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The estimation of relative water solubility for prodrugs that are unstable in water

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Summary

The pH 4.0 buffer solubilities (S_{H_2O}) of three series of *N*-acyl-5-fluorouracil (5-FU) prodrugs have been estimated from their solubilities in isopropyl myristate (IPM) and their very rapidly determined partition coefficient values between IPM and pH 4.0 buffer (K). These estimated values have been compared (1) with directly determined values for the two more hydrolytically stable series (1-alkyloxycarbonyl- and 1-alkylaminocarbonyl-5-FU), (2) with literature values and (3) with values calculated from (log water solubility) = −(log partition coefficient)−0.01(melting point) +1.05. There is good agreement between the absolute and relative values of the estimated and directly measured buffer solubilities for the two more hydrolytically stable series. There is also excellent agreement between the estimated values for the two more hydrolytically stable series and the literature values except for the 1-ethyloxycarbonyl-5-FU derivative where the literature value appears to be almost an order of magnitude too low. The calculated water solubilities give values that are one to three orders of magnitude too high and do not accurately reflect trends in the series. The partition coefficient and estimated buffer solubility values for the hydrolytically unstable series (1-alkylcarbonyl-5-FU) are reproducible and consistent with those of the two more stable series. Thus, the estimated buffer solubilities for the hydrolytically unstable series are reliable values that are accurate relative to other series of *N*-acyl prodrugs.

Introduction

The solubility of a drug in water is an extremely important determinant of its ultimate potency in a biological system. This is especially true where oral or rectal delivery of a drug is desired and the applied phase, or the phase external to the biological membrane, is water. The drug must exhibit an appropriate degree of water solubility in the applied phase before it can partition across the biological membrane. On the other hand, water solubility is also important where topical delivery of a drug is desired and the applied phase does not have to be water. In fact, in that case the external phase can be completely non-aqueous, or lipid-like. Then, since the stratum corneum, which is the rate-limiting biological barrier to percutaneous absorption, is also considered to be lipophilic in nature, dermal or

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transdermal delivery of a drug requires that the drug exhibit significant lipid solubility. However, even for the dermal or transdermal delivery of a drug from a non-aqueous, lipid-like applied phase, the drug must exhibit some appropriate degree of water solubility, in addition to its lipid solubility, in order for it to partition into and through at least the initial hydrophilic microphases of the lipophilic stratum corneum. In addition, if transdermal delivery of a topically applied drug is desired, solubility of the drug in the internal aqueous phases is essential for it to express its systemic potency.

Improvement in delivery of a drug frequently requires the design of transient derivatives of the drug which are called prodrugs. The design of prodrugs that exhibit the desired hydrophilic/lipophilic balance in their solubility which is necessary for the efficient dermal or transdermal delivery of their parent drug, requires precise knowledge of the relative aqueous and lipid solubilities of the members of the proposed series of prodrugs. In most instances this is a straight-forward process since many, if not most, prodrugs are reasonably soluble and stable in water and lipids. All that is usually necessary is to stir an excess of the pure prodrug in the lipid or in water at the appropriate pH so as to minimize ionization effects, filter the suspension and analyze the filtrate by UV or HPLC. This is a direct method of measuring water solubility. However, in those cases where the prodrug is not very stable in water, it has been virtually impossible to directly measure or accurately estimate water solubility. Byron et al. (1980) have described a kinetic method, but it requires that it is possible to determine partition coefficients (K) under conditions where the solute is stable. This condition is not often attainable.

The use of partition coefficient values to estimate or calculate water solubilities of water stable solutes has been described in several reports. Partition coefficients comprise an essential part of the general approach of Yalkowsky and co-workers (1980, 1983) to calculate water solubilities. Higuchi and co-workers (1979) used partition coefficient values and solubilities in lipoidal solvents to estimate the water solubilities of barely aqueous-soluble organic solids. Higuchi and co-workers (1979) also recommended the use of a partitioning driven process to facilitate dissolution of barely aqueous-soluble organic solids in water, and hence enable them to more accurately measure their water solubilities. However, there are no reports of the use of partition coefficient values to estimate the water solubilities of drugs or prodrugs that are unstable in water. This is probably because partition coefficient values have generally been obtained only after fairly lengthy equilibrium times which would be incompatible with measuring partition coefficients of solutes that were relatively unstable in the water phase.

