Improved Techniques for the Photometric Determination of Partition Coefficients, with Particular Reference to the Chloroform–Ammonia Solvent System

F. H. A. RUMMENS ˣ, R. F. BUJACZEK, M. PETERS, and R. D. GREEN

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Abstract □ Accurate partition coefficients $K = C_{org}/C_{aq}$ in chloroform–ammonia can be obtained by measuring the absorbance of the aqueous layer, replenishing with fresh aqueous solvent, and remeasuring the absorption after reequilibration. Provided the solute has a reasonably strong UV absorption, only 0.1–5 mg of material is required. Neither the amount, the extinction coefficient, nor (in most cases) the purity of the substrate material need be known. In general, K values ranging from 100 to 0.01 can be measured with a precision of ≥10%. A detailed analysis of the absorbance and volumetric error sources permits the optimum combination of experimental conditions such as volume ratios, cell lengths, and absorbance (either direct or differential, or with scale expansion) to be predicted *a priori*. Quantitative estimates for all primary error sources, such as photometric precision, baseline error, stray light, and volumetric errors were also experimentally determined. The theoretical error model was tested by determining K for aniline under widely ranging experimental conditions and by comparing the experimental standard deviations with those calculated from the theoretical model. The agreement was found to be satisfactory. The method described appears to be of particular usefulness for the determination of extraction or chromatographic parameters of basic drugs.

Keyphrases □ Partition coefficients—photometric determination in two-solvent systems, application to aniline in chloroform–ammonia □ Aniline—chloroform–ammonia solvent system, partition coefficients, photometric determination □ Photometry—determination of partition coefficients in two-solvent systems, application to aniline in chloroform–ammonia

During investigations on the extraction of narcotic drugs from body fluids one is often confronted with poor extraction yields and reproducibility. Chloroform–ammonia is the two-phase system generally used for basic drugs. Very little is known about the partition coefficients of drugs in this system, although knowledge of such partition coefficients would seem to be a prerequisite before even attempting extraction from biological samples. Persson *et al.* (1, 2) have reported on partition coefficients of amine-type drugs in the chloroform–water system, but their method involves complexation with inorganic anions and consequently requires additional information such as dissociation and polymerization constants of the complexes. A series of papers by Kemula *et al.* (3), a review paper by Sandell (4), and several studies by Korenman *et al.* (5) contain useful information on the partition coefficients of amines (and other compounds) and the dependence of these on concentration, degree of dissociation of the conjugate acid, and their relationship to chemical structure. The applicability of these studies to amine-type drugs (such as amphetamines and morphines) is limited, however, mostly because they relate to relatively high concentrations or rely on analytical techniques that require fairly large amounts of solute, both of which are often impractical in drug studies. Partition coefficients of these types of basic drugs have been reported, notably by Barfknecht *et al.* (6), Leffler *et al.* (7), Leo *et al.* (8), Kakemi *et al.* (9), Vree *et al.* (10), and Ramses and Fujimoto (11). Most of these studies lack experimental detail, employ in part ill-defined experimental conditions, and generally do not provide estimates of the reliability. In fact, the mutual agreement between the results of various authors is poor, wherever such a check can be made.

This paper attempts to provide a detailed and quantitative discussion, based on theory and experimental observations, of all the factors that contribute to the errors in the photometric determination of partition coefficients. The photometric method was chosen because of its sensitivity (normally requiring around 1–3 mg of material) and because most, if not all, basic drugs do show a distinct UV-absorption band. Since this paper deals exclusively with methodology, any basic, UV-absorbing solute could be employed; we have chosen to use aniline for this purpose because it has average UV-absorbing characteristics and because it is probably a weaker base than any of the amphetamine or morphine basic drugs.

THEORETICAL

Relationships Between the Partition Coefficient and Measured Absorbances—Consider a two-phase system, consisting of an aqueous layer of volume V_{aq} and an organic layer of volume V_{org}. Let an amount x of a solute be partitioned between the two phases so that an amount px is in the organic and qx in the aqueous layer ($p + q = 1$). The partition coefficient K of the solute will then be given (assuming equal activity coefficients in the two solvents) by:

$$K = \frac{C_{org}}{C_{aq}} = \frac{px/V_{org}}{qx/V_{aq}} = \frac{pV_{aq}}{qV_{org}} \qquad \text{(Eq. 1)}$$

where C_i is the solute concentration in volume V_i of layer i.

If one further assumes that it is the aqueous solution which lends itself best to spectrophotometric measurement, then the basic partition coefficient measurement consists of taking an absorption measurement of the aqueous solution in a cell of length l_1 at a suitable wavelength (usually λ_{max} of the solute):

$$A_1 = \epsilon l_1 qx/V_{aq} \qquad \text{(Eq. 2)}$$

To obtain q from Eq. 2, it appears that it is necessary to know the extinction coefficient ϵ. This, however, may pose severe problems, particularly if the solute is not available in pure form. In addition, literature data on ϵ may not be applicable to the instrumental conditions at hand. To obviate the necessity of knowing ϵ one may, however, proceed as follows. Assume that the aqueous layer is removed after partitioning of the solute, fresh aqueous solvent of volume V_{aq} is added, new equilibrium established, and the absorption of the aqueous layer remeasured. Since an amount px remained in the organic phase this will now be divided as p^2x in the organic layer and pqx in the aqueous layer. Therefore:

$$A_2 = \epsilon l_2 pqx/V_{aq} \qquad \text{(Eq. 3)}$$

Elimination of ϵ from Eqs. 2 and 3, solution of the resulting equation for

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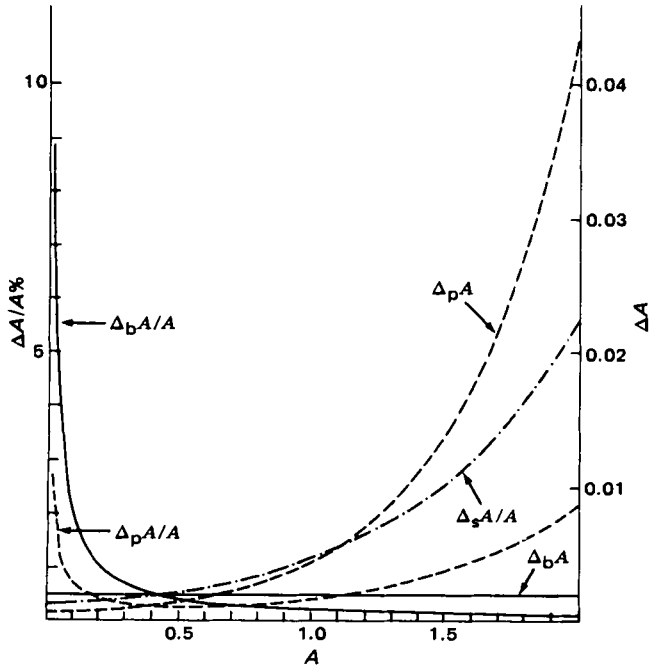

