

Drug Partitioning I

Nonemulsifying Method for Measuring Distribution Coefficients

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A simple rocking apparatus has been constructed for routine determination of distribution coefficients. With this apparatus, up to 36 two-phase samples in cylindrical tubes are equilibrated by rocking the horizontal tubes at 1 c.p.m. (cycle per minute) through an arc of 45°. This rocking causes the interface between the two immiscible phases to expand and contract slowly; it also causes the shape of each phase to vary constantly. These two actions facilitate uniform distribution of solute within each phase and facilitate drug transfer from one phase to the other. Emulsion formation is negligible since little turbulence is created. Considerable man hours are saved with some pharmaceutical systems if the rocking method, rather than the usual shake-out method, is used. The experimental method is simple and, where shake-out methods can be employed, gives results which are in agreement with results from shake-out methods. Data for the distribution of ephedrine, chlorpromazine and its salts, several benzoic acid derivatives, hexachlorophene, and bithionol are presented. Pharmaceutical applications of partitioning are discussed.

DETERMINING WITH convenience the partition coefficient or distribution character of drugs and related substances is becoming increasingly important in pharmacy and medicine. Beginning with Meyer (1) and Overton (2), in 1899, early workers (3-7) showed that the *degree* of biological action of many drugs is influenced by their oil/water partition coefficients. More recent workers (8-10) have shown that the *onset* and *duration* of action may also be affected by this property.

Brodie and co-workers (9, 11-13) and Nogami and co-workers (14-16) have shown that many compounds are absorbed from the gastrointestinal tract in their undissociated form. They have further shown that absorption may be related to the partition coefficient of the drug. Drug absorption from the oral cavity (17, 18) or through the skin (19, 20) is also influenced by the partition coefficient. Several authors (9, 21-23) have shown that the drug partitioning which occurs between various tissues and fluids is influenced by the drug's partition coefficient. Finally, it has been shown that metabolism in the liver (24) and renal tubule

transfer (25) are influenced by the drug's pKa and partition coefficient.

While there are these numerous references to the role of partitioning in the biological action of drugs, the references to partitioning in the pharmaceutical literature are mostly limited to drug analysis (26-29). Pharmacists should, however, be aware of not only the biological role of drug partitioning, but also of drug partitioning within dosage forms and between dosage forms and body fluids. Partitioning data have been used in the design of pharmaceutical dosage forms by Garrett and Woods (30) and Hibbott and Monks (31) in their studies of preservatives for emulsions, and by Lachman, *et al.* (32), in their studies of preservatives for multidose parenterals. A relationship exists between the pharmacologic action and partition coefficients of many drugs (33-37), but apparently few, if any, deliberate attempts have been made to alter a drug's partition coefficient in a predictable manner. In 1944, Walton (18) did suggest the synthesis of salts and other derivatives with partition coefficients favorable to sublingual absorption. More recently, Collander (38) has made a thorough attempt to correlate partition coefficient, chemical structure, and solvent nature. It will be shown in the present study, for example, that two salt forms may have different apparent partition coefficients—a fact which may be important in formulation.

Emulsion taste, drug chemical stability, and drug

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release from emulsions, ointments, or suppositories (39) are often dependent on the amount of drug in the aqueous phase or on the ability of the drug to partition from a nonaqueous phase. In preparing emulsions of amines, for example, use of the amine base and a slightly alkaline aqueous phase should keep virtually all of the amine in the nonaqueous phase and thereby improve the taste of the emulsion. Diffusion from ointment to the skin may depend on a favorable aqueous phase pH so that the drug can partition from the nonaqueous phase.

Most two-phase pharmaceutical products are complex, *i.e.*, they contain several components in each phase. Many opportunities, therefore, exist for one component to influence the partition coefficient of another component. Drugs, antioxidants (40), flavoring agents, and preservatives can influence the apparent partition coefficients of one another. The partition coefficients of these substances can also be influenced by more inert components, such as electrolytes and macromolecules. For this reason, it is important to study partitioning in the complete formulation, even if data from simple formulations predict satisfactory results.

