Extraction of Water-Soluble Vitamins from Pharmaceutical Preparations Using AOT (Sodium Di-2-ethylhexyl Sulfosuccinate)/Pentane Reversed Micelles

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Reversed micelles can be used to concentrate water-soluble materials in the water pool. In this study, the extraction of water-soluble vitamins into reversed micelles was attempted, and a flow system was used to determine the time-course of the vitamin extraction. The efficiency of extraction was strongly affected by the extraction temperature and the concentration of reversed micelles, and the selectivity depended on the size of micelles. Water-soluble vitamins could be efficiently and rapidly extracted. The selective extraction of a model mixture of vitamins from pharmaceutical preparations was also attempted. Moreover, the usefulness of the proposed method for the determination of vitamins in various commercial tablets was also demonstrated.

Using of this method, the surfactant remains mixed with the extracted compounds, and so we attempted to remove the surfactant from the extract by supercritical fluid extraction. Supercritical carbon dioxide containing 7.5% ethanol as entrainer was found to be the most efficient solvent for removing residual surfactant from the extract.

Key words reversed micelle; water-soluble vitamin; extraction; AOT (sodium di-2-ethylhexyl sulfosuccinate); pharmaceutical preparation; supercritical fluid extraction

In recent years, liquid-liquid extraction using reversed micelles has received much attention as an effective method for the concentration of water-soluble compounds such as peptides, proteins and enzymes.[1–3] Interestingly, it has even been reported that the activity of an enzyme can be preserved in a reversed micelle solution. Furthermore, Smith *et al.* have reported that it is possible to use sodium di-2-ethylhexyl sulfosuccinate (AOT)-propane or AOT-ethane under supercritical conditions for enzyme extraction in place of carbon dioxide (CO_2).[4] Usually, in the usual reversed micellar extraction, the analytes are extracted into reversed micelles by mixing the sample and reversed micellar solutions. For direct extraction of enzyme, another method has also been tried, in which the sample powder is added to a reversed micellar solution.[5,6] Both methods, however, have been used in batch processes and are too complicated for practical use as method of preparation. In addition, most of these methods have been applied to high molecular weight compounds, such as proteins, and few reports have appeared on the extraction of compounds of relatively low molecular weight.[1]

Vitamins are trace nutrients often found in various kinds of foods and medicines and are indispensable to human growth and health. Usually, titration or HPLC methods are used to determine vitamin concentrations. These methods often require extraction as a pretreatment process. Since water is used in large quantities for the extraction of water-soluble vitamins, its removal after extraction is usually difficult. In such a case, therefore, a sensitive detection method should be employed for determination of the analytes.

In this study, a reversed micellar flow system was used for the selective extraction of several water-soluble vitamins from the matrix of pharmaceuticals, and its extraction properties evaluated. In addition, removal of the surfactant from the extract with supercritical carbon dioxide was also studied.

Experimental

Materials Pentane was chosen as the organic solvent since it is easily evaporated after extraction. The surfactant used was AOT, which was obtained from Aldrich Chemical Co., U.S.A. (purity > 98%). Vitamin B_2 (riboflavin) and vitamin B_{12} (cyanocobalamin) were purchased from the National Institute of Hygienic Sciences, Japan. Vitamin B_6 (pyridoxine hydrochloride) and nicotinamide were purchased from Wako Pure Chemical Co., Japan. Vitamin E (tocopherol calcium succinate) was purchased from Eizai Co., Japan. Vitamin C (ascorbic acid) was purchased from Roche Co., Switzerland. L-HPC (low substituted hydroxypropyl cellulose), LH-21 grade, was purchased from Shinetsu Chemical Co., Japan. All other reagents and solvents were of reagent grade and were purchased from Kanto Chemical Co., Japan.

