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The solubility of benzodiazepines in sodium salicylate solution and a proposed mechanism for hydrotropic solubilization **

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Summary

The influence of simple structural modification on the solubility of a series of poorly soluble benzodiazepines in sodium salicylate solution was investigated. Results of solubility and spectral studies indicate that an electrostatic force of the donor–acceptor type plays an important role in the solubilization of these compounds by hydrotropy.

In an attempt to explain the characteristic positive deviation in hydrotropic solubilization diagrams, the solution properties of sodium salicylate were studied. Conductivity experiments indicate molecular aggregation of sodium salicylate at a concentration of 0.67 M at 25°C. The aggregation concentration increases as a function of temperature. Results obtained from density, viscosity and diffusion studies are consistent with those of conductivity measurements.

The remarkable increase in the solubilizing effect of sodium salicylate is probably associated with aggregate formation. Inclusion of the benzodiazepine molecules in the sodium salicylate aggregates is thought to be the mechanism responsible for the solubilization of these drugs. A donor–acceptor interaction between sodium salicylate and benzodiazepine molecules is assumed to stabilize such an inclusion and determine the degree of solubility of the benzodiazepines in sodium salicylate solution. Nevertheless, further studies are necessary to substantiate these results.

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Introduction

Hydrotropy is the term originally put forward by Neuberg (1916) to describe the increase in the solubility of a solute by the addition of fairly high concentrations of alkali metal salts of organic acids. There is a controversy concerning the mechanism by which hydrotropes act. For instance, Winsor (1950) considered hydrotropy a solubilization phenomenon while Ueda (1966a, b and c) proposed the formation of molecular complexes at low hydrotrope concentrations and a salting-in effect at high concentrations. Further, various forms of molecular interaction between insoluble compounds and hydrotrope molecules have been reported (Higuchi and Drubulis, 1961; Poochikian and Gradock, 1979). The proposed mechanisms fall short of giving an overall explanation of hydrotropy and a well-defined mechanism is of intrinsic interest.

In the present study, the influence of simple structural modification of the solute on hydrotropic solubilization and the solution properties of hydrotropic salts were investigated in an attempt to gain more insight into the mechanism of hydrotropy.

Materials and methods

The solubility of 5 benzodiazepine derivatives (Table 1) namely diazepam [1], medazepam [1], nitrazepam [1], clonazepam [1] and oxazepam [2] was determined in sodium salicylate [3] solutions of different concentrations (0–1.87 M). An excess amount of each drug was added to 10 ml of sodium salicylate solution in a screw-capped glass bottle. The bottles were covered with aluminium foil and shaken mechanically (100 spm) in a constant temperature water bath maintained at 25 ± 1°C for 24 h. The contents of the bottles were then equilibrated at the same temperature for 36 h. Aliquots of the filtered solutions were diluted 1 in 10 with ethanol and then suitably diluted with 0.1 N hydrochloric acid. The amount of drug in solution was determined spectrophotometrically [4] at 365 nm for medazepam and oxazepam, 360 nm for nitrazepam and clonazepam and 460 nm for medazepam.

Spectral study

The visible spectra of medazepam in freshly distilled water, sodium salicylate solutions of different concentrations (0.31–1.56 M) and in 0.067 M phosphate buffer pH [5] ranging from 5.0 to 8.0 were recorded [6].

[1] Courtesy of Hoffmann-La Roche Pharma Division, Basle, Switzerland.
[2] Courtesy of Wyeth Laboratories, Philadelphia, PA 19101, U.S.A.
[3] Ridel-De Haën AG, Seelze-Hannover, G.F.R.
[4] Unicam SP 1800 Ultraviolet Spectrophotometer.
[5] Corning pH meter 113.
[6] Carl Zeiss DMR 21 Spectrophotometer.

Conductivity measurement

The resistance of sodium salicylate solutions (0.1–1.2 M) and sodium benzoate [7] solutions (0.1–1.2 M) was determined at 25 ± 0.1°C using a conductivity bridge [8]. The specific conductivity was calculated using the constant of the conductivity cell employed. The specific conductivity of sodium salicylate solutions was also determined at 20, 30, 40 and 50°C.

Density and viscosity measurements

The density of sodium salicylate solutions (0.30–1.86 M) was determined at room temperature (23°C) using a 25 ml pycnometer.

The relative viscosity of these solutions was also measured at 23°C using an Ostwald viscometer.

