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57. Nobuo Tanaka, Goichi Hirata, and Isamu Utsumi: Interaction of Drugs with Polymers. II*2. The Phase Separation of Polyacrylic Acid by Cationic Drugs.*3

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In recent years the interaction of various drugs with natural or synthetic polyelectrolytes has been extensively studied,[1~7] but there were scarcely any reports on the study of the phase separation of polyelectrolytes by ionic drugs. Therefore, the investigation of the phase separation by the specific interaction of various ionic drugs with polyelectrolytes in an aqueous solution may lead us to find a new significance in a field of pharmaceutical research. Particularly, this problem is of interest in connection with stabilization of drugs, or prolongation and enhancement of action of drugs.

In the previous paper*2 the counter-ion (inorganic cation) effects on the complex coacervation of gelatin and various arabic acid salts as one example of pharmaceutical application of polyelectrolytes was discussed. This paper, moreover, describes that the phase separation depends upon drug concentration, polyelectrolyte concentration, temperature, and is highly specific with respect to the drug cations investigated. These effects were considered in terms of the phase separation temperature, Tp, *i.e.*, the temperature at which the polyelectrolyte-drug solutions either just become turbid or lose its turbidity when the temperature is lowered or raised. The polyelectrolyte used in this research was a polyacrylic acid (PAA) whose structure is linear and simple. On the other hand, the drugs used here were mainly hydrochlorides of organic compounds with more than one basic amino groups or heterocyclic nitrogens.

Experimental

Cationic Drugs——Aniline hydrochloride, pure reagent. N-Methylaniline hydrochloride, pure reagent. N,N-Dimethylaniline hydrochloride, pure reagent. Ephedrine hydrochloride, J. P. Ⅶ. N-Methylephedrine hydrochloride, J. P. Ⅶ. Pyridine hydrochloride, pure reagent. Pyridoxine hydrochloride, J. P. Ⅶ. Piperidine hydrochloride, pure reagent. Thiamine hydrochloride, J. P. Ⅶ. O-benzoylthiamine disulfied hydrochloride, pure reagent.

Polyacrylic Acid——Polyacrylic acid (PAA) was prepared by the method of Kagawa, *et al.*[8] Commercial sodium polyacrylate (low viscosity, Wakō Pure Chemical Industries, Ltd.) was purified by reprecipitation three times with MeOH adding NaOH. The average molecular weight, as determined viscometrically, was 272,000. PAA solution was prepared by passing the purified sodium polyacrylate through columns of ion-exchange resins of Amberlite IR-120 and those of Amberlite IR-4B several times alternately, and was dried in a vacuum oven at 60°. All concentrations were then expressed in terms of gram equivalent monomer per liter (eq./L.).

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*2 Part I: N. Tanaka, G. Hirata, T. Ida, I. Utsumi: Yakugaku Zasshi, **85**, 799 (1965).

*3 This paper was presented at Kinki branch meeting of Pharmaceutical Society of Japan. November, 1964.

1) L. Kennon, T. Higuchi: J. Am. Pharm. Assoc., **45**, 157 (1956).
2) *Idem*: *Ibid.*, **46**, 21 (1957).
3) H. Sulser: Arch. Klin. Exptl. Dermatol., **215**, 266 (1962); Chem. Abstr., **58**, 12789h (1963).
4) A. Packter: Kolloid-Z., **189** (2), 125 (1963).
5) P. Acred, D. M. Brown, T. L. Hardy, K. R. L. Mansford: Nature, **199**, 758 (1963).
6) M. Nakagaki, N. Koga, H. Terada: Yakugaku Zasshi, **83**, 586 (1963).
7) *Idem*: *Ibid.*, **84**, 516 (1964).
8) A. Takahashi, J. Hayashi, I. Kagawa: Kōgyō Kagaku Zasshi, **60**, 1059 (1957).

