The authors wish to thank Prof. T. Uno of the Kyoto University for many helpful discussions and suggestions during this work. Thanks are due to Mr. T. Sugimura, Miss K. Uchida and H. Hidaka for their assistances in the experimental work.

Summary

The infrared spectra of 44 kinds of benzenesulfonamide derivatives and a few naphthalenesulfonamide derivatives were measured and compared with the benzenesulfonic acid derivatives. The sulfonamide derivatives showed two characteristic bands near 900 cm^{-1} and 1090 cm^{-1} regions, but the latter didn't show the bands near 900 cm^{-1} region. The infrared spectra of deuterated benzenesulfonamide derivatives were examined. In the compound in which nitrogen atom is directly attached to hydrogen, the band near 900 cm^{-1} region shifted about 38~100 cm^{-1}. However, in case of the compounds where nitrogen atom is not attached to hydrogen, the band near 900 cm^{-1} region did not shift on deuteration work. These facts suggest that the band near 900 cm^{-1} region can be ascribed to S-N vibration.

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52. Keiji Sekiguchi and Keiji Ito : Studies on the Molecular Compounds of Organic Medicinals. I. Dissolution Behavior of the Molecular Compound of Sulfanilamide and Sulfathiazole.*1

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Up to the present, many organic systems have been investigated by thermal analysis, and it was widely recognized that molecular compounds were often formed between organic medicinals. Also, there have appeared in literatures a number of reports[1~3] concerning complex or molecular compound formation in solution with the intention of finding effective solubilizers for insoluble drug substances. These solubility studies, however, have been confined to the interactions at equilibrium, and none of them inferred the dissolution process before the system attained solution equilibrium. Moreover, the molecular compound isolated has never been employed as the starting material for investigation.

Since information obtained from these method of approach indicates little or nothing on the therapeutical efficacy of the molecular compound itself, it would be natural that only a few of them have been adopted for therapeutical purposes.

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*1 This work was presented at the Hokkaido Branch Meeting of Pharmaceutical Society of Japan, June 10, 1961.

*2 Kita-12-jo, Nishi-5-chome, Sapporo, Hokkaido, Japan (関口慶二, 伊藤圭二).

1) L. Irrera : Gazz. chim. ital., **61**, 614 (1931); C. A., **26**, 642 (1932).
2) T. Higuchi, D. A. Zuck : J. Am. Pharm. Assoc., **42**, 138 (1953); T. Higuchi, J. L. Lach : *Ibid.*, 43, 527 (1954).
3) M. Samejima : Yakugaku Zasshi, **80**, 95 (1960).

Such being the case, this series of investigations was undertaken to establish the fundamental significance of the molecular compound by comparing its physico-chemical properties, especially the dissolution behavior and its biological activity with those of the corresponding mixture containing the same component drugs.

In the present paper, the process of dissolution of the molecular compound of sulfanilamide and sulfathiazole and of the corresponding mechanical mixture are investigated and the results are discussed fully from the standpoint of physical pharmacy.

Experimental

Materials——Sulfanilamide and sulfathiazole were commercial products of J. P. grade. Since it has been confirmed that these sulfonamides are able to exist in several polymorphic forms,[4~7] identification of the sample materials will be necessary for dissolution experiments. By comparing their solubilities and IR absorption spectra with those of the polymorphs, isolated by the authors, both samples were found to be the most stable modifications of these sulfonamides at the experimental temperatures.*3

Preparation of the Molecular Compound——Equimolecular quantities of sulfanilamide and sulfathiazole were dissolved into a hot methanol solution which was previously saturated with either one of the components and the molecular compound at room temperature. On cooling, the molecular compound having a melting point at 178° crystallized out in good yield. For dissolution experiments, the compound is sieved to 100~200 mesh.

The combining ratio was confirmed by the visual thermal analysis of the system of these sulfonamides (Fig. 1), and by the spectrophotometric and the elemental analysis of the compound isolated. *Anal.* Calcd. for $C_{15}H_{17}N_5O_4S_3$: C, 42.14; H, 4.01; N, 16.38; S, 22.50. Found : C, 42.40; H, 4.05; N, 16.42; S, 23.00.

