Thermodynamic study of partitioning of sulfanilamide and sulfamethoxazole at two pH values

Fleming Martínez[*1]
Jaime Rojas[*]
Alfredo Gómez[**]

Summary

The thermodynamic functions free energy, enthalpy and entropy of transfer were determined for sulfanilamide and sulfamethoxazole over the temperature range of 20.0 – 40.0 ± 0.1 °C, from partitioning data in octanol/water at the isoelectric point and at pH 7.4 at 0.15 mole/L ionic strength. The actual calculated and experimental partition coefficients and the respective thermodynamic functions, were found to be very different for sulfamethoxazole, therefore caution must be taken in the use of these equations.
Key words: Thermodynamics of transfer - Sulfonamides - Partition coefficient.

Resumen

Estudio termodinámico del reparto de sulfanilamida y sulfametoxazol a dos valores de pH

Se determinó el coeficiente de reparto octanol/agua (*P*) de la sulfanilamida y el sulfametoxazol a cinco temperaturas entre 20.0 y 40.0 ± 0.1°C a pH isoeléctrico y pH 7.4 a fuerza iónica de 0.15 mol/L. A partir de los valores de reparto se calcularon la energía libre, la entalpía y la entropía de transferencia. En el caso del sulfametoxazol se encontró que el valor *P* verdadero calculado a partir de *P* aparente (pH 7.4) se desvía notoriamente del valor hallado experimentalmente y por lo tanto, también se afectan las correspondientes funciones termodinámicas, lo cual hace necesario utilizar con precaución las ecuaciones de estimación de este parámetro.
Palabras clave: Termodinámica de transferencia - Sulfonamidas - Coeficiente de reparto.

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* Universidad Nacional de Colombia, Departamento de Farmacia, A.A. 14490, Bogotá D. C., Colombia.

*1 E-mail: fmartine@ciencias.unal.edu.co.

** Universidad Nacional de Colombia, Departamento de Química, A.A. 14490, Bogotá D. C., Colombia.

Introduction

Sulfonamides are extensively used for the treatment of certain infections caused by gram-positive and gram-negative microorganisms, some fungi, and certain protozoa. Although the advent of antibiotics has diminished the usefulness of sulfonamides, they at present occupy a relatively small but important place in the therapeutic resources of the physician. Various physical and chemical parameters of these compounds have been related to chemotherapeutic activity: pK_a, protein binding, and electronic charge distribution, among others. Unfortunately, no single parameter can explain the action of sulfonamides (1, 2).

Up to now we do not have a complete information that may allows to propose suitable mechanisms for the transfer process of sulfonamides between immiscible liquid phases and between aqueous media and biologic membrane models, which would explain the different potency among them as a function of chemical structure (3). A first basic step should be the physicochemical study of the partition process between aqueous media and organic systems. This can be done by means of a more complete thermodynamic description of the system that includes the enthalpy and entropy contributions to the transfer (4).

The partition coefficient, P, is strongly dependent on pH. In acids, bases and ampholytes P and pH can be related by different equations (5). For sulfonamides the actual partition coefficient (without association or dissociation effect) can be calculated from Equation 1.

$$P = P_{app}(1 + 10^{pKa1 - pH} + 10^{pH - pKa2}) \qquad \text{(Eq 1)}$$

Where P_{app} is the apparent partition coefficient (considering the sulfonamide dissociation in aqueous media), and pK_{a1}, pK_{a2} are the ionization constants.

To evaluate the validity of Equation 1 in sulfonamides, in this work the partition coefficient of sulfanilamide and sulfamethoxazole between octanol and water, at the isoelectric point (pI), and at pH 7.4, at different temperatures was determined. From these values, the thermodynamic functions were calculated, and the effect of using the experimental or true calculated P was evaluated. These two sulfonamides are considered to be suitable compounds for this investigation, since their isoelectric points approximately differ from pH 7.4 in one and three pH units, respectively. The octanol-water system has been used as standard organic medium for partition experiments in QSAR studies (6).

