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Partitioning of oxytetracycline between aqueous and organic solvents
Effect of hydrogen bonding

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Abstract

Phase equilibrium behavior of antibiotics is important in drug design and for optimization of the recovery process in manufacturing. Aqueous/organic partitioning behavior of a clinically important antibiotic, oxytetracycline is measured. The organic phase includes pure solvent *n*-hexane, chloroform, diethyl ether and ethyl acetate. In addition, measurements are also made for mixed organic phase composed of *n*-hexane + ethyl acetate. Organic-phase partitioning increases with the hydrogen bonding (H-bonding) tendency of the solvent in the order *n*-hexane < chloroform < diethyl ether < ethyl acetate. An activity coefficient model that includes H-bonding, UNIQUAC-HB (Gupta, R.B., Kumar, R., Betageri, G.V., 1997. Ind. Eng. Chem. Res., 36, 3954–3959), is used to model the partitioning behavior between aqueous and organic phases. Single solvent-organic-phase data are correlated using the model to obtain the free-energy-of-H-bonding parameters for H-bonding between antibiotic and solvent. Predictions made for mixed-solvent-organic-phase systems agrees well with the experimental data without using any adjustable parameter.

Keywords: Hydrogen bonding; Aqueous and organic solvents; Oxytetracycline

1. Introduction

Investigation of phase equilibrium behavior of antibiotics is important both in understanding the partition mechanism and in the design and optimization of downstream recovery processes (Strong, 1986; Evans, 1988; Gupta and Heidemann, 1990; Zhu et al., 1990; Gupta et al., 1997; Kumar et al., 1998). The thermodynamic behavior of antibiotics also plays a key role in drug design in pharmaceutical science (Davis et al., 1974).

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Table 1
Partitioning of oxytetracycline between chloroform and water phases at 30°C

Oxytetracycline in organic phase		Oxytetracycline in aqueous phase		Partition coefficient	
C^{org} (mg/ml)	x^{org} (micro-mol/mol)	C^{aq} (mg/ml)	x^{aq} (micro-mol/mol)	C^{org}/C^{aq}	x^{org}/x^{aq}
0.0062	1.0843	0.0238	0.9313	0.261	1.16
0.0117	2.0462	0.0483	1.8900	0.242	1.08
0.0172	3.0081	0.0730	2.8515	0.236	1.05
0.0190	3.3229	0.1010	3.9521	0.188	0.84

Table 2
Partitioning of oxytetracycline between diethyl ether and water phases at 30°C

Oxytetracycline in organic phase		Oxytetracycline in aqueous phase		Partition coefficient	
C^{org} (mg/ml)	x^{org} (micro-mol/mol)	C^{aq} (mg/ml)	x^{aq} (micro-mol/mol)	C^{org}/C^{aq}	x^{org}/x^{aq}
0.0094	2.1579	0.0206	0.806	0.443	2.68
0.0193	4.4306	0.0407	1.593	0.475	2.78
0.0292	6.7033	0.0608	2.379	0.481	2.82
0.0360	8.2643	0.0837	3.275	0.434	2.52

Concepts of thermodynamics can be applied to biological systems because these processes are essentially physical, and chemical changes involving the exchange of energy. These concepts provide the basis for determining important parameters for rational drug design.

Almost all antibiotics of commercial importance are manufactured by large scale aerobic fermentation. After the production cycle, the fermentation broth is taken for harvesting. Microorganisms are removed by filtration or centrifugation. Antibiotic is recovered by solvent extraction, ion-exchange chromatography, precipitation, crystallization or a combination of these methods. In most cases solvent extraction is used (Belter et al., 1988; Chaubal et al., 1995). However, some highly-water-soluble antibiotics (e.g. streptomycin) require ion-exchange separation (Bartels et al., 1958). In addition, a new extraction process based on reverse micelles was recently proposed by Hu and Gulari (1996) for neomycin and gentamycin.

