Quantitative Analysis of Mixtures of L-Glutamic Acid Polymorphs by X-Ray Diffraction

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Two polymorphic forms of L-glutamic acid have been reported by a few authors. One form, α, reported by Bernal¹⁾, has an orthorhombic structure, with $a=7.06$, $b=10.3$, $c=8.75$ kX. The other form, β, which has been studied by Hirokawa²⁾, also has an orthorhombic structure, with $a=5.17$, $b=17.34$, $c=6.95$ Å. The X-ray diffraction patterns of these two polymorphic forms of L-glutamic acid are given in Fig. 1. L-Glutamic acid crystallizes in a mixture consisting of various proportions of the α- and the β-forms, from an aqueous solution under certain conditions. In industrial processes, it is necessary to control the formation of the L-glutamic acid polymorphs. To analyze the two forms quantitatively, usual chemical methods are useless. The X-ray diffraction method does, however, give satisfactory results for this purpose. The application of

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1) J. D. Bernal, *Z. Krist.*, **78**, 363 (1931).
2) S. Hirokawa, *Acta Cryst.*, **8**, 637 (1955).

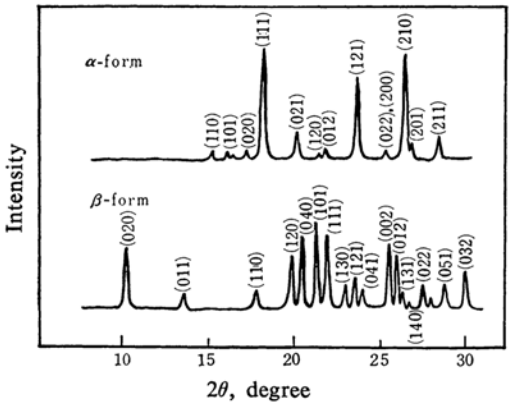

Fig. 1. X-Ray diffraction patterns of L-glutamic acid polymorphs. (CuK_α radiation)

such a diffraction method to the quantitative analysis of crystalline organic systems has been investigated by Christ et al.[3] This paper describes an analytical method for polymorphs of organic crystals using the X-ray diffractometer. Sufficiently accurate results can be obtained within 40 min.

A general theory of quantitative X-ray analysis by the diffractometer has been reported by Klug and Alexander[4] and by Copeland and Bragg[5]. They have shown that the integrated intensity of the $(h\,k\,l)$ line of the jth component in the diffraction pattern is given by

$$I_{(h\,k\,l)j}=K_{(h\,k\,l)j}\ (x_j/\rho_j\mu) \tag{1}$$

where $K_{(h\,k\,l)j}$ depends upon the nature of the component j and the geometry of the apparatus, x_j is the weight fraction, ρ_j, the density of the jth component, and μ, the total mass absorption coefficient of the sample. In the case of such mixtures of polymorphic forms, μ does not change with the composition. Equation 1 indicates that the intensity is directly proportional to the concentration of the component.

For a binary mixture (α- and β-forms), Eq. 1 can be written:

$$I_{(h\,k\,l)\alpha}=k_{(h\,k\,l)\alpha}x_\alpha,\quad I_{(H\,K\,L)\beta}=k_{(H\,K\,L)\beta}x_\beta \tag{2}$$

where $k_{(h\,k\,l)\alpha}$ and $k_{(H\,K\,L)\beta}$ are constant. The following selected lines were used:

$I_{(1\,1\,1)\alpha}$: Intensity of the 4.87Å line of the α-form, (1 1 1) reflection.

$I_{(1\,0\,1)\beta}$: Intensity of the 4.17Å line of the β-form, (1 0 1) reflection.

Each (1 1 1)α, (1 0 1)β line is the strongest in its respective pattern. Fig. 1 shows that the (1 0 1)β line is superposed with the (1 2 0) line of the α-form. In practice, Eq. 2 must be modified to:

$$\begin{aligned}I_{(1\,1\,1)\alpha}&=k_{(1\,1\,1)}x_\alpha\\ I_{(1\,0\,1)\beta}&=k_{(1\,0\,1)\beta}x_\beta+k_{(1\,2\,0)\alpha}x_\alpha\end{aligned} \tag{3}$$

Division of $I_{(1\,0\,1)\beta}$ by $I_{(1\,1\,1)\alpha}$ gives:

$$I_{(1\,0\,1)\beta}/I_{(1\,1\,1)\alpha}=K_1(x_\beta/x_\alpha)+K_2 \tag{4}$$

where K_1 and K_2 are constant. The plotted values of $I_{(1\,0\,1)\beta}/I_{(1\,1\,1)\alpha}$ vs. x_β/x_α give a straight line having the slope K_1 and the interception K_2. K_1 and K_2 can be obtained experimentally from the synthetic mixtures.

Equation 4 combined with $x_\alpha=1-x_\beta$, on solving for x_β, yields:

$$x_\beta=[K_1\{(I_{(1\,0\,1)\beta}/I_{(1\,1\,1)\alpha})-K_2\}^{-1}+1]^{-1} \tag{5}$$

Equation 5 gives the relation between the intensity ratio $I_{(1\,0\,1)\beta}/I_{(1\,1\,1)\alpha}$ and the concentration x_β. By this calibration curve, direct analysis is possible.