From such studies, it is clear that simple uncomplicated procedures for the routine determination of the solubilities and partition coefficients of compounds being screened for therapeutic activity would be useful.

Although a number of methods have been reported for determining partition coefficients (41-46), the one in common use is the shake-out method using separators. A simple rocking apparatus developed for two-phase systems, described in this report, overcomes or reduces the emulsification and foaming problems of the shake-out method and also avoids the possibility of anomalous equilibria as reported by Allen and McDowell (46). The second paper in this series (47) discusses the use of an inverted Y-tube apparatus to study partitioning of drugs from one aqueous phase through a nonaqueous phase into a second aqueous phase (*i.e.*, partitioning under simulated absorption conditions).

In this paper we will (*a*) present a brief review of partition coefficient theory, (*b*) describe an apparatus devised for routine measurement of drug distribution between liquid phases, (*c*) report some of the parameters studied, and (*d*) describe some of the applications evaluated.

THEORETICAL

If a solute is introduced into a system containing two immiscible or slightly miscible liquids, it will distribute itself between the two liquids until a definite equilibrium concentration ratio is attained. The distribution law requires that this ratio, at constant temperature, be a constant, regardless of the total quantity of solute present, *i.e.*,

$$\frac{C_1}{C_2} = K \quad (\text{Eq. 1})$$

where C_1 and C_2 are the solute concentrations in the two liquid phases and K is the distribution or partition coefficient.¹

This simple distribution law is exact only for ideal

¹ Although the distribution law does not refer to the composition of the phases, the less polar phase is usually assigned the designation C_1 and placed in the numerator. This convention will be followed in this report.

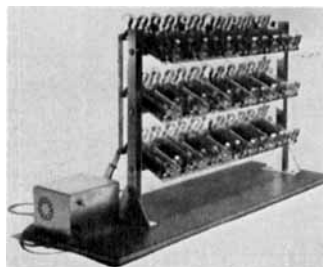


Fig. 1.—Photograph of the rocking device.

solutions. It is most closely approximated (*a*) when the liquid phases are completely immiscible, (*b*) when the solute neither associates nor dissociates in either phase, (*c*) when solute concentrations are relatively dilute, and (*d*) when the solute is only slightly soluble in either phase. When molecular association or other deviations from ideality occur, suitable modifications often can be applied to the distribution law to maintain a coefficient which is constant. Thus, for example, if a solute exists as undissociated single molecules in phase 2 and as a bimolecular species in phase 1, an equation may be written

$$\frac{\sqrt{C_1}}{C_2} = K' \quad (\text{Eq. 2})$$

When a solute partially dissociates in phase 2 and not in the other, then

$$\frac{C_1}{C_2(1-\alpha)} = K'' \quad (\text{Eq. 3})$$

where α is the degree of dissociation.

Equations can be derived for other cases which depart from ideality. The subject is covered adequately in accessible references (48). When systems being dealt with are nonideal, and corrections are not made for this, $\frac{C_1}{C_2}$ is sometimes designated as K_{apparent} .²

EXPERIMENTAL

Apparatus.—The rocking device is shown in Fig. 1. Its design, size, and construction materials are not critical. Our design has three interconnected shelves, each equipped with clips for holding 12 sample tubes. These shelves are connected to a synchronous motor which moves them from horizontal to 45° and back to horizontal with each revolution. Although the synchronous motor³ is available in various speeds and our design permits rapid interchange from one motor to another, a rate of 1 c.p.m. (cycle per minute) was used in all routine studies. The Pyrex sample tubes (Fig. 2) have a volume of 100 ml. and are designed to hold a sample of 50 ml.

² It is vital that the reader distinguish between the true (intrinsic) partition coefficient and the apparent (observed) partition coefficient. The true partition coefficient (TPC) for a drug is the one defined by the partition law and is, therefore, a measure of *only* the molecular species common to both phases. The apparent partition coefficient (APC) expresses the ratio of total drug observed in nonideal systems, regardless of the molecular species in which the drug exists. Since most drugs have the potential to ionize, associate, or complex, the partition coefficients reported for pharmaceutical systems are usually the apparent partition coefficients, which are thus valid only for the conditions under which they were measured. In pharmaceutical systems, when association or complexing occurs in the nonaqueous phase, APC is usually greater than TPC. When dissociation or complexing occurs in the aqueous phase, TPC is always greater than APC.