Theoretical

The partition coefficient allows to calculate the standard free energy of transfer of the solute from an aqueous phase to an organic medium. The enthalpy of transfer may be calculated by means of van't Hoff equation studying the temperature dependence of the process. The entropy may be calculated from the free energy and the enthalpy (7, 8). If the mole fraction scale is used, the free energy of transfer ($\Delta G^*_{w\to o}$) may be calculated according to Eq 2:

$$\Delta G^*_{w\to o} = -RT \ln P_X \qquad \text{(Eq 2)}$$

The term P_X is the actual partition coefficient, and is defined by Eq 3:

$$P_X = X_o / X_w \qquad \text{(Eq 3)}$$

Where X_o and X_w are the solute concentrations in the organic and aqueous media respectively. The enthalpy of transfer ($\Delta H^*_{w\to o}$) is obtained by studying the partition coefficient as a function of the absolute temperature at constant pressure by means of the Eq 4:

$$[\partial(-\ln P_X)/\partial(1/T)]_P = \Delta H^*_{w\to o} / R \qquad \text{(Eq 4)}$$

Then $\Delta H^*_{w\to o}$ is calculated from the slope of the $(-\ln P_X)$ vs. (T^{-1}) weighted curve using linear regression by least square methods. The entropy

of transfer is directly evaluated from the free energy and enthalpy by means of Eq 5:

$$\Delta G^*_{w\to o} = \Delta H^*_{w\to o} - T\,\Delta S^*_{w\to o} \qquad \text{(Eq 5)}$$

Experimental

Materials

Sulfonamides: sulfanilamide (SA) Merck; sulfamethoxazole (SMX) USP Quality (9). Solvents: octanol extra pure (ROH) Merck; distilled water (W) conductivity < 2 μS, Laboratory of Industrial Pharmacy, National University of Colombia. Others: absolute ethanol A.R. Merck; potassium chloride A.R. Merck; sodium mono and dihydrogen phosphates A.R. Merck; sodium acetate, and acetic acid A.R. Merck.

Equipments

Magni Whirl Blue M. Electric Company water baths; Wrist Action, Burrel, model 75 mechanical shaker; Mettler AE 160 sensitivity 0.1 mg digital analytical balance; DMA 35 Anton Paar digital densitometer; Unicam UV/Vis UV2 – 100 v 4.00 spectrophotometer; digital micro pipettes.

Methods

Preliminary essays

The effect of solvents on the UV maximum wavelengths (λ_{max}) and on the absorbances of the studied sulfonamides was evaluated as reported previously (10).

Partitioning studies

Before performing the experiments, both solvents were mutually saturated. Solutions of well known concentration, about $5 \cdot 10^{-5}$ M of sulfonamides were prepared in aqueous buffer solutions adjusted to the isoelectric points and pH 7.4 at ionic strength of 0.15 mole/L. Then 10.0 mL of octanol were added to 10.0 mL of the aqueous sulfonamide solution in glass flasks. The mixtures were then stirred in a mechanical shaker for one hour. Samples were allowed to stand in water baths kept at the appropriate temperature ± 0.1 °C at least for 72 hours. After this time the aqueous phases were isolated and the concentration values were determined by measuring the UV absorbances and interpolating in the previously constructed calibration curves for each sulfonamide (10). The partition coefficients were calculated by mass balance. All the partitioning experiments were repeated at least three times.

Density measurements

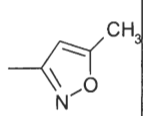
The density of each phase was determined using a digital densitometer according to a previously reported procedure to facilitate the conversion of the concentration scales (11).

Results and discussion

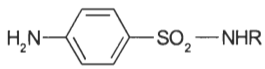
The molecular structures of each sulfonamide, their abbreviations, and some of their physicochemical properties are summarized in Table 1. The pK_{a1} and pK_{a2} were corrected to ionic strength values, $\mu = 0.15$ mole/L (similar to that of the gastrointestinal tract (12)), by means of Debye – Hückel equation (13) from Bell and Roblin data for sulfanilamide (14). For sulfamethoxazole only pK_{a2} has been published (15), then, pK_{a1} was calculated as an average of pK_a's of other sulfonamides. The above assumption is valid since Foernzler and Martin (16) showed that the electronic charge was approximately constant at the N4 position (primary amine group), by means of molecular orbital calculations.

Since the partitioning of sulfonamides in water is pH dependent (the studied compounds

Table 1. Sulfonamides, abbreviations, substituent, molecular weight (g/mole), pK_{a1}, pK_{a2}, pI, and λ_{max} (nm).

Sulfonamide	Abbr.	R [a]	MW	pK_{a1} [b]	pK_{a2} [b]	pI [c]	λ_{max} [d]
Sulfanilamide	SA	—H	172.2	2.54	10.28	6.41	258 258 262
Sulfamethoxazole	SMX		253.3	2.5	5.45	4.0	267 257 269

(a) Substituent over the basic structure of sulfanilamide:



(b) Corrected to $\mu = 0.15$ mole/L by means of the Debye-Hückel equation (13).