In this paper an experimental method for estimating the water solubilities of the members of a series of chemically unstable prodrugs (1-alkylcarbonyl-5-FU) is described which is based on the determination of their lipid solubilities and their rapidly determined partition coefficients between the lipid and pH 4.0 buffer. Buffer solubilities of two other series of prodrugs (1-alkyloxycarbonyl- and 1-alkylaminocarbonyl-5-FU), which are much more stable at pH 4.0, are also estimated using this experimental method. The estimated water solubilities for the latter two series are then compared with their directly measured water solubilities to assess the accuracy of the estimated values. In addition, water solubilities estimated from the proposed experimental method are compared with a standard method of calculating water solubilities from calculated or measured partition coefficients and melting points.

Experimental

Ultraviolet spectra were obtained using a Varian Cary 210 or a Shimadzu UV-265 spectrophotometer. The pH meter was a Radiometer pH meter 26. The HPLC system consisted of a Beckman Model 110A pump with a model 153 UV detector, a Rheodyne 7125 injector with a 20 μl loop, and a Hewlett-Packard 3392A integrator. The column was a Lichrosorb RP-8 10 μm reversed-phase column, 250 mm $\times$ 4.6 mm (inside diameter). Isopropyl myristate (IPM) was ob-

tained from Givaudan Corp of Clifton, N.J. The water was obtained from a Millipore Milli-Q water system. 1-Alkylcarbonyl-5-FU (Beall, 1991) and 1-alkyloxycarbonyl-5-FU prodrugs (Beall, 1991) were synthesized according to modifications of known procedures (Kametani et al., 1980) or according to the method of Ozaki et al. (1977) for the 1-alkylaminocarbonyl-5-FU prodrugs. The ^{1}H-NMR, UV and IR spectra of the prodrugs were consistent, within each series, with reported values. Elemental analysis were obtained for all new compounds and were consistent with assigned structures. The spectra and elemental analysis data have been reported in the dissertation of Beall (1991). HPLC was used to confirm the purity of the prodrugs.

Solubilities

The solubility of each prodrug in IPM was determined in triplicate by stirring an excess of each prodrug in three test tubes (16 × 100 mm) containing 2–6 ml of IPM for 48 h at room temperature (23 ± 1°C) using a magnetic stirrer and star-head stirring bars. The test tubes were insulated from direct contact with the surface of the stirrer. The suspensions were allowed to settle for 24–48 h, then each suspension was filtered through a 0.45 μm nylon filter. A 100 μl portion of each IPM filtrate was diluted with acetonitrile to 5, 25 or 100 ml and analyzed by UV spectrophotometry at 261 nm for the 1-alkylcarbonyl-5-FU prodrugs, at 254 nm for the 1-alkyloxycarbonyl-5-FU prodrugs and at 256 nm for the 1-alkylaminocarbonyl-5-FU prodrugs. Molar absorptivities were determined in triplicate in acetonitrile at the appropriate wavelength. Values for molar absorptivities for the first two series are reported in the dissertation of Beall (1991) while those for the later series will be reported elsewhere (Sloan et al., 1993). The solubilities of each 1-alkyloxycarbonyl- and the first three members of the 1-alkylaminocarbonyl-5-FU series of prodrugs in pH 4.0 acetate buffer were also determined in triplicate and duplicate, respectively, using the same procedure as above for the IPM solubilities except that the suspensions were only stirred for 1 hour at room temperature before they were filtered. UV spectrophotometry was used to determine the pH 4.0 buffer solubilities of each 1-alkyloxycarbonyl-5-FU prodrug. In addition, HPLC was used to determine the pH 4.0 buffer solubilities of the first three members of the 1-alkylaminocarbonyl- and 1-alkyloxycarbonyl-5-FU series of prodrugs ($\lambda_{anal} = 254$ nm). A mobile phase of 10% methanol and 90% 0.025 M acetate buffer, pH 5.0 (Buur and Bundgaard, 1985) at a flow rate of 1.0 ml/min was used to chromatograph the 1-alkyloxycarbonyl-5-FU prodrugs, while a mobile phase of 20% methanol and 80% buffer was used for the 1-alkylaminocarbonyl-5-FU prodrugs.

The solubility of each prodrug for all three series in pH 4.0 buffer was also estimated from the triplicate determinations of the IPM solubilities and from the triplicate values for the partition coefficients of each prodrug between IPM and pH 4.0 buffer that follows. Usually a 1.0 or 0.5 ml portion of each IPM filtrate, obtained from the determination of the IPM solubility above, was vigorously shaken by hand with an equal volume of pH 4.0 acetate buffer for 10 s. The two phases were allowed to separate for 1 min by gravity. Then a 100 μl portion of the IPM phase was removed, diluted with acetonitrile to 5, 25 or 100 ml and analyzed by UV spectrophotometry at 261, 256 or 254 nm as above. The IPM/pH 4.0 buffer partition coefficients (K) were calculated from:

$$K = A_a/(A_b - A_a)(V_{H_2O}/V_{IPM})$$

where A_b and A_a are the absorbance of the IPM phase before and after partitioning, respectively, V_{H_2O} is volume of the aqueous buffer phase and V_{IPM} is the volume of the IPM phase. The solubilities of each member of the three series of prodrugs in pH 4.0 buffer was then estimated from the mean of the IPM solubility divided by the means of the corresponding K:

$$S_{H_2O} = S_{IPM}/K$$

where S_{H_2O} and S_{IPM} are the saturated solubilities in pH 4.0 buffer and IPM, respectively.

