Application of the First Derivative Absorption Spectrum–Shift-Length Method to the Simultaneous Determination of Binary Mixture[1)]

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The FDAS-SL method, which is a means of measuring the shift-length (SL, nm) of the maximum absorption wavelength of a drug by utilizing the first derivative absorption spectrum (FDAS), was used in the simultaneous spectrophotometric determination of binary mixtures. This method utilized the shift of the absorption spectrum of a drug at surfactant concentration above the critical micelle concentration. The mixed molar ratio is obtained from the shift-length, and each concentration of drug is calculated by Beer's law. The technique is applicable to binary mixed systems such as the methylparaben–propylparaben mixture which show similar absorption spectra, and gave accurate results.

Keywords first derivative absorption spectrum; alkylparaben; sodium dodecyl sulfate; binary mixture; molar extinction coefficient; simultaneous determination; shift-length; Beer's law; dissolution medium

Introduction

Spectrophotometry is utilized in many fields, and recently has become particularly widespread owing to its use of the characteristics of the derivative absorption spectrum. We have reported[3-10)] some new spectrophotometric methods as well as data obtained by the first derivative absorption spectrum. In this report, we used the FDAS-SL method, which measures the shift-length (SL, nm) of the maximum absorption wavelength by the first derivative absorption spectrum (FDAS),[9)] on the simultaneous determination of binary mixtures. This method is effective for binary mixtures showing similar absorption spectra such as the methyl *p*-hydroxybenzoate (methylparaben)–propyl *p*-hydroxybenzoate (propylparaben) mixture. As alkylparabens show very similar absorption spectra, the simultaneous determination of the binary mixtures by the absorption spectra alone is very difficult. We carried out such determination of some binary mixed alkylparabens and obtained accurate results.

Experimental

Method of Simultaneous Determination Given M and N of two drugs. Then, M or N by the Beer's law is

$$A_m = e_m \times C_m \times L \quad (1)$$

$$A_n = e_n \times C_n \times L \quad (2)$$

where A_m (or A_n) is the absorbance of M (or N), e_m (or e_n) is the molar extinction coefficient of M (or N), C_m (or C_n) is the concentration (mole) of M (or N), and L is the path-length (cm).

The absorbance of the binary mixture (A_{mix}) is the sum of Eqs. 1 and 2.

$$A_{mix} = A_m + A_n = e_m \times C_m \times L + e_n \times C_n \times L \quad (3)$$

The total concentration of the binary mixture (C_{mix}) is

$$C_{mix} = C_m + C_n$$

C_m (or C_n) is

$$C_m = C_{mix} \times r_m \quad (4)$$

$$C_n = C_{mix} \times r_n \quad (5)$$

where r_m (or r_n) is the molar ratio of M (or N) in the M ~ N binary mixture ($r_m + r_n = 1$).

Substituting Eqs. 4 and 5 into Eq. 3, the following equation can be derived,

$$C_{mix} = A_{mix}/[(e_m \times r_m + e_n \times r_n) \times L] \quad (6)$$

In Eq. 6, we can obtain A_{mix}, e_m and e_n through the measurement of absorbance. Therefore, we obtain C_{mix} when we can compute r_m and r_n values, respectively.

On the other hand, the absorption spectrum of a drug in surfactant solution above the critical micelle concentration (cmc) often shifts. This phenomenon is due to the solvent effect on the drug incorporated into the micellar phase, and the absorption spectrum of the incorporated drug shows changes in both intensity and peak position. The shift-length of wavelength can be measured by the FDAS-SL method. The degree of the shift-length of wavelength depends on the concentration of surfactant, but not on the drug concentration. There should be a positive relation between the volume of the micellar phase and the amount of the drug incorporated into the micellar phase, and the shift-length should correspond to the latter. In this report, it has been assumed that the order of shift-length of M and N of two drugs in surfactant solution is M < N. Then, the order of shift-length of M, N and M ~ N binary mixture at an arbitrary ratio in surfactant solution is M < M ~ N < N. In the plots of the shift-length on the ordinate against the molar ratio on the abscissa, the plot of M ~ N binary mixture is on the M—N straight line of the shift-length of M and N. Therefore, r_m and r_n value can be obtained from this M—N straight line, which is the regression curve, and C_m and C_n can be calculated by using the mixed ratio and Eq. 6.