(c) pI = (pKa1 + pKa2) / 2.

(d) First value in water at pH = pI, second at pH = 7.4, and third in absolute ethanol.

Table 2. Actual and apparent octanol/water partition coefficients (molarity) of sulfonamides as a function of temperature (±0.1°C) (Values in parentheses: standard deviation).

Comp	t/°C	Partition coefficients		
		P (pI)	P_{app} (pH 7.4)	*P* (Calcd. by Eq 1)
SA	20	0.197 (0.005)	0.213 (0.009)	0.213
	25	0.192 (0.001)	0.200 (0.003)	0.201
	30	0.187 (0.002)	0.194 (0.003)	0.194
	35	0.177 (0.001)	0.184 (0.001)	0.185
	40	0.173 (0.002)	0.178 (0.003)	0.178
SMX	20	8.610 (0.047)	0.260 (0.004)	23.45
	25	8.222 (0.026)	0.247 (0.004)	22.23
	30	7.814 (0.154)	0.230 (0.009)	20.73
	35	7.273 (0.138)	0.191 (0.008)	17.22
	40	6.749 (0.074)	0.177 (0.006)	15.97

are amphoteric), this property was determined at pI, where pI = (pKa1 + pKa2) / 2, (analogous to the isoelectric point of amino acids). At this pH the sulfonamides exhibit their largest partition coefficient because the molecular compound without dissociation prevails (17); in addition, the partition coefficient was determined at pH 7.4 (blood pH). Each pH value

was regulated by acetate or phosphate buffers having β capacity between 0.01 and 0.02 using pKa values corrected to $\mu = 0.15$ mole/L.

The wavelengths of maximum absorption were determined in the respective buffers at constant ionic strength; it was observed that there are shifts with respect to the literature values in acid media (15); these values were also determined in absolute ethanol, in which different values were found.

Partitioning of sulfonamides

Actual and apparent partition coefficients of sulfonamides in the molarity scale at different temperatures are presented in Table 2; in addition, the calculated values for *P* (isoelectric point) by means of Equation 1 from experimental values at pH 7.4 are presented.

No differences were found for SA between *P* and P_{app},, which may be ascribed to pK_{a1} and pK_{a2} (2.54 and 10.28, respectively), and also because its pI (6.41) is separated of pH 7.4 by one unit. On the other hand the experimental and calculated partition coefficients at pI for SMX have large differences, the first values are almost three times greater than the second values. The partitioning of sulfamethazine at 25 °C and at different pH values was studied by Takacs-Novak et al (18), they found that P_{app} is almost constant in the pH range between 3.0 and 7.0. Nevertheless if *P* at the isoelectric point (about 5.0) is calculated from P_{app} at pH 8.0 using Equation 1, a different value is found, e.g. experimental *P* at pH 5.0 is 1.413 while the calculated value is 3.000. This behavior is analogous to that of sulfamethoxazole. Then, cautions must be taken when using Equation 1 and other related equations (5) to estimate the partition coefficients and the solubilities of drugs as function of pH, mainly in pharmacokinetical and biopharmaceutical studies. Nevertheless the experimental values in the present work are in agreement with those found in the literature, (as log *P*) for which

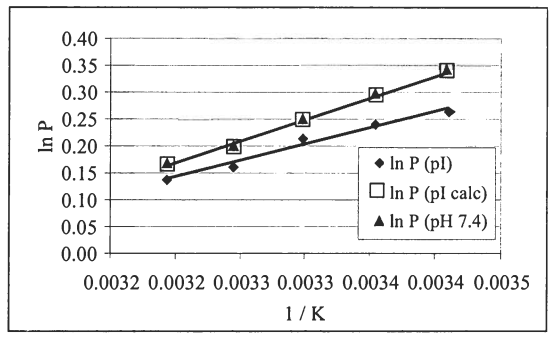


Figure 1. Partitioning – temperature dependence for SA at pI, calculated pI, and pH 7.4.

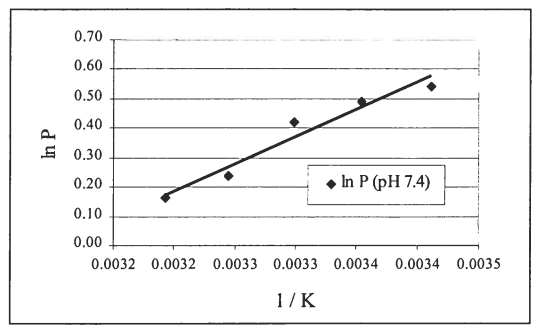


Figure 2. Partitioning – temperature dependence for SMX at pH 7.4.