Due to a very low concentration of antibiotic in the final fermentation broth, recovery constitute a major portion of the total manufacturing cost. Unfortunately, this area of research has not been given adequate attention and bio-separation units are designed empirically rather than on the basis of rational information. In general, bio-separation processes are governed by thermodynamics and kinetics. The most important of these two is thermodynamics, which has received the least attention. The bio-process, especially the two phase aqueous partitioning has great potential as an economical separation method for biochemical products and it offers the potential for strict product quality control (Hariri, 1989).

Although there are empirical equations (Tsuji et al., 1977, 1978, 1979; Bogardus and Palepu, 1979; Orella and Kirwan, 1987; Chen et al., 1989; Gupta and Heidemann, 1990; Taft et al., 1996) used for correlating some experimental data (Andrew and Weiss, 1959; Tomlinson and Regosz, 1985; Salvatore and Katz, 1993), comprehensive and predictive models for antibiotic solutions that can be used to scale up and optimize manufacturing processes do not exist (Zhu et al., 1990). Development of a predictive model for representing the phase equilibrium behavior of antibiotics has been difficult due to the lack of adequate experimental data (Zhu et al., 1990). Hence, there is clearly a need for a comprehensive study of

antibiotic thermodynamics. This work focuses on the phase behavior of a clinically important antibiotic, oxytetracycline.

Oxytetracycline, one of the widely used tetracyclines, is derived from *Streptomyces rimosus* (Kucers and Bennett, 1987). Oxytetracycline is a 'bacteriostatic', used extensively both for the treatment of infectious diseases and as an additive to animal feeds to facilitate growth. The tetracyclines are effective and may be life saving in rickettsial infections, including Rocky Mountain spotted fever, recrudescent epidemic typhus, muring typhus, scrub typhus, rickettsialpox and Q fever. Tetracyclines are also effective against pneumonia caused by *Mycoplasma pneumoniae*, in the treatment of Chlamydia caused by *Lymphogranuloma venereum*, psittacosis, inclusion conjuctivitis, trachoma, gonorrhea, Brucellosis, Actinomycosis and other Mycobacterial diseases, and acne (Gilman et al., 1990). A single intramuscular dose of a long-acting formulation of oxytetracycline is found to be effective in the treatment of anaplasmosis and in the prophylaxis of undifferentiated bovine respiratory disease occuring in feedlot cattle (Escudero et al., 1996). Britt et al. (1996) reported that oxytetracycline spray is effective in reducing the severity of sores (digital dermatitis) in cattle.

The objectives of this work are to study the phase behavior of oxytetracycline in single and multicomponent mixtures of industrially important solvents such as *n*-hexane, chloroform, diethyl ether and ethyl acetate, and to obtain parameters for an activity coefficient model based on molecular thermodynamics that can be used to extrapolate the experimental data conditions where data is not available. Thus, in addition to bioseparation, the understanding of molecular interactions obtained here can be extended to drug design.

2. Materials and methods

2.1. Chemicals

Oxytetracycline dihydrate, was purchased from Sigma (St. Louis, MO). Reagent grade chloroform, *n*-hexane, diethyl ether and ethyl acetate were obtained from Fisher (Fairlawn, NJ). All the chemicals were used as received.

2.2. Determination of partition coefficient

Oxytetracycline is dissolved in double distilled water (pH 6.6–7.0) to give a concentration of 0.12 mg/ml. From this, serial dilution was made to achieve concentrations of 0.03, 0.06 and 0.09 mg/ml. Then 5 ml each of the antibiotic solution and the appropriate solvent or solvent mixture are added to 20 ml scintillation vials having Teflon stoppers. The organic and aqueous phase are equilibrated for 4 h at 30 ± 0.1°C in a shaking water-bath (Dubnoff metabolic shaker). Concentration of oxytetracycline in the aqueous phase is determined by UV/VIS spectrophotometer (Beckman DU-15) at 356 nm with a pathlength of 1 cm. The concentration of oxytetracycline in the organic phase is estimated by mass balance. Measurements are made in triplicate and the variation is found to be less than 2%. In separate experiments, it is confirmed that 4 h contacting is necessary to achieve equilibrium. It is also found that oxytetracycline remained stable during the course of experiment.

