

Thermodynamic Dissociation Studies of dl-Cysteine at Different Temperatures and in Water-Organic Solvent Systems

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Summary: Thermodynamic dissociation constants of a dl-Cysteine were determined at varying temperatures ranging from 25°C to 50°C at 5°C interval and in 10, 20, 30 and 40 percent v/v dioxane/water, methanol/water and ethanol/water systems. The influence of the composition of the medium on the dissociation equilibria of dl-cysteine was also investigated. A computer program was written in GW-BASIC which is a modified version of earlier written program in FORTRAN IV, developed to be used with IBM PC XT/AT or compatible computers.

Introduction

Cysteine is not only a particularly prominent amino acid in the protein of hair, hoofs and the keratin of skin, but it is also a constituent of many other proteins, in which it establishes S-S bonds which are of great importance in maintaining the secondary structure of the protein. The function of glutathione are attributable to its cysteine contents. In addition to the function of cysteine/cystine in glutathione synthesis, this amino acid is important in conjugation with aromatic halogens to form mercapturic acids. Taurine, the cholic acid conjugate in bile which forms the bile acid, taurocholic acid is also derived from Cysteine [1].

A change in temperature causes a shift in equilibrium point which is of both practical and theoretical interest. While most tabulated ionization constants are reported at or near 25°C, one may now and again require the value of a constant near the freezing point of water, at body temperature or even above 100°C.

Acid-base equilibria are distributed by addition or removal of the species foreign to the equilibria. The nature of the solvent also effects the pK_a .

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Mixture of water with organic solvents, particularly alcohols and dioxane are popular media for studying acid behaviour. In the present case we used dioxane, methanol and ethanol solvents. Organic compounds that are not soluble in water are often brought into solution by addition of alcohol. Hydrogen ion probes, such as the hydrogen and the glass electrodes, continue to function in such mixtures, whereas it is frequently difficult to obtain a reproducible response from them in pure non-aqueous solvents.

The present study is a continuation of our previous work [2] which describes the method for the determination of dissociation constants.

The survey of literature shows that the ionization constant data in aqueous and non-aqueous solvents at different temperature are not frequently available [3-6]. It also indicates the importance of pK_a values both in chemical and pharmaceutical industries [3,4,7,8].

There are different methods which can be employed to find out the dissociation constant of acids and bases but all have certain limitations. For this purpose potentiometry is one of the faster and accurate method [3,4,9-12], while spectrophotometric methods are very accurate but are time consuming [10-12].

The purpose of this paper is to extend the study of effect of temperature and different composition of dioxane-water, methanol-water and ethanol-water solvent system on pK_a values of cysteine. Many chemist require to know the dissociation constant of organic acids and bases in non aqueous media. Therefore in recent years much attention has been paid to a quantitative explanation of the medium effect on the pK_a values [13-20].

The experimental data have been refined here by least squares method using the algorithm of Speakman [21]. The experimental data have been used in a computer program previously written in FORTRAN IV [3,22] to refine overlapping pK_a values and now modified in GW-BASIC language to work on IBM PC,PC-XT/AT or compatible computer [23].

Results and Discussion

Table-1 lists the values of pK_a of dl-cysteine at different temperatures. It is observed from Table-1 that pK_a values of dl-cysteine are affected with change in the temperature but not in a systematic manner. Fig. 1 and Fig. 2 further show that as temperature increases from 25 to 35°C, pK_1 and pK_2 values decrease upto 35°C but after 35°C they increase and increase is continued upto 50°C. The curves of pK_1 and pK_2 values vs temperature change is usually parabolic. Both pK_1 and pK_2 show minimum around 35°C.

Table-1: pK_a values of dl-cysteine at different temperature

Temp. °C	pK_a (evaluated)		pK_a (Reported)*	
	pK_1	pK_2	pK_1	pK_2
25	8.234±0.009	10.468±0.001		
30	30.193±0.005	10.411±0.01	8.14	10.34
35	8.141±0.007	10.379±0.009		
40	8.168±0.01	10.447±0.02		
45	8.238±0.008	10.520±0.007		
50	8.288±0.008	10.568±0.009		

*Hand book of Chemistry and Physics 67th edition 1968-87, edited by Robert C, Weast and Melvin J. Astle, Millian H. Bayer, CRC Press, U.S.A., pp (D-161).

