[Chem. Pharm. Bull. 15(1) 77 ~ 82 (1967)]

UDC 615.41[661.185.4] : 543.422.4[547.313]

10. Toyozo Uno, Koichiro Miyajima, Yoshie Miyajima : Determination of Surface-active Agents. Ⅷ.*2 Infrared Determination of the Proportion of Ethylene Oxide and Propylene Oxide in Pluronic.*3

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A new determination method of the proportion of ethylene oxide and propylene oxide in Pluronic was devised, the stretching bands of methyl and methylene group being used as the key bands. The proportion was determined within an error of ±5% without regard to the difference of molecular weight of propylene oxide condensate.

(Received March 22, 1966)

Nonionic surfactants of Pluronic type which have been used widely in textile and dye industries are the block polymer of ethylene oxide (EO) and isopropylene oxide (PO) as seen below.

Pluronic : $H(OCH_2CH_2)_{n_1}(OCH_2CH\cdot CH_3)_m(OCH_2CH_2)_{n_2}OH \quad (n_1+n_2=n)$

Polypropylene glycol (PPG) : $H(OCH_2CH\cdot CH_3)_mOH$

Polyethylene glycol (PEG) : $H(OCH_2CH_2)_nOH$

In this formula, PO-condensates, the central part of the molecule, have the lipophilic character, while EO-condensates, the end group of the molecule, the hydrophilic character. Therefore, the proportion of these condensates plays the important roll on the surface activity. However, there has not been reported the determination of the proportion of Pluronic except the oxidation method recently published by Kotzschmar.[1] This method is based on the determination of carbon dioxide and acetic acid produced by the decomposition of Pluronic with the mixture of chromic acid and sulfuric acid.

In general, the degrees of polymerization of such condensation polymer are determined by the increase in weight on preparation, or hydroxyl values[2] by acetylation of terminal hydroxyl groups.

However, the former method is applicable only for producer, and the latter method is applicable only in the case where the molecular weight of PO-condensates had been known. Hence, the new convenient method based on the differences of frequencies between methyl group of PO and methylene group of both PO and EO in infrared region was studied and developed. As shown in Fig. 1 and 2, Pluronic surfactants have the methyl C-H stretching band and the methylene C-H stretching band at 2975 cm^{-1} and 2870 cm^{-1} respectively. The relative intensity of these two bands changes in accordance with the change of the proportion of PO and EO. On the other hand, in polypropylene glycol (PPG), these two bands have nearly the same intensity, while in polyethylene glycol (PEG) the band at 2975 cm^{-1} is hardly observed as anticipated. In addition, the vibrational coupling between the CH_3-

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*2 Part Ⅶ : This Bulletin, **11**, 193 (1963).

*3 This work was presented at the symposium of Japan Society for Analytical Chemistry at Tokushima, October, 1962.

1) A. Kotzschmar : Z. anal. Chem., **183**, 30 (1961).

2) M. J. Rosen, H. A. Goldsmith : Systematic Analysis of Surface Active Agents. Interscience Publishers, Inc. (1960).

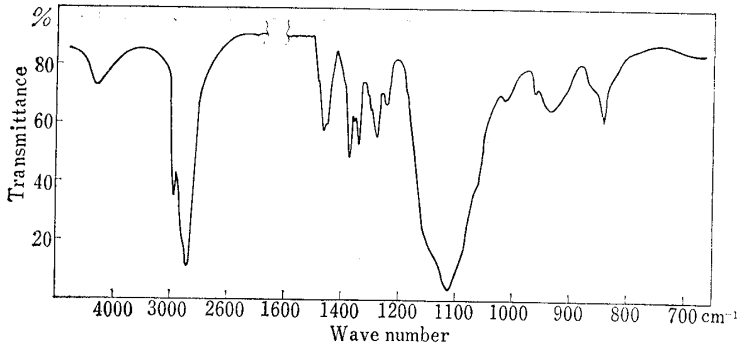

Fig. 1. Absorption Spectrum of Pluronic (liquid film)

