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Molecular recognition of phenobarbital in plasticizers Equilibrium investigations on the solubility of the barbiturate artificial receptor and its binding to phenobarbital in plasticizers

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Abstract

Environmental concern is renewing interest in selective, waste-free extractions. A recent report demonstrated an improved extraction of phenobarbital by means of a specifically designed molecular receptor. In that work, the solvent was $CHCl_3$. The current work is the first step in extending extractions based on molecular recognition to reusable solvents, namely plasticizers. Phenobartital aqueous/organic partition coefficients, receptor solubility, and phenobarbital–receptor-formation constants in several plasticizers and in their $CHCl_3$ solutions are reported. In addition, by a thermodynamic cycle, the free energy for transfer of the barbiturate–receptor complex from $CHCl_3$ to plasticizers has been calculated. Finally, the data have been displayed in coordinate systems representing extraction efficiency and selectivity. The most selective extraction medium yielding useful extraction efficiency is dioctyl phthalate.

1. Introduction

We have recently reported on the development of an enhanced extraction medium for barbiturates [1]. Through the use of a synthetic molecular receptor [2] the extraction efficiency of phenobarbital from serum was increased 40-fold, i.e., the phase ratio required for extraction of phenobarbital from serum into $CHCl_3$ is 40 times larger than into a receptor-enriched $CHCl_3$ solution. In effect, the free energy of binding to the receptor offsets the negative entropy due to concentration of the extracted species. This free energy arises from the presence of six H-bonds in the receptor–barbiturate complex. The receptor, essentially a condensation product of isophthalyldichloride and two 2,6-diamino pyridines, has a U-shaped cavity lined with H-bonding sites complementary to those of barbiturate [2].

While such selective and efficient extractions would be useful in chromatographic analysis, the shadow of solvent waste hangs over thoughts of such procedures. We have therefore begun to investigate solvents that are, in effect, reusable.

Plasticized polymers are just such solvents.

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Plasticizers are high-boiling organic solvents used chiefly to impart flexibility to a rigid plastic or polymer such as poly(vinylchloride) (PVC) [3]. The plasticization of PVC accounts for the single largest usage of plasticizers [4]. In analytical chemistry, plasticized PVC has found its way into the area of sensors. This material is used in the fabrication of ion-selective electrodes (ISEs) [5]. For use in ISEs, a plasticizer is normally chosen on the basis of its plasticizing ability, water immiscibility, viscosity, and receptor solubility [5]. Consequently, these properties of the plasticizer influence the overall performance of the membrane.

Thin-sheet-supported liquid membranes have been used in analytical chemistry for sample preparation and separation [6–11]. In the case of sample preparation, liquid membranes have been used to extract analytes selectively from solution [6–9]. The analytes are removed from the membrane by back-extraction prior to analysis. Another analytical approach for selectively isolating analytes from a sample matrix has been the selective transport of the analyte from one solution to another via a liquid membrane [10,11]. In a few of these analytical applications [6–9], supported liquid membranes [porous poly(tetrafluorethylene) (PFTE) membranes impregnated with organic solvents] were placed in a flow system which was connected either to a gas or liquid chromatograph [6–9]. The system allowed an analyte to be isolated, concentrated, and detected with a minimal amount of sample handling.

In this work, we have determined for a number of plasticizers three equilibrium properties relevant to the extraction of phenobarbital: the partition coefficient of phenobarbital between plasticizer and aqueous buffer, the receptor solubility, and the formation constant of the barbiturate–receptor complex. In addition, a fourth one has been calculated, viz., the partition coefficient of the receptor–barbiturate complex between plasticizers and a reference solvent. Finally, guided by the equilibrium expressions for extraction, we discuss the choice of the optimum plasticizer.

2. Experimental

2.1. UV studies

Reagents

Spectroscopic grade chloroform was purchased from Mallinckrodt Specialty Chemicals (Paris, KY, USA). Phenobarbital was purchased from Sigma Chemical (St. Louis, MO, USA). The details of the barbiturate receptor synthesis have been described elsewhere [2]. The solvents used in the UV studies were previously described. The indicators, 4-nitroanisole, Reichardt's dye, 4-nitroaniline (Aldrich) and N,N-dimethyl-4-nitroaniline (Lancaster, Windham, NJ, USA) were used as received.