Figure 1—*Relative and absolute baseline absorbance errors based on* $\Delta_b A = 0.0020$, *relative and absolute photometric error based on* $\Delta_p T = 0.1\%$, *and relative stray light error based on* s = 0.003, *all as function of absorbance.*

p (and for $q = 1 - p$), and substitution into Eq. 1 gives:

$$K\frac{V_{org}}{V_{aq}} = \frac{A_2}{A_1 l_2/l_1 - A_2} = \frac{p}{q} \qquad \text{(Eq. 4)}$$

With this method there is the practical problem of removing all of the aqueous layer after the first equilibration. Even after centrifugation this is difficult to achieve, particularly since none of the organic layer should be removed in the process.

Experiments in our laboratory established that this problem was the one of the major causes of irreproducibility of K values determined with this method using Eq. 4. A considerable improvement can be obtained, however, by removing only a fraction, $(1 - f)V_{aq}$, of the aqueous layer. If, for example, V_{aq} was 5.00 mL only 4.00 or 4.50 mL was removed with a calibrated pipet, and the same volume of fresh solvent was added so as to restore the original volume, V_{aq}. Since an extra amount (fqx) of solute is left behind, Eq. 4 requires modification. It can be shown that for this method one has:

$$K\frac{V_{org}}{V_{aq}} = \frac{A_2 - fA_1 l_2/l_1}{A_1 l_2/l_1 - A_2} = \frac{p}{q} \qquad \text{(Eq. 5)}$$

A further possible improvement might be achieved by differential measurements. For example $A_1^o - A_2^o$ can be determined directly by measuring A_1^o relative to the A_2^o solution placed in the reference cell, both with cell length l^o. It is similarly possible to determine $A_2^* - fA_1^*$ directly, by diluting the A_1 solution $1/f$ times, and using this solution as the reference for the A_2 solution, with both cells having length l^*. Equation 5 can then be restated as:

$$K\frac{V_{org}}{V_{aq}} = \frac{(A_2^* - fA_1^*)/l^*}{(A_1 - A_2^o)l^o} = \frac{p}{q} \qquad \text{(Eq. 6)}$$

Sources of Error—*Optimization of* V_{org}/V_{aq} *and* f—A preliminary experiment should reveal the order of magnitude of K. As will be discussed below, the best results can be obtained when $KV_{org}/V_{aq} = p/q$ is between 1 and 10. If K is substantially different from this, V_{org}/V_{aq} should be adjusted to bring p/q into the proper range. For example, if $K = 50$ then a V_{org}/V_{aq} ratio of about 1:5 is in order. In practice, V_{org}/V_{aq} can be allowed to range between 1:5 and 5:1 without introducing any extra error. Beyond these ratios one needs careful mutual equilibration of the phases at a precisely controlled temperature so as not to cause appreciable volume changes due to changing mutual solubility of the solvents. With such precautions taken, ratios of 10:1 or even larger are quite practicable.

In general $f = 0.2$ is a good compromise for all circumstances. Not much can be gained by using smaller f values, because $A_2 - fA_1$ is almost insensitive to changes of f between 0.0 and 0.2. In practice the f factor is often determined by the choice of pipets employed.

Absorbance Errors—If it can be assumed that V_{org}/V_{aq}, l^*/l^o (or l_2/l_1), and f are accurately known, then the relative error in K is equal to that in p/q and is, therefore, determined by the errors in the absorbance measurements. The major sources of error are the photometric imprecision, $\Delta_p A$, the baseline error, $\Delta_b A$, and the stray light error, $\Delta_s A$. The photometric error function is given by (12):

$$\left(\frac{\Delta_p A}{A}\right)^2 = \left(\frac{0.434}{A}\right)^2 (1 + 10^{2A}) \left(\frac{\Delta I_0}{I_0}\right)^2 \qquad \text{(Eq. 7)}$$

where $\Delta I_0/I_0$ is the relative uncertainty of the signal strength in the reference beam; it is usually a constant for a given instrument. The validity of Eq. 7 is predicted on the condition that $\Delta I_0/I_0$ is determined by the detector and associated amplifier, and by implication that the contribution to $\Delta I_0/I_0$ of the readout device (meter, recorder, digital readout) is negligible in comparison. However, this condition must be fulfilled in any case; high damping and/or low gain may result in aesthetically more pleasing spectral curves or stable readouts, but the systematic errors incurred in this fashion usually are very large in comparison with the random error that results from allowing some "noise" to be visible. In Fig. 1 $\Delta_p A$ and $\Delta_p A/A$ as a function of A are given for a photometric precision $(\Delta I_0/I_0)$ of ±0.1%. The latter figure is typical for good instruments; for a low-price instrument this may be as high as ±1%. Figure 1 can actually be used for all $\Delta I_0/I_0$ values by using a different scale factor.

The baseline error $\Delta_b A$ is, in part, an instrument constant, but more often is determined by incomplete solvent compensation between the cells, variable scattering caused by the cells and their contents, variable amounts of impurities in solvents, blanks, or solutions, and the incomplete correction for the difference in absorption of the cells themselves. This type of error can be determined simply by measuring blanks relative to the same reference cell, and repeating this a number of times after emptying, refilling, and repositioning the sample cell. The average of these measurements gives the systematic baseline correction, which should be applied to all measurements, whereas the variation obtained gives the combined error:

$$\Delta_{pb} A = \sqrt{(\Delta_p A)^2 + (\Delta_b A)^2} \qquad \text{(Eq. 8)}$$

from which $\Delta_b A$ can be obtained if $\Delta_p A$ is known. The influence of $\Delta_b A/A$ as a function of A is also given in Fig. 1 for $\Delta_b A = 0.002$; for other $\Delta_b A$ errors, the corresponding errors can be found from Fig. 1 by using a suitable scale factor.