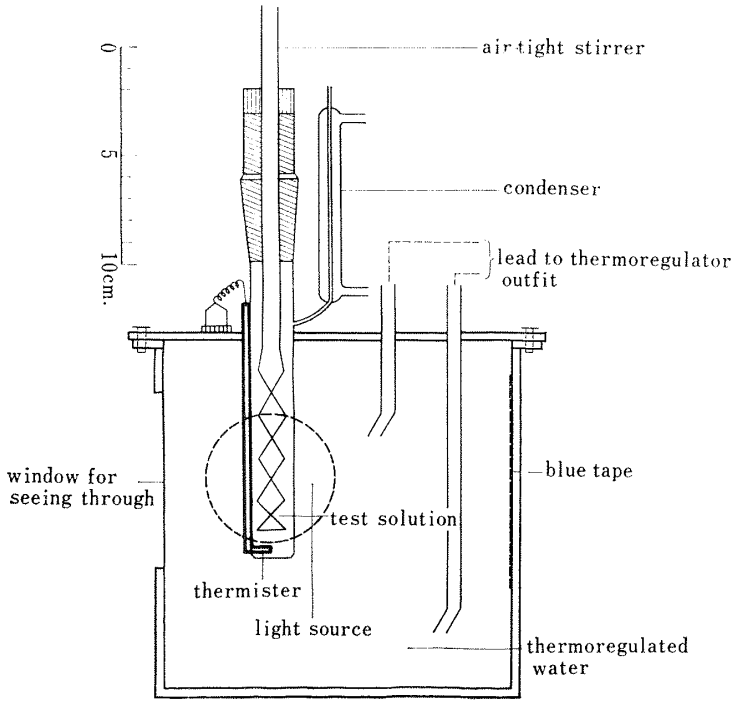


Fig. 1. Apparatus for Measuring Phase Separation Temperatures of the PAA-Drug System

Phase Separation Experiments—— The PAA-drugs solutions were prepared directly by pipetting solutions of PAA and those of drugs into the test tube (30 ml. capacity) with the condenser and the airtight stirrer, which was essentially similar to Kagawa's apparatus[9] (Fig. 1). The blue tape sticked on the wall of the water bath which is about 10 cm. distance from behind the test tube was observed visually through the stirred test solution with transverse illumination to the direction of observation. The PAA-drugs solutions were cooled or heated at the rate of 1° per 20 min. by circulating a thermoregulated water through the water bath until the blue tape appeared blurred or clear. The value of the temperature at which this phenomenon first became apparent was taken as the phase separation temperature Tp; Tp was measured by the thermister equipped at the bottom of the test tube, and was obtained within ±1°, being independent of whether approached from a high or a low temperature.

Results

The phase separation temperatures, Tp, were plotted against aniline, N-methylaniline, and N,N-dimethylaniline concentration for constant concentration of PAA in Fig. 2. All curves exhibit a distinct maximum, showing that the two phases (liquid-

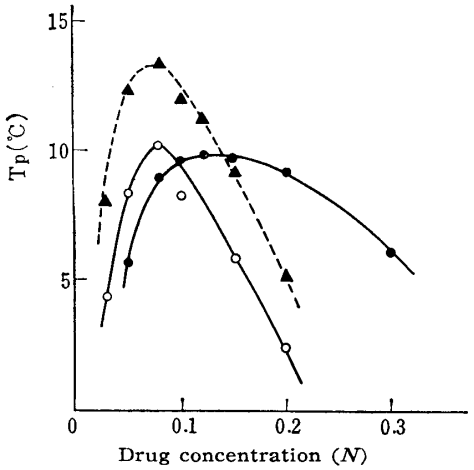


Fig. 2. Phase Separation Temperatures Tp, at Constant Concentration of PAA, as a Function of Drug Concentration

PAA=0.1 eq./L.

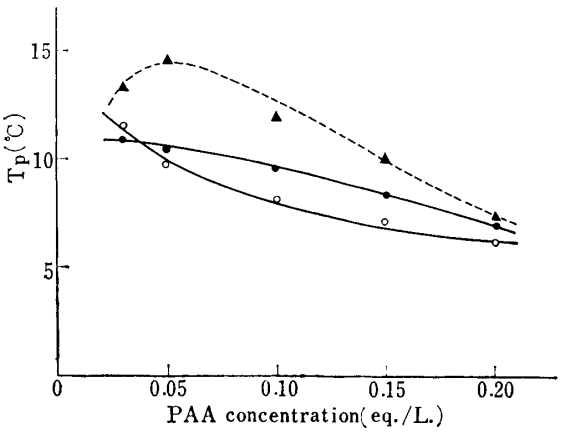


Fig. 3. Phase Separation Temperatures Tp, at Constant Concentration of Drug, as a Function of PAA Concentration