Diffusion study

A plexiglass diffusion cell supplied with a cellulose dialysis membrane [9] was used. Fifty milliliters of sodium salicylate solution (0.12–1.25 M) was transferred quantitatively to one compartment of the cell and an equal volume of water was placed in the other compartment. The contents of both compartments were stirred at 40 spm by means of teflon-coated magnetic stirrers. Samples were taken from the solvent side at intervals and assayed spectrophotometrically at 295 nm.

Results and discussion

The solubility diagrams for diazepam, nitrazepam, medazepam, oxazepam and clonazepam in sodium salicylate solution are shown in Fig. 1. Positive deviation of the curves is characteristic of hydrotropic solubilization (Saleh and Daabis, 1974). Consideration of the structural differences of these compounds (Table 1) in relation to the results in Fig. 1 indicates an obvious effect of the various substituents on the solubility of benzodiazepines in sodium salicylate solution. The influence of substituents affecting the electron density on the 7-membered ring of the benzodiazepine molecule seems to be the most important. A decrease in the electron density due to the carbonyl oxygen at C_2 may account for the greater solubility of diazepam compared to medazepam. However, the relatively low solubility of oxazepam may be attributed to the involvement of the carbonyl oxygen at C_2 and the additional hydroxyl group at C_3 in intermolecular hydrogen bonding (Gilli et al., 1978). The influence of a chloro-substituent in ring B can be noted when comparing the solubility of clonazepam and nitrazepam, the former being less soluble. This may be due to increased conjugation as a result of the hindrance of free rotation of the B-benzene ring in clonazepam (Karle and Karle, 1967) and the greater lipophilicity of this drug molecule. Results of this study tend to indicate that an electrostatic

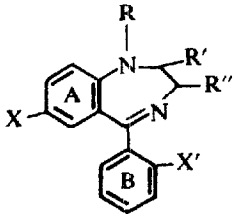
[7] BDH Chemicals, Poole, U.K.

[8] Conductometer E 518 'Metrohm Herisau'.

[9] Sigma Chemicals, St. Louis, MO 63178, U.S.A.

TABLE I

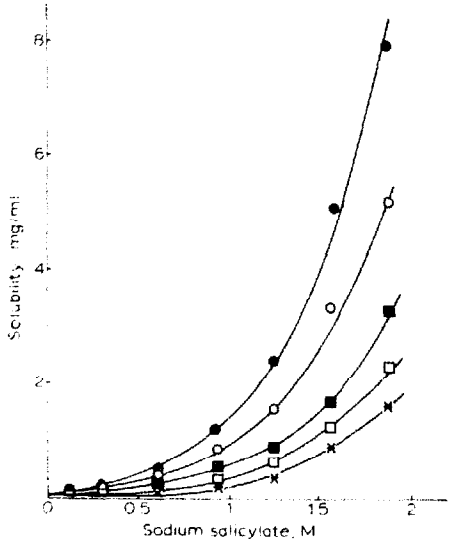
STRUCTURE OF BENZODIAZEPINE DERIVATIVES UNDER STUDY AND THEIR SOLUBILITIES IN 1.87 M SODIUM SALICYLATE SOLUTION



Name	X	X'	R	R'	R''	$M \times 10^3$
Diazepam	Cl	H	CH_3	O	H	27.6
Medazepam	Cl	H	CH_3	H	H	11.9
Oxazepam	Cl	H	H	O	OH	7.9
Nitrazepam	NO_2	H	H	O	H	18.4
Clonazepam	NO_2	Cl	H	O	H	5.1

force of the donor–acceptor type between sodium salicylate and the benzodiazepine molecules plays an important role in the solubility of these compounds in sodium salicylate solution.

To further investigate the possible mechanisms involved in the benzodiazepine–sodium salicylate interaction, a spectral study was carried out. Results in Table 2 show the wavelength of maximum absorption (λ_{max}) of medaze-



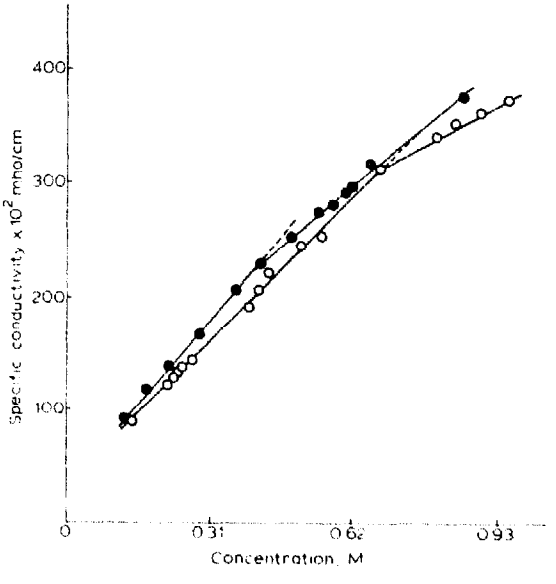


Fig. 1. Effect of sodium salicylate on the solubility of benzodiazepines at 25°C. Diazepam (●), nitrazepam (○), medazepam (■), oxazepam (□) and clonazepam (✱).