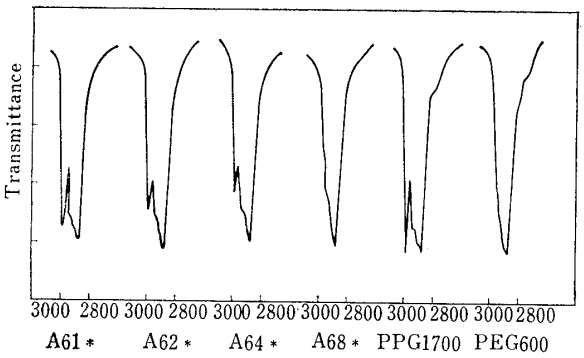

Fig. 2. Infrared-Spectra of Various Pluronic's, PPG and PEG in 2800~3000 cm^{-1}

* Molar percentage of EO of the compounds is shown in Table II.

and $-CH_2-$ group is hardly expected for the Pluronic type polymer. Considering these facts, various examinations were carried out with the mixture of PPG and PEG as the standard substances.

Method I (Graphical method):

Now let A_1 and A_2 be the absorbances at 2870 cm^{-1} and 2975 cm^{-1}, respectively. When the values of A_1/A_2 are plotted against the molar percentages*4 of EO according to the ordinary way of functional group analysis by infrared spectroscopy, curve I in Fig. 3 is obtained. As shown in curve I in Fig. 4, almost linear relation is obtained, when the values of log A_1/A_2 are plotted instead of A_1/A_2.

Method II (Calculative method):

Spectrophotometry of two components system was applied for this case. Introducing the abbreviations :

ε_1^E : Molar extinction coefficient of EO-monomer at 2870 cm^{-1}

ε_2^E : 〃 2975 cm^{-1}

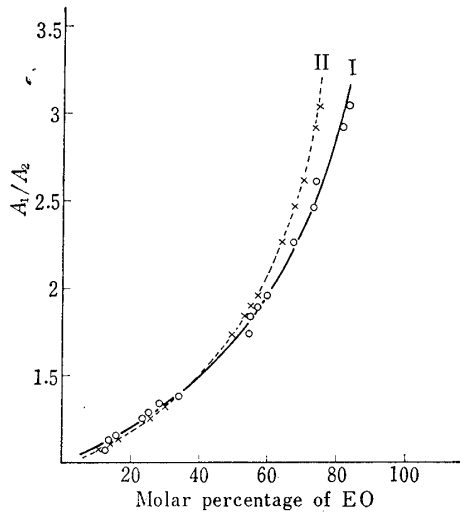

Fig. 3. Relation between the Molar Percentages of EO and A_1/A_2

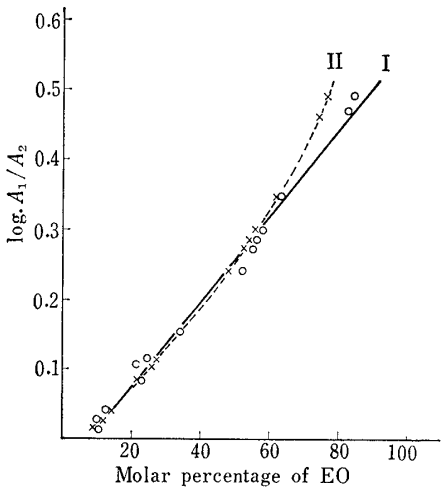

Fig. 4. Relation between the Molar Percentages of EO and log A_1/A_2

*4 Generally, commercially available compounds are classified by the weight percentage of EO, however this way is not suitable for IR-spectroscopic determination because of the difference of molecular weight of EO and PO.