Materials Methylparaben (minimum 99.0%), ethyl *p*-hydroxybenzoate (ethylparaben, minimum 99.0%), zinc sulfate heptahydrate (minimum 99.9%), boric acid (minimum 99.5%), sodium phosphate monobasic dihydrate ($NaH_2PO_4 \cdot 2H_2O$, minimum 99.0%), sodium phosphate dibasic 12 water ($Na_2HPO_4 \cdot 12H_2O$, minimum 99.0%) were obtained from Wako Pure Chemical Ind., Ltd., and propylparaben (minimum 99%), butyl *p*-hydroxybenzoate (butylparaben, minimum 99%) and sodium dodecyl sulfate (SDS, minimum 99%) were obtained from Nacalai Tesque Inc. These chemicals were used as received.

Measurement of Absorption Spectra and Transformation to First Derivative Absorption Spectra of Absorption Spectra A Shimadzu UV-2200 UV-VIS recording spectrophotometer equipped with a thermo-

TABLE I. Mixture Ratio

	A	B
Group 1	2.0	0
	1.6	0.4
	1.2	0.8
	1.0	1.0
	0.8	1.2
	0.4	1.6
	0	2.0
Group 2	2.0	0
	1.5	0.5
	1.0	1.0
	0.5	1.5
	0	2.0

A, B; A–B mixture.

electrically temperature-controlled cell holder TCC-240A was used. Measurement conditions of absorption spectra were: quartz path-length 1 cm or 0.1 cm, temperature 30 °C, scan speed low (about 180 nm/min), slit width 2 nm. Conditions of smoothing of absorption spectra[11] and transformation to the first derivative absorption spectra of absorption spectra were: point number 25, point space 1, scale expansion 26 times.

Preparation of Regression Curve The binary mixed alkylparaben solutions of mixed ratio shown in Table I were prepared using a stock solution of 0.5 mM of each alkylparaben. The solutions for the regression curve were prepared by mixing 2 ml of alkylparaben solution of each mixed ratio, 1 ml of 200 mM SDS solution and 7 ml of purified water. The solution without SDS was used as the reference whose volume was compensated by purified water. The absorption spectra of these mixed solutions were measured; the spectra were transformed to the first derivative absorption spectra after smoothing of them was done twice. The regression curve of the shift-length on the ordinate against the mixed ratio on the abscissa was drawn by the least-squares method.

Results and Discussion

Regression Curve The absorption spectrum of methylparaben in SDS solution above the cmc shifts in both the maximum absorption wavelength and the absorbance as compared with the absorption spectrum in aqueous solution (Fig. 1). We used the FDAS-SL method. As the wavelength of the maximum absorption is obtained directly from the crossing-point of the first derivative absorption spectrum and the baseline,[12] the measurement of the shift-length of wavelength is easy (Figs. 2, 3).

Figure 4 illustrates the first derivative absorption spectra of 0.05 or 0.1 mM methylparaben in 20 mM SDS solution, and shows that the wavelength shift does not depend on the drug concentration. The shift-length of methylparaben in 20 mM SDS solution was 0.85 nm. Similarly, the shift-lengths of ethylparaben, propylparaben and butylparaben in 20 mM SDS solution were 1.21, 1.50 and 1.71 nm, respectively. These are characteristic values of each alkylparaben.

Table II shows the shift-lengths of the binary mixed alkylparaben solutions. These shift-lengths were in a linear relationship to the mixed ratio.

Figure 5 shows plots of the shift-length of the binary mixed methylparaben–propylparaben. These plots were on a straight line (regression curve) with a correlation coefficient above 0.99. Similarly, all plots of the shift-length of the binary mixed ethylparaben–butylparaben, methylparaben–ethylparaben, methylparaben–butylparaben, ethylparaben–propylparaben and propylparaben–butylparaben showed straight lines with the correlation coefficient above 0.99 (Table III). In solution of the same SDS concentration, the mixed ratio of two alkylparabens influences the shift-length. Therefore, the mixed ratio of a drug of a binary system can be obtained from these straight lines, regardless of the concentration of alkylparaben. Furthermore, the concentration of alkylparaben can be calculated using this mixed ratio and Eq. 6.

Simultaneous Determination of the Binary Mixtures The binary mixtures are as follows: 1) the solution of alkylparabens mixed at arbitrary ratio experimentally, 2) the dissolution media A, B and C for ophthalmic

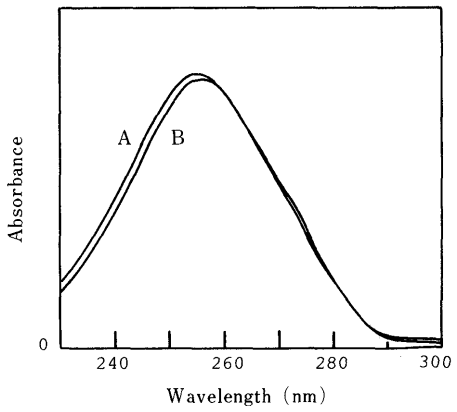

Fig. 1. Absorption Spectra of 0.1 mM Methylparaben
A, in H_2O; B, in 20 mM SDS solution.