Apparatus

UV absorbance spectrophotometers were either an IBM 9420 UV–Vis, a Hewlett-Packard 8450 diode array, or a Hewlett-Packard 8452A diode array. Quartz cells with path lengths of 0.1 and 1.0 cm were purchased from Fisher Scientific (Pittsburgh, PA, USA). An ultrasonicator bath (Cole–Parmer, Chicago, IL, USA) was used to aid in the dissolution of the barbiturate receptor in the various solvents. An Accumet pH meter equipped with an Orion Ross reference electrode (Fisher Scientific) was used to measure the pH of the water extracts.

Procedures and measurements

As discussed in Ref. [1], the absorbance difference at 318 nm is related to the amount of receptor–substrate (RS) complex present in solution. The effect of plasticizer on binding was determined by measuring the absorbance difference at 318 nm for a receptor solution (ca. 500 μM) in the presence and absence of phenobarbital (ca. 500 μM) as a function of plasticizer concentration (vol.%). The concentration of the RS complex is determined by measuring absorbance changes at 318 nm. In the dilution studies, the concentration of plasticizer–solvent ranged from 0–50% (v). The interfering absorbance bands arising from the solvents were minimized by acquiring the background absorbance of the

solvent prior to sample measurements. For these studies, quartz cells of path length 0.1 cm were used. All absorbance measurements were carried out at ambient temperature (22.0 ± 1°C).

The absorbance measurements were also used to calculate the binding constants (K_f) in pure solvents, some solvent mixtures, and in solvents–solvent mixtures diluted to 20% (v) in $CHCl_3$. The procedure is made clear by a consideration of the equilibrium

$$R + S \rightleftharpoons RS \quad (1)$$

and

$$K_f = [RS]/([R][S]) \quad (2)$$

where [R] is the receptor concentration, [S] is the substrate concentration (phenobarbital), and [RS] is the concentration of receptor–substrate complex. The concentration of the RS complex can be calculated for the single-wavelength measurements by using

$$[RS] = (A^T_{\lambda_1} - A^R_{\lambda_1})/(b\Delta\epsilon_{\lambda_1}) \quad (3)$$

where [RS] is the concentration of the phenobarbital–receptor complex, $A^T_{\lambda_1}$ is the baseline-corrected absorbance of a solution containing the barbiturate receptor, phenobarbital, and the receptor–phenobarbital complex at λ_1, b is the path length, $A^R_{\lambda_1}$ is the baseline corrected absorbance of a solution containing only the barbiturate receptor at λ_1, and $\Delta\epsilon_{\lambda_1}$ is the difference in molar absorptivities for the barbiturate–receptor complex and barbiturate receptor ($\epsilon^{RS} - \epsilon^R$) at λ_1. It is important to point out that it was assumed that $\Delta\epsilon_{\lambda_1}$ in the 20% dilutions of solvents–solvent mixtures in $CHCl_3$ was equal to $\Delta\epsilon_{\lambda_1}$ in chloroform (5430 l mol^{-1} cm^{-1} at 318 nm). The formation constant, K_f was calculated for the 20% solutions using

$$K_f = \frac{[RS]}{([R]_t - [RS])([S]_t - [RS])} \quad (4)$$

where [RS] is the concentration of the complex formed, $[R]_t$ is the total receptor concentration, and $[S]_t$ is the total phenobarbital concentration. These experiments could not be performed in many plasticizers, so an alternative method for K_f was used (see below).

Determination of λ_{max} and ϵ_{318}

The experimental procedure for the determination of λ_{max} and ϵ_{318} of receptor solutions in undiluted solvents was as follows. A stock solution of barbiturate receptor of known concentration in chloroform was prepared. An amount of 2 ml of the receptor solution was transferred to 2-ml volumetric tubes. Chloroform was evaporated by placing the tubes in a water bath (ca. 70°C). Once the tubes cooled, 2 ml of each solvent to be studied were placed in an individual tube. The weight of the solvent transferred was recorded because it is very difficult to pipet plasticizers accurately due to their high viscosities. The receptor solutions were then placed in an ultrasonicator bath for 10 min. Samples reconstituted with the more viscous, higher-boiling solvents [decanol, dioctyl phthalate, dioctyl phthalate–chloroparaffin (3:1, w/w), dioctyl phthalate–N-ethyl-*p*-toluene sulfonamide (9:1, w/w), epoxidized soya oil, tributyl phosphate, tributyl phosphate–chloroparaffin (3:1, w/w), tributyl citrate, dioctyl sebacate, and chloroparaffin] were heated in a water bath (75°C) for 2 h to further enhance progress towards equilibrium. Samples were examined by UV spectroscopy the following day using either 0.1- or 1.0-cm quartz cells depending on receptor concentration. The λ_{max} determinations were obtained using an IBM 9420 UV–Vis spectrophotometer while a HP8452 diode array was use to obtain ϵ_{318} measurements. Prior to recording the wavelength maximum for each of the samples, the spectrophotometer was calibrated using a holmium oxide filter. All absorbance readings were baseline corrected. Measurements were carried out at ambient temperature (22.0 ± 1°C).