Differential measurements are often quoted (13) as giving much improved precision, because the exponent $2A$ in Eq. 7 is now replaced by $2(A_1 - A_2)$. However, this is only correct if the reference absorption is known beforehand in an absolute sense (by preparing standard solutions). Since this is something the present method deliberately has set out to avoid, the main advantage disappears; $(A_1 - A_2)$ has to be substituted for A in all places in Eq. 7 so that Eq. 7 is directly applicable. Some advantage may still accrue because $(A_1 - A_2)$ may correspond to a more favorable position on the error curve of Fig. 1 than A_1 or A_2 separately.

Scale expansion can similarly improve precision, particularly for high absorbances. For an n-fold scale expansion, the exponent $2A$ in Eq. 7 is replaced by $2(A - n/10)$. In practice it means increasing the detector amplifier gain n times when the sample is in the beam, but decreasing it to the normal level with the reference in the beam. It means also, however, that much wider slits must be used (by a factor of $n^{1/2}$), since otherwise the higher gain would result in an excessively high noise level, which would in part, if not entirely, offset the improvement in signal level. Since scale expansion is only helpful at high absorbance, stray light may then introduce a systematic error larger than the gain in precision (see below).

Stray light (*i.e.*, signal caused by light wavelengths other than the one selected) is a systematic error in that it always causes the apparent absorbance A_a to be smaller than the true absorbance A_t. If s is the fraction of unwanted stray signal (*i.e.*, $s = \Delta I_s/(I_0 + \Delta I_s)$ then the error $\Delta_s A$ is given by (12):

$$\frac{\Delta_s A}{A} = \frac{\log T_t - \log\{T_t(1 - s) + s\}}{A} \qquad \text{(Eq. 9)}$$

where T_t is the true transmittance. Figure 1 gives $\Delta_s A/A$ for $s = 0.003$ (0.3%). Such error curves for different s values are only approximately different by a scale factor. As can be seen, $\Delta_s A/A$ can be very large, even for low values of s. The error curve given applies only to individual total absorbances including the absorbance of solvent and cell. For differential

measurements the $\Delta_s A$ errors of the two absorbances have to be determined separately first and then subtracted.

Influence of Absorbance Errors on K—$\Delta_p A$ and $\Delta_b A$, being random errors, can be combined for any measurement by applying Eq. 8. If Eq. 5 is applicable, then one has for the uncertainty in K, as caused by absorbance errors:

$$\left(\frac{\Delta_{pb}K}{K}\right)^2 = \frac{(\Delta_{pb}A_2)^2 + (\Delta_{pb}A_1 f l_2/l_1)^2}{(A_2 - A_1 f l_2/l_1)^2} + \frac{(\Delta_{pb}A_2)^2 + (\Delta_{pb}A_1 l_2/l_1)^2}{(A_1 l_2/l_1 - A_2)^2} \quad \text{(Eq. 10)}$$

If differential measurements are made both on the numerator and denominator of Eq. 6, one has:

$$\left(\frac{\Delta_{pb}K}{K}\right)^2 = \frac{[\Delta_{pb}(A_2^* - fA_1^*)]^2}{(A_2^* - fA_1^*)^2} + \frac{[\Delta_{pb}(A_2^o - A_1^o)]^2}{(A_1^o - A_2^o)^2} \quad \text{(Eq. 11)}$$

If only $(A_2 - fA_1)$ or $(A_1 - A_2)$ is measured differentially, suitable changes in Eqs. 10 and 11 need to be made.

The error in K due to stray light is given by $\Delta_s K = K_a - K_t$, where the apparent partition coefficient K_a is determined using Eq. 5 or 6, with the apparent absorbances A_a, and where the true partition coefficient K_t is similarly related to the true absorbances. Since $\Delta_s A$ is a systematic error, it can be measured, at least in principle; appropriate corrections can then be made *via* Eq. 9.

Influence of Volumetric Errors Δ_vK—In the experimental procedure discussed herein (see *Experimental*), there are four volumetric transfers involved. Each of these carries a possible error, which influences the value of K through the volumetric parameters V_{aq}, V_{org}, and f (Eqs. 5 and 6). Following the recommended experimental procedure and applying standard rules for the propagation of random errors it can be shown that:

$$\left(\frac{\Delta_v K}{K}\right)^2 = \frac{A_2^2[(\Delta V_{aq})^2 + (\Delta V'_{aq})^2 + (\Delta V''_{aq})^2] + A_1^2[(\Delta V_{aq})^2 + (\Delta V'_{aq})^2]}{(A_2 - fA_1)^2 V_{aq}^2} + \frac{(A_1^2 + A_2^2)(\Delta V_{org})^2}{(A_1 - A_2)^2 (V_{org})^2} \quad \text{(Eq. 12)}$$

where V'_{aq} and $V''_{aq} = (1 - f)V_{aq}$ are the removed and replenished volumes of aqueous solvent, respectively.