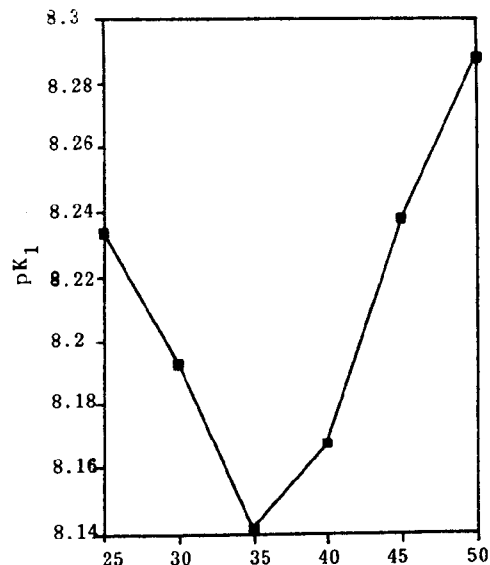


Fig.1. Graph showing the effect of temperature on pK_1 values of dl-cystein.

The influence of the composition of the medium on the dissociation equilibria of dl-

cysteine was also investigated. It was assumed that these effects could be described by simple equations for the dependence of pK_n on particular variable related to the solvent composition, pK_n vs percent composition has been plotted in Fig. 3.

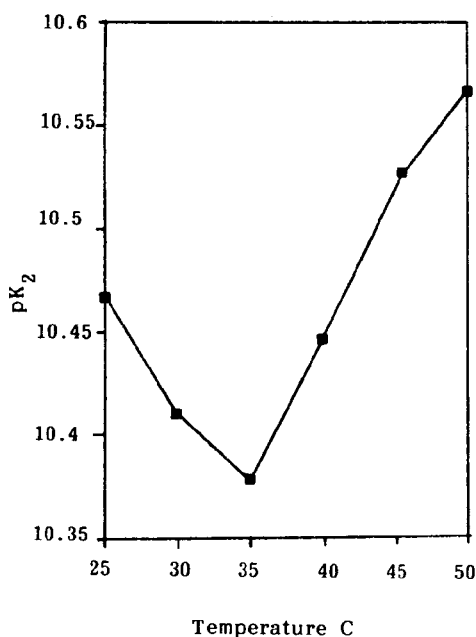


Fig. 2: Graph showing the effect of temperature on pK_2 values of dl-cysteine.

Fig. 3 shows that as composition of organic solvent increases, pK_1 values also increases for the three systems under investigation. At 20 percent composition pK_1 values in dioxane and ethanol lies close to each other. Fig. 4 shows the effect of percent composition of water-dioxane, water-methanol and water-ethanol system on pK_2 values. Effect on pK_2 values are some what different from pK_1 . pK_2 values for ethanol and methanol lie close to each other and in 35 percent composition they overlap on each other, while in 35 percent dioxane pK_2 values are higher. This difference in pK values may be due to different in dielectric constant values which are 2.21, 32.6 and 24.3 for dioxane, methanol and ethanol respectively. The dielectric constant (ϵ) of a solvent expresses its effect on the electrostatic force between ions. The work of separation of ions will vary inversely with the dielectric constant of the solvent. We therefore expect that the reaction occurs to a greater extent in water than in alcohol. When the dielectric constant is very low, the ions produced by proton transfer may not separate at all but persist as an ion-pair.