Receptor solubility determinations

A number of approaches have been given in the literature [12] for determining the solubility of solids in liquids. One approach is to weigh out a large quantity of the solid and place the sample in a solvent of known volume. Samples are

heated well above the final analysis temperature to approach equilibrium from the side of supersaturation. Once a sample has reached equilibrium, the sample is removed, filtered, and analyzed by an appropriate method. Equilibrium is said to be reached when successive samplings yield identical results. We followed this general approach with the exception that a receptor stock solution in chloroform was prepared, transferred, and the chloroform was evaporated. The samples were reconstituted with the appropriate solvent and placed in an ultrasonicator bath for 20 min. The higher-boiling solvents were placed in a water bath (ca. 75–80°C) for two 1-h intervals separated by 10 min in the ultrasonicator bath. The first sampling was taken after 2 days and periodic samplings were taken throughout the week until two or three reproducible receptor concentration measurements were obtained. The samples were filtered through Teflon filters (0.45 μm pore size, Acrodisc CR PTFE, Gelman Sciences) prior to UV analysis.

The receptor concentrations were calculated using Beer's Law. Absorbance measurements were recorded at 318 nm (baseline corrected) and ϵ_{318} values for the barbiturate receptor were obtained from prior experiments.

For some solvents, solubilities were very high, and to conserve material we determined a lower limit on the solubility.

2.2. Chromatographic studies

Reagents

The mobile-phase buffer was prepared from stock solutions of 0.50 *M* potassium phosphate, monobasic (EM Science, Gibbstown, NJ, USA) and 0.45 *M* phosphoric acid (EM Science, Gibbstown, NJ, USA). The pH of the buffer solution was adjusted to pH 4.2 using a 1 *M* NaOH solution. The mobile phase consisted of 65% 20 m*M* phosphate buffer–21% methanol (Mallinckrodt Chemical Specialty, Paris, KY, USA)–14% acetonitrile (EM Science, Gibbstown, NJ, USA) (v/v/v). All of the solvents were filtered prior to use. The organic solvents were filtered through Teflon filters (0.45 μm pore size), while the aqueous solvents were filtered through cellulose ester filters (0.45 μm pore size). The aqueous solutions were prepared daily with water passed through a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Apparatus

The chromatographic system consisted of a Waters 600E pump, Hewlett-Packard 1050 autosampler, an Alltech precolumn (Brownlee RP-18 New Guard Cartridge), a Hypersil ODS C_{18} 15 cm × 4.6 mm column with 5 μm packing, and a Waters 990 diode-array detector. Data were acquired using a Waters 900^{+} Version 6.22A data acquisition program. Experiments were carried out at ambient temperature. The chromatographic conditions were as follows: wavelength of detection for phenobarbital, 196 nm; flow-rate, 1.0 ml min^{-1}; and injection volume, 50 μl. The average chromatographic run time was 10 min except for extractions carried out with the mixture dioctyl phthalate–N-ethyl-*p*-toluene sulfonamide. The plasticizer, N-ethyl-*p*-toluene sulfonamide, is fairly water soluble and has a strongly absorbing peak eluting at approximately 15 min. The chromatographic run time for N-ethyl-*p*-toluene sulfonamide extractions was 25 min.

2.3. Procedures and measurements

Partitioning studies (K_p)

RP-HPLC was used to determine the partition coefficients (K_p) for phenobarbital and the formation constants (K_f) for the phenobarbital–receptor complex in the plasticizers. For the K_p studies, aqueous solutions (pH 5.05) of phenobarbital (ca. 100 μM) were transferred to a scintillation vial containing a known volume of extracting solvent. The contents of the vials were gently mixed for a few seconds using a Vortex-Genie (speed control: 1; Scientific Industries, Bohemia, NY, USA), vented, and placed on a shaker (setting: 4; Eberbach, Ann Arbor, MI, USA) for 30 min. The samples were removed from the shaker and after a short period of time, the layers separated. The aqueous layer was filtered, transferred to a vial, and examined by RP-HPLC. All of the extractions were carried out at room temperature (22.0 ± 1°C) and ex-

amined in duplicate. The chromatographic procedure for the phenobarbital determination was adapted and modified from a method previously described in the literature by Gerson et al. [13].

