Temperature and concentration dependent partitioning of three tetracyclines between phosphate buffers and octanol

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Colaizzi & Klink (1969) analysed in detail the pH-dependence of the apparent partition coefficients (P) of several tetracyclines at 25° and one initial concentration of the drugs in the aqueous phase in an attempt to interpret qualitatively the differences in biological activities of this homologous series of compounds. The partition coefficients of the zwitterionic form have been calculated from these data, and incorporated in a review by Leo, Hansch & Elkins (1971). We have found that the values of P may vary significantly with temperature, and also with drug concentration in the region studied by Colaizzi & Klink (1969). Such a dependence may be of importance in quantitative correlations of 'lipophilicity' with drug transport properties, protein binding and biological activity.

In our work the drugs were dissolved in octanol-saturated phosphate buffer of defined ionic strength, pH 7·5 at 25°; any minor deviation in pH was corrected at this stage. The solutions were shaken vigorously with buffer-saturated octanol in a shaking bath at the required temperature for 2 h. Samples from the aqueous phase were then diluted suitably, and analysed spectrophotometrically at 274 nm (tetracycline and doxycycline hydrochlorides) and 282 nm (methacycline hydrochloride), using phosphate-saturated buffer as reference. The values of P were calculated as the ratio of the concentration in the octanol phase divided by the concentration in the aqueous phase, the former value obtained from mass balance.

Table 1. *Apparent partition coefficients of methacycline hydrochloride (I) between phosphate buffer (pH 7·5 at 25°, ionic strength 0·09* M*) and octanol and of doxycycline hydrochloride (II) between phosphate buffer (pH 7·5 at 25°, ionic strength 0·045* M*) and octanol at various initial concentrations of the drugs in the buffer, and at several temperatures.*

Initial concn of drug (mg %)	Temperature (°C)	Apparent partition coefficients I	II
20	25	0·45	0·67
	29	0·42	—
	31	—	0·59
	33	0·37	—
	37	0·33	0·57
30	25	0·51	0·70
	29	0·44	—
	31	—	0·59
	33	0·38	—
	37	0·34	0·58
40	25	0·50	0·75
	29	0·47	—
	31	—	0·65
	33	0·46	—
	37	0·35	0·63

Table 1 shows a decrease in P of methacycline and doxycycline hydrochlorides with increasing temperature and decreasing drug concentration. In Table 2, the difference between P values at 25° and 37° is indicated for the three tetracyclines, the conditions used being otherwise similar to those of Colaizzi & Klink (1969).

As shown by Colaizzi & Klink (1969), a small decrease from pH 7·5 results in higher values of P. The pH of our buffer decreases by about 0·03 units as a result of the temperature increase of 12°. It is evident that the trends in Tables 1 and 2 cannot be explained on the basis of this change of pH. In fact, Cooke (1976) concluded that the temperature dependence of the second ionization constant of tetracyclines, which determines the overall contribution of the most lipophilic ionic species of these compounds, i.e., the zwitterion concentration (Leeson, Krueger & Nash, 1963; Colaizzi & Klink, 1969), is unlikely to account fully for the observed dependence of the apparent partition coefficients.

The temperature dependence of the intrinsic (i.e. single species) octanol/water partition coefficients has been reported to be small, of the order of 0·01 log P

Table 2. *Apparent partition coefficients of three tetracyclines between phosphate buffer (pH 7·5 at 25°) and octanol at 25° and 37°.*

Drug	Initial concn in buffer (mg %)	Ionic strength of buffer (M)	Apparent partition coefficient 25°	37°
Tetracycline HCl	52	0·18	0·038	0·026
Methacycline HCl	20	0·09	0·45	0·33
Doxycycline HCl	20	0·09	0·60	0·51

For comparison Colaizzi & Klink (1969) found the following P values at pH 7·5, 25°, ionic strength of the phosphate buffer 0·1M, drug concentration $5{\cdot}203 \times 10^{-4}$ M (approx. equivalent to 25 mg %): tetracycline hydrochloride 0·036, methacycline hydrochloride 0·43, and doxycycline hyclate 0·60. Full details with results can be found in Cooke (1976).

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(intrinsic) per °C (Leo & others, 1971). Care must be exercised, however, in applying this statement. Firstly, for compounds with small absolute values of log P, the difference between the *in vitro* values (usually determined at room temperature) and the *in vivo* magnitude at 37° can lead to a large error for the purpose of quantitative *in vitro—in vivo* correlations Secondly, many compounds of medicinal interest have pKa values with temperature dependences (Ballard, 1974) that can have a profound effect on the proportion of their most lipophilic forms present, and thus on partitioning results. Such effects have been reviewed by Ballard (1974). Recently, temperature dependence of partition coefficients has been reported by Kaufman, Semo & Koski (1975), Davis, Elson & others (1976) and Dearden (1976).

Finally, in view of the complex ionization scheme (Leeson & others, 1963) and partitioning behaviour (Colaizzi & Klink, 1969) of tetracyclines, it would be rather tedious to extract thermodynamic values of the relevant physicochemical parameters for the purpose of correlating these quantitatively with biological activity of these compounds. We suggest that, instead, the apparent partition coefficients of tetracycline and its analogues should be measured under conditions closely resembling the biological activity studied.

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The effects of solubility and method of drying on the drug content of various size fractions of tablet granules

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Variations of drug content of different size fractions of granules have been reported (Lachmann & Sylwestrowicz, 1964; Cox, Ambaum & Wijnand, 1968; Travers, 1974; Selkirk, 1976). These variations have been considered to be due to solvent migration during drying, and abrasion of the granule surface during subsequent handling. We find that this is an insufficient explanation of some results we have obtained.

To examine the effects of the solubility of the minor component and the method of drying, a series of 1 kg batches of granules were made using lactose B.P. (Whey Products Ltd.) as diluent and either sulphanilamide (ICI) or sulphacetamide sodium (Ward Blenkinsop) as drug. The damp granules were divided into two sub-batches and then dried using either a fluid-bed drier or a standard laboratory oven with a fan. A 100 g sample of each sub-batch of dried granules was sieved, and one coarse and some fine sieve fractions were assayed for drug content.

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Preparation of granules. The granules were made by one of two methods. In method 1, the component powders were screened together. For the 0·02 and 1% levels of sulphacetamide sodium, and the 0·02% level of sulphanilamide, the required weight of drug for a 1 kg batch was dissolved in 120 ml of the binder solution, 5% w/v aqueous polyvinylpyrrolidone (K29-32Gaf). For sulphanilamide 1 and 2%, drug equivalent to 0·02% was dissolved in the binder solution and the remaining sulphanilamide was mixed with the lactose in a Morton Z blade mixer for 2 min before binder was added. The binder (plus drug) was added in two 60 ml portions with 1 min massing between additions; there was a further 5 min massing following the addition of the second portion of the binder. The damp mass was forced through a 1·0 mm screen using a Jackson-Crockatt granulator and divided into 4 portions by quartering. Two opposite quarters were combined for fluid bed drying at 50° for 25 min and one quarter was placed in an enamel tray (20 cm × 30 cm) for oven drying at 50° for 90 min. The dried granules, which had