Drug=0.1 N

--▲-- C_6H_5-$N(CH_3)_2$HCl
—•— C_6H_5-NH_2HCl
—○— C_6H_5-$NHCH_3$HCl

9) A. Takahashi, I. Kagawa : Kōgyō Kagaku Zasshi, **64**, 1637 (1961).

liquid or liquid-solid) recombine with excess drug at constant temperature. From the above fact it was found that excess drug cations lower Tp, and that at a constant temperature and polymer concentration there exist an upper limited and a lower limited drug concentration between which phase separation occurs. Because, as seen in Fig. 2, methylation of amino group of aniline raises Tp, for the N-methyl derivatives of aniline we found Tp to rise in the following order; aniline ≒ N-methylaniline < N,N-dimethylaniline. It is to be noted that the phase separation follows thus the reverse order of ionization potential of aniline derivatives.[10] The effect of change of PAA concentration on Tp at constant drug concentration is shown in Fig. 3. Curves of Fig. 3 show specificity of the drug cations with respect to phase separation, *i.e.*, a maximum in the Tp against PAA concentration curves for aniline derivatives except N,N-dimethylaniline was not observed.

Further a series of the phase separation experiments on ephedrine derivatives were performed to investigate the influence of the degree of methylation of amino group on Tp. At 0.1 eq./L. of PAA the above mentioned relations between Tp and concentration of aniline derivatives are also satisfied in this series. The phase separation follows the degree of methylation of amino group; N-methylephedrine > ephedrine (Fig. 4). An examination of Fig. 4 reveals that, at higher added drug concentration region, the curves will converge, and this phenomenon suggests that specific effects become less. The curves in Fig. 5 seem to converge at higher concentration region of added PAA, but to diverge markedly at lower concentration of added PAA, and consequently Tp rises exponentially with decreasing PAA concentration.

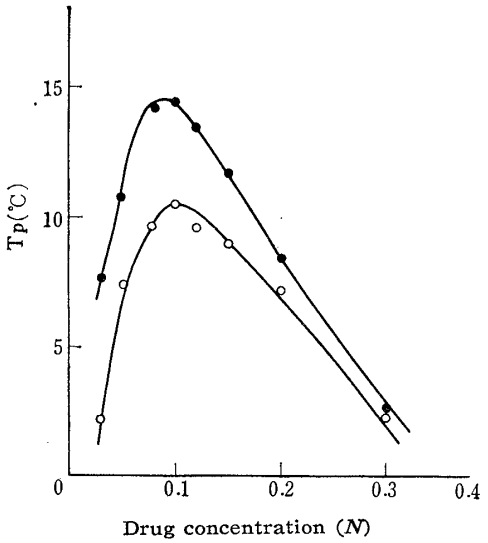


Fig. 4. Phase Separation Temperatures Tp, at Constant Concentration of PAA, as a Function of Drug Concentration

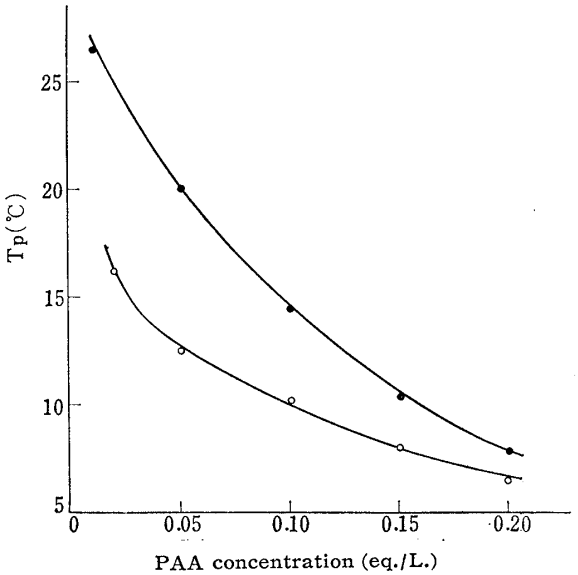


Fig. 5. Phase Separation Temperatures Tp, at Constant Concentration of Drug, as a Function of PAA Concentration

PAA=0.1 eq./L. Drug=0.1 N

—•— C_6H_5-CH(OH)CHCH$_3$ (N(CH$_3$)$_2$HCl)

—○— C_6H_5-CH(OH)CHCH$_3$ (NHCH$_3$HCl)

10) K. Kimura, H. Tsubomura, S. Nagakura : Bull. Chem. Soc. Japan, 37, 1336 (1964).