In order to determine if the time that the IPM and buffer phases were shaken together had an effect on the values of K, IPM phases containing a representative unstable prodrug (1-acetyl-5-FU, **2**) were shaken with pH 4.0 buffer for 10, 20 or 30 s. The rest of the procedure for determining K remained the same.

In those instances where the UV absorbance of the IPM phase after partitioning was not significantly different from the background absorbance, the ratio of the volume of the IPM phase to that of the buffer was increased up to a ratio of 10:1 to obtain the most reproducible values for K. In those instances where the UV absorbance of the IPM phase after partitioning was not significantly different from the UV absorbance before partitioning, the ratio of the volume of the buffer phase to that of IPM phase was increased up to a ratio of 10:1. Also, phase volume ratios were varied for each member of the 1-alkyloxycarbonyl- and 1-alkylcarbonyl-5-FU prodrug series over a range of an order of magnitude. The phase volume ratios were varied from 1:1, 1:2, 1:6 to 1:10 (IPM/buffer) for each member of the 1-alkylcarbonyl series (**2**–**7**) and for **12** and **13** of the 1-alkyloxycarbonyl series; from 1:1, 2:1, 6:1 to 10:1 for **8**–**10** of the 1-alkyloxycarbonyl series; from 1:2, 1:1, 2:1, to 3:1 for **11** of the 1-alkyloxycarbonyl series. This was done to determine if there were any trends in changes in values of K with changes in phase volume ratios. Only a single determination of K was made at each phase volume ratio different from the ratios given in Tables 1–3.

Statistical analysis was performed using Student's t-test where indicated.

Results and Discussion

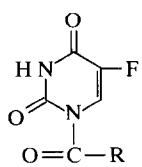
The IPM solubilities, the IPM/pH 4.0 buffer partition coefficients (K) and the estimated pH 4.0 buffer solubilities for the three series of N-acyl prodrugs of 5-FU are given in Tables 1–3. The IPM solubilities of the members of all three series are reproducible to $< \pm 3\%$ except for the 1-propionyl derivative **3**, which exhibited a variation of $\pm 5\%$.

Unless otherwise indicated in the S_{H_2O} column, the experimental K values for the distribution of the prodrugs between IPM and pH 4.0 buffer that are given in Tables 1–3 were obtained from experiments where a phase volume ratio of 1:1 was used. Alternately, the lowest phase volume ratio that gave reproducible results was used. This was done to achieve the fastest possible distribution of solute between the two phases, and then separation of the phases. Also, in order to achieve the fastest possible measurement of the partition coefficient, the two phases were shaken for only 10 s. In a separate experiment using prodrug **2**, there was no significant effect on the partition coefficient if the two phases were shaken for 10, 20 or 30 s ($K = 0.183 \pm 0.006$).

Speed is important where the desired K value is that of a prodrug which is unstable in water or protic solvents in general. This is particularly true if the method of analysis can be non-specific, such as UV analysis frequently is, and only the organic phase is analyzed. The faster the solute distributes between the two phases and the phases are separated, the less opportunity the solute has to decompose in the buffer phase. The more

TABLE 1

Solubilities in IPM, partition coefficients (K) and solubilities in pH 4.0 buffer estimated from K for 1-alkylcarbonyl-5-FU prodrugs [a]



R	S_{IPM} [b]	K (±SD) [c]	S_{H_2O} [b,d]
2 CH_3	22.1	0.185(0.0131)	120
3 C_2H_5	36.4	0.764(0.0176)	47.6
4 C_3H_7	17.4	2.69(0.215)	6.50
5 C_4H_9	39.2	11.3(1.14)	3.48
6 C_5H_{11}	112.7	38.2(2.40)	2.95 (1:10)
7 C_7H_{15}	110.7	759 (94.6)	0.146 (1:10)

[a] Beall (1991).
[b] Solubility in mM.
[c] Partition coefficient between IPM and pH 4.0 buffer.
[d] Determined from solubility ratio: $S_{H_2O} = S_{IPM}/K$ (IPM/buffer phase volume ratio different from 1:1).