To analyse the quantities of the α- and the β-forms of L-glutamic acid when these forms are mixed with other substances, the internal standard method should be used.

A known amount of the internal standard is added to the sample. Let the weight fraction of the internal standard be x_s, and let the weight fraction of the component j after adding the internal standard be x_j'. Then the intensity of the jth component $I_{(h\,k\,l)j}$ and that of the internal standard $I_{(H\,K\,L)s}$, are given from Eq. (1):

$$\begin{aligned}I_{(h\,k\,l)j}&=K_{(h\,k\,l)j}(x_j'/\rho_j\mu),\\ I_{(H\,K\,L)s}&=K_{(H\,K\,L)s}(x_s/\rho_s\mu)\end{aligned}$$

Dividing $I_{(h\,k\,l)j}$ by $I_{(H\,K\,L)s}$ gives:

$$I_{(h\,k\,l)j}/I_{(H\,K\,L)s}=K(x_j'/x_s)$$

where K is constant. As an internal standard, sodium chloride was suitable, because of the following properties:

i) Satisfactory purity.
ii) Sharp diffraction lines.
iii) It has a suitable line free from interference and superposition (see Fig. 2).

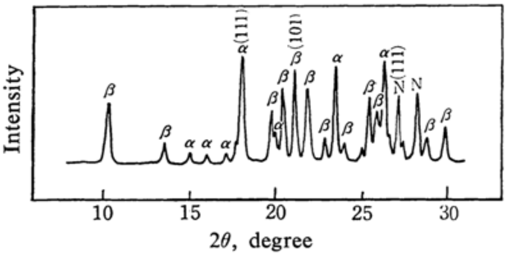

Fig. 2. Diffraction pattern of a sample for the internal standard method.

α: α-form L-glutamic acid, β: β-form L-glutamic acid, N: sodium chloride

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[3] C. L. Christ, R. B. Barnes and E. F. Williames, *Anal. Chem.*, 20, 789 (1948).

[4] L. Alexander and H. P. Klug, ibid., 20, 886 (1948).

[5] L. E. Copeland and R. H. Bragg, ibid., 30, 196 (1958).

Calibration curves were prepared by measuring the following intensities:

$I_{(111)\alpha}$: Intensity of the 4.87Å line of the α-form, (1 1 1) reflection.
$I_{(101)\beta}$: Intensity of the 4.17Å line of the β-form, (1 0 1) reflection.
$I_{(111)s}$: Intensity of the 3.26Å line of NaCl, (1 1 1) reflection.

To determine the concentration of the α- or the β-form, Eq. 6 is modified as (the (1 0 1) line of the β-form is superposed with the (1 2 0) line of the α-form),

$$I_{(111)\alpha}/I_{(111)s}=K_\alpha'(x_\alpha'/x_s)$$

$$I_{(101)\beta}/I_{(111)s}=K_{\beta 1}'(x_\beta'/x_s)+K_{\beta 2}'(x_\alpha'/x_s)$$

When x_s is held constant, the weight fractions of the components α and β-forms, in the original sample, x_α and x_β, are given by:

$$\begin{aligned} x_\alpha&=x_\alpha'/(1-x_s)=K_\alpha(I_{(111)\alpha}/I_{(111)s})\\ x_\beta&=x_\beta'/(1-x_s)=K_{\beta 1}(I_{(101)\beta}/I_{(111)s})\\ &\quad -K_{\beta 2}(I_{(111)\alpha}/I_{(111)s}) \end{aligned} \tag{7}$$

where K_α, $K_{\beta 1}$, $K_{\beta 2}$ are constant. Thus, when the internal standard is added in a constant proportion, the concentration of the component α or β is a linear function of the intensity ratio $I_{(111)\alpha}/I_{(111)s}$ and $I_{(101)\beta}/I_{(111)s}$.

Experimental

Instrumentation. — The diffractometer used was a "Geigerflex" D-3F type (Rigakudenki Co.) using CuK_α radiation (filtered through Ni). The operating conditions were as follows: CuK_α, 30 kV. 15 mA.; Divergence slit, 1°; Scatter slit, 1°; Receiving slit, 0.2 mm.; Time constant, 2 sec.; Counter speed, 1/2° per minute; Chart speed, 1 cm. per minute.

The intensity ratio was obtained by measuring relative peak heights above their backgrounds. It is essential in accurate work to measure peak areas rather than peak heights, although the latter may be sufficient for simple analysis.