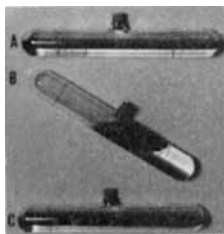
³ Merkle-Korff Gear Co., Chicago, Ill.

(normally, 25 ml. of each phase). Their inner surfaces are smooth to minimize turbulence. Cork stoppers, with and without aluminum foil liners, were used to seal the ports.

Materials.—Ephedrine (hydrous) N.F.; benzoic acid U.S.P.; salicylic acid U.S.P.; *o*-chlorobenzoic acid and *o*-methylbenzoic acid, Matheson Coleman and Bell; *o*-nitrobenzoic acid, Eastman Organic Chemicals; bithionol, Winthrop-Stearns; hexachlorophene U.S.P.; chlorpromazine base, hydrobromide, hydrochloride, hydroiodide, maleate, succinate, hemisulfate, sulfate, and sulfanilate, Smith Kline and French Laboratories; and *N,N*-dimethyl oleamide, The C. P. Hall Co., were utilized.

Skellysolve B (petroleum naphtha), Skelly Oil Co.; spectroanalyzed cyclohexane, Fisher Scientific

Fig. 2.—Pyrex sample tubes for the rocking device. Assuming a rocking rate of 1 c.p.m., then *A*, *B*, and *C* indicate the position of the sample tubes at 0, 1/2, and 1 minute, respectively. The change in interfacial area is easily observed. Outside dimensions of the tubes are 1 1/8 × 8 in., and they will conveniently hold 50 ml. of sample when tilted as in position *B*.



Co.; cottonseed oil (winterized), E. F. Drew and Co.; peanut oil, Planters Co.; isopropyl myristate, Kessler Chemical Co.; glyceryl tripalargonate (redistilled), Emery Industries, Inc.; castor oil U.S.P.; and mineral oil U.S.P. were also employed.

Analyses.—Concentrations of ephedrine in the aqueous and nonaqueous phases were determined volumetrically. Aqueous phase concentrations were also determined spectrophotometrically. Volumetric analysis of the aqueous phases followed the procedure described in N.F. XI. Volumetric analysis of the nonaqueous phases was accomplished by titration with 0.1 *N* perchloric acid in glacial acetic acid to the α -naphtholbenzein end point. The aqueous phases were analyzed spectrophotometrically by diluting aliquots in 0.1 *N* hydrochloric acid and reading the absorbance at 256.5 $m\mu$. The nonaqueous phases were not analyzed spectrophotometrically, but the concentrations were calculated from the initial and final aqueous phase concentrations by difference. Volumetric analyses were used in the studies with ephedrine-cyclohexane-water systems as a check on the method and the results. Spectrophotometric analyses were used in the studies with ephedrine-fixed-oil-water systems.

Aqueous concentrations of benzoic acid and its derivatives were determined by titration with freshly standardized sodium hydroxide to the phenolphthalein end point. Nonaqueous concentrations were determined by titration with freshly standardized alcoholic sodium hydroxide to the α -naphtholbenzein end point.

Aqueous concentrations of bithionol were determined spectrophotometrically in 0.1 *N* sodium hydroxide using the absorbance maximum at 307 $m\mu$. Aqueous concentrations of hexachlorophene were determined spectrophotometrically in 0.1 *N* sodium hydroxide using the absorbance maximum at 320 $m\mu$.

Aqueous concentrations of chlorpromazine base

and its salts were determined spectrophotometrically in 0.1 *N* hydrochloric acid using the absorbance maximum at 254.5 $m\mu$. Concentrations of these materials in cyclohexane were determined spectrophotometrically using the absorbance maximum at 258 $m\mu$.