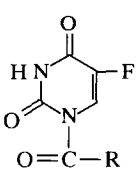
opportunity the solute has to decompose in the buffer phase while vigorous agitation and intimate mixing of the phases is occuring, the greater the opportunity for more solute to partition from the IPM phase into the buffer phase to compensate for its decomposition in the buffer phase. The result would be a lower apparent value of K and a higher apparent value of water solubility.

The standard deviation for each K value is also given in Tables 1–3. In most cases that deviation was less than 10%, and in about one-third of all the cases it was less than 5%. In three cases, though, the deviation was greater than 10% (**7**, **17** and **18**). All three cases correspond to the more lipophilic members of their respective series, and two were determined from the lowest IPM to buffer ratios (1:10). In two series (**2–7** and **8–13**) K values were determined where the phase volume ratios were varied by a factor of 10 for each member of the series to give three additional values of K (data not given). Although there was some deviation ($< 12\%$ except for **6**) in those values of K, no consistent trends were observed. Also, if those additional values for K and the values for K reported in Tables 1–3 were combined to calculate a mean value of K for each prodrug from all the experiments, those K were not significantly different ($P < 0.05$) from the values reported in the tables. The exception was the value for **7**. In that case, reliable values for K could not be obtained using phase volume ratios of IPM/buffer less than 1:10.

The buffer solubilities in Tables 1–3 were determined by dividing the IPM solubility values by the respective K values. Buffer solubilities for most of the members of the more stable series (**8–13** and **14–16**) were also determined directly by stirring an excess of the prodrug in pH 4.0 buffer, but only for 1 h. The direct buffer solubilities determined by UV (Table 2) are reproducible to $< \pm 8\%$ and those determined by HPLC (Tables 2 and 3) are reproducible to $< \pm 3\%$. This method is in direct contrast with most reported methods of directly determining water solubility

TABLE 2

Solubilities in IPM, partition coefficients (K) and solubilities in pH 4.0 buffer estimated from K or measured directly for 1-alkyloxycarbonyl-5-FU prodrugs [a]



R	S_{IPM} [b]	K (±SD) [c]	S_{H2O} [b,d]	Direct S_{H_2O} [b]		
				UV [c]	HPLC [e]	HPLC [f]
8 OCH_3	2.13	0.0192(0.00078)	111 (6:1)	120	110	124
9 OC_2H_5	13.1	0.0750 (0.0088)	174	263	269	34.2
10 OC_3H_7	15.2	0.357(0.0319)	42.6	55.1	54.6	–
11 OC_4H_9	33.8	1.44(0.132)	23.3	29.0	–	25.7
12 OC_6H_{13}	153.5	30.5(0.63)	5.04 (1:6)	5.39	–	5.81 [g]
13 OC_8H_{17}	36.4	285 (25.2)	0.128 (1:10)	0.140	–	–

[a] Beall (1991).
[b] Solubility in mM.
[c] Partition coefficient between IPM and pH 4.0 buffer.
[d] Determined from solubility ratio: $S_{H_2O} = S_{IPM}/K$ (IPM/buffer phase volume ratio different from 1:1)
[e] Determined by analyzing filtrate from suspension of prodrug in pH 4.0 buffer stirred at 23 ± 1°C for 1 h.
[f] Buur and Bundgaard (1986).
[g] Buur and Bundgaard (1987).

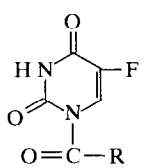
where excess solute is stirred with water for at least 24 h, but is reasonably similar to the reported methods for directly determining water solubility of *N*-acyl prodrugs of 5-FU. In those reports, suspensions of 1-alkylaminocarbonyl (Buur and Bundgaard, 1985) and 1-alkyloxycarbonyl-5-FU prodrugs (Buur and Bundgaard, 1986) in pH 4.0 buffer were placed in an ultrasonic bath for 30 and 15 min, respectively, before they were rotated for 1 h at room temperature.

In Tables 2 and 3 the pH 4.0 buffer solubilities of the 1-alkyloxycarbonyl- and 1-alkylaminocarbonyl-5-FU prodrugs, respectively, that were determined directly by UV and by HPLC are compared with (1) the pH 4.0 buffer solubilities which were estimated from K values and (2) the literature values. There is very good agreement ($< 5\%$ deviation) between the available litarature values, the directly measured values and those estimated from K values for the first three members of the 1-alkylaminocarbonyl series of derivatives in Table 3 (**14–16**). Since there was such good agreement for **14–16** and they were the members of the series whose water solubilities were the more interesting (see below), the direct solubilities of **17** and **18** were not determined here. Although the agreement between the pH 4.0 buffer solubility estimated from K and that reported by Buur and Bundgaard (1985) for **17** is poor, there is good agreement with the pH 4.0 buffer solubility reported by Sasaki et al. (1990) for **17**. On the other hand, the agreement between the buffer solubility estimated from K and that reported by Sasaki et al. (1990) for **18** is poor.