Phenobarbital standards were prepared daily in mobile phase and placed randomly on the autosampler. A calibration curve was prepared for each set of runs. There were day to day variations in retention times but these variations on the average were less than 30 s.

The fraction of phenobarbital remaining (q) was calculated based on changes in peak height prior to and after each extraction. The distribution coefficient (D_c) and the partition coefficient (K_p) were calculated using

$$D_c = (1 - q)/q\Phi \quad (5)$$

and

$$K_p = D_c[1 + K_a/(H^+)] \quad (6)$$

where q is the fraction of phenobarbital remaining following extraction, Φ is the volume phase ratio, (H^+) is the proton activity, and K_a is the first acid dissociation constant of phenobarbital.

Formation constant (K_f) determinations.

The general procedure described above was followed. The only changes were (1) the initial [phenobarbital] was ca. 50 μM, (2) the pH of the aqueous solution was either 5.05 or 7.96, and (3) the extracting solvent was enriched with ca. 550 μM of barbiturate receptor. In the case of dioctyl sebacate, which has low receptor solubility, the concentration of receptor was ca. 77 μM. The volume phase ratio ranged anywhere from 0.05 to 2.0.

In this set of experiments, Eq. 7 was used to calculate the formation constant, K_f.

$$K_f = (1 - q - qD_c\Phi)/qD_c\Phi[R]_{t,org} \quad (7)$$

where K_f is the equilibrium formation constant, q is the fraction of phenobarbital remaining in the aqueous phase following extraction, D_c is the distribution coefficient, Φ is the volume phase ratio, and $[R]_{t,org}$ is the total concentration of receptor present in the extracting solvent.

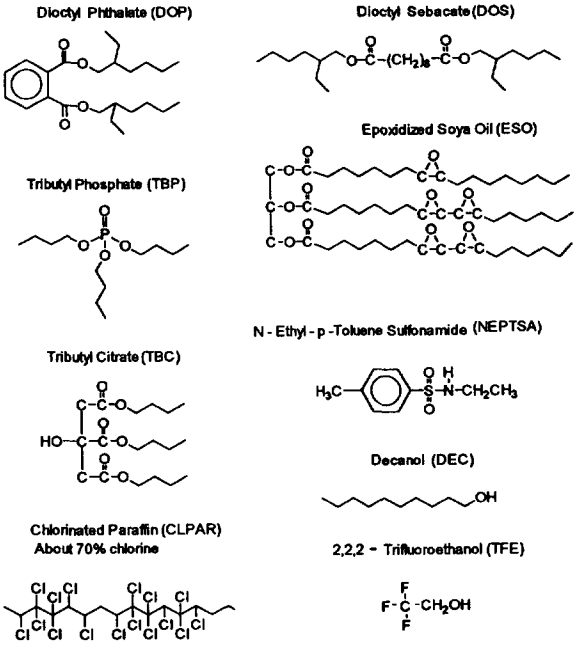

Fig. 1. Structures of plasticizers and solvents.

3. Results and discussion

3.1. Influence of solvent on binding

As shown in Fig. 1, plasticizers vary in size as well as in their hydrogen bonding capabilities. Dioctyl sebacate (DOS), dioctyl phthalate (DOP), epoxidized soya oil (ESO), and tributyl phosphate (TBP) were chosen to represent examples of hydrogen bond acceptor solvents. Plasticizers having both hydrogen bond acceptor and donor properties are represented by decanol (DEC), N-ethyl-*p*-toluene sulfonamide (NEPTSA), and tributyl citrate (TBC). Chloroparaffin (CLPAR) was chosen to represent plasticizers with little or no hydrogen bonding ability. Even though 2,2,2-trifluoroethanol (TFE) is not a plasticizer, it was used in this study because it is a hydrogen bond donor but not an acceptor. Since binding occurs in chloroform as well as methylene chloride, these solvents were included in this study as well.

The first step in understanding the influence of these plasticizers on phenobarbital–barbiturate receptor interactions was to determine the effect

on binding of various concentrations of the plasticizers in chloroform. In these dilution studies, the concentration of plasticizer ranged from 0–50% (v) in chloroform. The effect of plasticizer on binding was determined by measuring the absorbance difference at 318 nm for a receptor solution in the presence and absence of phenobarbital. The absorbance difference is proportional to the receptor–substrate complex concentration. Representative results for each hydrogen bonding class of plasticizers and their effects on binding are given in Fig. 2.