Determination of Partition Coefficients by the Rocking Method.—With the rocker device in the 45° position (Fig. 2*B*), the desired volume of the aqueous phase was pipetted into each of the tubes. The less dense water immiscible phase was then carefully pipetted into the tubes with the stream directed against the tube wall just above the level of the aqueous phase and with slow draining to minimize mixing and emulsification.

The ports were stoppered with corks, and the rocking was generally carried out overnight. Normally, aliquots of each phase were analyzed after removing samples *via* pipets. The apparent oil/water (*o/w*) partition coefficient (*K*) was then calculated. True partition coefficients were obtained by buffering the aqueous phase to a pH at which virtually no dissociation of the drug could occur. The TPC's of ephedrine, chlorpromazine, and *o*-hydroxybenzoic acid were also calculated by the method of Butler (49) which utilizes two APC values obtained by altering the pH of the aqueous phase.

Determination of Partition Coefficients by the Shake-Out Method.—To avoid emulsion formation and the possibility of anomalous equilibria, the separators were gently inverted rather than vigorously shaken. A study of the systems employed showed

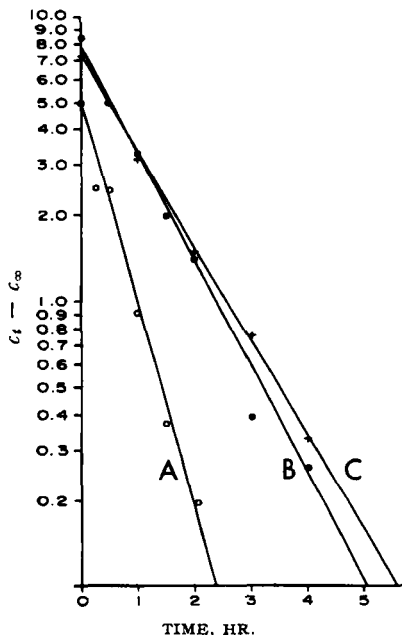


Fig. 3.—Rate of approach to equilibrium for systems containing ephedrine, water, and various nonaqueous phases, using the rocking device of Fig. 1 at 25°C. and rocking at 1 c.p.m. The ephedrine was initially all in the aqueous phase and its disappearance from this phase was followed. From the slopes of the curves, the half-lives for the approach to equilibrium for ephedrine in various nonaqueous phases were: *A*, cyclohexane, $t_{1/2}$ 0.4 hours; *B*, isopropyl myristate, $t_{1/2}$ 0.8 hours; *C*, peanut oil, $t_{1/2}$ 0.9 hours.

that 50 inversions were adequate to reach equilibrium.

RESULTS AND DISCUSSION

Rocking Method.—Our original partitioning studies utilized the separator shake-out technique. The frequent occurrence of emulsification led to a search for a better method for routine studies. The Craig countercurrent apparatus (44) and one modification of the Gershberg-Stoll disintegration apparatus (43) employ a rocking action. The rocking apparatus used in this study (Figs. 1 and 2) was constructed to facilitate solute transfer from one phase to the other and to facilitate uniform distribution of solute within each phase. These two actions are achieved, respectively, by (a) slow expansion and contraction of the interface and (b) constant variation in the shape of each phase.

In cylindrical tubes, rocking from horizontal to 45° causes almost as much change in interfacial area and phase shape as does rocking from horizontal to 90° (vertical). Preliminary studies showed that rocking rates faster than 1 or 2 c.p.m. often produced emulsification. Thus, the useful angle and rate of rocking were found to be 45° and 1 c.p.m. The port hole on the sample tube was placed in the center rather than on one end to avoid surface irregularities which might produce emulsification. This position of the port hole limits the volume the tube will hold, but this limitation has not been a problem.

All experiments were conducted in an air-conditioned room ($25 \pm 1^\circ$). The results, therefore, may have some variation, but are estimated to be not more than $\pm 2-3\%$.

