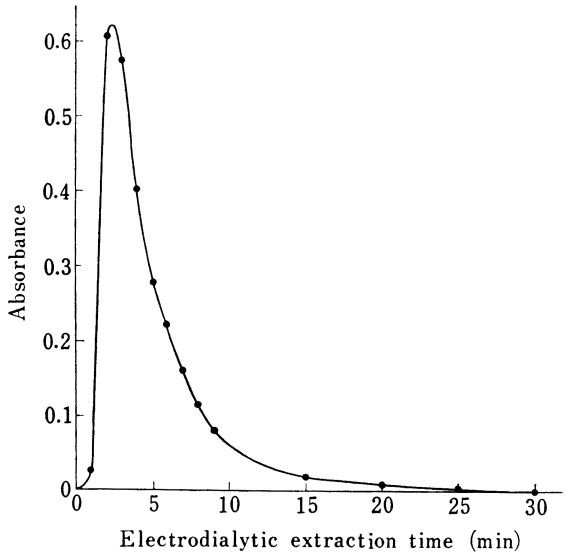

Fig. 5. Electrodialytic Extraction Curve

In order to investigate the extracting power of electrodialytic extraction, data of electrodialytic extraction and shaking extraction were compared in Table I. The recovery with shaking extraction did not exceed 90% even when the volume of the solvent was increased and the time of extraction was prolonged, but with the electrodialytic extraction, quantitative extraction was achieved in the relatively short time and by the limited volume of extractant and in the present instances they are 30 minutes and 60 ml respectively.

Preliminary experiment could be carried out very rapidly using a liquid sample instead of a solid sample to find out whether or not the test component is extractable by electrodialytic extraction. As shown in Fig. 4 no essential differences were observed in the rate of extraction between a liquid sample and a solid sample.

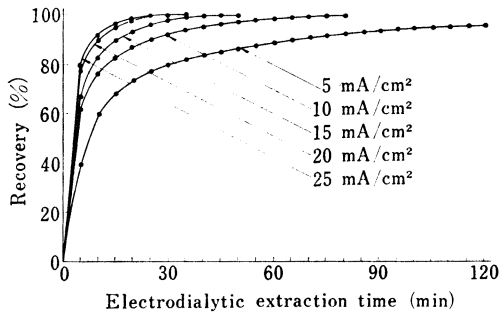

Fig. 6. Effect of Current Density on Electrodialytic Extraction Rate

The extraction data of the liquid sample shown in Fig. 4 were calculated into absorbance–time curve in Fig. 5, from which it is evident that electrodialytic extraction has high efficiency.

The effect of current density on extraction rate is shown in Fig. 6. The acrinol base was extracted from the sample within 1 hour, if a current density of more than 15 mA/cm² was supplied, although it should be possible to extract this component quantitatively by extracting for longer time with a current density less than 10 mA/cm². It has been further observed that there was no great difference in extraction rate by increasing the current density 20 mA/cm² to 25 mA/cm² so that the optimum current density was suggested to be 20 mA/cm².

When various amounts of the sample were taken into the sample chamber, the time required for quantitative extraction of this component did not indicate large change as shown in Table II, although there was a little tendency of longer time with increasing sample weight.

Table II. Relation between Quantities of Acrinol Base and Electrodialytic Extraction Rate

Electrodialytic extraction time (min)	Recovery (%)				
	Quantities of acrinol base (μg)				
	50	100	200	250	300
5	67.8	64.5	65.9	64.5	67.8
10	87.4	85.5	85.1	86.9	89.0
15	96.6	94.5	93.9	94.4	96.1
20	99.7	98.2	97.5	97.3	99.0
25		99.9	99.1	98.7	100.1
30			99.4	99.3	100.6

The effect of particle size of the sample in electrodialytic extraction was examined and the result is shown in Table III, from which it could be seen that the extraction rate was reduced with the increase of the particle size of the sample. The suitable particle size was about 100 mesh considering the labor required for crushing the preparation and the efficiency of extraction from it.