Fig. 2 shows that plasticizers that are good hydrogen bond donors (e.g. decanol and trifluoroethanol) diminish complex formation and eventually destroy complex formation as the level of plasticizer increases in solution. On the other hand, dioctyl phthalate and chloroparaffin have little effect on complex formation. However, Fig. 2 does not give a complete picture of hydrogen bond acceptor plasticizer effects on complex formation. While dioctyl phthalate and dioctyl sebacate were found to have minimal effect on complex formation, epoxidized soya oil and tributyl phosphate, also hydrogen bond acceptors, were found to have a dramatic influence on binding as a function of plasticizer concentration. These results can be found in Fig. 3. These results indicate the need for determining quantitatively the differences in hydrogen bonding capabilities. Such studies are underway and will be reported in the future.

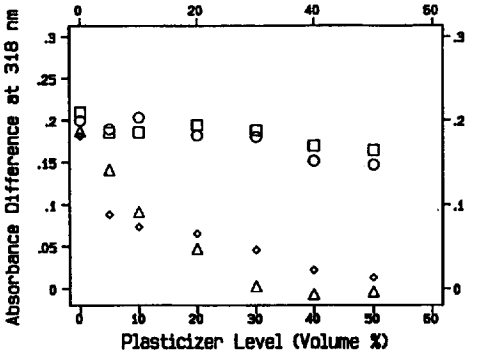

Fig. 2. Plot of the absorbance difference at 318 nm (which is proportional to the concentration of receptor–substrate complex) versus plasticizer concentration (vol.%) in $CHCl_3$ for each class of solvent: dioctyl phthalate (□), chloroparaffin (○), decanol (△), and 2,2,2-trifluoroethanol (◇). Conditions: [receptor] $\cong$ 500 μM and [phenobarbital] $\cong$ 500 μM.

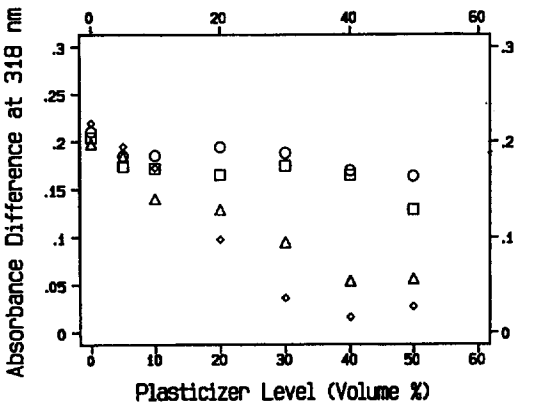

Fig. 3. Plot of the absorbance difference at 318 nm (which is proportional to the concentration of receptor–substrate complex) versus plasticizer concentration (vol.%) in $CHCl_3$ for the hydrogen bond accepting solvents: dioctyl sebacate (○), dioctyl phthalate (□), epoxidized soya oil (△), and tributyl phosphate (◇). Conditions: [receptor] $\cong$ 500 μM and [phenobarbital] $\cong$ 500 μM.

RP-HPLC and UV spectroscopy were used to measure quantitatively the degree of complex formation. The results have been summarized in Table 1. The results in Table 1 show that complex formation is more favorable in chloroform and less favorable in tributyl phosphate and decanol.

3.2. Influence of solvent on receptor solubility

The extraction enhancement factor is $K_f[R]_{t,org} + 1$. This represents the factor by which the quantity of analyte left behind in the aqueous phase is decreased due to complex formation. It depends on not only on the formation constant, but on the concentration of the receptor in the extracting solvent as well. We needed to know if an analytically useful concentration of barbiturate receptor can be obtained in plasticizers. This is an important piece of information for device development. Thus, the solubility of the receptor in the plasticizers was explored. Table 2 summarizes the experimental results of the receptor solubility study. Of particular interest are

Table 1
A summary of K_f values for solutions containing 20% (v) and 100% levels of solvent