Equilibration Time.—After establishing that the rocking apparatus had little tendency to produce emulsions, it was necessary to establish the minimum time of rocking which would produce equilibrium in the systems under study. Nine tubes, containing cyclohexane and 0.1 *M* aqueous ephedrine as the two phases, were rocked; periodically, one of the tubes was removed, and the aqueous phase was analyzed for ephedrine. The final tube was allowed to rock for 24 hours. Thus, nine values were obtained at nine different time intervals. To plot the data as a first-order approach to equilibrium, the concentration (C_∞) in the 24-hour sample, considered to be the equilibrium concentration, was subtracted from the concentration at each time interval (C_t), and the difference plotted on a log scale against time. When, however, the study was conducted starting with the drug in cyclohexane, the plot was made by subtracting the concentration at each time from the equilibrium concentration and plotting the log of the difference against time.

The same procedure was used to obtain the log ($C_t - C_\infty$ or $C_\infty - C_t$) versus time plots for the ephedrine-isopropyl myristate-water and the ephedrine-peanut oil-water systems (see Fig. 3). Since the plots in Fig. 3 are straight lines, the approach to the equilibrium is first order and the half-lives for the three systems containing cyclohexane, isopropyl myristate, and peanut oil are 0.4, 0.8, and 0.9 hours, respectively. (The half-lives were approximately the same regardless of the direction from which equilibrium was approached.) From these data, it appears that viscosity has some influence on the rate of partitioning. After seven

TABLE I.—INFLUENCE OF THE NONAQUEOUS PHASE ON THE APPARENT PARTITION COEFFICIENT OF EPHEDRINE

Nonaqueous Phase	APC
Mineral oil	0.18
Skellysolve B	0.28
Cyclohexane	0.34
Isopropyl myristate	0.98
Cottonseed oil	1.03
Glyceryl tripelargonate	1.07
Peanut oil	1.32
Castor oil	3.76

half-lives, systems following first-order kinetics are within 1% of true equilibrium. Thus, for the systems mentioned above, partition coefficients could be determined within 1% after approximately 6 hours of rocking.

Precision.—The precision of the rocking method was determined by comparing data obtained with the rocking method and the separator shake-out method using a system which does not readily emulsify. Thus, the two Skellysolve/water partition coefficients of ephedrine were determined by using 25 ml. of each phase in the rocking apparatus (16 hours) and in a 125-ml. separator (50 inversions), respectively. The partition coefficients determined by the two methods are the same within experimental error, *i.e.*, both values were 0.28. As expected, it was also found that the direction from which equilibrium is approached (oil to water and water to oil) had no effect on the observed partition coefficient.

Drug Concentration and Phase Volume.—Macy (50) and Collander (38) have shown that the only compounds which change appreciably in their partition coefficient with change in concentration are those in which a change in concentration also produces significant change in degree of association. In other words, the apparent partition coefficient may change, but not the true partition coefficient. The main effect of varying phase volume, other than this concentration effect, is mechanical, *i.e.*, sampling is made more difficult. With the system containing ephedrine, cyclohexane, and pH 11 aqueous buffer (to prevent dissociation), altering the ratio of the phase volumes from 20:80 to 80:20 had no effect on the ephedrine true partition coefficient. Using the same system with equal phase volumes, but varying the ephedrine concentrations from 0.01 to 0.1 moles/L., also did not affect the ephedrine true partition coefficient. It should be kept in mind, however, that higher concentrations may influence the activity—and thus the apparent partition coefficient—of a drug.

Nature of the Nonaqueous Phase.—In pharmaceutical partitioning studies the nonaqueous phase is usually lipoidal in nature, although the exact lipoidal composition of the various membranes affecting drug absorption is not known. For convenience, organic solvents are often used in *in vitro* partitioning studies to simulate these lipoidal materials; this is a practice which cannot always be justified. For this reason, oil/water partition coefficients of ephedrine were determined using both organic solvents and fixed oils as the nonaqueous phase. In this study, the samples were rocked for 48 hours, and the results are shown in Table I.

As might be expected, the partition coefficients

tend to parallel the ephedrine solubility in the nonaqueous phase. It is of interest that the castor oil partition coefficient is about four times higher than the partition coefficients obtained with the other fixed oils. Probably the hydroxyl group in the fatty acid chain of castor oil permits additional association between castor oil and ephedrine and thus increases the ephedrine solubility. The free fatty acid content of the oils may also be important.