Preparation of the Extractant Solutions of reversed micelles were prepared by dissolving AOT in pentane in a flask with shaking. The size of a reversed micelle is generally expressed by the surfactant-to-water ratio W_0(=[H_2O]/[AOT]). Therefore, experiments were performed involving changing the concentration of water. In all cases, the water content in the AOT was checked by means of a Karl-Fischer titrator (model NKA-3P, Kyoto Electron Industry Co., Japan). The AOT concentration is expressed as

$$[\mathrm{AOT}] = \frac{\frac{W_{\mathrm{AOT}}(100-a)}{100}}{M_{\mathrm{AOT}}}$$

and the water concentration is given by

$$[\mathrm{H_2O}] = \frac{W_{\mathrm{H_2O}} + \frac{a \cdot W_{\mathrm{AOT}}}{100}}{M_{\mathrm{H_2O}}}$$

Here, W_{H_2O} and W_{AOT} are the weights of added water and AOT per liter of pentane, respectively, M_{H_2O} and M_{AOT} are the molecular weights of water and AOT, and a is the water content (%) determined by the Karl-Fischer method. W_0 was obtained by dividing the H_2O con-

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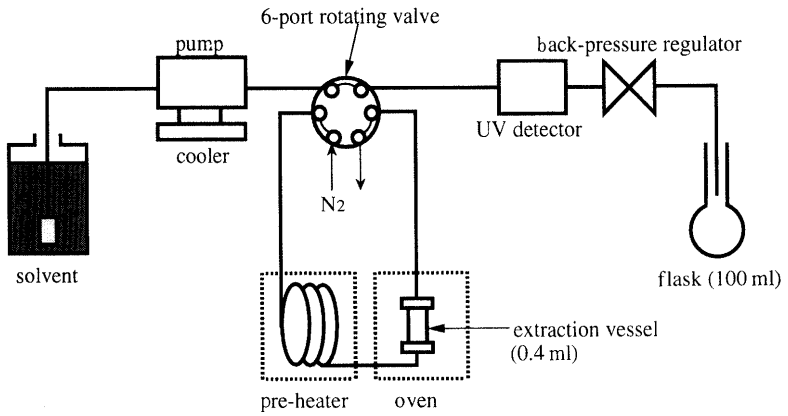

Fig. 1. Extraction System

Table 1. HPLC Conditions

Sample	Mobile phase	Internal standard	Detection
Vitamin B_2	AcCN–H_2O–H_3PO_4 (10:90:0.1)	Salicylic acid	Fluorescence (ex. 350 nm, em. 450—800 nm)
Vitamin B_6, nicotinamide	AcCN–H_2O–H_3PO_4–SDS (30:70:0.1:0.5)	L-Phenylalanine	UV (270 nm)
Vitamin E	MeOH–H_2O–H_3PO_4 (90:10:0.1)	Di-2-ethylhexyl phathalate	UV (280 nm)
Vitamin C	AcCN–H_2O–H_3PO_4 (92:8:0.1)		UV (245 nm)
Vitamin B_{12}	0.04 M Tartaric acid (pH 3)–MeOH (80:20)	Benzoic acid	UV (280 nm)

AcCN, acetonitrile; SDS, sodium dodecyl sulfate; MeOH, methanol.

centration by the AOT concentration.

Extraction Apparatus and Its Operation The flow extraction system illustrated in Fig. 1 was used.

A 0.4 ml stainless-steel empty column was used as the extraction vessel. A sample containing 20 mg of vitamin was placed in the vessel for extraction. In order to stabilize the sample, the vessel with nitrogen was flushed continuously until the oven had reached a specified temperature. The extractant was delivered by means of a HPLC pump (Model LC-9A, Shimadzu Co., Japan). The concentration of vitamin in the exit stream from the vessel was monitored with a UV detector (Model SPD-6AV, Shimadzu Co., Japan). To determine the recovery, pentane was removed under a stream of nitrogen at room temperature. The residue was then dissolved in methanol–water–phosphoric acid (50:50:0.1), and, after the addition of internal standard, an aliquot was injected into the HPLC.

Sample Preparation Six kinds of vitamins were chosen as model compounds, and mixed 1:19 by weight with matrix. L-HPC was selected as the matrix for the dispersion of these vitamins.