The infrared spectrum of the molecular compound given in Fig. 2 exhibited characteristic peaks, with which the compound appeared in the solid residue was identified throughout the dissolution experiment.

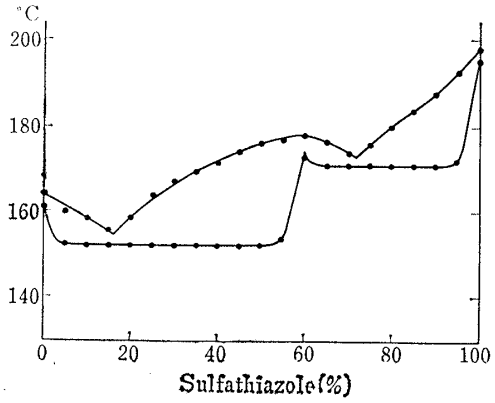


Fig. 1. Thermal Analysis of Sulfanilamide and Sulfathiazole

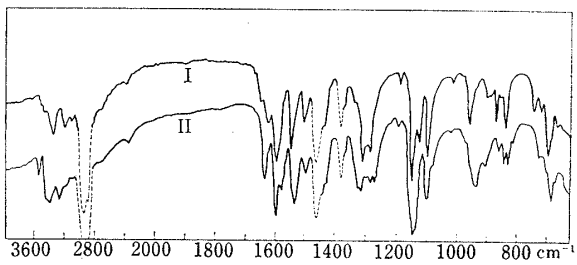


Fig. 2. Infrared Absorption Spectra of the Molecular Compound of Sulfanilamide and Sulfathiazole (I) and the Corresponding Mechanical Mixture (II) in Nujol

Preparation of the Mechanical Mixture——Particle size of each sulfonamide was arranged from 100 to 200 mesh or from 20 to 50 mesh, and these were mixed in equimolecular ratio.

Determination of Sulfanilamide and Sulfathiazole——Sulfanilamide and sulfathiazole in their single saturated solutions were determined by spectrophotometric measurement of their suitable dilutions at the wave length of 258 and 283 mμ, respectively (Table I).

*3 Investigations on solubilities and on infrared absorption of these polymorphs, involving the hydrate form of sulfanilamide were presented at the Hokkaido Branch Meeting of the same society on June 10, 1961 and March 3, 1962. Details will be reported later.

4) A. Watanabe, H. Kamio : Yakugaku Zasshi, **62**, 501, 503 (1942).
5) D. C. Grove, G. L. Keenan : J. Am. Chem. Soc., **63**, 97 (1941).
6) P. N. Leech : J. Am. Med. Assoc., **116**, 307 (1941).
7) H. Miyazaki : Yakugaku Kenkyu, **19**, 133 (1947).

Table I. Solubilities of Sulfanilamide and Sulfathiazole in Water

Temp. ±0.05°	Sulfanilamide $M\times10^3$	Sulfathiazole $M\times10^3$
15	23.111	1.047
25	41.925	1.837
35	77.928	3.122

When the sample solution contained both of the sulfonamides, extinction values of a proper dilution of the aliquot were measured both at 258 and 283 mμ, and by solving the simultaneous equation thus introduced, each concentration could be determined.[8] A high degree of reliability of this method was confirmed with a number of known solutions in which the ratios and the total amounts of the components were varied. Although sulfanilamide and sulfathiazole in a concentrated solution would partly be present as the molecular compound, the absorption curve of the diluted mixture was found to be a mere composite of individual curves of the components, and neither a new absorption nor a shift was observed (Fig. 3).