If all volume errors (ΔV) are equal, a substantial simplification results:

$$\left(\frac{\Delta_v K}{K}\right)^2 \simeq \frac{(3A_2^2 + 2A_1^2)(\Delta V)^2}{(A_2 - fA_1)^2 V_{aq}^2} + \frac{(A_1^2 + A_2^2)(\Delta V)^2}{(A_1 - A_2)^2 V_{org}^2} \quad \text{(Eq. 13)}$$

On substitution of $A_2 = (p + qf)A_1$, this further reduces to:

$$\left(\frac{\Delta_v K}{K}\right)^2 = \frac{2 + 3(p + qf)^2}{p^2(1 - f)^2}\left(\frac{\Delta V}{V_{aq}}\right)^2 + \frac{1 + (p + qf)^2}{q^2(1 - f)^2}\left(\frac{\Delta V}{\Delta V_{org}}\right)^2 \quad \text{(Eq. 14)}$$

Eq. 14 shows that $\Delta_v K$ is fundamentally independent of the absorbances. It also shows why accurate K measurements are limited to a K range of 10 to 0.1; beyond these limits either of the denominator terms $p^2V_{aq}^2$ or $q^2V_{org}^2$ becomes very small. For example, if K is very large one needs a large V_{aq}/V_{org} ratio to keep $(A_1 - A_2)$ accurately measurable. Since the largest volume V_{aq} is limited by the physical dimensions of extraction and centrifugation equipment, a small V_{org} will have to be taken, combined with a small q value. The same reasoning applies for very small K values, with the roles of V_{aq} and V_{org} and of p and q interchanged.

Numerical Results—The implications of Eqs. 10–14 will be considered for three typical cases: $p/q = 10$, $p/q = 1.0$, and $p/q = 0.10$. The combined absorbance and volumetric errors in K can be calculated according to:

$$\left(\frac{\Delta K}{K}\right)^2 = \left(\frac{\Delta_{pb}K}{K}\right)^2 + \left(\frac{\Delta_v K}{K}\right)^2 \quad \text{(Eq. 15)}$$

The Case of p/q = *10*—Only a small fraction of the solute is extracted into the phase to be measured, and A_1 and A_2 will be fairly similar since $A_2 = l_2(10 + f)A_1/l_1$. For that reason equal cell lengths ($l_1 = l_2$) are used. K is likely to be >10 so that a ratio of V_{aq}/V_{org} larger than unity must be assumed to have been used to reduce p/q as much as possible. Typical volume parameters for this case are $V_{aq} = 8$ mL, $V'_{aq} = V''_{aq} = 6.4$ mL (*i.e.*, $f = 0.2$), and $V_{org} = 2$ mL. Most likely Mohr pipets have to be used throughout for which an average error of $\Delta V = \pm 0.012$ mL (see *Results*) can be taken. The first term of Eq. 12, 13, or 14 is negligible, and the second term gives a constant error of $(\Delta_v K)/K = 11.2\%$. With slightly different volume parameters (such as $V_{aq} = 10$ mL, $V'_{aq} = V''_{aq} = 8$ mL, and $V_{org} = 2$ ml), volumetric pipets could be used which have a typical error of $\Delta V = \pm 0.007$ mL. This would reduce $\Delta_v K/K$ to 7.1% (Fig. 2).

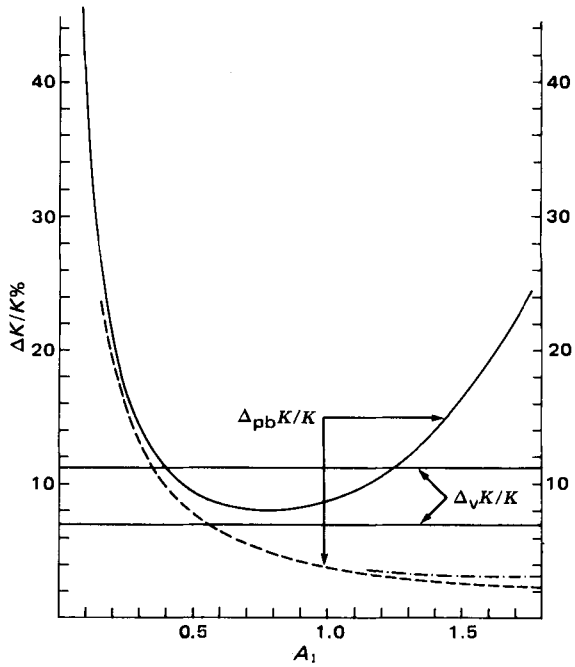

Figure 2—*Theoretical relative error in the partition coefficient* (K) *for the case* KV_{org}/V_{aq} = p/q = *10. Calculations are based on* $\Delta_b A$ = *0.0020,* $\Delta_p T$ = *0.1%,* f = *0.2, and* $l_1 = l_2$. *Calculations of* Δ_{pb}K/K *are for* A_1 *and* A_2 *separate (—);* A_1 *and* fA_2 *separate, but* $A_1 - A_2$ *measured in differential mode (- - - -); and for* A_1 *and* A_2 *measured with 10× scale expansion (- · - · -).* Δ_vK/K *is given for* ΔV = *0.012 mL (upper line) and* V_{org} = *2 mL,* ΔV = *0.007 mL (lower line). Total error* ΔK/K *can be calculated using Eq. 15.*

Figure 2 also shows the absorbance-related errors $\Delta_{pb}K$ based on $\Delta_b A = 0.0020$ and a photometric error of $\Delta_p T = 0.1\%$. It is seen that for $A_1 < 0.3$ the error in K is dominated by absorbance error, in particular $\Delta_b A$, whereas for $0.3 < A_1 < 1.0$ the $\Delta_p K$, $\Delta_b K$, and $\Delta_v K$ are of approximately the same magnitude. Differential measurement of $A_1 - A_2$ is only beneficial at $A_1 > 0.8$. Scale expansion measurement of A_1 and A_2 appears to give a similar improvement, but since this technique assumes that the scale expansion factor is errorless, the differential $(A_1 - A_2)$ technique should be preferred. The best overall result with $\Delta K/K = 8\%$ is obtainable for $1.0 < A_1 < 1.5$, using volumetric pipets and differential $(A_1 - A_2)$. No gain in accuracy is attainable at higher absorbance; $\Delta K/K$ is dominated by the constant $\Delta_v K/K$ term, and one would additionally run the risk of considerable stray light error.