HPLC System The HPLC apparatus consisted of a pump (Model LC-9A, Shimadzu Co., Japan), a UV detector (Model SPD-6AV, Shimadzu Co., Japan), a fluorescence detector (Model FLD-6A, Shimadzu Co., Japan), an injector (Model 7125, Rheodyne, Cotati, U.S.A.), a separation column for vitamin C (TSK-gel NH_2-60, 250 × 4.6 mm i.d., Tosoh Co., Japan), a separation column for other vitamins (TSK-gel ODS-120A, 150 × 4.0 mm i.d., Tosoh Co., Japan), a column oven (Model CTO-6A, Shimadzu Co., Japan) and an integrator (Model C-R4A, Shimadzu Co., Japan). HPLC separation was performed at 50 °C, and the sample injection volume was 10 μl.

Measurement of Vitamins The HPLC conditions used for the measurement of extracted vitamins are shown in Table 1.

Results and Discussion

Effect of Pressure The effect of extraction pressure on the recovery of vitamin B_2 was examined at different temperatures. All extractions were performed above the critical pressure (33.3 atm) in order to prevent the vaporization of pentane. Recovery was unaffected by pressure. Thereafter, 50 atm was employed as the extraction pressure.

Effect of Micellar Concentration The effect of micellar concentration (numbers of micelles) on the recovery was examined, while keeping micellar size constant. Since the number of surfactant molecules required to form a micelle (*As*) is also constant, micellar concentration can be assumed to be more or less proportional to the concentration of AOT. The relationship of micellar concentration ([Mic]) to AOT concentration ([AOT]) is given by

$$[\mathrm{Mic}] = \frac{[\mathrm{AOT}] - cmc}{As}$$

When *cmc* (critical micellar concentration) is negligibly small (about 5×10^{-4} M)[7] compared with AOT concentration, this becomes

$$[\mathrm{Mic}] = \frac{[\mathrm{AOT}]}{As}$$

Therefore, the effect of a change in AOT concentration on recovery was examined. As shown in Fig. 2, for vitamin B_2, the relationship between AOT concentration and recovery over the temperature range 40—70 °C was linear.

Using these results, an approximately linear relationship was found between temperature and recovery when the two were plotted at a constant concentration of AOT. Since extraction can be performed in oxygen-free conditions, no decomposition of vitamin B_2, even at 80 °C, was observed.

Results similar to those for vitamin B_2 were obtained for the other vitamins studied. When 100% pentane was used as the extractant, none of the water-soluble vitamins used in this experiment were extracted. Therefore, it seems that almost all water-soluble vitamins dissolved in the

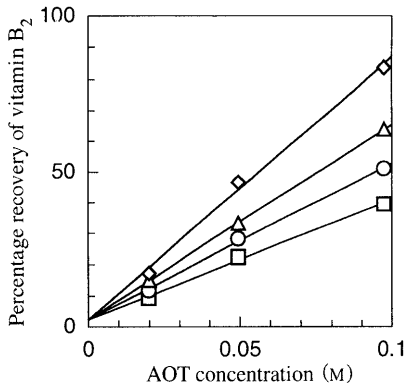

Fig. 2. Effect of Micellar Concentration on Recovery at Constant W_0

◇, 70°C; △, 60°C; ○, 50°C; □, 40°C. Solvent, H_2O/AOT/pentane; pressure, 50 atm, $W_0=5.9$; flow rate, 2.0 ml/min; extraction time, 30 min; sample, vitamin B_2 (1 mg) in L-HPC (20 mg).

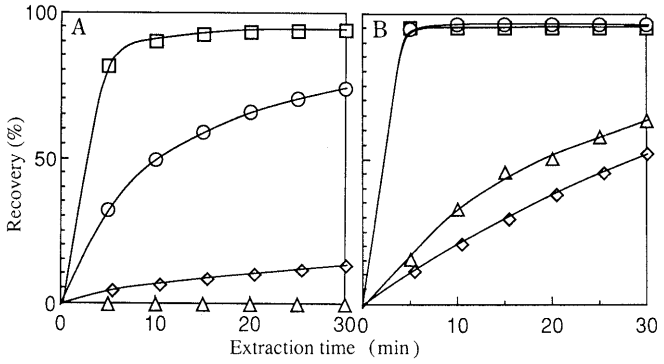

Fig. 3. Effect of W_0 on Recoveries of Vitamins

□, vitamin B_6; ○, vitamin C; ◇, vitamin B_{12}; △, vitamin B_2. Solvent, H_2O/AOT/pentane, [AOT]=0.05 M; pressure, 50 atm; flow rate, 2.0 ml/min; extraction time, 30 min; temperature, 50°C; sample, vitamin B_2 (1 mg) in L-HPC (20 mg).