ε_1^P : Molar extinction coefficient of PO-monomer at 2870 cm^{-1}
ε_2^P : 〃 2975 cm^{-1}
n' : Molar number of EO obtained by the equation (Ⅺ) in PPG-PEG system
n : Molar number of EO in Pluronic
m : Molar number of PO in both PPG-PEG system and Pluronic
x : Weight (gr.) of PEG in 100 ml CCl_4 on PEG-PPG system
y : Weight (gr.) of PPG in 100 ml CCl_4 on PEG-PPG system
PPG (mol. wt.) : Molecular weight of PPG
PEG (mol. wt.) : Molecular weight of PEG
Equation (I) is derived by Lambert-Beer's law.

$$\left.\begin{aligned} A_1&=(\varepsilon_1^E n'+\varepsilon_1^P m)10y/\text{PPG(mol. wt.)}\\ A_2&=(\varepsilon_2^E n'+\varepsilon_2^P m)10y/\text{PPG(mol. wt.)}\end{aligned}\right\} \qquad (\text{I})$$

Equation (I) may be rewritten as equations (Ⅱ), (Ⅱ′) and (Ⅲ).

$$n'=\frac{\varepsilon_2^P A_1-\varepsilon_1^P A_2}{\varepsilon_1^E\varepsilon_2^P-\varepsilon_2^E\varepsilon_1^P}\cdot\frac{\text{PPG(mol. wt.)}}{10y} \qquad (\text{Ⅱ})$$

$$m=\frac{\varepsilon_1^E A_2-\varepsilon_2^E A_1}{\varepsilon_1^E\varepsilon_2^P-\varepsilon_2^E\varepsilon_1^P}\cdot\frac{\text{PPG(mol. wt.)}}{10y} \qquad (\text{Ⅱ}')$$

$$\frac{n'}{n'+m}=\frac{\varepsilon_1^P A_2-\varepsilon_2^P A_1}{A_1(\varepsilon_2^E-\varepsilon_2^P)+(\varepsilon_1^P-\varepsilon_1^E)A_2} \qquad (\text{Ⅲ})$$

By substituting the experimental values of ε ($\varepsilon_1^E=60$, $\varepsilon_2^E=8$, $\varepsilon_1^P=52$, $\varepsilon_2^P=55$) into equation (Ⅲ), we obtained equation (Ⅳ)

$$\frac{n'}{n'+m}=\frac{55A_1-52A_2}{47A_1+8A_2} \qquad (\text{Ⅳ})$$

In Pluronic n' equals n, therefore equation (Ⅳ) is rewritten

$$\frac{n}{n+m}=\frac{55A_1-52A_2}{47A_1+8A_2} \qquad (\text{Ⅴ})$$

When the molar percentages of EO $100n/(n+m)$ obtained by substituting the experimental values for A_1 and A_2 are plotted against A_1/A_2, curve Ⅱ in Fig. 3 is obtained. And if the molar percentages of EO are plotted against $\log A_1/A_2$, curve Ⅱ in Fig. 4 is obtained. The good correspondences of each pair of curves in Fig. 3 and 4 corroborate the reliability of graphical method.

The three molar percentages of EO shown in Table I were obtained from (I) increase in weight on preparation; (Ⅱ) the curve I in Fig. 4; and (Ⅲ) the equation (Ⅴ).

Table I. Determination of the Proportion of PO and EO in Pluronic

Pluronic	Molar % of EO		
	from the increase in weight on preparation (I)	from the graphical method in Fig. 4. (Ⅱ)	from the calculative method [equation (Ⅴ)] (Ⅲ)
A61	13	12	11
A62	34	36	38
A64	47	52	52
A68	84	90	85
B710	13	11	10
B720	25	25	25
B740	47	46	49
B750	58	57	57
B785	88	86	76

They agreed with each other within an error of ±5%. The determination is not interfered by the differences of molecular weight of PO, and about 10% differences may be allowed on the concentration of Pluronic.

The linear relation between the molar percentages of EO and $\log A_1/A_2$ may be explained as follows. From equation (Ⅳ) we obtained equation (Ⅶ) and (Ⅷ) applying the Maclaurin's expansion, where $x=n/(n+m)$ and $y=A_1/A_2$.

$$x=\frac{55y-52}{47y+8} \qquad (Ⅵ)$$

$$y=\frac{52+8x}{55-47x}\fallingdotseq\frac{52}{55}+0.945x+0.8x^2+\cdots\cdots \qquad (Ⅶ)$$

$$\log y=\log\left(\frac{52+8x}{55-47y}\right)\fallingdotseq\log\frac{52}{55}+1.02x+0.35x^2+\cdots\cdots \qquad (Ⅷ)$$

The curvature of equation (Ⅷ) is smaller than that of equation (Ⅶ) in the range $x=0\sim1$. Therefore, it may be considered that better lineality was obtained on the case where the molar percentages of EO were plotted against $\log A_1/A_2$.