The Case of p/q = *1*—A number of subcases arise in this situation. $K = 4$ with $V_{aq} = 8$ mL, $V_{org} = 2$ mL, and $V'_{aq} = V''_{aq} = 6.4$ mL requires Mohr pipets with $\Delta V = \pm 0.012$ mL. The second term in Eq. 14 is still dominant and $\Delta_v K/K = 1.9\%$. For $K = 1$, $V_{aq} = V_{org} = 5$ mL, $V'_{aq} = V''_{aq} = 4$ mL, and $\Delta V = 0.007$ mL, one finds $\Delta_v K/K = 1.2\%$, whereas for $K = 0.25$ with $V_{aq} = 2$ mL, $V_{org} = 8$ mL, and $V'_{aq} = V''_{aq} = 1.6$ mL with $\Delta V = \pm 0.012$ mL, one has $\Delta_v K/K = 2.5\%$. In either of these cases, however, the effect of volumetric errors on K is much smaller than with $p/q = 10$. The absorbance-caused errors in K are also substantially reduced because both p and q are relatively large ($p = q = 0.5$). Since A_2 is now about half that of A_1 (for $l_1 = l_2$), the use of a longer cell for A_2 ($l_2 = 2l_1$) might be contemplated. As Fig. 3 indicates this would indeed decrease $\Delta_{pb}K$ substantially at low A_1, but this improvement is illusory since one must assume that in practice a low A_1 is encountered despite having already used the longest available cells. Differential measurement of $(A_1 - A_2)$ is still of noticeable advantage, particularly at $A_1 > 1$, although the gain in precision is less than with $p/q = 10$. The optimum result will be around $\Delta K/K = 1.5\%$ and can be achieved at high A_1 ($1.0 < A_1 < 1.6$) with $(A_1 - A_2)$ in differential mode, provided $K = 1$ and provided all transfers can be done with volumetric pipets. Provided A_1 is at least 0.4, a precision of $\Delta K/K$ of 4% is always attainable. Scale expansion is of little use; it can be applied only to A_1 (because A_2 will always be smaller than unity); in Fig. 3 it would show predicted errors intermediate between those for A_1 and A_2 separately ($l_1 = l_2$ and without scale expansion) and for $(A_1 - A_2)$ in differential mode. Calculations show that an additional improvement results if $(A_1 - fA_1)$ is also measured differentially; but, this is almost quantitively balanced by a loss in precision due to the extra dilution required.

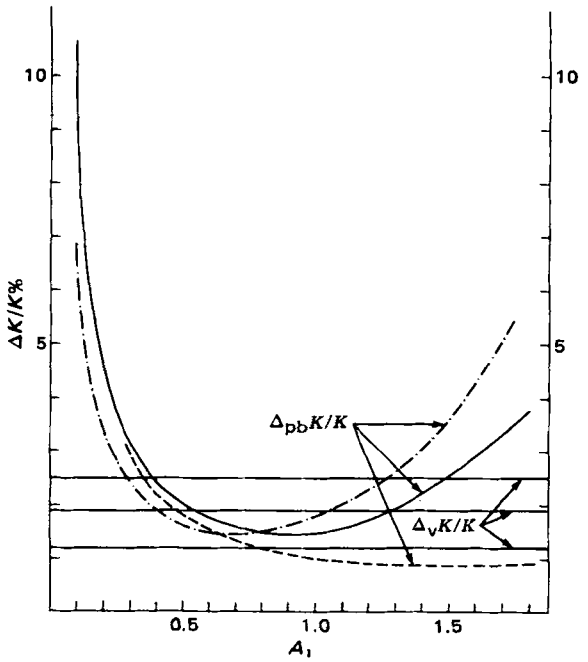

Figure 3—*Theoretical relative error in the partition coefficient (K) for the case* KV_{org}/V_{aq} = p/q = *1. Calculations are based on* $\Delta_b A$ = *0.0020,* $\Delta_p T$ = *0.1%, and* f = *0.2. Calculations of* $\Delta_{pb}K/K$ *are for* A_1 *and* A_2 *measured separately combined with* $l_2 = l_1$ *(—) and* $l_2 = 2l_1$ *(- · - · -), as well as for* $A_1 - A_2$ *measured in differential mode (- - - - -).* $\Delta_v K/K$ *is given for* V_{org} = *8 mL,* V_{aq} = *2 mL, and* ΔV = *0.012 mL (upper line), for* V_{org} = *2 mL,* V_{aq} = *2 mL, and* ΔV = *0.012 mL (middle line), and for* $V_{org} = V_{aq}$ = *5 mL and* ΔV = *0.007 mL (lower line). Total error* $\Delta K/K$ *can be calculated using Eq. 15.*

The Case of p/q = *0.1*—The phase to be measured has the smaller volume on this case. This, combined with the facts that p is also small and that the extra transfers required for the A_2 measurement are carried out on the small volume V_{aq}, means (Eq. 14) that the volumetric error $\Delta_v K/K$, even under the most careful experimental conditions will be very large and will dominate the total error (except for $A_1 \leq 0.2$). If accurate K values are required, all volumes need to be determined by weighing. If this is done, $\Delta_v K/K$ will be <1%, and the absorbance errors will become dominant. The absorbance A_1 is now much larger than A_2; as a result $(A_1 - A_2)$ is large, but $(A_2 - fA_1)$ is small so that the second term in Eq. 10 or 11 becomes negligible. There is, thus, no point in using a differential $(A_1 - A_2)$ measurement. Although A_2 and fA_1 are now of approximately the same magnitude, a differential measurement of $(A_2 - fA_1)$ does not give any improvement; on the contrary, careful analysis of the first terms of Eqs. 10 and 11 shows that for corresponding cell lengths (*i.e.*, $l^* = l_2$), the differential measurement of $(A_2 - fA_1)$ will, in fact, always produce larger errors. As Fig. 4 shows, some gain in precision can be obtained if l_2 is larger than l_1 at least for small A_1. Again, this is generally not a practical advantage: if A_1 is small, it will have to be measured in the largest available cell. Also, since V_{aq} is small there may not be enough liquid volume available to fill large cells. Without scale expansion, a best $\Delta_{pb}K/K$ of 3–4% can be obtained for $0.8 < A_1 < 1.6$. Scale expansion is useful in all cases where $A_1 > 1.0$, but to take full advantage of the technique, the l_2/l_1 ratio should be taken such that $A_1 = A_2$, so that both can be measured with scale expansion. Not only can $\Delta_{pb}K/K$ then be reduced to ~1%, but in addition, the stray light errors almost cancel if $A_1 = A_2$.