A, $W_0=2.45$; B, $W_0=11.6$.

water pool rather than the oil phase.

Effect of Micellar Size In order to compare the efficiencies of extraction at different value of W_0, the concentration of water was changed while the AOT concentration was held constant. Figure 3 shows the extraction curves obtained in this way for four vitamins.

Vitamin B_{12} was not extracted when $W_0=2.45$. This may have been because the molecular size of vitamin B_{12} is fairly large compared with that of the other vitamins. Thus, the size of the water pool in the reversed micelle is too small to dissolve vitamin B_{12} when $W_0=2.45$. It is known that a linear relationship exists between the size of reversed micellar AOT and W_0, and that the following equation can be used[8]:

$$R(\text{nm}) \fallingdotseq 0.16 \cdot W_0 + 1.2\,\text{nm}$$

Here, R is the radius of the AOT reversed micelle. Since the value of 1.2 nm in this formula corresponds to the length of the AOT molecule, the value obtained by multiplying W_0 by 0.16 corresponds approximately to the radius of the water pool. Thus, when $W_0=2.45$, the diameter of the water pool is estimated as 0.8 nm. Since the diameter of the vitamin B_{12} molecule is larger than this, our results appeared to be reasonable.

On the other hand, when $W_0=11.6$, the efficiency of extraction of vitamin B_{12} was higher than that of vitamin B_2. When $W_0=11.6$, the micellar size is sufficiently larger than the molecular sizes of the vitamins and, therefore, the extraction efficiency becomes higher as the solubility in water increases. In addition, others may be slightly more extractable at $W_0=11.6$. In this experiment, as W_0 was changed while the AOT concentration was kept constant, the water concentration in the extractant at $W_0=11.6$ became about five times higher than that with $W_0=2.45$. Since it is thought that the water-soluble vitamins mostly dissolve in the water pool, a higher water content results in a higher efficiency of extraction.

Table 2. Recoveries (%) of Vitamin Extractions

	Extraction time (min)		
	0—20	20—70	70—120
Vitamin E	101.2	0	0
Vitamin B_6	0	94.7	0
Vitamin B_{12}	0	5.2	89.3

Selective Extraction of Mixed Vitamins Based on the results shown in Fig. 3, selective extraction from tablets containing equal amounts (1 mg/20 mg) of vitamin E, vitamin B_6 and vitamin B_{12} was attempted by changing the micellar size of the extractant step by step. The results are shown in Table 2.

When pentane was used as the extractant for the first 20 min, efficient extraction of vitamin E alone was obtained. Then, an additional extractant with reversed micelles at $W_0=2.38$ was fed into the vessel, and vitamin B_6 was almost completely extracted in 50 min. In this fraction, a small amount of vitamin B_{12} was found, but no vitamin E was detected. The remaining vitamin B_{12} was recovered almost completely when W_0 was changed to 22.4.

In the reported extraction experiments[1,4,5] using reversed micelles, in which the size of the sample protein molecules was much larger than the size of the reversed micelles, and no such discrimination phenomena were detected. Proteins could be extracted by stirring with the extractant and no selectivity due to the size of the reversed micelles was observed. It might be that reversed micelles are repeatedly broken and enlarged due to longer contact time of reversed micelles with the sample molecules. As a result, the protein molecules are enclosed in the reversed micelles and extracted together. In our method, which features shorter contact time, molecules larger than reversed micelles cannot be extracted because of the initial size of the reversed micelles. Water-soluble polymers, which could not be eliminated by ordinary extraction, could be eliminated by our method.