If the porosity of the membrane has any effect on the mobility of the test component, the apparatus with the membrane of the bigger pore size should be more efficient. The result

TABLE III. Effect of Particle Size of Sample on Electrodialytic Extraction Rate

Electrodialytic extraction time (min)	Recovery (%) Particle size of sample (mesh)					
	40—50	50—60	60—80	80—100	100—200	>200
1	12.4	14.9	13.8	14.4	16.5	16.2
2	32.3	36.5	36.4	36.3	39.7	38.8
3	45.7	50.4	51.2	50.2	54.2	52.5
4	54.8	59.7	60.6	59.2	63.0	61.3
5	61.6	66.5	67.4	65.8	69.2	68.1

TABLE IV. Effect of Pore Size of Membrane on Electrodialytic Extraction Rate

Electrodialytic extraction time (min)	Membrane Pore size (nm) Current density (mA/cm²)	Recovery (%) Millipore's membrance VF 10		VM 50		VC 100		Cellulose membrane 2.4	
		20	0	20	0	20	0	20	0
1		13.8	1.9	12.4	2.0	13.5	3.4	11.0	0
2		35.0	3.1	33.1	3.2	34.9	4.9	33.4	0
3		52.1	3.9	51.5	3.9	52.1	5.7	52.1	0
4		65.1	4.7	63.2	4.4	64.7	6.4	66.1	0
5		74.6	5.3	73.3	4.8	74.7	6.9	76.3	0

TABLE V. Relation between Different Concentrations of Carrier Solutions and Electrodialytic Extraction Rate

Carrier solution (M)		Recovery (%) Extraction time (min) 1	2	3	4	5
HCl	0.001	28.9	53.8	66.5	73.2	78.1
	0.003	9.3	25.8	38.3	48.3	56.1
	0.005	5.5	16.7	26.4	34.7	41.8
	0.007	3.2	8.9	14.9	20.7	26.2
	0.01	0.8	4.0	8.5	13.7	19.1
Citric acid	0.01	13.4	36.7	53.9	66.9	76.9
	0.03	6.0	20.4	32.6	42.8	50.8
	0.05	3.6	17.0	30.5	41.5	50.8
	0.1	1.9	9.3	18.5	27.1	34.7
	0.5	0.4	1.6	3.7	6.1	8.8
AcOH	0.1	19.6	50.2	70.6	82.4	90.5
	0.2	23.8	51.7	69.0	80.4	84.6
	0.3	14.3	40.5	60.8	74.7	84.3
	0.4	12.8	37.1	56.3	70.2	80.4
	0.5	11.0	33.3	52.1	66.1	76.3
	0.6	11.4	32.7	49.8	62.9	72.9
	0.7	10.6	30.2	46.7	59.6	69.4
	0.8	10.9	31.4	47.0	59.4	68.6
	0.9	7.9	26.4	42.6	56.0	65.6
	1.0	7.2	24.9	39.5	51.8	61.7

of the experiment using millipore's filters of various pore size is compared with that of cellulose membrane in Table IV. It is apparent from Table IV that the low molecular weight compounds like acrinol could pass through the fine pore (2.4 nm) of cellulose membrane quite rapidly with the aid of the applied potential. Consequently the cellulose membrane found to be suitable for electrodialytic extraction of low molecular weight drugs.

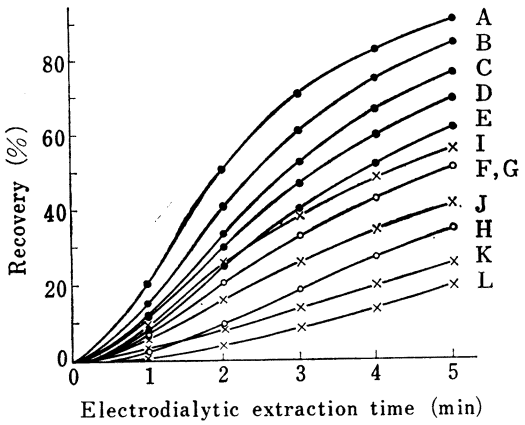

Fig. 7. Relation between Different Concentrations of Carrier Solutions and Electrodialytic Extraction Rate

A: 0.1M AcOH B: 0.3M AcOH C: 0.5M AcOH
D: 0.7M AcOH E: 1.0M AcOH F: 0.03M citric acid
G: 0.05M citric acid H: 0.1M citric acid I: 0.003M HCl
J: 0.005M HCl K: 0.007M HCl L: 0.01M HCl

The carrier solution used in electrodialytic extraction must have the ability to dissolve the test component from the sample effectively. The composition of the carrier solution has a significant influence on electrodialytic extraction rate. The relationship between the compsition of the carrier solution and electrodialytic extraction rate was investigated from various points of view. The electrodialytic extraction rate using various concentrations of hydrochloric acid, citric acid and acetic acid as the carrier solutions are shown in Table V and Fig. 7. With regard to the same kind of the carrier solution, the extraction rate was faster at the lower concentration. It was found that acetic acid is an excellent carrier solution because it provided a stable constant current in wide range of concentrations, *i.e.* 0.2—1.0 M, and has high extraction rate.