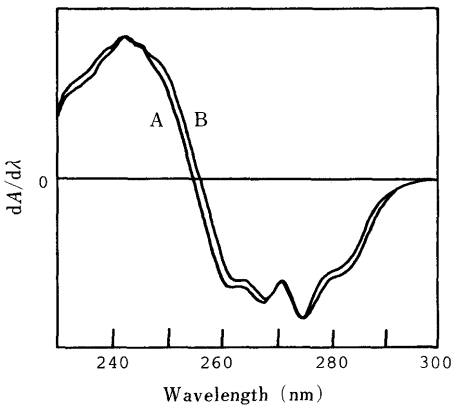

Fig. 2. First Derivative Absorption Spectra of 0.1 mM Methylparaben
A, in H_2O; B, in 20 mM SDS solution.

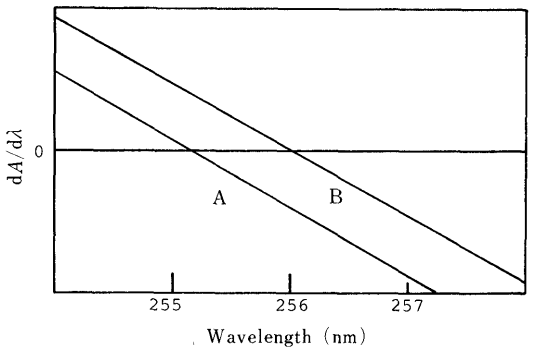

Fig. 3. Magnified First Derivative Absorption Spectra (×26) of 0.1 mM Methylparaben
A, in H_2O; B, in 20 mM SDS solution.

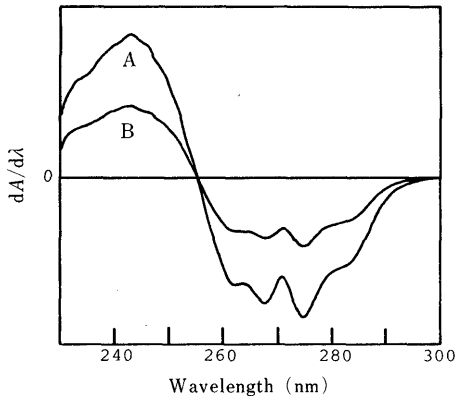

Fig. 4. First Derivative Absorption Spectra of Methylparaben in 20 mM SDS Solution
A, 0.1 mM methylparaben; B, 0.05 mM methylparaben.

Table II. Shift-Length of Binary Mixed Alkylparaben Solutions

MR	MP–EP mixture			MR	EP–PP mixture		
	MP (mM)	EP (mM)	SL (nm)		EP (mM)	PP (mM)	SL (nm)
10:0	0.103	—	0.85	10:0	0.101	—	1.21
8:2	0.082	0.022	0.92	8:2	0.081	0.020	1.27
6:4	0.062	0.043	1.00	6:4	0.061	0.040	1.32
5:5	0.051	0.054	1.03	5:5	0.051	0.050	1.35
4:6	0.041	0.065	1.06	4:6	0.040	0.060	1.40
2:8	0.021	0.087	1.15	2:8	0.020	0.080	1.46
0:10	—	0.109	1.21	0:10	—	0.100	1.50

MR	MP–PP mixture			MR	EP–BP mixture		
	MP (mM)	PP (mM)	SL (nm)		EP (mM)	BP (mM)	SL (nm)
10:0	0.051	—	0.85	10:0	0.098	—	1.21
8:2	0.041	0.010	1.00	8:2	0.078	0.020	1.31
6:4	0.031	0.020	1.12	6:4	0.059	0.040	1.41
5:5	0.026	0.029	1.19	5:5	0.049	0.049	1.48
4:6	0.021	0.035	1.27	4:6	0.039	0.059	1.50
2:8	0.021	0.080	1.37	2:8	0.020	0.079	1.60
0:10	—	0.058	1.50	0:10	—	0.098	1.71

MR	MP–BP mixture			MR	PP–BP mixture		
	MP (mM)	BP (mM)	SL (nm)		PP (mM)	BP (mM)	SL (nm)
10:0	0.099	—	0.85	10:0	0.101	—	1.50
8:2	0.079	0.020	1.02	8:2	0.081	0.020	1.53
6:4	0.059	0.040	1.17	6:4	0.061	0.040	1.58
5:5	0.049	0.049	1.26	5:5	0.051	0.049	1.60
4:6	0.039	0.059	1.35	4:6	0.040	0.059	1.63
2:8	0.020	0.079	1.54	2:8	0.020	0.079	1.69
0:10	—	0.099	1.71	0:10	—	0.099	1.71

MR, mixture ratio; MP, methylparaben; EP, ethylparaben; PP, propylparaben; BP, butylparaben; SL, shift-length. Concentration of source solution; 0.5 mM of each alkylparaben.