Preparation of Calibration Curves. — The compositions of the mixtures prepared for the calibration curves for the analysis in the binary system and in the multicomponents system are given in Tables I and II respectively. Each sample was ground in an agate mortar to about a 5 μ particle size. The β-form crystal has a tendency to crystallize in various habits. When crystallites were ground, they were frequently oriented in a preferred

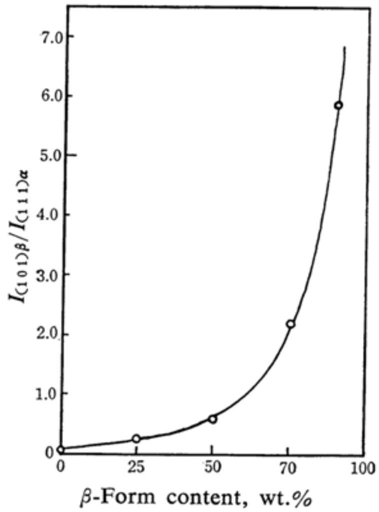

Fig. 3. Calibration curve for the analysis of the β-form concentration by the direct method.

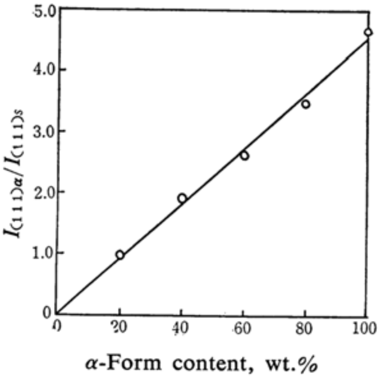

Fig. 4. Calibration curve for the analysis of the α-form concentration by the internal standard method.

TABLE I. COMPOSITION OF SYNTHETIC MIXTURES FOR BINARY SYSTEM

Sample	B-1	B-2	B-3	B-4	B-5
α-Form (g.)	1.00	0.753	0.502	0.248	0.101
β-Form (g.)	0	0.249	0.499	0.745	0.902
β-Form (wt.%)	0	25	50	75	90

TABLE II. COMPOSITION OF SYNTHETIC MIXTURES FOR MULTI-COMPONENT SYSTEM

Sample	M-1	M-2	M-3	M-4	M-5	M-6
α-Form (g.)	0	0.201	0.400	0.601	0.801	1.001
β-Form (g.)	0.999	0.800	0.600	0.400	0.201	0
NaCl (g.)	0.500	0.499	0.501	0.500	0.500	0.500
β-Form (wt.%)	100	80	60	40	20	0

direction, and the preferred orientation caused an abnormal increase in the intensity of the $(0\,k\,0)$ lines in the diffraction pattern. However, sufficient grinding reduced this effect, and the intensity of the line $(1\,0\,1)$ for the analysis in question was scarcely effected by the preferred orientation. The data for the calibration curves are given in Table III. Each value is an average of 7—8 specimens of each sample. From these data, using the method of least squares, the values of K_1 and K_2, and of K_α, $K_{\beta 1}$ and $K_{\beta 2}$ are obtained as follows:

$$K_1=0.673, \qquad K_2=0.037$$

$$K_\alpha=21.7, \qquad K_{\beta 1}=32.1, \qquad K_{\beta 2}=2.08$$

From Eqs. 5 and 7, each combined with these experimental values, the calibration curves can be obtained (Figs. 3 and 4).

Results and Discussion

Three samples of different concentrations were analyzed by the internal standard method and the direct method. These results are given in Table IV. From these data the standard deviations in analytical work were estimated at about 1 wt.% by the direct method. The reproducibility of the analytical results by the internal standard method was generally poorer than by the direct method. This may be due to the lower peak heights caused by the X-ray absorption of sodium chloride and the imperfection of mixing of the standard with the samples. In practice, glutamic acid crystallites have good purity, so polymorphic analysis can be carried out by the method of the binary system. Many experiments suggest that the lower limit of detection of each component in practical samples is about 2%. To obtain more accurate results, it seems necessary to repeat the experiment. Quantitative analysis based on the intensities of two or more selected lines of one component seems to be more accurate, but in organic substances, it is difficult, because of their complex diffraction patterns, to choose lines free from the superposition of other materials. High stabilization of the tube current and voltage is important in obtaining reproducible data.

TABLE III. DATA FOR CALIBRATION CURVES

Sample	B-1	B-2	B-3	B-4	B-5
I_β/I_α	0.0456	0.259	0.557	2.18	5.89
x_β/x_α	0	0.331	0.995	3.00	8.93

Sample	M-1	M-2	M-3	M-4	M-5	M-6
I_α/I_s	0	0.96	1.91	2.62	3.47	4.63
I_β/I_s	3.19	2.45	1.96	1.46	0.91	0.269

TABLE IV. ANALYTICAL RESULTS FOR SAMPLES X, Y AND Z

Sample	Method direct β-Form wt.%	Internal standard method α-Form wt.%	Internal standard method β-Form wt.%
X	90.1	10.1	90.5
	88.3	13.1	98.3
	88.9	10.1	87.7
	90.4	11.0	102
	90.6	9.7	92.1
S. D.[a]	1.0	1.1	5.9
Y	47.6	55.6	47.8
	47.6	53.4	44.5
	47.4	50.2	49.1
	47.4	58.2	72.3
	45.7	55.8	62.7
S. D.[a]	0.8	3.1	11.6
Z	10.0	84.7	9.2
	10.1	97.0	10.2
	11.3	119	13.5
	12.0	90.3	9.9
	12.2	78.6	10.9
S. D.[a]	1.0	15.7	1.6

a Standard deviation

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