The value reported by Sasaki et al. for **18** is the more believable value. An average π value for CH_2 of about 0.61 ± 0.03 is obtained for the comparison of their log K_{OCT} (octanol/pH 4.0 buffer) values for their butyl and hexyl derivatives with that for their octyl derivative. That π value is consistent with π values reported here for the remaining members of the 1-alkylaminocarbonyl series and the members of the other series as well (Table 4). The IPM solubility of **18** from their report and that value from this work are not significantly different ($p < 0.05$), so their reported buffer solubility together with the S_{IPM} value from Table 3 gives a much larger value of log K for **18**, +3.19 vs +1.93, from which the

TABLE 3

Solubilities in IPM, partition coefficients (K) and solubilities in pH 4.0 buffer estimated from K or measured directly for 1-alkylaminocarbonyl-5-FU prodrugs [a]



R	S_{IPM} [b]	K (±SD) [c]	S_{H_2O} [b,d]	Direct S_{H_2O} by HPLC [b]		
14 $NHCH_3$	0.299	0.081(0.0013)	3.69 (10:1)	3.48 [e]	3.37 [f]	–
15 NHC_2H_5	2.79	0.36(0.010)	7.76	7.71 [e]	7.46 [f]	–
16 NHC_3H_7	12.4	1.39(0.049)	8.98	9.44 [e]	–	–
17 NHC_4H_9	24.6	4.80(0.66)	5.11	–	3.58 [f]	4.80 [g]
18 NHC_8H_{17}	46.9	84.8(13.3)	0.553 (1:10)	–	–	0.0302 [g]

[a] Sloan et al. (1993).
[b] Solubility in mM.
[c] Partition coefficient between IPM and pH 4.0 buffer.
[d] Determined from solubility ratio: $S_{H_2O} = S_{IPM}/K$ (IPM/buffer phase volume ratio different from 1:1).
[e] Determined by analyzing filtrate from suspension of prodrug in pH 4.0 buffer stirred at 23 ± 1°C for 1 h.
[f] Buur and Bundgaard (1985).
[g] Sasaki et al. (1990).

more reasonable π value of 0.63 for CH_2 in **18** can be calculated (Table 4). The high pH 4.0 buffer solubility of **18** estimated from the K value of the present work is probably a consequence of the fact that it was not practical to use lower IPM/buffer phase volume ratios than 1 : 10 when it was also necessary to use a short time for separation of the phases.

The agreement between the directly measured pH 4.0 buffer solubilities of the members of the 1-alkyloxycarbonyl-5-FU series of prodrugs of the present work and those reported in the literature is generally good, but somewhat poorer than the agreement observed for the 1-alkylaminocarbonyl-5-FU series. However, the difference between the buffer solubility value for **9** reported in the literature and that determined here is almost an order of magnitude. The much higher direct buffer solubility of **9** was obtained by both UV and HPLC analysis. Also, there is no question that **9** is authentic 1-ethyloxycarbonyl-5-FU, since its melting point is consistent with the reported value (Buur and Bundgaard, 1986) and its ^{1}H-NMR and UV spectra were consistent the other members of the series (Beall, 1991). Although the reason for the discrepancy in the pH 4.0 buffer solubility values for **9** is not clear (there is no definitive evidence that it is not due to differences in crystallinity of polymorphs), the higher value reported here is highly reproducible.

There is very good agreement between the pH 4.0 buffer solubilities determined directly by UV and by HPLC (variation is $< \pm 5\%$) for the 1-alkyloxycarbonyl-5-FU series (**8–13**). On the other hand, the buffer solubilities estimated for **8–13** from K values are less than those determined by direct measurements. This is especially true for derivatives that are very soluble in buffer, e.g., **9**. This result may be the consequence of increased self-association of the more polar and more buffer

TABLE 4

Log partition coefficients (K) and associated methylene π values, log pH 4.0 buffer solubilities estimated from log K, melting points, and log solubilities in pH 4.0 buffer calculated from Eqn 1 for N-acyl-5-FU prodrugs [a]