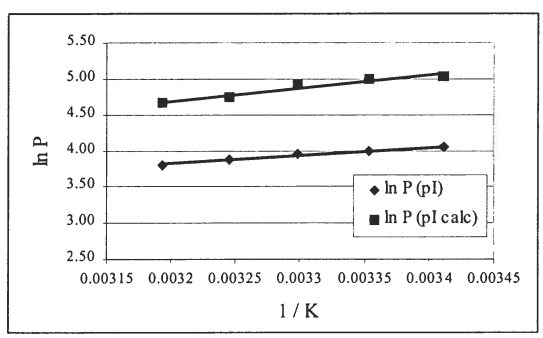


Figure 3. Partitioning – temperature dependence for SMX at pI and calculated pI.

no pH and ionic strength values are reported (15, 19).

In Table 3 the partition coefficients in the mole fraction scale are presented; this concentration scale is more adequate for thermodynamic studies (7). For scale conversion, the experimental densities of the solutions and the octanol-water liquid-liquid equilibria data presented by Dallos and Liszi were used (20).

The partitioning – temperature dependence for SA is showed in Figure 1 ($\ln P$ vs T^{-1}), and the same information for SMX is showed in Figs 2 and 3. Straight lines with r values greater than 0.95 were obtained in all cases.

Thermodynamic aspects of transfer

Table 4 summarizes the thermodynamic functions relatives to the transfer process of sulfonamides at experimental pI, pH 7.4 and calculated pI. These values were calculated from partitioning data of Table 3 by means of Gibbs and van't Hoff equations using weighted values and error propagation

Table 3. Actual and apparent octanol/water partition coefficients of sulfonamides (mole fraction) as a function of temperature (± 0.1°C). (Values in parentheses: standard deviation).

Compd	t / °C	Partition coefficients		
		P (pI)	P_{app} (pH 7.4)	*P* (Calcd. by Eq 1)
SA	20	1.301 (0.033)	1.406 (0.057)	1.408
	25	1.271 (0.007)	1.327 (0.022)	1.328
	30	1.238 (0.013)	1.288 (0.021)	1.290
	35	1.174 (0.007)	1.223 (0.005)	1.225
	40	1.148 (0.013)	1.180 (0.021)	1.181
SMX	20	56.86 (0.31)	1.720 (0.027)	155.05
	25	54.38 (0.17)	1.635 (0.025)	147.38
	30	51.70 (1.02)	1.525 (0.059)	137.45
	35	48.15 (0.91)	1.268 (0.051)	114.30
	40	44.65 (0.49)	1.176 (0.038)	105.94

Table 4. Thermodynamic functions relatives to sulfonamides partitioning process (DS and DG at 25.0 ± 0.1 °C), (Values in parentheses: standard deviation).

Compd	pH	Enthalpy / kJ/mole	Entropy / J/mole.K	Free energy / kJ/mole
SA	pI	-5.02 (0.40)	-14.85 (1.35)	-0.594 (0.014)
	7.4	-6.59 (0.57)	-19.76 (1.93)	-0.701 (0.042)
	Calcd (pI)	-6.59	-19.77	-0.704
SMX	pI	-9.21 (0.47)	2.33 (1.59)	-9.905 (0.008)
	7.4	-15.46 (1.24)	-47.76 (4.16)	-1.219 (0.038)
	Calcd (pI)	-15.46	10.33	-12.376

methods (21). The standard free energy is negative at the isoelectric point (experimental and calculated) in two sulfonamides, therefore the process of transfer from water to octanol is spontaneous. The enthalpy of transfer of SA and SMX is also negative, while the entropy of transfer of SA is negative, but is positive in SMX. It is concluded that the transfer of SA is enthalpy driven, while the transfer of SMX is driven by a combination of enthalpy and entropy, which may explain the large free energy of transfer.

With respect to pH effect on the thermodynamics of transfer it may be seen that the values for all functions of SA are almost equally. On the other hand SMX shows some differences: e.g. at pH 7.4 the entropy is highly negative (– 47.76 J/mole.K), thus, the transfer is enthalpy driven, while at pI this property is positive. The enthalpies of transfer at pI (obtained from experimental and calculated partition coefficients at pI), are very different (– 9.21 and –15.46 kJ/mole, respectively); additionally, the second value (calculated) is coincident with that experimentally obtained at pH 7.4. The above also lead to differences in the entropy of transfer (Table 4).