EXPERIMENTAL

A single-beam manual spectrophotometer of the electrical compensation type was used throughout[1]. It was equipped with one quartz glass prism and with a photomultiplier as detector. The slit width was set at 0.15 mm at λ = 239 nm; at other wavelengths, corresponding equal-energy slit widths were employed. Plastic-stoppered quartz glass cells of 10, 20, and 40 mm were used; the cell compartment was thermostated at 25°C. All photometer readings were repeated six times, and the average reading

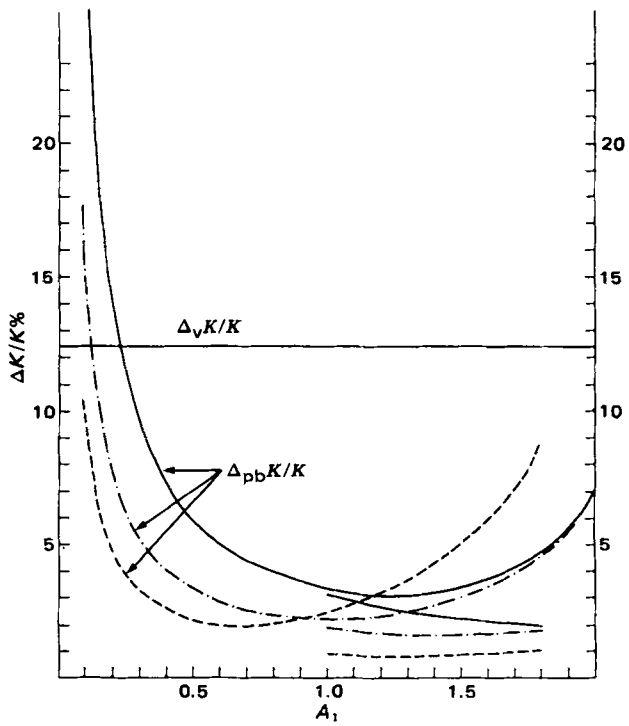

Figure 4—*Theoretical relative error in the partition coefficient (K) for the case* KV_{org}/V_{aq} = p/q = 0.1. Calculations of $\Delta_{pb}K/K$ *are based on* $\Delta_b A$ = *0.0020,* $\Delta_p T$ = *0.1%,* f = *0.2,* A_1 *and* A_2 *measured separately and for cell lengths* $l_2 = l_1$ *(—)* $l_2 = 2l_1$ *(- · - · -), and* $l_2 = 4l_1$ *(- - - - -); the extra curves for* $A_1 > 1.0$ *refer to the corresponding cell lengths ratios, but with a 10× scale expansion.* $\Delta_v K/K$ *is calculated for* V_{aq} = *2 mL and* ΔV = *0.005 mL. Total error* $\Delta K/K$ *can be calculated using Eq. 15.*

was calculated. The aniline absorbances were determined at λ = 281 nm. The instrument was equipped with a 10× scale expansion switch for measurements above A = 1.0.

A weighed amount of aniline hydrochloride was placed in a volumetric flask and dissolved in ammonia, the latter being presaturated with chloroform (IR spectrometric quality; *i.e.*, without ethyl alcohol) having a pH of 10.5. By adding ammonia (pH 11.5) the solution was brought to pH 10.5. To a standard 15-mL centrifuge tube[2] the appropriate volumes of aqueous solution and presaturated chloroform were added. The same volumes of chloroform and ammonia solvents were added to a second centrifuge tube to form the blank. Wherever necessary (particularly with the larger cells) two centrifuge tubes each for the solution and the blank were used. Plastic stoppers, designed specifically for this purpose with sides machined in such a way as to leave only three raised flanges in contact with the glass, were fitted into the centrifuge tubes ~2 cm. This guaranteed a tight fit with a minimum of liquid being trapped between the stopper and the glass wall of the centrifuge tube. The stoppered tubes were hand shaken for ~10 s, the stopper was then released, and the two phases were allowed to settle; this procedure was repeated three times. It was observed that with a 1:1 ratio of V_{aq}/V_{org} the two-phase system settled very quickly, whereas with other volume ratios a larger proportion of very fine droplets formed, requiring a much longer settling time. (These small droplets are more efficient in the extraction process as is evident from the results; with hindsight it can now be said that the above described extraction-by-shaking procedure is not adequate for volume ratios close to 1:1.) The tubes were then centrifuged at 2400 rpm for ~1 min. The second extraction (for the A_2 solution) was done in a similar fashion. Where possible, volumetric pipets were used, but for some volume ratios and for most removals and replenishing of volumes V'_{aq} and V''_{aq}, Mohr pipets were required. The extraction and centrifugation were carried out at room temperature (24–26° C).

RESULTS AND DISCUSSION

Error Determination—*Determination of the Photometric Error* $\Delta_p A$—Standard solutions of potassium chromate in 0.05 M NaOH, measured at the absorption maximum of ~230 nm, were used. Sixteen

[1] Unicam SP-500 series 2.

[2] Pyrex.

Table I—Random Baseline Absorbance Error $\Delta_b A$

Cell Length, mm	Number of Data Sets	Average Standard Error $\Delta_b A$
10	16	±0.0020
20	12	±0.0019
40[a]	9	±0.0043
40[b]	6	±0.0024

[a] Free-standing cell. [b] Positioned with spacer.

Table II—Random Errors of Volumetric Experiments

Type of Pipet	Average Standard Deviation ΔV, ml
1-mL Volumetric	0.0026
2-mL Volumetric	0.0038
5-mL Volumetric	0.0065
10-mL Volumetric	0.0090
2-mL Mohr	0.0057
5-mL Mohr	0.0079
10-mL Mohr	0.0110

different concentrations were used covering a range of 0.5–98% T. At each concentration six readings were taken, and the standard error was calculated. It was found that there was no correlation between these standard errors and the $\%T$, meaning that the photometric error $\Delta I/I$ is independent of $\%T$. The average of the individual standard errors was 100 $\Delta I/I = 100\ \Delta I_0/I_0 = 0.087\%\ T$. In all further calculations as well as in Figs. 1–4 this $\%T$ photometric error was rounded off to 0.1% T.