Experimental

1) Apparatus : Kôken Model DS 301 IR-Spectrophotometer. Prism : NaCl prism, Cell : 0.25 mm NaCl cell.

2) Materials and Reagents : Pluronic surfactants are commercially available compounds manufactured by Sanyo Kasei Co., Ltd. and Daiichikogyo Seiyaku Co., Ltd. The molar percentages of EO of these compounds are calculated by the following way.

Calculation for the molar percentages of EO from the weight percentages of EO in Pluronic.

$(OCH_2OCH_2)=44$ (mol. wt.) $\qquad$ $(OCH_2OCH_3)=58$ (mol. wt.)

Molar number of PO in PO-condensate

$$m=\frac{\text{PPG (mol. wt.)}-18}{58}$$

Weight percentage of EO :

$$a\cdot100=\frac{44n}{44n+\text{PPG (mol. wt.)}}\cdot100$$

Molar number of EO in EO-condensates

$$n=\frac{\text{PPG (mol. wt.)}\cdot a}{44(1-a)}$$

Molar percentage of ED :

$$\frac{n}{n+m}\cdot100 \qquad (Ⅸ)$$

Table Ⅱ. Molar Percentages of Pluronic

Pluronic	PPG (mol. wt.)	Molar numbers of PO (m)	Molar numbers of EO (n)	W/W % of EO	Molar % of EO
A61	1700	29	4.5	10.5	13.4
A62	1700	29	15.0	28	34.1
A64	1700	29	25.8	40	47.1
A68	1700	29	154.5	80	84.2
B710	2000	34.2	5.1	10	13.0
B720	2000	34.2	11.4	20	25.0
B740	2000	34.2	30.3	40	47.0
B750	2000	34.2	45.5	50	57.1
B785	2000	34.2	257.6	85	88.3

PPG and PEG are also the compounds prepared by Sanyo Kasei Co., Ltd. The molecular weight cf these compounds were measured by acetylation method in our laboratory.

Calculation for the molar percentage of EO on mixed system.

x : Weight of PEG (gr.) y : Weight of PPG (gr.)

Molar number of EO in PEG taken :

$$\alpha=\frac{\text{PEG(mol. wt.)}-18}{\text{PEG(mol. wt.)}\cdot 44}$$

Molar number of PO in PPG taken :

$$\beta=\frac{[\text{PPG(mol. wt.)}-18]\cdot y}{\text{PPG(mol. wt.)}\cdot 58}$$

Molar percentage of EO :

$$b\cdot 100=\frac{\alpha}{\alpha+\beta}\cdot 100 \qquad \text{(X)}$$

Molar number of ED in Pluronic form :

$$n'=\frac{b\cdot m}{1-b} \qquad \text{(XI)}$$

$$m=\frac{\text{PPG(mol. wt.)}-18}{58}=29 \quad [\text{PPG(mol. wt.)}=1700]$$

TABLE III. Molar Percentages of EO on Mixed System

Mean-mol. wt.[a] of PEG	PEG(Taken) x (mg.)	PPG(Taken) y (mg.)	W/W % of PEG	Molar % of EO	Molar numbers of EO (n')
300	16.23	133.43	10.8	13.2	4.4
300	30.77	123.33	20.0	23.8	9.1
300	79.55	83.80	48.7	54.3	34.5
300	120.30	28.70	80.7	84.0	152.1
600	35.78	130.06	21.6	26.3	10.4
600	73.28	76.48	48.9	55.4	36.9
600	102.75	44.45	69.8	75.0	87.0
1540	14.95	141.00	9.6	12.3	4.1
1540	45.05	109.93	29.1	35.1	15.7
1540	77.70	72.15	51.9	58.7	41.2
1540	106.09	46.53	69.5	75.0	87.1
4000	16.31	138.37	10.6	13.6	4.6
4000	30.08	118.94	20.2	25.1	9.7
4000	77.06	78.06	49.7	56.7	38.0
4000	91.33	60.92	60.0	67.4	57.7
4000	120.54	32.08	80.0	83.3	144.5

a) PPG : 1700 (mol. wt.)
These values are designated as the mean molecular weight by manufacturing company. However, molecular weight measured by acetylation method in our laboratory was used in these experiments.