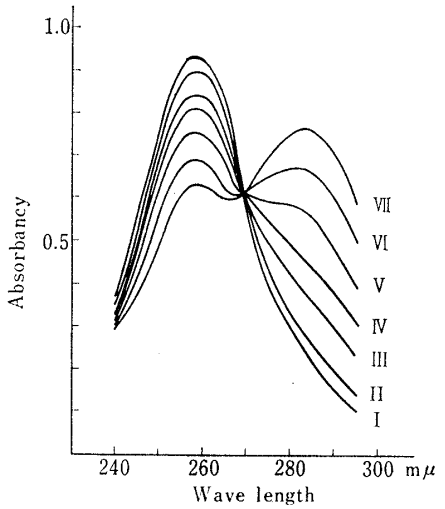


Fig. 3. Ultraviolet Absorption Spectra

I : sulfanilamide, 1.0+sulfathiazole, 0 (μg./0.1 ml. water)
II : 〃 0.9+ 〃 0.1(〃)
III : 〃 0.7+ 〃 0.3(〃)
IV : 〃 0.6+ 〃 0.4(〃)
V : 〃 0.4+ 〃 0.6(〃)
VI : 〃 0.2+ 〃 0.8(〃)
VII : 〃 0 + 〃 1.0(〃)
The spectrum of the mol. compd. (1.0 μg./0.1ml. water) was the same as that of V.

As a check, aliquots of the sample solutions were evaporated at about 70°, and the dried residues were weighed for total amounts of the two sulfonamides dissolved. The results agreed well with those by spectrophotometry.

Procedure for Dissolution Study——In a 200 ml. egg-plant type flask, immersed in a thermostat at the experimental temperature (±0.05°), a certain excess quantity of the molecular compound or the mixture prepared as above was added with 100 ml. of redistilled water (pH 5.7～5.9) which was previously kept at the same temperature (see Figs. 4, 5, and 6). Immediately after the addition, vigorous agitation by an electric stirrer was applied to them. At certain time intervals, aliquot portions were removed through a pipette with a filter, and the concentrations of the solutes in the filtrate were then determined by the method just described.

Results and Discussion

Dissolution curves of the molecular compond and the corresponding mixture at 15, 25, and 35° are shown in Figs. 4, 5, and 6, where the total concentrations of sulfanilamide in solution and those of sulfathiazole are plotted against time in hour. At every temperature of the experiment, difference in dissolution behaviors was distinctly observed.

In the case of the mechanical mixture, the total concentration of sulfanilamide increases rapidly and a maximum (point p) is reached in an initial stage of dissolution. Then, it falls rapidly, though the rate of decrease becomes progressively slower. In the curve for sulfathiazole, a maximum (p′) appears simultaneously with the one for sulfanilamide. Soon later, the curve passes through a minimum (q) and rises very gradually.

The first rapid rise in concentration will be attributed to the tendency of the mechanicl mixture to show individual solubilities of its constituents. However, along with the dissolution, interaction between the two sulfonamides will occur. Since at this time the molecular compound formed in the solution exists in a super-saturated condition, the two maxima p, p′ will be produced by deposition of the molecular com-

8) D. T. Englis, D. A. Skoog : Ind. Eng. Chem., Anal. Ed., **15**, 748 (1943).

pound excessively dissolved. Although sulfanilamide added dissolves rapidly and completely, considerable amount of sulfathiazole still remains in the solid residue and it continues to pass into the solution with the deposition of the molecular compound; accordingly, equimolar decrease in the total concentrations of both sulfonamides will not be observed. The minimum in the curve for sulfathiazole is supposed to be the point where the rate of deposition of the molecular compound becomes equal to that of dissolution of sulfathiazole. Thus, under these experimental conditions, the processes which proceed after the disappearance of sulfanilamide in solid phase will be described as follows.