Fig. 2. Specific conductivity of sodium salicylate (○) and sodium benzoate (●) solutions at 25°C.

TABLE 2

λ_{max} OF MEDAZEPAM IN SODIUM SALICYLATE SOLUTION AND PHOSPHATE BUFFER

Sodium salicylate solution			Phosphate buffer	
M	pH	λ_{max}	pH	λ_{max}
0.000	7.0	448	8.0	443
0.313	6.5	456	7.0	448
0.625	6.7	460	6.0	458
0.938	6.7	462	5.0	463
1.250	6.8	462		
1.565	6.9	462		

pam in solutions of different sodium salicylate concentrations (pH ranging from 6.5 to 6.9) and in phosphate buffer of pH values ranging from 5.0 to 8.0. A bathochromic shift is noticed when the concentration of sodium salicylate is increased or the pH of the buffer solution is lowered. The almost constant pH of sodium salicylate solutions under study eliminates any pH effect. Interaction of medazepam with the buffer species can also be excluded as the λ_{max} of the drug in water (pH 7.0) and phosphate buffer (pH 7.0) are identical. Consequently, it can be assumed that the conjugation of medazepam molecules in sodium salicylate solution (0.93 M and above) and in acid medium (pH 5.0) is more or less the same. This supports the

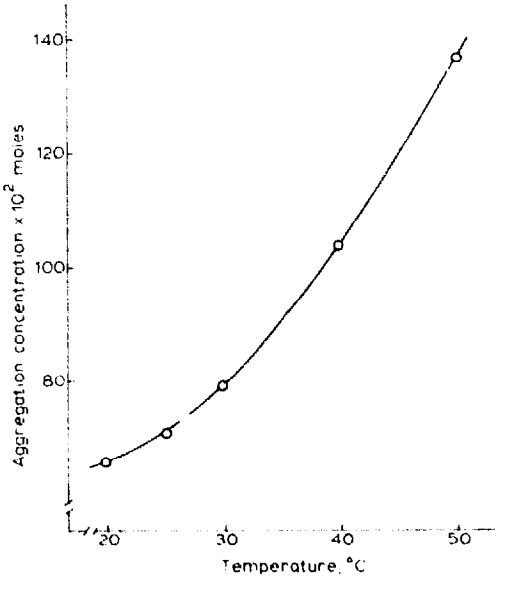


Fig. 3. Effect of temperature on the aggregation concentration of sodium salicylate.

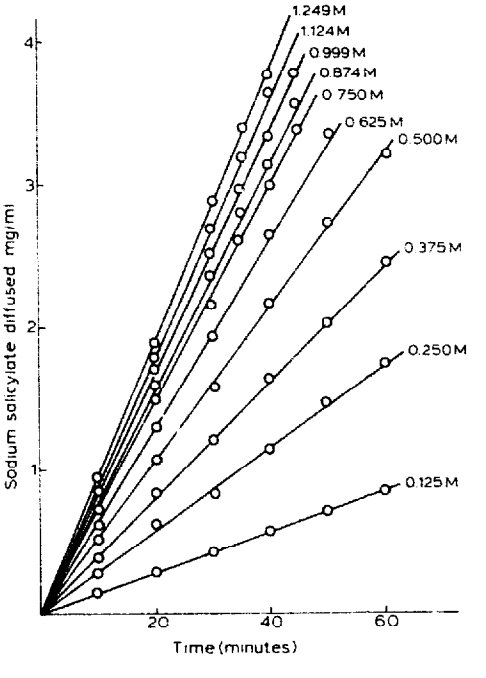


Fig. 4. Diffusion of sodium salicylate.

donor–acceptor mechanism of benzodiazepine–sodium salicylate interaction. However, such a mechanism cannot be solely responsible for the sharp increase in solubility particularly after a certain sodium salicylate concentration range (0.6–0.8 M) is exceeded. The obvious increase in solubilizing effect of sodium salicylate in the present study as well as in previous reports (Saleh and Daabis, 1974; Poochikian and Gradock, 1979) initiated the investigation of the solution properties of sodium salicylate over a wide concentration range.