It is concluded that in amphoteric compounds, if it is possible, it is more convenient to obtain directly the partition coefficient values at the isoelectric point, since like in SMX, large deviations may be encountered when the *P* values are calculated from P_{app} determined at other pH values. This in turn, may lead to larger errors in thermodynamic studies by the van't Hoff method.

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References

1. A. Korolkovas, "Essentials of Medicinal Chemistry", 2nd edition, John Wiley & Sons, Inc., New York, 1988.
2. G.L. Mandell and M.A. Sande, Antimicrobial agents, In: "Goodman & Gilman. The Pharmacological Basis of Therapeutics", 8th edition, Ed. by Gilman A.G., Rall, T.W., Nies N.S., Taylor P, McGraw-Hill, Singapore, Vol 2, 1992.
3. U. Ucucu, I. Isikdag, and H. Dogu Studies of the determination of the physicochemical parameters of some commercially available sulfonamide drugs in Turkey, *Acta Pharm. Turc.*, **37**, 134 (1995).
4. E. Tomlinson, Enthalpy – entropy compensation analysis of pharmaceutical, biochemical and biological systems, *Int. J. Pharm.*, **13**, 115 (1983).
5. A. Kristl, A. Mrhar, and F. Kozjek, Intermolecular interactions of association and/or solvation type of guanine derivatives, In: "Proc. 11th Pharmaceutical Technology Conference", Manchester, Vol 1, 1992.
6. J. Sangster, "Octanol – Water Partition Coefficients: Fundamentals and Physical Chemistry", John Wiley & Sons, Inc., Chichester, England, 1997.
7. I.M. Klotz and R.M. Rosenberg, "Chemical Thermodynamics: Basic Theory and Methods", 5th edition, John Wiley & Sons, Inc., New York, 1995.
8. F. Martínez, M. Tello, and A. Gómez, Solventes orgánicos como sistemas de

reparto en modelación QSAR, *Rev. Col. Cienc. Quím. Farm.*, **29**, 16 (2000).

9. USP23 – NF18. The United States Pharmacopeia (23rd edition) and the National Formulary (18th edition). The United States Pharmacopeial Convention, Inc., Easton, USA, 1994.
10. F. Martínez, "Aspectos Termodinámicos de la Transferencia de Sulfonamidas entre Medios Acuosos y Sistemas Orgánicos", Ph.D. Thesis. Department of Chemistry National University of Colombia, 2001.
11. F. Martínez and J.H. Rojas, Un método refracto-densimétrico para el análisis de mezclas líquidas ternarias homogéneas, *Rev. Col. Cienc. Quím. Farm.*, **28**, 45 (1999).
12. C. Best and N. Taylor, "The Physiological Basis of Medical Practice", 7th edition, Williams & Williams, Baltimore, Md., 1961.
13. A.N. Martin, P. Bustamante, and A.H.C. Chun, "Physical Pharmacy, physical chemical principles in the pharmaceutical sciences", 4th edition, Lea & Febiger, Philadelphia, 1993.
14. P.H. Bell and R.O. Roblin, Studies in chemotherapy. VIII. A theory of the relation of structure to activity of sulfanilamide type compounds, *J. Am. Chem. Soc.*, **64**, 2905 (1942).
15. A.C. Moffat, J.V. Jackson, M.S. Moss, and B. Widdop, "Clarke's Isolation and Identification of Drugs, in pharmaceuticals, body fluids, and post-mortem material", 2nd edition, The Pharmaceutical Press, London, 1986.
16. E.C. Foernzler and A.N. Martin, Molecular orbital calculations on sulfonamide molecules, *J. Pharm. Sci.*, **56**, 608 (1967).
17. A.T. Florence and D. Attwood, "Physicochemical Principles of Pharmacy", 3rd edition, MacMillan Press Ltd, London, 1998.
18. K. Takacs-Novak, M. Jozan and, G. Szasz, Lipophilicity of amphoteric molecules expressed by the true partition coefficient, *Int. J. Pharm.*, **113**, 47 (1995).
19. A. Leo, C. Hansch, and D. Elkins, Partition coefficients and their uses, *Chem. Rev.*, **71**, 525 (1971).
20. A. Dallos and J. Liszi, (Liquid + liquid) equilibria of (octan-1-ol + water) at temperatures from 288.15 K to 323.15 K, *J. Chem. Thermodynamics*, **27**, 447 (1995).
21. P.R. Bevington, "Data Reduction and Error Analysis for the Physical Sciences", McGraw-Hill Book Co., New York, 1969.