$$\begin{array}{ccc} S\,(\text{soln.}) + T\,(\text{soln.}) & \longrightarrow & ST\,(\text{soln.}) \\ \uparrow \text{dissolution} & & \downarrow \text{deposition} \\ T\,(\text{solid}) & & ST\,(\text{solid}) \end{array}$$

where S, T, and ST represent free sulfanilamide, sulfathiazole and the molecular compound in the solution or in the solid residue, respectively. Since the rate of interaction between the two sulfonamides is thought to be rapid in solution, the attainment of equilibrium will be influenced by the two phase reactions, dissolution of sulfathiazole and the deposition of the molecular compound. In the actual practice, the equilibrium state was not realized even with vigorous stirring for 12 or 36 hours. The decrease and increase in the total sulfanilamide and sulfathiazole concentrations become very small from this stage of dissolution. Therefore, it is evident that under the same experimental conditions, concentrations of the two sulfonamides differ from their final equilibrium values attained by the molecular compound, though the curves for the mechanical mixture exhibit a strong tendency to approach the equilibrium.

The difficulty of attainment to the equilibrium state is attributed to the fact that sulfathiazole particles in the residue are easily coated by the molecular compound deposited and thus the dissolution of the excess sulfathiazole is extremely hindered. As shown in Fig. 7 (A) and (B), apparent size of sulfathiazole particles grows bigger by this coating effect. Since the solution is not saturated with respect to sulfathiazole, the concentration gradient will present around these particles; consequently, the molecular compound will be formed much greater in the very vicinity of these particles than in the remaining portion of the solution, where the concentrations of all the solutes will be uniform throughout by vigorous stirring. Thus, part of the compound formed in the solution will not diffuse but deposit onto sulfathiazole particles yielding a kind of envelope, by which the dissolution of sulfathiazole will be to a large extent hindered and the nonequilibrium state will last longer. When the size of sulfathiazole particles in the sample mixture is large, the equilibrium becomes practically unattainable because of the smaller surface area available for the dissolution of sulfathiazole before and particularly after accumulation of the molecular compound.

While the changes in concentrations were measured, the kinds of solid phase in the residue were examined by both melting point determination and infrared absorption measurement. As is expected from the dissolution curves, the solid residue before the maximum is found to be sulfathiazole only, and after the maximum, additional solid phase of the molecular compound begins to appear with those of the former.

When the molecular compound is dissolved into water at a constant temperature, equimolar increase in concentrations of the components occurs rapidly just like a simple substance, and in a soon, both concentrations attain a constant and the same value. Thus, the first horizontal part appears in the curve. By further stirring of the solution and the residue, the concentration of sulfanilamide is again increased, while at the same time, that of sulfathiazole begins to decrease, and after certain period of

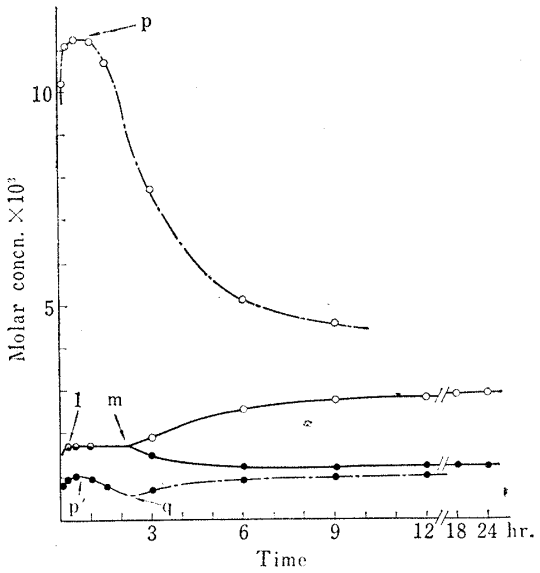


Fig. 4. Dissolution Curves of the Molecular Compound of Sulfanilamide and Sulfathiazole (solid lines) and the Corresponding Mechanical Mixture (chain lines) at 15°

sample amount: each 0.5 g. per 100 ml. of water
particle size: each 100~200 mesh
open circles: total concentrations of sulfanilamide
closed circles: total concentrations of sulfathiazole
l-m: metastable equilibrium state