Partition Coefficients of Various Drugs.—Both apparent and true partition coefficients for a number of substances have been obtained and representative results are shown in Table II. Partition coefficients over the range 0.03 to 5000 have been determined with the rocking device. No difficulty from the technique or apparatus was experienced in obtaining these values. The magnitude and precision of the values are limited only by the sensitivity of the analytical procedures. True partition coefficient

TABLE II.—PARTITION COEFFICIENTS OF VARIOUS DRUGS AND CHEMICALS

Drug or Chemical	Cyclohexane/Water Partition Coefficient	
	APC	TPC
Ephedrine	0.34	0.41
Chlorpromazine	300	15,000
Benzoic acid	1.05	1.18
<i>o</i> -Nitrobenzoic acid	0.05	0.13
<i>o</i> -Hydroxybenzoic acid	0.16	0.32
<i>o</i> -Chlorobenzoic acid	0.31	0.46
<i>o</i> -Methylbenzoic acid	3.54	4.45
Hexachlorophene	350 ^a	Not done
Bithionol	>80,000 ^b	Not done

^a Peanut oil/water partition coefficient. ^b Chloroform/water partition coefficient. No detectable bithionol in the aqueous phase.

(TPC) values up to 15,000 have been calculated from the APC data by the method of Butler (49). It should be remembered that the APC may vary considerably with a change in the pH of the aqueous phase.

Ephedrine was used in all the studies previously discussed and was selected as a model drug primarily because it is an amine and, in most of the systems employed, has a TPC near 1.⁴ *Ephedrine N.F.* is commercially available as the hemihydrate, and it was frequently observed that some water of hydration is stripped during dissolution of ephedrine in nonaqueous solvents. Rosin, *et al.* (51), reported a difference in the liquid petrolatum solubility of hydrous and anhydrous ephedrine. Anhydrous ephedrine was prepared in our laboratory and had the same cyclohexane/water partition coefficient as the hydrated form. This result was anticipated, despite the data of Rosin, since the partition coefficients were equilibrium values obtained in the presence of a large quantity of water.

Chlorpromazine base has a very high partition coefficient compared with ephedrine, even when the aqueous phase is unbuffered. Under optimum conditions, as much as 15,000 times more chlorpromazine can be placed in the nonaqueous phase as in the aqueous phase.

Benzoic acid dimerizes in some organic solvents (48, 52); therefore, the APC of the monomer was found by dividing the square root of the concentration in cyclohexane by the concentration in water.

⁴ A partition coefficient near 1 is desirable because it enables one to assay either phase conveniently.

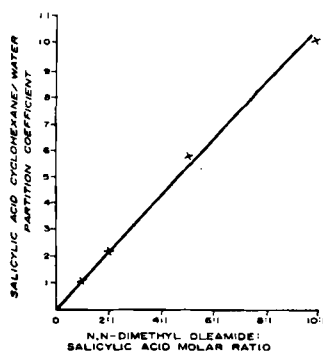


Fig. 4.—Influence of *N,N*-dimethyl oleamide on the cyclohexane/water partition coefficient of salicylic acid. The initial aqueous phase concentration of salicylic acid was 0.01 *M* in all of the samples.

The value for the APC is 1.05. The TPC for the monomeric form of benzoic acid is 1.18 and was obtained in a similar manner, but using an acidified aqueous phase, so that no dissociation occurred.⁵ The similarity of the APC and TPC indicates that very little dissociation occurs in the aqueous phase under normal conditions.

The ability to predict the effect of structure modification on the TPC of a drug could be very useful. For this reason, several *benzoic acid derivatives*, substituted in the *ortho* position, were studied to determine the effect of various substituent groups on the TPC of benzoic acid. No association of these derivatives was apparent in the cyclohexane phase at the concentrations used. The results are shown in Table II. As with benzoic acid, the TPC's were obtained by using acidified aqueous phases. The three polar substituent groups caused a shift in the cyclohexane/water partition coefficient of benzoic acid in favor of the aqueous phase (nitro > hydroxyl > chloro). In contrast, the nonpolar *o*-methyl group caused a shift in favor of the nonaqueous phase. The relative order of these results is not surprising if one considers (a) the electronic effects of the substituents (53), (b) the ability of adjacent hydroxyl and carboxyl groups to hydrogen-bond, (c) the over-all steric effects, and (d) the polarity of the two phases. Collander (38) further indicates that not only the nature of the groups but also their position and size are important.

