Experimantal

The animals used in these studies were male ddY mice of about 5 weeks old, weighing 19—23 g. They were fed with standard diet CE-2 (CLEA Japan Inc., Tokyo) and with unlimited supply of water. The mice were supplied from an animal farm in Shizuoka Prefecture, Japan. Ehrlich ascites carcinoma cells used were the LP-12 cell line[11] which have been maintained by a serial intraperitoneal transplantation into ddY male mice. Tumorcells obtained from ddY mice 7 days after inoculation were employed as a material for the experiment. A 0.2 ml $NaHCO_3$-free Tyrode suspension of the LP-12 cells was transplanted into tail vein of the mice. Hydrocortisone acetate was injected subcutaneously and other antitumor drugs intraperitoneally. The treatment with the drugs performed 24 hours before, after or simultaneously with the transplatation of the tumor cells. Six mice in a group were employed for each trial.

11) Y. Hasegawa, Y. Irikura, M. Ishidate, Jr. and D. Mizuno, *GANN*, **61**, 73 (1970).

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Factors Affecting Dissolution Rate of Cellulose Acetate Phthalate in Aqueous Solution[1,2]

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Various polyelectrolytes which are insoluble in acidic and soluble in neutral or weakly alkaline media have been available to enteric coating. However, precise dissolution studies of them have never been done, which should be important in discussing the drug availability or in finding the means useful to the quality control of enteric coated preparations.

In previous papers,[1,4] the dissolution of polyvinylpyrrolidone (PVP) was investigated in acetone-water system to make an approach to an understanding of general dissolution behaviors of synthetic polymers. Although generally PVP is not included in the category of polyelectrolytes, its dissolution rate was influenced by the addition of NaCl,[4] and from the viscosity measurement in water the low molecular weight fractions of PVP was considered to behave as a polyelectrolyte.[1] Accordingly, it seems significant to investigate the dissolution behaviors of intrinsic polyelectrolytes, analyzing the factors affecting the charges of solute molecules, such as pH and ionic strength of solution.

From the above points of view, in the present study, the dissolution of cellulose acetate phthalate J.P. VII Part II (CAP), which is popular and commercially available as an enteric coating material and is quantitatively determinable by ultraviolet (UV) absorption method, was investigated in various buffer solutions according to the rotating disk method.

Experimental

Materials——CAP obtained commercially was purified by a Soxhlet's extractor with ether, and the dried fraction passing through a 150 mesh (104 μ) sieve was used as the sample, containing less than 0.5% of free

1) This paper forms Part XV of "Physico-chemical approach to Biopharmaceutical Phenomena." Preceding paper, Part XIV: H, Nogami, T. Nagai, and A. Kondo, *Chem. Pharm. Bull.* (Tokyo), **18**, 2290 (1970).
2) This work is outlined in Abstracts of Papers, 90th Annual Meeting of the Pharmaceutical Society of Japan, Sapporo, July 1970, No. OB10-6.
3) Location: *Hongo, Bunkyo-ku, Tokyo.*
4) H. Nogami, T. Nagai, and A. Kondo, *Chem. Pharm. Bull.* (Tokyo), **18**, 1185 (1970).

phthalic acid according to the J.P. VII purity test. This sample consisted from 32.2% of carboxybenzoyl and 18.8% of acetyl groups according to the J.P. VII assay methods, corresponding to 0.72 pieces of carboxybenzyl, 1.45 of acetyl and 0.83 of hydroxyl groups per glucose unit.

Apparatus and Procedure——Rotating disk method as described in a previous paper[5] was employed. Experiments were carried out under the following conditions: 200 ml of buffer solution at 37°; the rotating velocity of disk at 300 rpm; the disk of 3 cm diameter compressed under 3.5 or 5 ton/cm². It was recognized preliminarily by X-ray diffractometry that the sample was amorphous and no phase transition took place during the compression. Every 15 min, 1 ml of the solution was sampled out, the resultant want was being made up by adding the same buffer solution of the same temperature as that before the beginning of the dissolution.

Quantitative Determination of the Dissolved Amount of CAP——After diluting the sample solution with the same buffer solution as used in the dissolution experiment, the concentration of CAP was determined according to ultraviolet (UV) absorption method, using a Hitachi 124 spectrophotometer at 282 mμ. The optical density was recognized to follow Lambert–Beer rule, being independent of pH and the concentration of buffer solution above pH 6.

Result and Discussion

Dissolution Curve of CAP

In previous papers,[1,4] there were found three stages of dissolution of PVP with the lapse of time, *i.e.*, the initial, the main and the final ones, the main one being explained according to Noyes–Nernst equation. However, in the present study, the dissolution curve of CAP showed no initial stage, as shown in Fig. 1, and moreover it was not explained according to Noyes–Nernst equation. In other words, both the plots of C_2 against C_1 and of log (C_2-C_1) against t_1, where C_1 is the concentration at the time t_1 and C_2 the concentration after a given time, Δt, *i.e.*, at the time $(t_1+\Delta t)$, gave no exact straight lines and thus the exact values of the saturated concentration, C_0 and the dissolution rate constant, K_T, were not obtainable.