The two components of the medium can also differ in their solvating power. It is widely assumed on the basis of electrostatic theory [24] that ions are preferentially solvated by water rather than by organic solvents. The chemical potential of an ion varies inversely with the dielectric constant of the

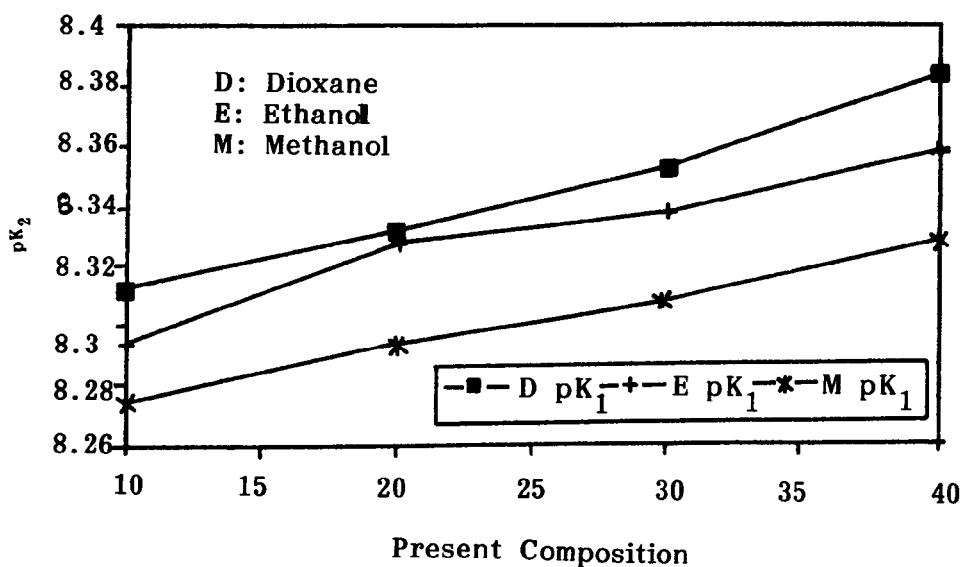


Fig.3: Graph showing the effect of percent composition of solvent on pK_1 values of dl-cystein.

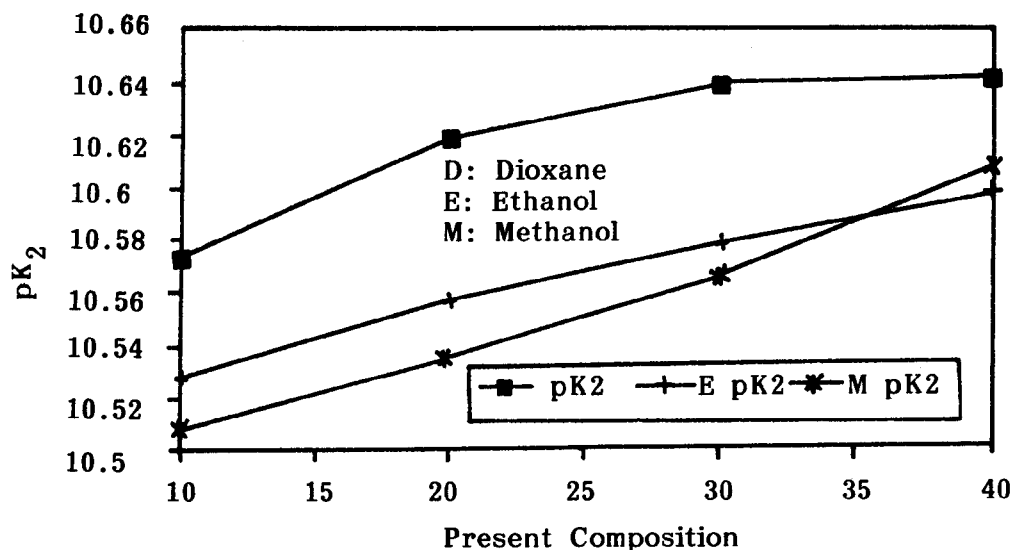


Fig. 4: Graph showing the effect of percent composition of solvent on pK₂ values of dl-cystein.

medium. Most of the loss in energy in transferring an ion from empty space to solution occurs in the immediate neighborhood of the ion. The solvent component of higher dielectric constant (water) will concentrate around the ion to make its chemical potential as low as possible [25].