Tetrachloromethane : Commercially available CCl_4 (reagent grade) was directly used as a solvent.

3) Procedure : To 0.25 mm thick NaCl cell, 1.5% CCl_4 solution of sample is taken and the transmittances at 2975 cm^{-1} and 2870 cm^{-1} are measured. The same procedure is repeated with only CCl_4 to conpensate the absorbance of the solvent. From the transmittances $\log A_1/A_2$ are calculated. These values are calibrated by the calibration curve obtained by Pluronic or mixture of PPG and PEG with known molecular weight.

4) Measurement of molar extinction coefficients of PPG and PEG : 1% CCl_4 solution of PPG and PEG was used and the results are shown in Table IV.

5) Influence of the concentration of sample in CCl_4 : The concentration of sample in CCl_4 was varied from 1.3% to 1.6% and the relative intensity was not much affected in the range 1.4~1.6%.

Table IV. Molar Extinction Coefficients of PEG and PPG

Compound	ε_1 (2870 cm^{-1})	ε_2 (2975 cm^{-1})	Degree of polymerization (m, or n')	ε_1/m or n'	ε_2/m or n'
PPG 200	183.1	189.6	3.2	57.2	59.2
〃 400	322.4	367.8	6.6	48.9	55.7
〃 1200	1075.9	1115.9	20.4	52.7	54.5
〃 1700	1492.0	1553.1	29.0	51.4	53.4
〃 2000	1798.7	1832.5	34.2	52.3	53.2
PEG 200	269.4	37.5	5.4	49.7	6.9
〃 300	342.3	42.9	6.3	54.0	7.7
〃 600	933.1	115.6	14.9	62.5	6.8
〃 1000	1683.1	234.5	26.9	62.7	7.7
〃 1540	2408.1	260.5	36.4	66.2	7.2
〃 4000	5552.5	662.3	87.7	63.3	7.6
〃 6000	8143.0	1048.2	137.5	59.2	7.6

$\varepsilon_1^P = \varepsilon_1/m$ (average)=52.5 $\varepsilon_1^E = \varepsilon_1/n$ (average)=59.7
$\varepsilon_2^P = \varepsilon_2/m$ (average)=55.2 $\varepsilon_2^E = \varepsilon_2/n$ (average)=7.5

Table V. Influence of the Concentration of Sample in CCl_4

Pluronic	Concentration (W/V %)	log A_1/A_2	Molar percentage of EO by method I
A64	1.62	0.216	43.0
〃	1.51	0.224	44.5
〃	1.43	0.225	44.5
〃	1.26	0.250	50.0

The authors express their thanks to Dr. K. Machida for his useful advices and also thank to Sanyo Kasei Co., Ltd. and Daiichikogyo Seiyaku Co., Ltd. for the gifts of surfactants.

[Chem. Pharm. Bull. 15(1) 82 ~ 87 (1967)]

UDC 547.964.4.07 : 615.74

11. Noboru Yanaihara and Minoru Sekiya : Synthesis of l-Glutamic Acid Kallidin.*1,*2

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The Synthesis of l-glutamic acid kallidin was performed. The azide and *p*-nitrophenyl ester methods were selectively employed in the coupling stages. The biological activity of this peptide compared to that of synthetic bradykinin is reported.

(Received March 26, 1966)

Since amino acid sequences of bradykinin[1] and kallidin[2] were elucidated, a number of their analogs have been synthesized in an attempt to discover relationships between structure and activity.

*1 The peptides and peptide derivatives mentioned in this paper are of the L-configuration.
*2 l-Glutamic acid kallidin=Glutamylarginylprolylprolylglycylphenylalanylserylprolylphenylalanylarginine.
*3 160 Oshika, Shizuoka (矢内原 昇, 関屋 実).

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