2.3. Experimental results

The results of partitioning of aqueous solution of oxytetracycline in equilibrium with pure solvents chloroform (Table 1), diethyl ether (Table 2), and ethyl acetate (Table 3) are presented. The data shows that the partitioning of oxytetracycline increases with increasing polarity and hydrogen bonding (H-bonding) tendency of the solvent in the order: *n*-hexane < chloroform < diethyl ether < ethyl acetate. Tables 4–6 show the partitioning of antibiotic in mixed-organic phase, ethyl acetate + *n*-hexane with varying ethyl acetate content. From the data it is clear that as the *n*-hexane content is increased, the antibiotic partitioning decreased due to a decrease in solvent-antibiotic H-bonding.

Table 3
Partitioning of oxytetracycline between ethyl acetate and water phases at 30°C

Oxytetracycline in organic phase		Oxytetracycline in aqueous phase		Partition coefficient	
C^{org} (mg/ml)	x^{org} (micro-mol/mol)	C^{aq} (mg/ml)	x^{aq} (micro-mol/mol)	C^{org}/C^{aq}	x^{org}/x^{aq}
0.0090	1.9200	0.0210	0.8217	0.428	2.34
0.0210	4.4801	0.0390	1.5216	0.539	2.94
0.0323	6.8908	0.0577	2.2578	0.560	3.05
0.0377	8.0428	0.0820	3.2087	0.460	2.51

Table 4
Partitioning of oxytetracycline between *n*-hexane + ethyl acetate (57 mol.% ethyl acetate) and water phases at 30°C

Oxytetracycline in organic phase		Oxytetracycline in aqueous phase		Partition coefficient	
C^{org} (mg/ml)	x^{org} (micro-mol/mol)	C^{aq} (mg/ml)	x^{aq} (micro-mol/mol)	C^{org}/C^{aq}	x^{org}/x^{aq}
0.0010	0.2436	0.0290	1.1348	0.034	0.215
0.0012	0.2922	0.0589	2.3048	0.020	0.127
0.0037	0.9012	0.0863	3.3769	0.043	0.267
0.0060	1.4614	0.1140	4.4608	0.052	0.328

2.4. Theory

Liquid–liquid (organic-aqueous) phase equilibrium is determined by equating the activity (a) in the organic (org) and aqueous (aq) phases for all components in the solution

$$a_i^{org} = a_i^{aq} \quad \text{for all } i \tag{1}$$

The activity of component i is written in terms of activity coefficient (γ_i) based on mole fraction (x_i) as

$$a_i = \gamma_i x_i \tag{2}$$

Activity coefficient is a function of solution composition, temperature and pressure. Here we use UNIQUAC-HB model (Gupta et al., 1997; Kumar et al., 1998) for calculation of activity coefficient. Similar to the concept proposed by Fu et al. (1995), in UNIQUAC-HB model, the activity coefficient for a compound is expressed as the sum of combinatorial (γ_i^{comb}), residual (γ_i^{res}) and H-bonding (γ_i^{hb}) contributions. Contribution by the combinatorial term is due to differences in molecular size and shape, the residual term is due to weak energetic interactions, and the H-bonding term is due to strong attractive force between proton donor and acceptor sites on the molecules.

$$\ln \gamma_i = \ln \gamma_i^{comb} + \ln \gamma_i^{res} + \ln \gamma_i^{hb} \tag{3}$$

The combinatorial and residual terms are from original Universal Quasi-Chemical (UNIQUAC) activity coefficient model (Abrams and Prausnitz, 1975).