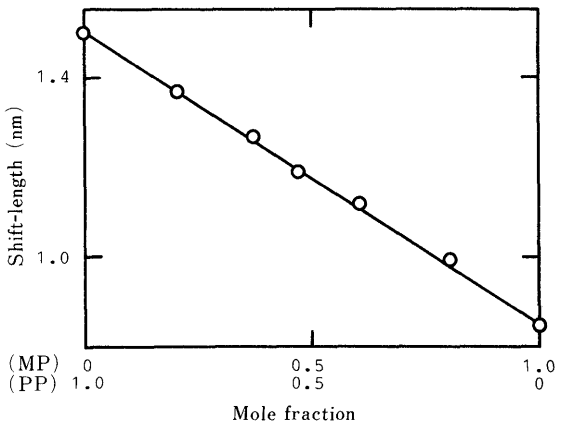

Fig. 5. Regression Curve of Methylparaben–Propylparaben Mixed Solutions

MP, methylparaben; PP, propylparaben.

preparation, and the preservatives in zinc sulfate ophthalmic solution appearing in "Chyozaishishin, 9th ed." and elsewhere[13,14] (Table IV).

1) The simultaneous determination of methylparaben–propylparaben and ethylparaben–butylparaben mixed solutions at arbitrary ratio were carried out experimentally.

Table V shows the analytical values by this simultaneous determination method. The differences of the theoretical values and the experimental values were less than 2.81%.

2) The simultaneous determination of the dissolution media A, B and C for ophthalmic preparation, and the preservatives in zinc sulfate ophthalmic solution was made.

The mixed ratio of the dissolution medium B for ophthalmic preparation, which contained sodium phosphate (NaH_2PO_4 and Na_2HPO_4), was obtained from the regression curve, but was not an accurate value. Owing to Na^+ in the dissolution medium B for ophthalmic preparation, the cmc value of SDS is lowered, and SDS micelles are easily formed. Consequently, the difference of the shift-length of alkylparabens becomes small, and the slope of the regression curve becomes gentle. For this reason, the accurate mixed ratio cannot be obtained. Therefore, the regression curve was obtained from the solution of the same Na^+ concentration. In the method using this regression curve, measurement at slightly higher SDS concentration than cmc is the best condition. As the cmc of SDS solution containing Na^+ is about 1.7 mM,[15] the regression curve was obtained from the binary mixed alkylparabens in 5 mM SDS solution (Table VI and Fig. 6). This regression curve is shown in Table III. The results are shown in Table VII.

Table III. Regression Equation of Binary Mixed Alkylparaben Solutions

Mixture	Regression equation	Correlation coefficient
Methylparaben–ethylparaben	$Y=-0.36X+1.21$	−0.9990
Methylparaben–propylparaben	$Y=-0.64X+1.51$	−0.9986
Methylparaben–propylparaben[a]	$Y=-0.64X+1.02$	−0.9979
Methylparaben–butylparaben	$Y=-0.86X+1.70$	−0.9993
Ethylparaben–propylparaben	$Y=-0.30X+1.51$	−0.9962
Ethylparaben–butylparaben	$Y=-0.49X+1.71$	−0.9983
Propylparaben–butylparaben	$Y=-0.23X+1.72$	−0.9930

Y, shift-length; X, mole fraction. *a*) Solution contains sodium dihydrogenphosphate (0.560 g/l) and sodium phosphate (0.284 g/l).