Compound	Log K	π	Log S_{H_2O} [b]	m.p.	Log $S_{H_2O(c)}$ [b]
2	−0.73	–	−0.92	127	+0.51
3	−0.12	0.61	−1.32	128	−0.12
4	+0.43	0.55	−2.19	143	−0.
5	+1.05	0.62	−2.46	118	−1.18
6	+1.58	0.53	−2.53	99	−1.52
7	+2.88	0.65	−3.82	82	−2.65
8	−1.71	–	−0.95	160	1.17
9	−1.12	0.59	−0.76	128.5	0.89
10	−0.45	0.67	−1.37	126	0.23
11	+0.16	0.61	−1.63	98	−0.09
12	+1.48	0.66	−2.30	67	−1.10
13	+2.45	0.52	−3.89	98	−2.38
14 [c]	−1.08	–	−2.45	170 [d] (212) [e]	+0.43 (0.01) [e]
15 [c]	−0.44	0.64	−2.11	165 [d] (180) [e]	−0.16 (−0.31) [e]
16 [c]	+0.14	0.58	−2.05	145 [d] (139) [e]	−0.54 (−0.48) [e]
17 [c]	+0.68	0.54	−2.29	137 [d] (133) [e]	−1.00 (−0.96) [e]
18 [c]	+1.93	0.31	−3.26	100 [d] (91) [e]	−1.88 (−1.79) [e]
18	+3.19	0.63	−4.52 [f]		−3.14 (−3.05) [e]

[a] Beall (1991).
[b] Log solubility in M.
[c] Sloan et al. (1993).
[d] Ozaki et al. (1977).
[e] Endotherm peaks from DSC: Sloan et al. (1993) and log $S_{H_2O(c)}$ generated from DSC data.
[f] Sasaki et al. (1990).

soluble prodrugs in IPM which gives increased values of K and hence decreased values for their buffer solubilities estimated from K (Yalkowsky et al., 1983). However, the K values from which the buffer solubilities were calculated give π values for CH_2 that average 0.61 ± 0.06 (Table 4). This is consistent with the π value for CH_2 from the first four members of the 1-alkylaminocarbonyl series ($\pi = 0.59 \pm 0.05$, Table 4) where there was excellent agreement between buffer solubilities that were directly measured and those that were estimated from K values. Thus, although there may be a tendency to underestimate absolute buffer solubility values derived from K, the relative buffer solubilities derived from K are reproducible and reliable values.

It was not possible to directly measure the pH 4.0 buffer solubilities of the 1-alkylcarbonyl-5-FU prodrugs because they hydrolyze too quickly in water ($t_{1/2} = 7$ min at pH 4–7) (Buur and Bundgaard, 1984). However, the π values obtained from the log K values given in Table 4 give an average π value for the CH_2 group of 0.59 ± 0.05 for the series. This π value is entirely consistent with those for the other two series of hydrolytically stable N-acyl prodrugs and verifies the accuracy of these K values for the hydrolytically unstable series relative to the other, stable series. Since the 1-alkylcarbonyl prodrugs are stable in IPM, their IPM solubilities should be, and are, as accurate as those for the other, more hydrolytically stable series of prodrugs, and together with the K values should generate equally accurate relative buffer solubilities.

Frequently, when it is impractical or impossible to directly measure the water solubility of a drug or prodrug (S_{H_2O}), the water solubility can be calculated from a semi-empirical equation such as the following (Yalkowsky and Valvani, 1980):

$$\log S_{H_2O} = -\log K_{OCT} - 0.01MP + 1.05 \qquad (1)$$

where S_{H_2O} is the solubility of the drug or prodrug in water, K_{OCT} denotes the octanol/water partition coefficient, MP represents the melting point, and 1.05 is a constant. Hence, it was of interest to see how well such an approach would predict the aqueous solubilities of a series of prodrugs that were unstable in water, where no direct method of estimating the water solubility was available. Since one of the assumptions made in the derivation of Eqn 1 (Yalkowsky et al., 1983) is that the solubility ratio ($SR = S_{IPM}/S_{H_2O}$) is equal to the partition coefficient (C_{IPM}/C_{H_2O} for dilute solutions) and this is also the basis for estimating the pH 4.0 buffer solubility from K and S_{IPM} in this work, it was also of interest to see how closely the results from the two approaches were related.

When the IPM/pH 4.0 buffer partition coefficients (K) for each member of the three series of N-acyl prodrugs were substituted for the octanol/water partition coefficient term in Eqn 1, log solubilities of the prodrugs in pH 4.0 buffer ($S_{H_2O(c)}$) could be calculated (Table 4) using the literature values for their melting points given in Table 4. The melting point data for **14–18** were all taken from Ozaki et al. (1977) for consistency, although the melting points for **14** and **15** could not reproduced (Sloan et al., 1993). Alternative values of log $S_{H_2O(c)}$ for **14–18** calculated from differential scanning calorimetry (DSC) data (Sloan et al., 1993) are included in Table 4. Those log calculated pH 4.0 buffer solubilities calculated from melting point and K data ($S_{H_2O(c)}$) were then plotted against the log pH 4.0 buffer solubilities (S_{H_2O}) estimated from the same values of K. The results are shown in Fig. 1. The alternative values of $S_{H_2O(c)}$ for **14–18** in Table 4 are not plotted in Fig. 1. The alternative values for **14** and **15** are the only ones that are significantly different from those calculated from the literature melting points, but they do not change the general relationship between S_{H_2O} and $S_{H_2O(c)}$.