Mobility is a primary factor of extraction rate in electrodialytic extraction when the same carrier solution is used and the same current density is supplied. When diluted sample is used for high mobility substance, the ratio of specific conductivity of carrier solution to test component can be used for estimating the electrodialytic extraction rate. This fact was demonstrated with sample of nicotinic acid in sodium hydroxide solution as shown in Table VI. After the above experiment, the relationship between specific conductivity of carrier

Table VI. Measurement of Electrodialytic Extraction Rate in Nicotinic Acid–Sodium Hydroxide System

Concn. of nicotinic acid (M)	Current density (mA/cm²)	Amount of electricity carried by nicotinic ion (%)		
		Calcd.	Found	
			1	2
0.0588	20	10.25	15.02	14.00
	40		12.17	11.66
	60		10.95	10.41
0.0294	20	3.78	4.04	4.00
	40		3.10	3.16
	60		3.08	3.15
0.0059	20	0.63	—	0.74
	40		0.49	0.53
	60		0.35	0.46

TABLE VII. Electrodialytic Extraction Rate of Phenobarbital, using (Tris-hydroxymethyl) aminomethane–Sodium Chloride as Carrier Solution

Composition of carrier solution		Specific conductivity of carrier solution	Current density	Recovery (%)				
Concn. of A (M)	Concn. of B (M)	(mho/cm)	(mA/cm²)	Extraction time (min) 5	10	15	20	30
0.05	0.005	0.623×10^{-3}	*a*)					
0.05	0.006	0.751×10^{-3}	*a*)					
0.05	0.007	0.864×10^{-3}	*a*)					
0.05	0.008	0.978×10^{-3}	20	49.9	79.0	91.3	95.7	98.5
0.05	0.009	1.089×10^{-3}	20	49.9	80.1	91.9	96.4	99.0
0.05	0.010	1.187×10^{-3}	20	48.0	75.8	88.2	94.1	97.5
0.05	0.011	1.298×10^{-3}	20	47.8	76.4	89.5	94.9	97.9
0.05	0.012	1.411×10^{-3}	20	42.4	70.1	86.0	92.9	97.2
0.05	0.013	1.523×10^{-3}	20	44.6	72.5	88.0	94.3	97.3
0.05	0.014	1.696×10^{-3}	20	40.5	66.0	81.9	90.8	95.7
0.05	0.015	1.806×10^{-3}	20	38.1	62.7	79.6	89.2	95.0

A: (tris-hydroxymethyl)aminomethane
B: NaCl
a) The current (20mA/cm²) could not be obtained in these carrier solutions so that electrodialytic extraction was discontinued.

TABLE VIII. Electrodialytic Extraction Rate of Sulfadiazine, using Sodium Hydroxide as Carrier Solution

Carrier solution		Current density	Recovery (%)					
Concn. of NaOH (M)	Specific conductivity (mho/cm)	(mA/cm²)	Extraction time (min) 5	10	15	20	30	40
0.005	1.197×10^{-3}	*a*)						
0.007	1.646×10^{-3}	20	23.5	58.9	85.4[b]			
0.010	2.324×10^{-3}	20	20.3	46.8	71.5	85.0	97.4[c]	
0.050	11.724×10^{-3}	20	4.0	8.1	12.7	17.8	28.9	49.3
		40	6.7	16.5	27.0	37.2	55.8	72.5
0.100	22.400×10^{-3}	20		4.4		8.8	13.3	18.3
		40	3.7	4.4	9.1	14.4	24.8	35.7

a) See *a*) of Table VII.
b) This value was the rate for 16 minutes, and constant current (20 mA/cm²) was not maintained longer than 16 minutes.
c) Constant current (20 mA/cm²) was not maintained longer than 30 minutes.

solution and electrodialytic extraction rate was examined with (tris-hydroxymethyl)aminomethane–sodium chloride–phenobarbital as shown in Table VII, and with sodium hydroxide–sulfadiazine as shown in Table VIII. In Table VII, the (tris-hydroxymethyl)aminomethane solution which has low specific conductivity has good extraction property and the specific conductivity was increased by addition of sodium chloride. The specific conductivity in a range from 1.0×10^{-3} to 1.5×10^{-3} mho/cm of this carrier solution was suitable for electrodialytic extraction. As shown in Table VIII, sodium hydroxide solution has poor extraction property since it has high specific conductivity. Electrodialytic extraction was not possible when carrier solution of sodium hydroxide with specific conductivity below 1.5×10^{-3} mho/cm was used. Consequently weak electrolytes are superior to strong electrolytes as the carrier

solution, and 0.5 M acetic acid, 0.05 M citric acid and 0.005 M hydrochloric acid indicated similar extraction rates.

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