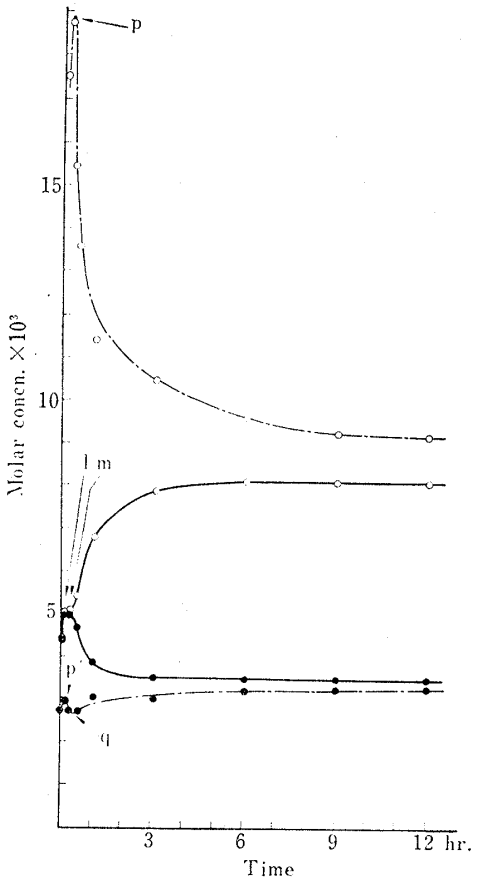


Fig. 6. Dissolution Curves at 35°

sample amount: each 1.0 g. per 100 ml. of water
particle size: each 100~200 mesh

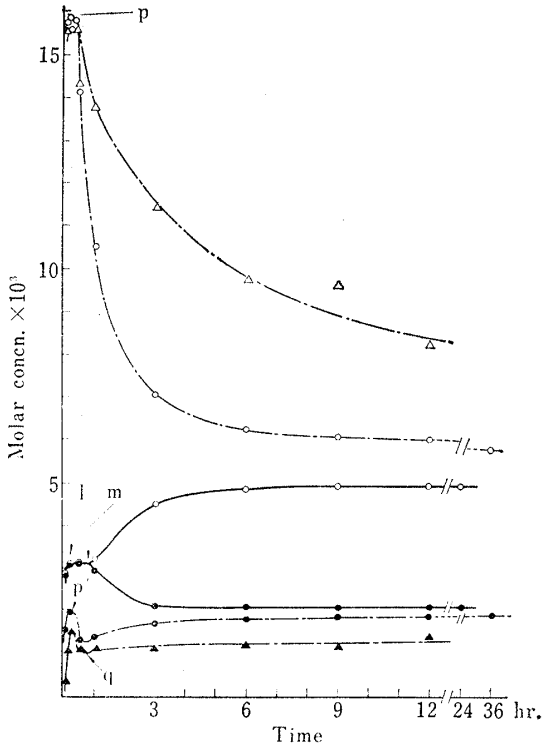
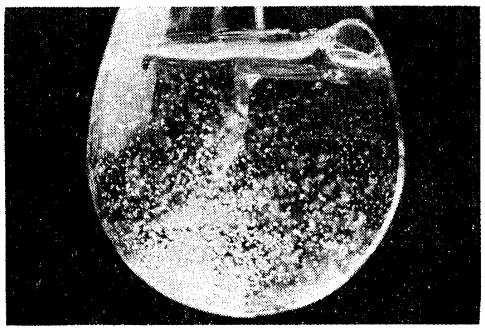
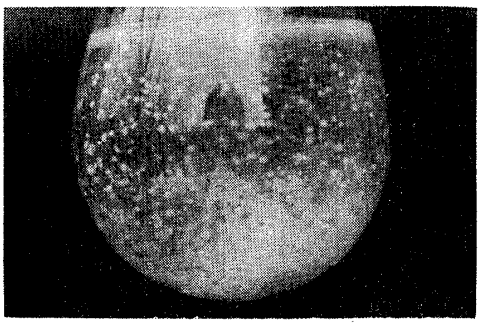


Fig. 5. Dissolution Curves at 25°

sample amount: each 0.7 g. per 100 ml. of water
particle size: 100~200 mesh and 20~50 mesh
open triangles: total concns. of sulfanilamide during dissolution of the mixture containing larger particles of both components
closed triangles: total concns. of sulfathiazole during dissolution of the mixture containing larger particles of both components



(A): initial state; Suspended particles are sulfathiazole.