The specific conductivity of sodium salicylate solutions versus concentration shows a definite break at 0.67 M (Fig. 2). Such a discontinuity in conductivity plots is strongly indicative of molecular aggregation at a well-defined concentration (Mukerjee, 1967). A similar behaviour has also been observed in the conductivity plot of a structurally related hydrotrope, sodium benzoate, at 0.42 M. This indicates that a simple structural difference in hydrotrope molecules affects the aggregation of these molecules.

The effect of temperature on the aggregation concentration of sodium salicylate (Fig. 3) is similar to the temperature effect on the critical micelle concentration of ionic surfactants. The increased thermal motion of the molecules may be responsible for the increase of the aggregation concentration in both cases at higher temperatures.

Results of the diffusion study (Fig. 4) exhibit deviation from linearity when the rate of transfer of sodium salicylate is plotted as a function of concentration (Fig. 5). Further, plots of density and relative viscosity of sodium salicylate solution versus concentration (Fig. 5) show discontinuities at a similar concentration (about 0.7 M).

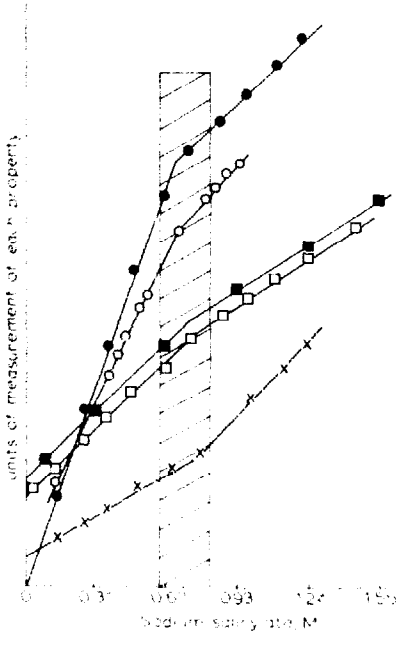


Fig. 5. Properties of sodium salicylate showing changes which occur sharply at the aggregation concentration. Diffusion rate (●), conductivity (○), log diazepam solubilized (■), density (□) and relative viscosity (○).

Distinct breaks in the above-mentioned plots indicate clearly the onset of aggregation. The negative deviation of the diffusion rate of sodium salicylate as a function of concentration can be explained by the expected slower rate of transfer of the aggregates. Moreover, an increase in the partial molal volume of sodium salicylate is indicated by the negative deviation of the density plot. The increase in partial molal volume upon aggregation may be attributed to the expansion of the hydrocarbon portion of the molecule, on its partial removal from the high compressive force of water (Florence, 1966). On the other hand, positive deviation of the relative viscosity plot indicates that aggregate formation is associated with an increase in viscosity of sodium salicylate solutions.

From this study, it can be concluded that sodium salicylate molecules probably associate into aggregates at a critical concentration. Aggregate formation is indicated by the deviation in the solution properties of sodium salicylate when the concentration surpasses 0.7 M approximately at 25°C which can be considered as the aggregation concentration. These results are illustrated in Fig. 5. The figure shows the narrow concentration range over which a distinct change in the solution properties of sodium salicylate occurs. The remarkable increase in the solubilizing effect of sodium salicylate at concentrations higher than 0.7 M can, hence, be associated with the formation of aggregates (Fig. 5). Sodium salicylate being a planar molecule, can be included in class II of the classification proposed by Mukerjee (1974). The planar structure of these molecules allows a stacking type association. In this stacking, each monomer can lie flat on top of a stack and there are no geometric restrictions to open-ended self-association. Interaction of sodium salicylate aggregates with the solute molecule, a portion of which should be planar, is thought to be the mechanism of solubilization by hydrotropy. Although the nature of such an interaction is not yet well defined, it is considerably influenced by an interaction between the solute and the hydrotrope.

In the present study, benzodiazepine molecules having a planar ring system (Sjöholm and Sjödin, 1972) are solubilized in sodium salicylate solution probably due to the inclusion of these molecules in the sodium salicylate stack. The different degree of solubility of these compounds is assumed to be the result of the donor–acceptor interaction which contributes to the overall stability of the benzodiazepine–sodium salicylate system.

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