The scale readability standard error was found to be ~0.04% T so that Eq. 7 is still applicable for the calculation of $\Delta_p A$ or $\Delta_p A/A$; the latter are given in Fig. 1. For other instruments or for the same instrument under different energy conditions, the $\Delta_p A/A$ curve of Fig. 1 can be used by applying a simple scale factor; this is correct because of the proportionality between $\Delta_p A/A$ and $\Delta I_0/I_0$ (Eq. 7).

Determination of the Baseline Error Δ_bA—Systematic baseline errors were taken care of by running a blank sample (*i.e.*, one that did not contain the absorbing test compound, but was otherwise treated in identical fashion through the extraction and equilibrium procedures) with each test sample and by measuring its absorbance in the same cell as the test sample relative to the same reference cell. By subtracting the absorbance of the blank from that of the corresponding test sample, all absorbances were corrected for systematic differences between the measurement and reference cells, including their differing nonsolute contents.

There remains, however, the variability in the baseline correction, which is of a random nature and is caused by such things as variation in cell cleaning, drying efficiency, centrifugation efficiency, sample cleanliness (small particles, including small $CHCl_3$ bubbles, cause scattering and hence increase the apparent absorbance), and solvent composition. The determination of K for aniline automatically provided us with the necessary data. Since each K value was determined at least six times, the standard deviation in the baseline correction could be calculated from each six-fold set of absorbance measurements. These individual standard errors varied slightly, but their average value is thought to be a good measure of $\Delta_b A$ (Table I). Whereas the 10- and 20-mm cells were positioned with copper blade springs, the cell holder employed did not have the space to provide such a spring for the 40-mm cells. As Table I shows, tight cell positioning is necessary, as was found when a spacer was subsequently constructed to fit the 0.5-mm free space between the 40-mm cells and the cell holder rear wall. With this modification installed it appears that $\Delta_b A$ is virtually independent of cell length. In this case, therefore, the important contributor to $\Delta_b A$ can only have been variations in the cell cleaning efficiency. A value of $\Delta_b A = 0.0020$ has hence been taken for all further calculations (except for those referring to the free-standing 40-mm cells) and for Figs. 1–4.

The baseline variability $\Delta_b A$ is clearly an important source of error; it should be determined in conjunction with any absorbance measurements. The above determined baseline error also includes, in principle, the photometric error $\Delta_p A$. However, with $\Delta I_0/I_0 = 0.1\%$, the error $\Delta_p A$ is only equal to 0.0004 at the 99% T level which is relevant to the baseline measurements. This, however, is negligible compared with $\Delta_{pb} A = 0.0020$ in view of the quadratic progression of errors as given in Eq. 8.

Determination of the Scale Expansion Factor—On a total of 16 test samples with absorbance just over unity, the absorbance was measured (six times) with and without the 10× scale expansion. Both the average scale factors and their standard errors were then used to determine a weighted overall average and the overall uncertainty. It was found that the real scale factor was 10.11 (±0.02) which corresponds in absorbance to a scale term $\Delta A = 1.0047 \pm 0.0019$. Therefore, 1.0047 rather than unity was added to all absorbances measured with the 10× scale factor. Our impression is that the scale factor is not quite constant in time (presumably due to changes in contact resistance in the switch). The error given above includes both the photometric error and the possible nonconstancy of the scale factor itself.

Determination of the Stray Light Error Δ_sA—Since the chromate ion is known to accurately follow Beer's law (12), the stray light factor can be determined from an observed apparent nonlinearity of absorbance *versus* concentration (Eq. 9). Thirteen standard solutions of potassium

Table III—Partition Coefficient of Aniline in Chloroform–Ammonia at 25° C and its Experimental and Theoretical Standard Deviation as a Function of Experimental Conditions

	V_{aq}, mL	$V'_{aq} = V''_{aq}$, mL	V_{org}, mL	p/q	f	l_1, mm	l_2, mm	A_1	K	Standard Error, % Experimental	Standard Error, % Theoretical
1	5	4	5	20	0.2	10	10	0.10	22.4	62	79
2	6	4	6	20	0.33	10	20	0.10	16.6	46	63
3	10	8	10	20	0.2	10	40	0.10	14.3	41	63[a]
4	10	8	10	20	0.2	20	40	0.21	12.95	31	41[a]
5	10	8	10	20	0.2	20	20	0.19	12.9	25	35
6	10	8	10	20	0.2	40	40	0.38	12.4	53	44[a]
7	6	4	3	10	0.33	10	10	0.12	20.2	15	44
8	6	4.5	3	10	0.25	10	10	0.86	21.1	7.9	9.3
9	6	4.5	3	10	0.25	10	10	1.25	20.2	14.1	12.3
10	6	4.5	3	10	0.25	10	10	1.25[b]	20.8	12	5.7
11	6	4.5	3	10	0.25	10	10	1.35	18.9	9.7	14
12	6	4.5	3	10	0.25	10	10	1.35[b]	19.5	7.3	5.7
13	6	4.5	3	10	0.25	10	10	1.35[c]	19.5	6.4	5.4
14	6	4.5	3	10	0.25	10	10	1.75	22.5	28	26
15	6	4.5	3	10	0.25	10	10	1.75[b]	21.6	16	5.3
16	6	4.5	3	10	0.25	10	10	1.75[c]	25.4	7.3	5.8
17	6	4	3	10	0.33	10	20	0.13	18.2	30	28
18	12	9	6	10	0.25	10	40	0.16	19.2	33	24[a]
19	12	9	6	10	0.25	20	20	0.23	17.4	21	20
20	12	9	6	10	0.25	20	40	0.34	17.9	20	14[a]
21	12	9	6	10	0.25	40	40	0.50	26.2	15	19[a]
22	8	6	2	5	0.25	10	10	0.21	21.8	15	24
23	8	6	2	5	0.25	10	20	0.13	24.3	18	19
24	16	12	4	5	0.25	10	40	0.09	19.3	8.8	19[a]
25	16	12	4	5	0.25	20	20	0.21	21.0	6.1	12.3
26	16	12	4	5	0.25	20	40	0.24	20.2	81	10.3[a]
27	16	12	4	5	0.25	40	40	0.50	22.0	8.9	10.5[a]