Linear regression analyses of the plots for the 1-alkylcarbonyl- (**2–7**) and 1-alkyloxycarbonyl-5-FU prodrugs (**8–13**) show reasonably good correlations between $S_{H_2O(c)}$ and S_{H_2O} for those two sets of data (slopes = 1.07 and 1.13; and S_{H_2O} intercepts = −1.31 and −1.63, respectively). The only outlier is the data for **8**. On the other hand, there is no apparent correlation between the two sets of data for the 1-alkylaminocarbonyl-5-FU prodrugs (**14–18**) except for the last three mem-

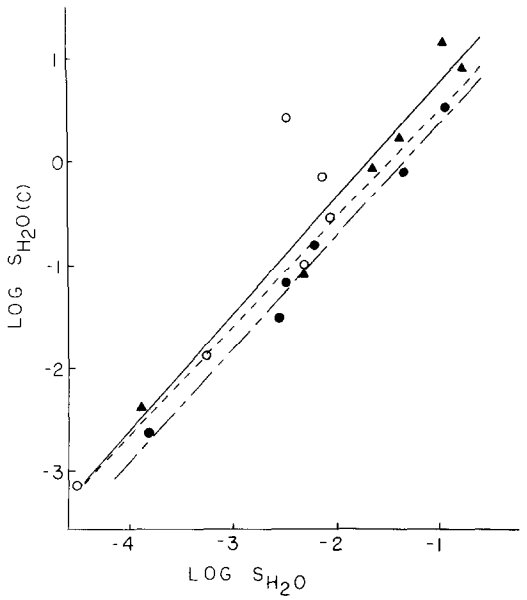


Fig. 1. A plot of log $S_{H_2O(c)}$ vs log S_{H_2O} for 1-alkylcarbonyl-5-FU prodrugs (●) (—·—), 1-alkyloxycarbonyl-5-FU prodrugs (▲) (———) and 1-alkylaminocarbonyl-5-FU prodrugs (○) (— — —).

bers of the series (slope = 1.05; S_{H_2O} intercept = −1.45). If the literature buffer solubility data for **18** is used to generate a value for K which is then used to generate $S_{H_2O(c)}$, the inclusion of that second set of data for **18** in Table 4 does not significantly affect the slope of the plot for the last three members of the series in Fig. 1.

These results would be the same regardless of which variation of Eqn 1 was used. The reason for this lack of correlation of the log calculated buffer solubilities ($S_{H_2O(c)}$) with directly measured or estimated pH 4.0 buffer solubilities for the first member of the 1-alkyloxycarbonyl- and the first two members of the 1-alkylaminocarbonyl-5-FU prodrug series is that in each series the first one or two members, respectively, of each series are less soluble than a subsequent member of the series. As long as the partition coefficients for an homologous series of prodrugs increases in a regular fashion and the corresponding melting points decrease in a regular fashion, semi-empirical treatments such as Eqn 1 will fail to predict a trend of initially increasing, followed by decreasing water solubilities in an homologous series. On the other hand, in spite of the facts that their melting points fail to exhibit as regular a decrease, and their IPM solubilities fail to exhibit as regular an increase with increasing chain length as do the members of the other series, the correlation between S_{H_2O} and $S_{H_2O(c)}$ for the members of the 1-alkylcarbonyl-5-FU series are comparable ($r = 0.993$ vs 0.980 and 0.988) to those for the other two series. This outcome may be the result of the dominance of K as a variable in Eqn 1, and that values of K for the 1-alkylcarbonyl series increase in a regular fashion.