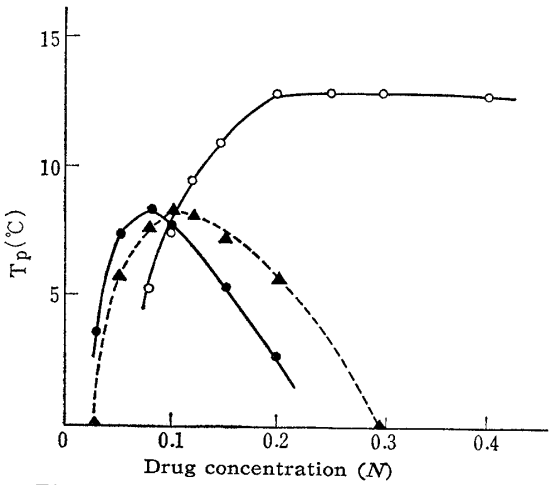


Fig. 6. Phase Separation Temperatures Tp, at Constant Concentration of PAA, as a Function of Drug Concentration
PAA=0.1 eq./L.

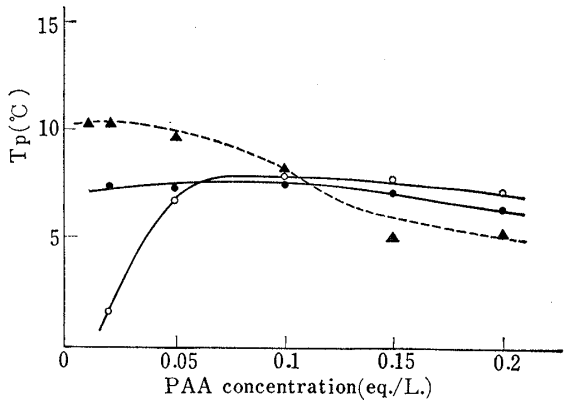
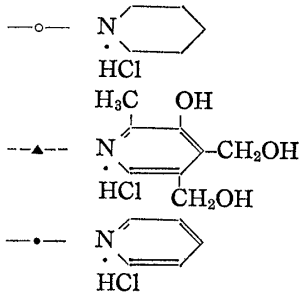


Fig. 7. Phase Separation Temperatures Tp, at Constant Concentration of Drug, as a Function of PAA Concentration
Drug=0.1 N



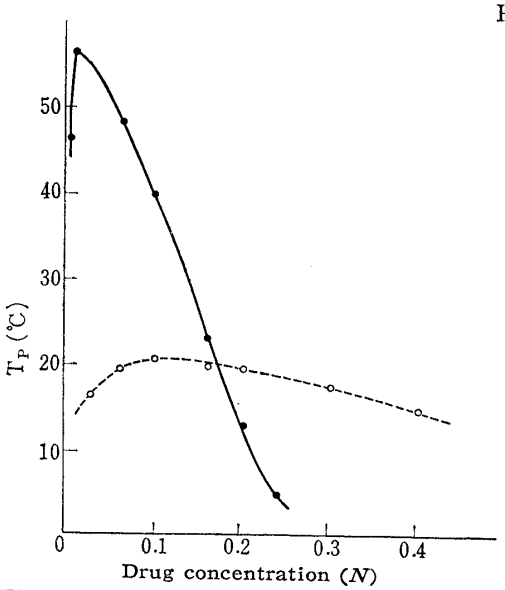


Fig. 8. Phase Separation Temperatures Tp, at Constant Concentration of PAA, as a Function of Drug Concentration
PAA=0.1 eq./L.