(B): after 3 hrs.; Particle size of sulfathiazole is apparently enlarged by the accumulation of the molecular compound formed.

Fig. 7. The Coating Phenomenon during Dissolution

time, each concentration reaches to the second constant value of its own. No further changes in concentrations were observed until the end of the experiment.

The first constant value is attributable to the metastable solubility of the molecular compound itself, caused by the suspended deposition of sulfathiazole. Since at this period, the system is in the metastable equilibrium, the duration interval can not precisely be defined, though the results indicate qualitatively that the lower the temperature, the longer the state is maintained.

The discrepancy in concentrations of both sulfonamides caused subsequently is regarded as the process to approach the stable equilibrium, and sulfathiazole begins to appear in the solid residue of the molecular compound. The kinds of solid phase are also identified by the melting point and infrared absorption measurement. Since in this case, the influence by the coating effect as described above will hardly be expected, the dissolution of the molecular compound toward stable equilibrium will proceed smoothly. Under the present conditions of the experiments,*4 when the system attains the stable equilibrium, the state is now expressed by the following:

$$\begin{array}{ccc} \text{S (soln.)} + \text{T (soln.)} & \rightleftarrows & \text{ST (soln.)} \\ \updownarrow & & \updownarrow \\ \text{T (solid)} & & \text{ST (solid)} \end{array}$$

The time taken for the equilibrium to appear varies with the experimental temperature. At 35°, it takes roughly about 6 hours, and at 25°, about 9 hours; while at 15°, even after 24 hours stirring, the total concentrations of the two sulfonamides are still changing slightly, though the system is much nearer to the equilibrium state than in the case of the mechanical mixture.

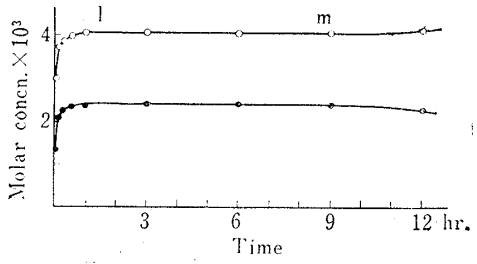


Fig. 8. Prolongation of the Metastable Solution Equilibrium at 25°

sample amount: molecular compound 0.7 g. and sulfanilamide 0.03 g. per 100 ml. of water
open circles: total concentrations of sulfanilamide
closed circles: total concentrations of sulfathiazole
l-m: metastable equilibrium state. During this period, difference in total concn. between sulfanilamide and sulfathiazole corresponds to the amount of sulfanilamide added.

When the same amount of the molecular compound as in the previous experiment is dissolved at 25° into the same volume of water with a small quantity of sulfanilamide, it is expected that the process of dissolution will resemble to that of the molecular compound itself. Although the inference is made from the ternary phase diagram of the two sulfonamides and water, on which the authors will discuss in the successive paper, the dissolution curve at the specified composition shows, however, the actual stability of the metastable equilibrium state and the further decomposition of the molecular compound to form solid sulfathiazole is considerably hindered even after 9 hours stirring (Fig. 8).

The stability constant for the reaction in water solution is written as follows:

$$K=\frac{[\mathrm{ST}]}{[\mathrm{S}]\,[\mathrm{T}]}$$

where K is the stability constant for the molecular compound and [S], [T], and [ST] represent concentration of free sulfanilamide, sulfathiazole and the molecular compound.

*4 The results obtained by using various amounts of the molecular compound will be discussed in the next paper.