Extraction of Water Soluble Vitamins from Commercial Tablets A conventional batch extraction method and the method proposed here were used for the determination of vitamins in commercial tablets. The conditions of extraction for each case are shown in Table 3.

In the batch method, a methanol–water–phosphoric acid (50:50:0.1) mixture was used as the extraction solvent. After heating and shaking, the aqueous supernatant was obtained by centrifugation. These operations

were repeated three times and the final supernatants were combined. In the proposed method, extraction was performed using the solvent with $W_0 = 11$ and [AOT] = 0.1 M for 30 min at 80 °C. Crushed tablets containing three types of vitamins were chosen as the test sample. Reversed-phase LC was used for the determination of the vitamins using an internal standard method. Almost identical results were obtained with the two methods, as shown in Table 4.

The reproducibilities of both methods were also satisfactory. The value obtained for vitamin B_2 by our method was slightly lower than that obtained with the batch method; however, it appeared that the extraction time was too short for complete extraction. Since monitoring the concentrations of extracted compounds in real time is possible, optimization of the conditions of extraction could be much more easily accomplished.

Removal of AOT from Extract In the ordinary reversed micellar extraction technique,[9] a back-extraction is generally used to collect the extracted substances. This, however, has the disadvantage that the target component is diluted. Pentane, which was used as the extractant in this study, can easily be evaporated. For the selective removal of AOT from the mixture of AOT, vitamin and the small amount of water which remains after pentane has been removed, it is necessary that AOT hardly forms reversed micelles and that it can dissolve in the extractant. We found that carbon dioxide with a small amount of alcohol can dissolve AOT under supercritical conditions. Thus, AOT alone can be extracted from the mixture of AOT, attached to this extraction system, using the similar procedures as for the extraction of vitamins. Therefore, we devised a two-step method of collection in which pentane is vaporized first and then AOT is removed by supercritical fluid extraction (SFE), as illustrated in Fig. 4.

A 0.1 M AOT/pentane solution (20 ml) containing 0.35 mg of vitamin B_2 was poured into a high-pressure vessel, and pentane was removed by a stream of nitrogen. Then the vessel was placed in the SFE apparatus and extraction was performed for 40 min using supercritical CO_2 (250 atm), which was added with an appropriate entrainer at a total flow rate of 4 ml/min. The extracted AOT was dried in a rotary evaporator and its recovery was determined by weighing. Under these conditions, when only CO_2 was used, very little AOT could be removed. Then 7.5% methanol, ethanol or hexane was added as an entrainer and extraction carried out for 40 min. The results are presented in Table 5.

It is clear from Table 5 that AOT was almost completely removed when methanol or ethanol was used as an entrainer. The loss of vitamin B_2 was relatively high (38.1%) with methanol, and the most efficient entrainer

Table 3. Conditions of Extraction of Vitamins from Pharmaceutical Preparations

Conditions	Extraction method	
	Batch	Proposed
Extraction solvent	MeOH–H_2O–H_3PO_4 (50 : 50 : 0.1)	H_2O/AOT/pentane ($W_0 = 11.3$, [AOT] = 0.1 M)
Solvent volume	90 ml (30 ml × 3 times)	60 ml (2 ml/min × 30 min)
Temperature	65 °C	80 °C
Shaking	5 min × 3 times	—
Centrifugation	3000 r.p.m., 5 min	—

Table 4. Analysis of Vitamins from Pharmaceutical Preparations

Extraction method	Components	Labeled amount (mg/4 tablets)	Detected amount (mg/4 tablets)					Percentage of label
			1	2	3	Av.	RSD (%)	
Batch	Vitamin B_2	24.0	24.4	24.1	24.0	24.2	0.9	100.8
	Vitamin B_6	100.0	102.3	101.7	103.6	102.5	0.9	102.5
	Nicotinamide	60.0	58.2	58.7	58.9	58.6	0.6	97.7
Proposed	Vitamin B_2	24.0	20.9	21.5	20.8	21.1	1.8	87.9
	Vitamin B_6	100.0	101.4	102.0	102.1	101.8	0.4	101.8
	Nicotinamide	60.0	58.2	58.7	58.8	58.6	0.5	97.7