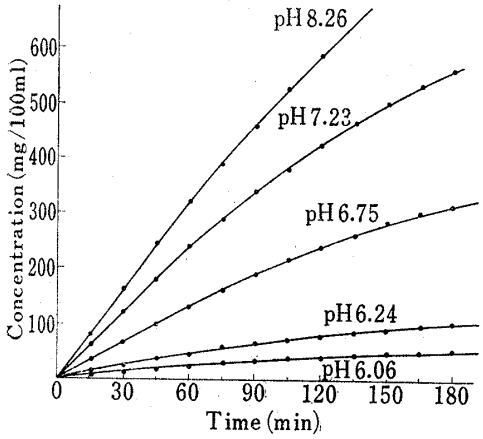

Fig. 1. Dissolution Curves of CAP in 200 ml of 1/30M Phosphate Buffer Solution at 37° from a Disk compressed under 5 ton/cm² of 3 cm Diameter Rotating at a Velocity of 300 rpm

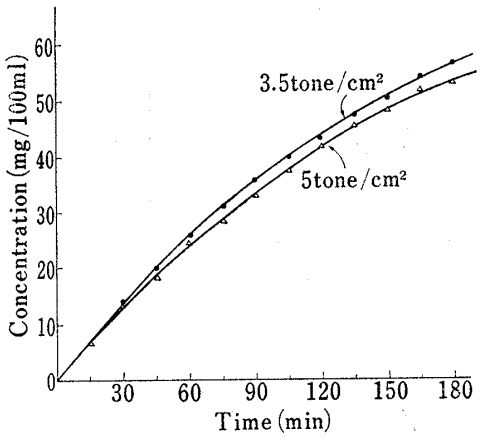

Fig. 2. Dissolution Curves of CAP in 200 ml of 1/30M Phosphate Buffer Solution (pH 6.06) at 37° from a Disk compressed under Different Compressional Pressures of 3 cm Diameter Rotating at a Velocity of 300 rpm

Dissolution of low molecular weight electrolytes has been explained on the basis of the consideration that the dissociation takes place quickly compared with the diffusion and the respective species such as undissociated molecules or ions diffuse independently. However, in the cases of polyelectrolytes, the dissociation is considered to be much complicated and

5) H. Nogami, T. Nagai, and A. Suzuki, *Chem. Pharm. Bull.* (Tokyo), **14**, 329 (1966).

gives effect on the dissolution rate, as will be described later. Accordingly it seemed difficult to apply the existing theory of rotating disk method to the dissolution of CAP.

Dependence of Dissolution Rate on the Compressional Pressure to Make Disk

Fig. 2 as an example shows that the dissolution rate of CAP from a disk compressed under 5 ton/cm² was a little lower than that from a disk compressed under 3.5 ton/cm², as was observed in any case with a satisfactory reproducibility. In the cases of low molecular weight compounds, the dissolution rate did not depend on the intrinsic surface area,[5] as was considered to be characteristic of the diffusion controlled dissolution.[6] Therefore, the above result gave an additional demonstration so that the dissolution of CAP might not be diffusion-controlled, and the swelling process seemed to play an important role in the dissolution.

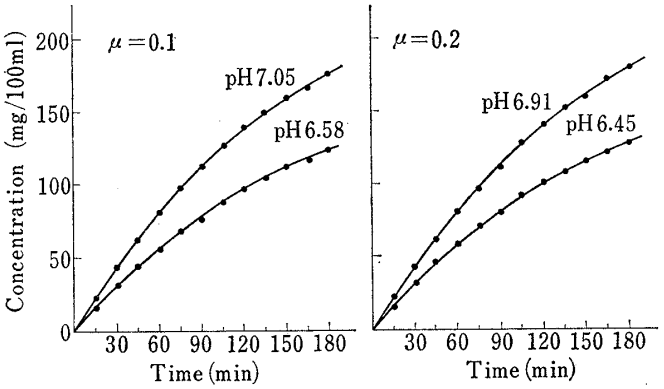

Fig. 3. Dissolution Curves of CAP in 200 ml of Phosphate Buffer Solutions of Different Ionic Strengths (μ) of Different pH's at 37° from a Disk compressed under 5 ton/cm² of 3 cm Diameter Rotating at a Velocity of 300 rpm

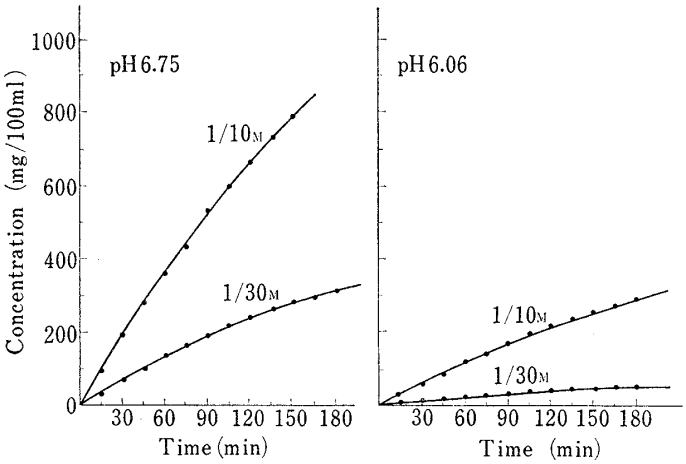

Fig. 4. Dissolution Curves of CAP in 200 ml of Phosphate Buffer Solution of Different pH's of Different Concentrations at 37° from a Disk compressed under 5 ton/cm² of 3 cm Diameter Rotating at a Velocity of 300 rpm

Considering that enteric coated films practically may vary in physical properties according to the treatment, the variety of the dissolution rate may result. Therefore, the more precise quality control should be taken into consideration upon the treatment of coating.