Since these concentration in the second term are relatively small, even at saturation, it is reasonably considered that both the incongruently and the congruently saturated solutions will behave similarly as the ideal solutions. Thus, at the stable equilibrium where the solution is saturated with sulfathiazole and the molecular compound, [T] may be taken as being equal to the solubility of sulfathiazole in water; hence,

[ST]=total concentration of sulfathiazole−[T]
[S]=total concentration of sulfanilamide−[ST]

and the stability constants can be computed, using solubility values determined at several temperatures (Tables I and II).

When the system of the molecular compound and water attains to the metastable equilibrium, [T] can not be regarded as the solubility of sulfathiazole, since in this case, the solution is saturated merely with the molecular compound. However, [ST] may be taken as equal to the value as determined above, so that,

[S]=[T]=total concentration of sulfanilamide or sulfathiazole−[ST]

For the metastable eqilibrium resulted with the mixture of the molecular compound and a small amount of sulfanilamide, [S] and [T] become as follows:

[S]=total concentration of sulfanilamide−[ST]
[T]=total concentration of sulfathiazole−[ST]

Table II. The Stability Constants for the Molecular Compound in Water Solution

Temp. (°C)	Sample used	Sample amount, g. per 100 ml. of water	State of equilibrium	Molar concn. × 10^3					Stability constant
				Total S	Total T	Free S [S]	Free T [T]	[ST]	
15	ST	0.5	metastable	1.784	1.785				
25	ST	0.7	stable	4.942	2.068	4.711	1.837	0.231	26.7
	ST	0.7	metastable	3.078	3.055	2.847	2.824	0.231	28.7
	ST S	0.7 0.03	metastable	4.175	2.432	3.944	2.201	0.231	26.6
35	ST	1.0	stable	8.115	3.534	7.703	3.122	0.412	17.1
	ST	1.0	metastable	5.082	5.083	4.670	4.671	0.412	18.9

S, T, and ST represent sulfanilamide, sulfathiazole and the molecular compound, respectively.

Hence, in both cases, the constant K is also determined.

The stability constants thus calculated are given in Table II. The fact that there is a satisfactory agreement between the values obtained at the stable equilibria and those at the metastable ones will support the validity of the assumptions made for the determination of the constant.

The plot of the logarithm of the solubility against the reciprocal of the absolute temperature yields a straight line as shown in Fig. 9. Since from the calculated values of K and [T], $[ST]=K[T]^2 \ll [T]$, the fact means that the logarithm of the concentration of free sulfanilamide or sulfathiazole in the metastably saturated solution is proportionale to the reciprocal of the absolute temperature.

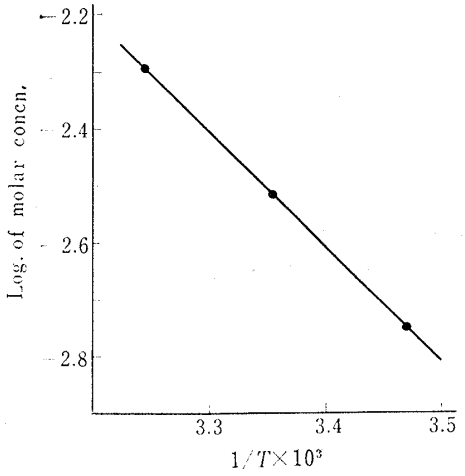


Fig. 9. The Relation between Temperature and the Total Concentration of Sulfanilamide (or Sulfathiazole) at the Metastable Solution Equilibrium obtained with the Molecular Compound

Conclusion

It is widely known that drug substances often interact with each other to form molecular compounds; nevertheless, their dissolution behaviors by which the absorption of the component drugs is influenced, have hardly investigated. For this reason, the authors tried here to obtain experimental evidences for the difference in the process of dissolution between the molecular compound of sulfanilamide and sulfathiazole and the corresponding mixture of them. Although this is only one example, it will be generally admitted from the results that a molecular compound differs more or less from the mechanical mixture of the same composition in the behavior during dissolution.