In 1962, Higuchi and co-workers (54) reported that dimethyl amides complex with organic acids such as salicylic acid and thereby increase the solubility of the acid in organic solvents, such as cyclohexane and olive oil. In our laboratory, the effects of oil-soluble dialkyl amides on the partition coefficients of acidic drugs were studied. For example, the influence of *N,N*-dimethyl oleamide on the cyclohexane/water partition coefficient of salicylic acid was determined with the rocking device (Fig. 4). As expected, the partition coefficient increases as the amide-salicylic acid molar ratio increases. For example, a 10:1 amide:acid molar ratio shifts the salicylic acid partition coefficient between cyclohexane and water from 0.16 to 10.0. Thus, salicylic acid can be shifted from the aqueous phase to the nonaqueous phase by the use of complexing principles.

High partition coefficients would seem to be advantageous in germicides, since most microorganisms are enclosed by a lipid-like membrane. *Hexachlorophene* and *bithionol* are used for their germicidal action in many pharmaceutical products.

⁵ Both the APC and TPC for benzoic acid were calculated by Eq. 2.

TABLE III.—CYCLOHEXANE/WATER PARTITION COEFFICIENTS AND CYCLOHEXANE SOLUBILITIES OF SEVERAL CHLORPROMAZINE SALTS AT 25°C.

Salt	1	2	3	4	5
	APC	pH Aqueous Phase	Concn. in Cyclohexane Phase, ^a mcg./ml.	Solubility in Cyclohexane, ^b mcg./ml.	Concn. in Cyclohexane Phase Solubility in Cyclohexane
Hydrobromide	0.46	3.8	99	0.9	110
Hydrochloride	0.47	3.8	97	9.5	10
Hydriodide	0.47	3.8	103	2.7	40
Maleate	0.44	3.8	94	3.2	30
Succinate	1.71	4.2	205	72.0	3
Sulfanilate	0.67	3.9	129	0.7	180
Base	300	6.5	318	>5000	<<1

^a From partitioning data; values expressed in terms of the base. The initial aqueous phase concentration of each salt was $1 \times 10^{-2} M$ or 319 mcg./ml. in terms of the base. Therefore, if all the drug partitioned into the cyclohexane phase, the value in this column would be 319. ^b From separate solubility determinations in pure cyclohexane. The values are expressed in terms of the base.

From their low aqueous solubilities, it might be expected that the partition coefficients of these substances highly favor the nonaqueous phase. In Table II, the high apparent partition coefficients of hexachlorophene and bithionol are illustrated.

In studying *chlorpromazine* and other *phenothiazine salts* in our laboratories, it became readily apparent that some of the salts had unusual solubilities in lipids, e.g., glyceryl monostearate. This lipid solubility may have a significant effect on formulation with phenothiazines and on their *in vivo* absorption. The cyclohexane/water partition coefficients for these phenothiazine salts were determined and representative results are shown in column 1 of Table III. The ratios in column 5 show that the chlorpromazine present in the cyclohexane phase (column 3) greatly exceeded the solubilities of the salts (column 4) in cyclohexane. This indicates that in each case virtually all of the chlorpromazine in the cyclohexane phase was the free base, not the salt.

From the data in column 1, it would appear that the succinate and sulfanilate salts have higher APC's than the other salts. Actually, the slight differences in aqueous phase pH (column 2) are enough to produce the observed differences in partition coefficient, again indicating that the base, (not the salt) is partitioning. This can be verified mathematically by using the observed pH's and the TPC of chlorpromazine.

Because some chlorpromazine salts are lipid soluble, cyclohexane may not properly simulate an *in vivo* absorption barrier. Studies are currently underway in our laboratory to investigate the influence of salt forms on the partitioning of phenothiazine type drugs, to investigate the influence of the lipid phase composition on the solubility and partition coefficient of the salts, and to determine the significance of these variables as they relate to the absorption properties of the drugs.