Dependence of Dissolution Rate of CAP on pH and Ionic Strength

Fig. 3 and 4 show that the dissolution rate increased with the increases in pH and in ionic strength of buffer solutions.

On the other hand, in distilled water of pH 5.5—5.8, CAP was so slightly soluble that the amount dissolved was less than 1 mg/100 ml after 10 hr. Therefore, it was considered that the dissociation of CAP was the most important factor affecting the dissolution rate.

Although the dissociation of polyelectrolytes is much complicated, Kern,[7] Kagawa and Tsumura,[8] and Katchalsky[9] proposed different equations to express the dissociation constant or the degree of dissociation, which substantially express the same matter. Moreover, Kagawa proposed another equation by considering the effect of concentration of the poly-

6) D.P. Gregory and A.C. Riddiford, *J. Chem. Soc.*, **1956**, 3756.
7) W. Kern, *Z. Physik. Chem.*, **181A**, 249 (1938).
8) R. Kagawa and K. Tsumura, *Kogyo Kagaku Zasshi*, **47**, 437 (1944).
9) A. Katchalsky and P. Spitnik, *J. Polymer Sci.*, **2**, 432 (1947).
10) R. Kagawa, *Kogyo Kagaku Zasshi*, **47**, 574 (1944).

electrolyte itself,[10] which may not be important in the cases of low molecular weight electrolytes.

Regarding the dissolution process of CAP, the dissociation constant is considered to change with the change in concentration of CAP itself from the above Kagawa's proposal, and also in pH and in ionic strength of solution, reflecting upon the dissolution rate. Thus, it is difficult to explain the phenomenon according to Noyes–Nernst equation.

Aside from the quantitative discussion which is difficult as described above, the dissociated form of carboxyl group of CAP was considered to increase with the increase in pH and thus the solubility increased, resulting in the increase in dissolution rate. The increase in dissolution rate with the buffer concentration at a given pH was explained on the consideration that such gegen ions as Na^+ and K^+ were fixed by the ion exchange, resulting in the increase in dissociation, as was discussed in the cases of carboxymethylcellulose[11] and alginic acid.[12]

Conclusively, the factors affecting the charges of polyelectrolyte molecules are very important in dissolution rate or in drug availability of enteric coated preparations and thus should be taken into consideration in detail by manufactures and the committee for pharmacopoeia upon establishing a method available to the quality control of such preparations.

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11) R. Kagawa and K. Katsuura, *Kogyo Kagaku Zasshi*, **53**, 79 (1950).
12) T. Kiyoyama, *Kogyo Kagaku Zasshi*, **53**, 244 (1950).

[Chem. Pharm. Bull. 18(11)2353—2356(1970)]

UDC 547.773.02.07 : 547.234.04 : 547.339.2.04

Sur la Formation d'un Dérivé Pyrazolique Original à partir d'Hydrazine et d'Ethoxyméthylène Malononitrile

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Plusieurs auteurs ont étudié la réaction de condensation entre l'hydrazine et l'éthoxyméthylène malononitrile (I). En chauffant en milieu alcoolique ces deux réactifs dans des proportions variées, ils n'ont obtenu qu'un seul produit de condensation[2]: l'amino-3 pyrazole carbonitrile-4 (II).

Cependant, au cours d'essais systématiques de cette réaction, nous avons pu obtenir, à côté du pyrazole II, un autre dérivé pyrazolique. Le présent travail a pour objet de déterminer la structure de ce nouveau composé.

Comme nous l'indiquons sur le Tableau I, en traitant le réactif I par l'hydrate d'hydrazine en milieu alcoolique, nous avons pu former un ou deux composés pyrazoliques selon les proportions relative des réactifs que nous avons utilisées et selon la température à laquelle nous

1) Adresse: *5–Chome, Shirogane, Minato–ku, Tokyo.*
2) *a*) R.K. Robins, *J. Am. Chem. Soc.*, **78**, 784 (1956); *b*) G.H. Hitching et E.A. Falco, Brevet américain 2759949 (1956) [*C.A.*, **51**, 1139 (1959)]; *c*) E.B. Towne, W.H. Moore et J.B. Dickey, Brevet américain 3336285 (1967) [*C.A.*, **68**, 14072r (1968)]; *d*) Wellcome Foundation Ltd, Brevet anglais 798662 (1958) [*C.A.*, **53**, 1328 (1959)].