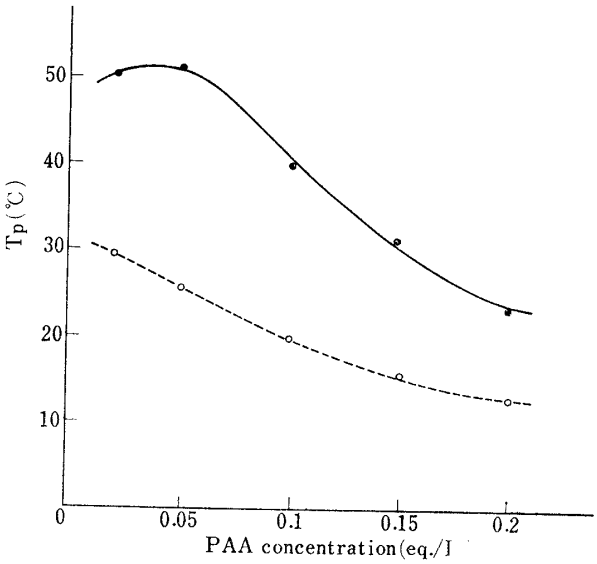


Fig. 9. Phase Separation Temperatures Tp, at Constant Concentration of Drug, as a Function of PAA Concentration
Drug=0.1 N

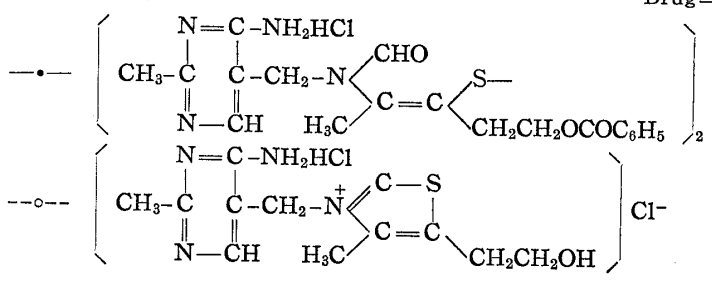


Fig. 6 exhibits a plot of Tp against drug concentration for the pyridine derivatives dissolved in 0.1 eq./L. PAA aqueous solution. The plots show that among the pyridine derivatives the phase-separating action of piperidine is quite different from that of pyridine and pyridoxine. The Tp rises linearly with the increasing of piperidine concentration ($<0.2N$), and then is constant above piperidine concentration of $0.2N$. On the contrary, the Tp *vs.* drug concentration curves for pyridine and pyridoxine exhibit a distinct maximum at about $0.1N$. When added drug concentration is constant (Fig. 7), the Tp for pyridine and pyridoxine is scarcely changed, being independent on the added PAA concentration. However the Tp for piperidine rises linearly at low PAA concentrations ($<0.05N$).

As seen in Fig. 8 and 9, the Tp for the divalent drug cations (thiamine, O-benzoylthiamine) is much higher than that for the above mentioned monovalent drug cations (aniline derivatives, ephedrine derivatives, pyridine derivatives). At constant PAA concentration (Fig. 8) both Tp *vs.* drug concentration curves exhibit a distinct maximum, at approximately $0.1N$ of thiamine and at about $0.01N$ of O-benzoylthiamine disulfide, respectively. On the other hand, no maximum in the Tp *vs.* PAA concentration curve for thiamine was observed at constant drug concentration.

Discussion

In summarizing the results of these studies, at a constant polymer concentration there exists a distinct maximum in the Tp *vs.* drug concentration curves except piperidine system, and the maximum Tp appears when drug concentration is nearly equal to polymer concentration. On the other hand, at a constant added drug concentration there dose not exist, in most cases, a maximum in the Tp *vs.* polymer concentration curves.

As the results of Tp measurements above mentioned, it was found that the phase separation is highly specific with respect to the species of drug cations, especially, to the basic structure of drug molecules, and to valences of drug cations.