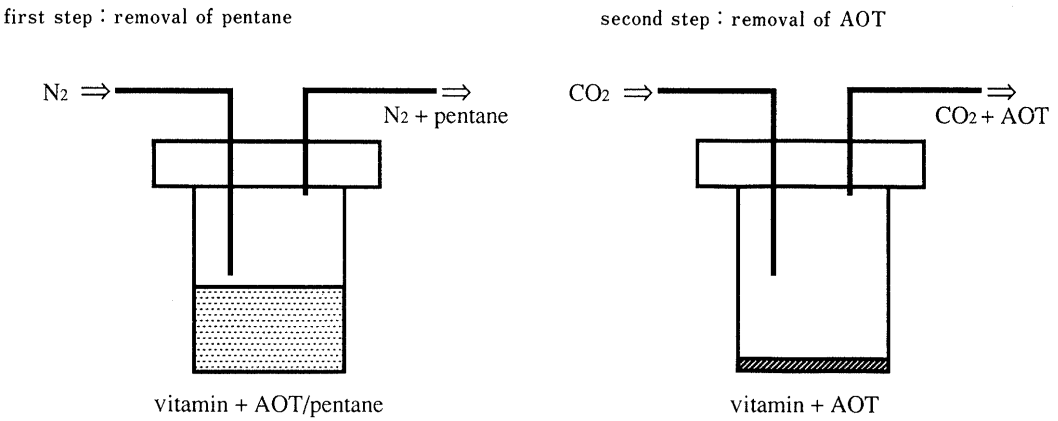

Fig. 4. Schematic Presentation of Experimental Apparatus

Table 5. Recoveries of AOT from Extract with Various Extractants

Solvent	AOT recovery (%)	Vitamin B_2 loss (%)
CO_2 100%	1.2	0
7.5% Hexane–CO_2	1.3	0
7.5% MeOH–CO_2	97.8	38.1
7.5% EtOH–CO_2	97.5	11.1

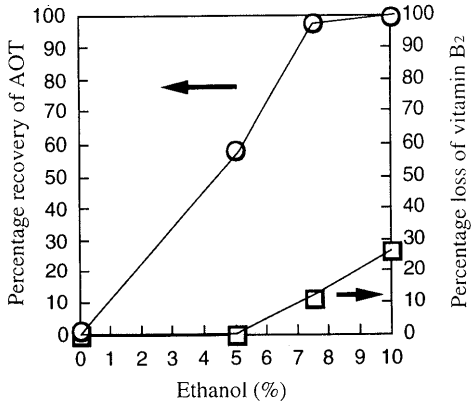

Fig. 5. Effect of Addition of Entrainer on AOT Recovery

Solvent, ethanol/CO_2; pressure, 300 atm; flow rate, 4.0 ml/min; temperature, 60°C; extraction time, 40 min.

was ethanol.

Figure 5 shows the recoveries at different ethanol concentrations.

When ethanol was mixed at a concentration of less than 5% no loss of vitamin B_2 resulted after extraction for 40 minutes, but this was not sufficient for AOT removal. However, 7.5% ethanol appeared to be suitable for removal of AOT.

The AOT removal ratio may be independent of the kind of vitamin tested. On the other hand, the loss of substrate (vitamin) may differ between vitamins. Therefore, it may be necessary to study other samples, containing different vitamins, using our method.

Conclusion

The proposed method has been proven to be useful, particularly when only small amounts of sample are available, since water-soluble vitamins could be extracted rapidly and recovered in high concentrations. Our method is effective for the extraction of various kinds of vitamin if micellar concentration and size are carefully chosen. Our findings also suggest that size-selective extraction can be accomplished by proper adjustment of micellar size. Moreover, our method is useful for the extraction of selected constituents from various matrices of medicinal preparations and foods. Furthermore, it has been shown that removal of the surfactant AOT from the extract can be achieved by the use of supercritical CO_2.

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