SUMMARY

1. A rocking apparatus, holding up to 36 samples, has been developed for studying drug partitioning. Illustrations of the application of this apparatus are given with data. The paper is introductory and is to be followed by more detailed studies of some of the areas of application described.

2. The rocking method, using an ephedrine-Skellysolve B-water system, was as reliable as the

separator shake-out method. The rocking method described in this study does not tend to produce emulsions.

3. Phase volume and drug concentration did not influence the partition coefficient as long as these parameters were kept within reasonable limits, and association or dissociation of the drug was considered.

4. The influence of the nonaqueous phase on the partition coefficient of ephedrine was shown. With ephedrine, castor oil/water produced the highest observed partition coefficient and mineral oil/water the lowest.

5. The following cyclohexane/water TPC's were reported: ephedrine (0.41), chlorpromazine (15,000), benzoic acid (1.18), *o*-nitrobenzoic acid (0.13), *o*-hydroxybenzoic acid (salicylic acid) (0.32), *o*-chlorobenzoic acid (0.46), and *o*-methylbenzoic acid (4.45).

6. The cyclohexane/water partition coefficients of several salts of chlorpromazine varied fourfold. This variation was due to slight differences in the pH of the aqueous phase. It was shown that in the cyclohexane/water system, chlorpromazine base (but not its salts) partitions into the cyclohexane phase. Since some chlorpromazine salts are known to have appreciable solubility in selected lipids, cyclohexane may not be a suitable simulated *in vivo* absorption barrier for these salts.

7. Pharmaceutical applications of drug partitioning were discussed, and the advantages of more extensive studies of partition coefficients of new and old drugs were pointed out. In addition to the usual analytical and absorption applications, these were (a) deliberately attempting to alter the partition coefficients of drugs in a predictable manner, (b) improving emulsion taste, (c) improving drug chemical stability, (d) influencing drug release from emulsions, ointments, suppositories or other dosage forms, and (e) studying the influence of aqueous phase components, such as electrolytes, surfactants and macromolecules, on the partitioning of drugs, antioxidants, flavors, sweetening agents, and preservatives.

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Drug Partitioning II

In Vitro Model for Drug Absorption

By JAMES T. DOLUISIO and JOSEPH V. SWINTOSKY

An *in vitro* model to simulate some factors involved in the absorption process is described. It consists of a tube containing two aqueous phases separated by an immiscible phase. A rocking apparatus agitates the fluids while causing the liquid interfaces to expand and contract. Rates of drug transfer and equilibrium drug distribution were determined under conditions where one aqueous phase was maintained at pH 7.4 and the other buffered at various pH values. Salicylic acid, barbital, antipyrine, aminopyrine, and tetracycline were studied in this manner. The initial drug transfer simulated a first-order rate process. Results of the equilibrium studies are in general agreement with predictions of the pH-partition theory. Tetracycline did not undergo transfer from one aqueous phase to the other at any pH condition of the study.

PREVIOUS INVESTIGATIONS (1-5) have demonstrated that the gastrointestinal absorption of drugs is often dependent upon their ability to penetrate a lipoidal barrier, and that for some compounds absorption is accomplished by passive diffusion of the unionized moiety. The following equations derived by Shore, *et al.* (1), give the theoretical ratios, R , of drug concentrations, C , in aqueous solutions of differing pH separated by a

barrier which is selectively permeable to the unionized moiety:
for a base,

$$R = \frac{C_{gut}}{C_{plasma}} = \frac{1 + 10^{(pK_a - pH_{gut})}}{1 + 10^{(pK_a - pH_{plasma})}} \quad (\text{Eq. 1})$$

and for an acid,

$$R = \frac{C_{gut}}{C_{plasma}} = \frac{1 + 10^{(pH_{gut} - pK_a)}}{1 + 10^{(pH_{plasma} - pK_a)}} \quad (\text{Eq. 2})$$

In situ experiments (1-5) have shown that the distribution of some drugs approximates these equations. However, it is possible that an ionized