Aside from the obvious inconsistencies in the calculated buffer solubilities of the first few members of two of the series, the calculated values also overestimate the water solubilities by one to three orders of magnitude. This outcome may be the result of substituting the IPM/buffer partition coefficient for K_{OCT} in Eqn 1. However, a comparison of the respective solubility ratios (S_{IPM}/S_{H_2O} vs S_{OCT}/S_{H_2O}) for the S^6,9-bisacyloxymethyl-6-MP series of prodrugs (Waranis and Sloan, 1987) suggests that the K_{OCT} values are very similar to K. Since that is the only available comparison of K with K_{OCT}, the large difference between S_{H_2O} and $S_{H_2O(c)}$ may be attributable to factors other than the substitution of K for K_{OCT}. Then, if the estimated and calculated buffer solubilities for **8**, **14** and **15**, which are outliers, are excluded from the calculation, the mean difference between the log S_{H_2O} and log $S_{H_2O(c)}$ values is 1.37 ± 0.18 for all three series. Thus, Eqn 1 would more accurately calculate the buffer solubilities of these 5-FU N-acyl prodrugs if the constant was −0.32 instead of +1.05. However, even with that correction, the use of semi-empirical approaches such as Eqn 1 would still not predict the S_{H_2O} values for **8**, **14** and **15** relative to the other members of their respective series.

Conclusion

A method of estimating buffer solubilities of N-acyl prodrugs of 5-FU has been developed

which is based on the determinations of the solubilities of the prodrugs in a representative lipid (S_{IPM}) and then of their partition coefficients (K) between IPM and the buffer. Since the partition coefficients are determined very rapidly by very vigorous manual shaking of the two phases (10 s) and then by allowing the two phases to separate by gravity for only 1 min, partition coefficients can be determined even for hydrolytically unstable prodrugs such as 1-alkylcarbonyl-5-FU prodrugs which exhibit a half-life of only about 7 min at pH 4.0. Buffer solubilities can subsequently be estimated from S_{IPM}/K. These estimated buffer solubilities reproduced directly measured buffer solubilities for representative more hydrolytically stable *N*-acyl prodrugs such as the 1-alkyloxycarbonyl- (half-life of about 23 h at pH 4.0) and the 1-alkylaminocarbonyl-5-FU prodrugs (half-life of about 46 h at pH 4.0). Thus, the buffer solubilities estimated for the hydrolytically unstable 1-alkylcarbonyl-5-FU prodrugs by the same method should be equally reliable.

The buffer solubilities calculated from K values and melting points according to the general method of Yalkowsky et al. (180), even when corrected for *N*-acyl type prodrugs of 5-FU, do not predict the most buffer soluble member of two of the three series of prodrugs that were examined. Thus, in order to predict the more water soluble member of a series of prodrugs, it is better to measure the corresponding K values and lipid solubilities, and estimate the S_{H_2O} values than it is to try to calculate S_{H_2O} on the basis of K and melting point. The correct relative water solubilities for a series of more lipid soluble prodrugs is particularly important for predicting the most effective member of the series with which to enhance transdermal delivery. For that application of prodrugs, the more water soluble member of the series is usually the more effective member (Sloan, 1989), and the present method is the only one that accurately predicts which member of the series it is. However, the present method is even more important for estimating the relative water solubilities of a series of hydrolytically unstable prodrugs where it is the only reported method of accurately estimating their water solubilities.

The use of IPM or similar esters as the lipid phase in the partitioning experiments described here is important for two reasons. First, since drugs or prodrugs that are unstable in water may also be unstable in protic solvents, octanol can not be used as the lipid phase for stability reasons. On the other hand, drugs or prodrugs should exhibit easily measurable solubility values in the lipid phase in order to facilitate their quantitation. Thus, an unstable and relatively polar drug or prodrug should not only be sufficiently stable, but also soluble in an aprotic lipid which contains an ester functional group, e.g., IPM, whereas it may not be sufficiently soluble in an alkane solvent. Second, since the partitioning is conducted with very vigorous shaking and required rapid separation of the phases, octanol cannot be used as the lipid phase because of its tendency to form emulsions upon vigorous shaking with an aqueous phase. These emulsions are very difficult to separate. On the other hand, the IPM phases have been found to separate quickly from the aqueous phases in these experiments. This is in contrast to the difficulty reported by Pozzo et al. (1991). Thus, the use of IPM or similar types of lipids is essential to the success of estimating the water solubility of hydrolytically unstable drugs or prodrugs from their partition coefficient values.

Although this method of estimating buffer solubilities enjoys certain advantages, it is important to remember its limitations. The basis for its use is the assumption that the solubility ratio, determined from saturated solutions, is equal to the partition coefficient, determined from dilute solution. Self-association of more polar solutes in the lipid phase at higher concentrations may lead to anomalously high values of K, whereas self association of more lipophilic solutes in the aqueous phase at higher concentrations may lead to anomalously low values of K compared to K determined from dilute solutions. Care should also be exercised in choosing phase volume ratios to keep the ratio as close to 1 : 1 as possible while ensuring that accurate and reproducible K values are obtained for the more buffer and the more lipid soluble members, such as **18**, of an homologous series.

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