For the contribution due to H-bonding, a lattice-fluid H-bonding theory (Veytsman, 1990; Gupta and Prausnitz, 1991; Panayiotou and Sanchez, 1991; Gupta and Johnston, 1994; Veytsman and Gupta, 1996) is used because this theory has well defined H-bonding parameters with a clear physical meaning and which can be measured spectroscopically. In addition, lattice-fluid H-bonding theory can easily account for complex H-bonding equilibria such as in antibiotic mixtures presented here.

Oxytetracycline has low solubility in the solvents studied, for example antibiotic mole fractions in this study are of the order of 10^{-5}. An approximation that the antibiotic is in infinitely dilute state, where one antibiotic molecule does not interact with another one, can safely be made. For infinitely dilute concentrations, combinatorial acitivity coefficient is written as

Table 5
Partitioning of oxytetracycline between *n*-hexane + ethyl acetate (80 mol.% ethyl acetate) and water phases at 30°C

Oxytetracycline in organic phase		Oxytetracycline in aqueous phase		Partition coefficient	
C^{org} (mg/ml)	x^{org} (micro-mol/mol)	C^{aq} (mg/ml)	x^{aq} (micro-mol/mol)	C^{org}/C^{aq}	x^{org}/x^{aq}
0.003	0.6822	0.027	1.0565	0.111	0.646
0.008	1.8192	0.052	2.0348	0.154	0.894
0.0117	2.6605	0.0783	3.0639	0.149	0.868
0.016	3.6383	0.104	4.0695	0.154	0.894

Table 6
Partitioning of oxytetracycline between *n*-hexane + ethyl acetate (92 mol.% ethyl acetate) and water phases at 30°C

Oxytetracycline in organic phase		Oxytetracycline in aqueous phase		Partition coefficient	
C^{org} (mg/ml)	x^{org} (micro-mol/mol)	C^{aq} (mg/ml)	x^{aq} (micro-mol/mol)	C^{org}/C^{aq}	x^{org}/x^{aq}
0.0062	1.3560	0.0238	0.9313	0.261	1.456
0.0136	2.9745	0.0464	1.8151	0.293	1.638
0.0218	4.7680	0.0682	2.6687	0.320	1.787
0.0300	6.5614	0.0900	3.5217	0.333	1.863

$$\ln \gamma_1^{comb} = \ln\left(\frac{r_1}{r_2}\right) + 5q_i \ln\left(\frac{q_1}{q_2}\frac{r_2}{r_1}\right) + 5(r_1 - q_1) - (r_1 - 1) - \left(\frac{r_1}{r_2}\right)[5(r_2 - q_2) - (r_2 - 1)] \quad (4)$$

where subscript 1 refers to antibiotic and subscript 2 refers to solvent. Molecular size and shape parameters r and q are obtained from UNIFAC, a group contribution approach (Fredenslund et al., 1977). These parameters are given in Table 7.

In order to reduce the number of adjustable parameters, residual contribution to the activity coefficient is assumed to be negligible in comparision with H-bonding contribution, hence

$$\ln \gamma_1^{res} = 0 \quad (5)$$

Activity coefficient contribution due to H-bonding depends upon number of the H-bonding sites on antibiotic and solvent and on the strength of the H-bonds formed, i.e. free energy of H-bonding (G^0) or parameter A

$$A = \frac{r_2}{0.9}\exp\left(\frac{G^0}{RT}\right) \quad (6)$$

The numbers of H-bonding donor and acceptor sites on each molecule are listed in Table 7. Oxytetracycline has eight proton donor sites and 14 proton acceptor sites. For oxytetracycline molecule, all H-bond donor sites are assumed to be identical. The same is assumed for the acceptor sites. The assumption is needed in order to reduce the number of H-bonding parameters needed. The oxytetracycline–oxytetracycline H-bonding, i.e. self association, is negligible due to the low concentration.