In the case of the molecular compound, whether it is decomposed by the solvent or not, the concentrations of the components will rise in the same ratio as their molar composition, at least in the initial period of dissolution. Especially, when the decomposition by the solvent does not occur or is retarded appreciably as with the molecular compound of the above sulfonamides, the compound will show the solubility of its own, though in the latter case, the solubility is the metastable one and the stable solution equilibrium will ultimately be attained by the succeeding decomposition. It will therefore, be natural that the components in the molecular compound are ready to be taken up simultaneously, when administered orally, because the ease of absorption of drugs depends largely upon their dissolution patterns into the intestinal fluid. Thus, one may sometimes expect synergistic action of two drugs more effectively by using the molecular compound than by the mixture.

When the mechanical mixture in which the composition of both components is the same as that of the molecular compound is dissolved into water, dissolution curves for the two components differ considerably from those for the molecular compound, and at the same time, they are largely influenced by the experimental conditions, such as the degree of agitation and the particle sizes of the components.

The most characteristic phenomenon encountered in the dissolution of the mixture of sulfanilamide and sulfathiazole is the fact that particles of the latter are coated with the molecular compound formed in solution and as the results, apparent size enlargement is observed during dissolution. The coating effect first observed by the authors will not be peculiar to the mixture of these sulfonamides, but is supposed to be a general tendency for those mixtures of which the constituents can form less soluble molecular compounds in solution. Making use of this effect, it will perhaps be possible to develope a new type of preparation for modifying the duration of action of drugs.

Solid preparations such as powders, granules and tablets usually contain several drug substances. When there is no interaction between the ingredients, the concentration of each one of them at saturation in water, will be nearly equal to its intrinsic solubility. This is especially true, if all the ingredients have low solubilities, since in such a case, the saturated solution can be treated as an ideal solution. "Sulfa-combination principle"[9] concerning the additivity in concentrations of sulfonamide mixtures will therefore, be applicable only when no molecular compound is formed between the sulfonamides involved.

If there is any interaction, however, the additivity rule will no more be applied and the dissolution of the drugs to be reacted will proceed in a more complicated manner, as is illustrated with the mixture of sulfanilamide and sulfathiazole. Also, the attainment of the final solution equilibrium will become difficult, and in some cases, it will practically be impossible. Although the rate of molecular compound formation is

9) B. Sjögren, B. Örtenblad: Acta Chem. Scand., **1**, 605 (1947).

thought to be very slow in solid state, nevertheless, appreciable amount of the compound may be formed at the interface of reactive ingredients during manufacturing processes or storage of the preparations, and thus variations in the drug action may be caused. For this reason, it will be very important to make a test on whether any ingredient to be incorporated does form molecular compounds with other ones, before a solid pharmaceutical preparation is produced.

The authors express their hearty gratitude to Prof. Dr. H. Nogami of University of Tokyo for his great encouragement throughout this work. They are also indebted to Mr. K. Narita for the elemental analysis.

Summary

In order to establish the therapeutical significance of the molecular compound composed of organic medicinals, dissolution behaviors of both the one-to-one molecular compound of sulfanilamide and sulfathiazole and the corresponding mechanical mixture were investigated at 15, 25, and 35°.

Soon after the dissolution of the molecular compound, both components showed a fixed and the same concentrations for several time which is attributable to the metastable solubility of the molecular compound itself. By further stirring of the solution and the solid residue, the compound was gradually decomposed by water, and the stable equilibrium of the system was finally attained, at which the total concentration of sulfanilamide was larger than that of sulfathiazole. Stability constants of the compound at these temperatures were determined. As was expected, the constants at the stable equilibria agreed well with the ones at the metastable equilibria.

In the case of the mechanical mixture, the dissolution curves were completely different from those of the former, and neither of the components showed a constant concentration during the course of experiment. The difficulty of attainment to the solution equilibrium would be mainly attributed to the fact that sulfathiazole particles in the residue were coated with the molecular compound deposited from the solution.

Thus, it may be concluded that the absorption pattern of the molecular compound will be different from that of the mechanical mixture, since dissolution into the intestinal fluid prior to absorption will take place in an analogous manner to that observed in the above.

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