These results, being compared with the change of the Tp of sodium polyacrylate by inorganic monovalent salts,[9,11,12] and with the salting-out phenomena of PAA by inorganic polyvalent salts,[13] suggest that the Tp is closely related not only to the direct interaction between polyions or to the chain configuration of polyions, but also to the specific drug ion binding to polyions, the hydration of polyions, or drug ions, or polyion-drug ion complexes, inter- or intra-ion hydrogen bonds, and the arrangement of water molecules. However, at present for lack of the data on these respects, the above results can not quantitatively be interpreted. The results of more detailed investigation of phase separation will be reported in a future paper.

Summary

Phase separation of PAA by various cationic drugs has been investigated as a function of temperature, polymer and added drug concentration. There exists a temperature Tp at which the PAA-drug aqueous solution separates into two phases (liquid-liquid or solid-liquid). The Tp changes remarkably not only with PAA or added drug concentration, but also with the basic structure of drug molecules or valences of

11) A. Takahashi, S. Yamori, I. Kagawa : Nippon Kagaku Zasshi, **83**, 11 (1962).
12) P. J. Flory, J. E. Osterheld : J. Phys. Chem., **58**, 653 (1954).
13) A. Ikegami, N. Imai : J. Polymer Sci., **56**, 133 (1962).

drug cations. The decreasing order of the Tp is O-benzoylthiamine > thiamine > N-methylephedrine > ephedrine > piperidine > N,N-dimethylaniline > N-methylaniline ≒ aniline > pyridoxine ≒ pyridine.

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58. Yutaka Morita : Studies on Phenazines. XXVII.*1 Nuclear Magnetic Resonance Studies. I.*2

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Recently, the dilution method and the double resonance technique have been applied to determine the chemical shift and coupling constant of ring protons in polycyclic aromatic compounds, such as triphenylene, chrysene, and pyrene.[1] The multiplicity of spin-spin coupling in these cases made unfavorable to analyse those data by using the ordinary theoretical treatment.

In some aromatic compounds, however, successful results have been obtained; naphthalene, lutidine, and alkylated benzenes.[2,3]

Among them, the NMR (nuclear magnetic resonance) spectrum of naphthalene has been studied by Pople, *et al.*[3] clarifying the existence of A_2B_2 system. In naphthalene nucleus, though eight protons look actually A_4B_4 type, they can approximately be dealt as a superposition of two sets of A_2B_2 type. In fact, the theoretical treatment of naphthalene protons assigned as A_2B_2 system has been shown in good agreement with the obtained data.

In the case of phenazine, which has also two pairs of equivalent protons in its molecule, the interrelation of protons appears quite similar to naphthalene. The present and following papers are dealing with the NMR analyses of phenazine and its derivatives with the complete assignment of their ring protons, thus expecting the possible elucidation of the chemical structures of phenazine derivatives, especially to determine the positions of ring substituents. First of all, the validity of A_2B_2 system in phenazine nucleus and the evaluation of their coupling constants (J) were examined using phenazine (I), phenazine mono-N-oxide (II), phenazine di-N-oxide (III). Secondly, by the inspections of the spectra of methoxy derivatives, the calculated various J values were demonstrated their correctness. These data are applicable to the analyses of numerous phenazine derivatives obtained by syntheses and from natural source. For these purposes, following methoxyphenazines were dealt with; *i.e.*, 1,3-dimethoxy- (V), 2,3-dimethoxy- (VI), 1,4-dimethoxy- (VII), 1,6-dimethoxy- (VIII), 2,8-dimethoxy- (IX), 1,9-dimethoxy- (X), and 1,6-dimethyl-4,9-dimethoxy-phenazine (IV). The latter compound

*1 This is one of the series of Studies on Phenazines (I. Yosioka). Part XXVI. Y. Kidani, K. Ukai: This Bulletin, **14**, 293 (1966).

*2 A part of this was presented at the 85th Annual Meeting of the Pharmaceutical Society of Japan (Tokushima, October, 1965).

*3 Toneyama, Toyonaka, Osaka-Fu (森田 豊).

1) R.H. Martin, N. Defay, F. Geerts-Evrard, S. Delavarenne : Tetrahedron, **20**, 1073 (1964).
2) H.J. Bernstein, J.A. Pople, W.G. Schneider : Can. J. Chem., **35**, 65 (1957).
3) J.A. Pople, W.G. Schneider, H.J. Bernstein : *Ibid.*, **35**, 1060 (1957).