Experimental

The dl-cysteine solution was of analytical grade (Fluka) and was used without further purification. Double distilled water was used throughout the experiments. Fresh distilled dioxane, methanol and ethanol were used. Stock solution of sodium hydroxide of 0.1 M and dl-cysteine of 0.002 M were prepared in CO₂ free distilled water. The solution of compound was kept in a cell coupled with calibrated Ingold combined glass and Ag/AgCl reference electrode with potassium hydrogen phthalate buffer (pH = 4.001 25°C) and borax buffer (pH = 9.00 at 25°C). The temperature of solution was maintained at constant temperature by circulating water from JULABO HC thermostate accurate to $\pm 0.01^\circ\text{C}$, through double glass jacket cell. The pH of the cell was measured with PHILIPS PW 9420 digital pH meter. Mettler DV 210 micro burette readable to ± 0.01 ml was used as dispenser of titrant. Efficient mixing of sample was obtained by magnetic stirrer. Before and during measurement, sample was kept

at inert atmosphere by bubbling purified nitrogen gas. The experimental data was used for calculation of pK_a values were of the mean of three experimental sets. Theory of the program is reported elsewhere [23].

References

1. H.A. Harper, "Review of Physiological Chemistry", Lange Medical Publisher (1971)
2. A.T. Mufti, Saeeduddin, K.M. Bhatti and A.W. K. Khanzada *Physical Chemistry Pakistan* (in press).
3. B. Gutbenzalic and E. Grun Wald, *J. Am. Chem. Soc.*, **75**, 559 (1953).
4. A. Albert and E.P. Serjeant "The Determination of ionization constants, A Laboratory manual" 3rd Edn, Chapman and Hall London, p. 1, 65, 11 (1984).
5. E.P. Serjeant, "Potentiometry and Potentiometric Titration", John Wiley, New York Chap. p. 305 (1984).
6. G. Papanastasiou, I. Ziogas and I. Moutzias, *Anal. Chim. Acta*, **1**, 86, 213 (1986).
7. N.W. Tietz and Olc Siggaard-Anderson "Acid base and Electrolytic balance in Fundamentals of Clinical Chemistry" N.W. Tietz, Saunders Co., U.S.A., (1976).
8. M. Meloun, J. Hovel and E. Hogfeldt, "Computation of Solution Equilibria", Ellis

- Horwood, Chichester, New York (1988).
9. R.W. Ramette, *J. Chem. Educ.*, **44**, 647 (1967).
 10. F.R. Cookson, *Chem. Revs.*, **74**, 5 (1974).
 11. W.A.E. Mc Bryde, *Talanta*, **21**, 187 (1974).
 12. A.G. Asuero, M.J. Nawas and J.L. Jimenez-Trillo, *Talanta*, **33**, 2 (1986).
 13. V. Nummert and V.A. Palm, *Org. React. (Tartu)*, **17**, 331 (1980).
 14. I.A. Koppel and M.M. Karelson, *Org. React. (Tartu)*, **11**, 1975 (1985).
 15. V. Nummert, *Org. React. (Tartu)*, **18**, 274 (1981).
 16. G. Launay, B. Wojtkowiak and T.M. Krygowski, *Can. J. Chem.*, **57**, 3065 (1979).
 17. M. Ludwig, O. Pytela and M. Vecera, *Collect. Gzech. Chem. Common.*, **49**, 2593 (1984).
 18. M. Ludwig, O. Pytela and M. Vecera, *Collect. Gzech. Chem. Common.*, **51**, 1948 (1986).
 19. M. Ludwig, O. Pytela, H. Javurkow and M. Vecera, *Collect. Gzech. Chem. Common.*, **52**, 2900 (1987).
 20. M. Ludwig, V. Baron, K. Kalfus and M. Vecera, *Collect. Gzech. Chem. Common.*, **51**, 2135 (1986).
 21. J. C. Speakman, *J. Chem.Soc.*, 855 (1940).
 22. T.R. Dickson "The Computer and Chemistry an Introduction to Programming and Numerical methods," Freeman San Francisco, p. 141 (1969).
 23. Saeeduddin A.T. Mufti, K.M. Bhatti and A.W.K. Khanzada *Physical Chemistry* (submitted for review)
 24. H.S. Frank, *J. Phy. Chem.*, **23**, 2023 (1955).
 25. E.A. Guggenheim and J.E. Mayer. *The International Encyclopedia of Physical Chemistry and Chemical Physics*, Pergamon Press, England (1965).