Table IV. Prescriptions of the Dissolution Medium A, B and C for Ophthalmic Preparation, and Zinc Sulfate Ophthalmic Solution

Medium A, pH 5.0 (=solution A)	
Boric acid	20.0 g
Methylparaben	0.26 g
Propylparaben	0.14 g
Sterile purified water	Total 1000.0 ml
Medium B, pH 6.5 (=solution B)	
Anhydrous monosodium phosphate	5.60 g
Anhydrous disodium phosphate	2.84 g
Methylparaben	0.26 g
Propylparaben	0.14 g
Sterile purified water	Total 1000.0 ml
Medium C, pH 5.7—6.0 (=solution C)	
Methylparaben	0.26 g
Propylparaben	0.14 g
Sterile purified water	Total 1000.0 ml
Zinc sulfate ophthalmic solution	
Zinc sulfate	0.3 g
Preserved water A	Total 100.0 ml

TABLE V. Analysis of Binary Mixed Alkylparaben Solutions

Methylparaben–propylparaben mixture								
Methylparaben mixture ratio			Methylparaben concentration			Propylparaben concentration		
Additive (%)	Found (%)	Recovery (%)	Additive (mM)	Found (mM)	Recovery (%)	Additive (mM)	Found (mM)	Recovery (%)
75	75.00	100.0	0.37	0.38	102.7	0.13	0.13	100.0
45	42.19	93.76	0.23	0.22	95.65	0.30	0.30	100.0
40	39.06	97.63	0.20	0.20	100.0	0.30	0.31	103.3
30	27.60	92.00	0.15	0.15	100.0	0.38	0.38	100.0
Ethylparaben–butylparaben mixture								
Ethylparaben mixture ratio			Ethylparaben concentration			Butylparaben concentration		
Additive (%)	Found (%)	Recovery (%)	Additive (mM)	Found (mM)	Recovery (%)	Additive (mM)	Found (mM)	Recovery (%)
70	71.43	102.0	0.36	0.38	105.6	0.15	0.15	100.0
65	63.27	97.34	0.33	0.32	96.97	0.18	0.18	100.0
30	30.61	102.0	0.15	0.16	106.7	0.36	0.35	97.22
25	26.53	106.1	0.13	0.14	107.7	0.37	0.38	102.7

TABLE VI. Analysis of Solutions for Ophthalmic Preparation and Ophthalmic Solution

	Methylparaben mixture ratio			Methylparaben concentration			Propylparaben concentration		
	Additive (%)	Found (%)	Recovery (%)	Additive (g/l)	Found (g/l)	Recovery (%)	Additive (g/l)	Found (g/l)	Recovery (%)
Solution A	68.67	67.19	97.84	0.26	0.25	96.15	0.14	0.15	107.1
		68.75	100.1		0.26	100.0		0.14	100.0
		68.75	100.1		0.26	100.0		0.14	100.0
Solution B	68.67	70.31	102.4	0.26	0.25	96.15	0.14	0.13	92.86
		70.31	102.4		0.25	96.15		0.13	96.86
		67.19	97.84		0.24	92.31		0.14	100.0
Solution C	68.67	65.63	95.57	0.26	0.25	96.15	0.14	0.16	114.3
		68.75	100.1		0.26	100.0		0.14	100.0
		68.75	100.1		0.26	100.0		0.14	100.0
Zinc sulfate ophthalmic solution	68.67	68.75	100.1	0.26	0.26	100.0	0.14	0.14	100.0
		71.88	104.7		0.27	103.8		0.12	85.71
		71.88	104.7		0.27	103.8		0.13	92.85

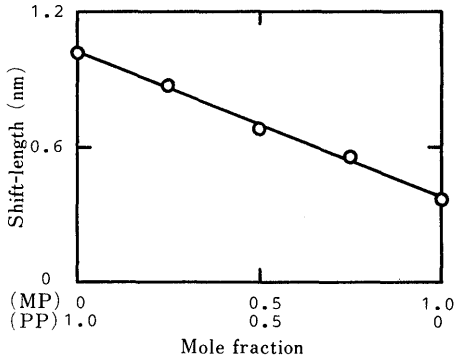

Fig. 6. Regression Curve of Methylparaben–Propylparaben Mixed Solutions

Solution contains sodium dihydrogenphosphate (0.560 g/l) and sodium phosphate (0.284 g/l); MP, methylparaben; PP, propylparaben.

In conclusion, this method is shown to be applicable to the simultaneous determination of binary mixtures which have similar absorption spectra. We propose to use this technique to analyze various binary mixed preparations.

TABLE VII. Shift-Length of Methylparaben–Propylparaben Mixture[a]

Mixture ratio	Methylparaben (mM)	Propylparaben (mM)	Shift-length (nm)
8:0	0.098	—	0.37
6:2	0.074	0.025	0.56
4:4	0.049	0.050	0.68
2:6	0.025	0.075	0.87
0:8	—	0.100	1.01

a) Solution contains sodium dihydrogenphosphate (0.560 g/l) and sodium phosphate (0.284 g/l). Concentration of source solution; 0.5 mM methylparaben, 0.5 mM propylparaben.

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References and Notes

1) A part of this paper was presented at the 110th Annual Meeting of

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