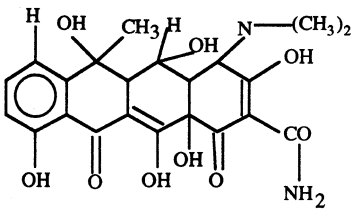
For each antibiotic + solvent mixture studied here, a separate activity coefficient expression is obtained. For a detailed derivation of Eqs. (7)–(11), see Gupta et al. (1997).

2.5. Water and oxytetracycline mixture

Water has two acceptor and two donor sites. In addition to water-oxytetracycline H-bonding, water–water H-bonding is also present in the mixture (Gupta et al., 1997). The activity coefficient contribution is given as

$$\ln \gamma_1^{hb} = 22 \ln\left(\frac{A_{water-oxytetracycline}}{0.17138 + A_{water-oxytetracycline}}\right) \quad (7)$$

Table 7
Molecular parameters and H-bonding sites

Component	Molecular structure	Volume parameter (r)	Surface-area parameter (q)	No. of H-bond donor sites	No. of H-bond acceptor sites
Oxytetracycline		15.0718	11.668	8	14
Chloroform	$CHCl_3$	2.870	2.410	1	0
Diethyl ether	$(C_2H_5)_2O$	3.395	3.016	0	2
Ethyl acetate	$CH_3COOC_2H_5$	3.479	3.116	0	4
n-Hexane	$CH_3(CH_2)_4CH_3$	4.500	3.856	0	0
Water	H_2O	0.920	1.400	2	2

2.6. Chloroform-oxytetracycline H-bonding

Chloroform has only one proton donor site and no proton acceptor site. The activity coefficient contribution is

$$\ln \gamma_1^{hb} = 14 \ln\left(\frac{A_{\text{chloroform}-\text{oxytetracycline}}}{1 + A_{\text{chloroform}-\text{oxytetracycline}}}\right) \quad (8)$$

2.7. Diethyl ether-oxytetracycline H-bonding

Diethyl ether has two proton acceptor sites. H-bonding contribution to the activity coefficient is

$$\ln \gamma_1^{hb} = 8 \ln\left(\frac{A_{diethylether-oxytetracycline}}{2 + A_{diethylether-oxytetracycline}}\right) \quad (9)$$

2.8. Ethyl acetate-oxytetracycline H-bonding

Ethyl acetate has four proton acceptor sites. H-bonding contribution to the activity coefficient is

$$\ln \gamma_1^{hb} = 8 \ln\left(\frac{A_{\text{ethylacetate}-\text{oxytetracycline}}}{4 + A_{\text{ethylacetate}-\text{oxytetracycline}}}\right) \quad (10)$$

2.9. Hexane and ethyl acetate-oxytetracycline H-bonding

Hexane does not participiate in H-bonding. Only ethyl acetate can form H-bonds with oxytetracycline. H-bonding contribution to the activity coefficient is

$$\ln \gamma_1^{hb} = 8 \ln \left(\frac{A_{\text{ethylacetae}-\text{oxytetracycline}} r_2}{4x_{\text{ethylacetate}} r_{\text{ethylacetate}} + A_{\text{ethylacetate}-\text{oxytetracycline}} r_2}\right) \quad (11)$$

In the case of above mixed solvent, r and q parameters are calculated as

$$r_2 = x_{\text{ethylacetate}} r_{\text{ethylacetate}} + x_{\text{hexane}} r_{\text{hexane}} \quad (12)$$

and

$$q_2 = x_{\text{ethylacetate}} q_{\text{ethylacetate}} + x_{\text{hexane}} q_{\text{hexane}} \quad (13)$$

3. Calculations

Values of the free energy of H-bonding (Table 8) are obtained by fitting UNIQUAC-HB model to the experimental data for binary systems (Tables 1–3). Calculated phase equilibria (Fig. 1) are in good agreement with the experimental data.