[a] Calculated with $\Delta_b A$ (40 mm) = 0.0043 (Table II). [b] Scale expansion employed on A_1 and A_2. [c] Differential measurement on $(A_1 - A_2)$.

chromate in 0.05 M NaOH were prepared by dilution and weighing from a 0.003 M stock solution. In the conversion to mol/L units, corrections were applied for the varying density of the chromate solutions. Concentrations were such as to give absorbances at 230 nm in 10-mm cells ranging from 0.25 to 2.0. Where applicable (*i.e.*, $A = >1.0$) measurements were taken with and without 10-fold scale expansion. All readings were made sixfold, and at each concentration several completely separate measurements were made: 3 at absorbances ≤ 0.8, 6 between 0.9 and 1.8, and 12 for absorbances >1.8. For each concentration the average (baseline corrected) absorbance and its standard deviation were determined. With these data points a least-squares best straight line through the origin was fitted, with each point weighted according to the inverse of its relative standard error. With this best slope, absorbances at each of the actual concentrations were calculated and compared with those obtained experimentally. As it turned out each experimental point was on this straight line well within twice its own standard error, without any systematic deviation from linearity being apparent. Consideration of the standard errors in conjunction with Eq. 9 showed that the stray light must have been $\leq 0.03\%$. Stray light is wavelength dependent, but this result is likely correct for 220–300 nm. With other instruments or at wavelengths near the limit of the wavelength range of the instrument a much more severe stray light effect is possible. In all further calculations $s = 0.0003$ was used.

Volumetric Errors—These were determined experimentally by weighing and transferring water by pipet (for V_{aq} and V'_{aq}) and chloroform (for V_{org}) into an Erlenmeyer flask. For V'_{aq} water was transferred by pipet from out of an Erlenmeyer flask. The results are given in Table II. Each reported error ΔV is the average of at least four standard deviations, with each of the latter determined on six separate weighing experiments. There was no significant difference between water or chloroform.

Experimental Tests—The partition coefficient of aniline at 25°C and pH 10.5 was determined under a wide range of experimental conditions to test the theoretical error model developed herein (Table III). Each K value and its experimental standard deviation was determined from at least six independent determinations. The theoretical standard deviation was calculated according to Eqs. 10–15 with the numerical error data as given in the present section. Comparison of the experimental and theoretical standard errors indicates that, apart from the unavoidable statistical scatter, the agreement is good. In fact, the average ratio s(experimental)/s(theoretical) is 1.03, which proves that the error model is basically correct.

As predicted, the precision improves generally if p/q is lowered from 20 to 10 to 5 by changing the V_{aq}/V_{org} ratio. Also, as A_1 increases from 0.1, the precision first improves and then deteriorates (at high A_1), generally following the trend indicated by Fig. 2. Scale expansion on A_1 and A_2 does improve the precision, although not as much as predicted. This lends further credence to the aforementioned possibility that the scale factor is perhaps not constant. Differential ($A_1 - A_2$) measurement improves the precision approximately as predicted. Varying cell length has no effect other than that of varying the absorbances. Table III contains some data for $l_1 \neq l_2$. This, in itself, is not necessary for the $p/q = 10$ case; but, it is interesting to note that for, say $l_1 = 10$, $l_2 = 20$ mm, the resulting error (both experimental and theoretical) is intermediate between those for $l_1 = l_2 = 10$ mm and those for $l_1 = l_2 = 20$ mm. Several other such combinations yielded the same result.

The average K value for the $V_{aq}/V_{org} = 4$ case is 21.4 ± 1.7 and for the $V_{aq}/V_{org} = 2$ case, $K = 20.6 \pm 2.5$; for $V_{aq}V_{org} = 1$, $K = 15.2 \pm 4.9$. The decreasing K value with associated increase in standard error reveals a systematic error which was identified as being due to incomplete equilibration. Particularly a 1:1 chloroform–water system does not mix well; the phases separate immediately and completely when shaking is halted. With other mixing ratios one observes after shaking a mist of fine droplets in both phases, which clears up only slowly. Clearly, for $V_{aq} = V_{org}$ a much longer shaking period is required.

CONCLUSIONS

The above described technique and its error analysis indicates that K values ranging from 100 to 0.01 can be measured with an accuracy of $\geq 10\%$; in the K range of 10–0.1 an accuracy of 2% should be attainable. Several provisos must be made, however. First, the substrate of interest must have a reasonably strong absorption band in the UV or visible light region. For an extinction coefficient of ~10,000 (such as the p-band of aromatics in the 200–300 nm region) this translates to a requirement of ~1–5 mg if $K = 100$ and 0.01–0.05 mg if $K < 1$. The sample material need not be pure; impurities will cause erroneous results only if the following four conditions apply simultaneously: (*a*) the partition coefficient of the impurity is equal to or smaller than that of the compound of interest, (*b*) the impurity in question is present at a considerable level (*i.e.*, at least 10%), (*c*) the impurity has an extinction coefficient at the wavelength of maximum absorption of the compound of interest which is comparable in magnitude with that of the latter, and (*d*) the impurity has solubility in the two-phase system equal to or higher than that of the compound of interest. Second, to obtain accurate K values, careful attention has to be paid to the experimental conditions. Order-of-magnitude errors in the determination of K can easily occur if volumes, volume ratio, cell length, cell length ratio, initial absorbance, and photometric technique (separate, differential, and/or scale expansion) are not optimally chosen. In this regard, it is important to know the error characteristics of the photometric and volumetric equipment to be used. A final limitation to the use of this method may be posed by very low solubility of the substrate in either solvent. However, the method described is not limited to chloroform–ammonia. Any solvent pair will do, provided that at least one solvent is transparent at the wavelength to be used.

In applying this method there is no need for running multiple determinations. It may still be advisable to run duplicates in order to spot obviously erroneous results, but if an estimate of the accuracy of the partition coefficient is required, this can be calculated from the equations given in this paper. This is possibly even better than using experimental standard deviations, because the latter are not a reliable measure of imprecision for small numbers of repeated experiments.

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