Table 8
Free energy of H-bonding

Type of H-bonding (donor–acceptor, or acceptor–donor)	Free energy of H-bonding (G^0) at 30°C (kJ/mol)
Water–water	−10.467
Oxytetracycline–water	−3.36
Chloroform–oxytetracycline	−0.199
Oxytetracycline–diethyl ether	−1.922
Oxytetracycline–ethyl acetate	−0.36

Using the parameters obtained from Fig. 1, predictions are made for liquid–liquid phase equilibria for oxytetracycline + water/oxytetracycline + ethyl acetate + *n*-hexane system. The predicted phase equilibria are compared with the ternary data shown in Fig. 2. A good agreement is obtained between the predictions and experiment, given that there are no adjustable parameters used.

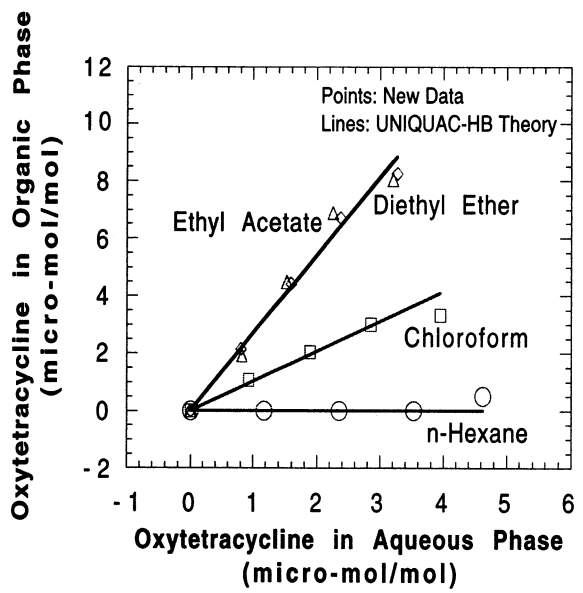


Fig. 1. Oxytetracyline partitioning between aqueous and organic phases at 30°C.

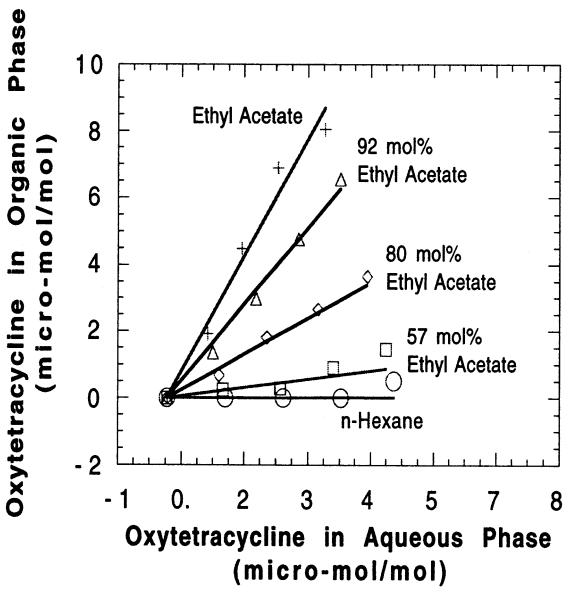


Fig. 2. Oxytetracycline partitioning between mixed ethyl acetate + *n*-hexane phase and aqueous phase at 30°C. Points are new data and lines are UNIQUAC-HB calculations. For mixed solvent cases, lines are theoretical predictions without any adjustable parameter.

4. Conclusion

From the new experimental data obtained for partitioning of oxytetracycline between organic and aqueous phases, for both pure organic solvent and mixed-organic solvent cases, it appears that H-bonding plays an important role in the phase behavior of oxytetracycline. Partitioning increases with the increasing H-bonding tendency of the organic solvent in the order: *n*-hexane < chloroform < diethyl ether < ethyl acetate. The activity coefficient model, UNIQUAC-HB, can accurately represent the observed phase behavior. Predicted phase equilibria for the mixed-organic-phase system is in good agreement with the experimental data, without any adjustable parameters. Free energy of H-bonding parameters are obtained. The model can be used in the design of a bioseparation unit and in drug design to predict the drug activity.

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