Solvent	K_f (20% solutions) (M^{-1})	K_f (100%) (M^{-1})
Methylene chloride	3.7×10^{4} [a]	7.6×10^{3}
Chloroform	$(2.5 \pm 1.0) \times 10^{4}$ [b]	$(2.5 \pm 1.0) \times 10^{4}$
Dioctyl phthalate	$(1.4 \pm 0.20) \times 10^{4}$	$(7.1 \pm 0.19) \times 10^{2}$
Chloroparaffin	$(1.2 \pm 0.46) \times 10^{4}$	4.9×10^{3}
Dioctyl phthalate–chloroparaffin (3:1, w/w)	$(1.1 \pm 0.22) \times 10^{4}$	$(4.6 \pm 0.30) \times 10^{3}$
Dioctyl sebacate	7.4×10^{3} [a]	$(5.6 \pm 2.4) \times 10^{3}$
Dioctyl phthalate–N-ethyl-*p*-toluene sulfonamide (9:1, w/w)	$(6.1 \pm 0.78) \times 10^{3}$	3.6×10^{2}
Tributyl phosphate–chloroparaffin (3:1, w/w)	3.1×10^{3} [a]	[c]
Epoxidized soya oil	$(2.5 \pm 0.65) \times 10^{3}$	2.4×10^{1} [a]
2,2,2-Trifluoroethanol	8.0×10^{2} [a]	9.4×10^{1} [a]
Decanol	$(5.6 \pm 1.8) \times 10^{2}$	5.9×10^{2} [a]
Tributyl phosphate	$(3.5 \pm 0.63) \times 10^{2}$	$(2.3 \pm 0.057) \times 10^{2}$

[a] Single point measurements.
[b] Pure chloroform.
[c] Value was not determined.

Table 2
A summary of receptor solubility results for plasticizers and their 20% solutions (v) in chloroform

Solvent	20% Solutions (mM)	100% Solvent (mM)
Dioctyl sebacate	1.6	$(76 \pm 0.01) \times 10^{-3}$
Chloroparaffin	2.5 ± 0.21	$(330 \pm 0.12) \times 10^{-3}$
Dioctyl phthalate	3.0 ± 0.14	1.2 ± 0.04
Methylene chloride	3.6 ± 0.21	1.4 ± 0.22
Dioctyl phthalate–chloroparaffin (3:1, w/w)	3.7 ± 0.28	1.3 ± 0.07
Chloroform	3.8 ± 0.19 [a]	3.8 ± 0.19
Tributyl phosphate	4.2 ± 0.14	13^{++}
Tributyl phosphate–chloroparaffin (3:1, w/w)	4.4 ± 0.14	10^{++}
Dioctyl phthalate/N-ethyl-*p*-toluene sulfonamide (9:1 by wt.)	4.7 ± 0.07	1.1 ± 0.01
Epoxidized soya oil	7.6 ± 0.14	2.4 ± 0.06
Tributyl citrate	10^{++}	10^{++}
Decanol	12^{++}	2.4 ± 0.5
2,2,2-Trifluoroethanol	12^{++}	13^{++}

[a] Pure chloroform.
$^{++}$ indicates that solubility is greater than this concentration.

the solubility results for dioctyl phthalate and its mixtures. Initially, receptor solubility in dioctyl phthalate and its mixtures was found to be ca. 200 μM, which is much lower than the values reported in Table 2. The receptor solubility was found to be highly dependent on how much excess receptor was present in solution. Receptor solubility increased as the amount of receptor in excess was reduced. We have not been able to explain adequately the relationship between receptor solubility and the quantity of excess receptor present in solution.

3.3. Influence of solvent on phenobarbital partition coefficient

Table 3 lists the partition coefficients, K_p, for phenobarbital in each of the plasticizers along with the 20% solutions of plasticizers in chloroform. 2,2,2-Trifluoroethanol was omitted from this study due to its high water solubility. The results in Table 3 show that phenobarbital can be extracted more efficiently with tributyl phosphate and decanol while the extraction becomes much more difficult with chloroparaffin.

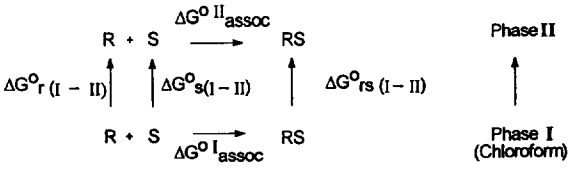

Fig. 4. The thermodynamic cycle involving the transfer of the phenobarbital barbiturate receptor complex from chloroform (phase I) into a second solvent phase (II).

3.4. Influence of solvent on complex partition coefficient

We can determine the relative stability of the phenobarbital–barbiturate receptor complex in a solvent phase (II) by calculating the free energy required to transfer the complex from chloroform (phase I). The thermodynamic cycle for this process is given in Fig. 4. The free energy of transfer ($\Delta G^0_{RS(I\rightarrow II)}$) is determined by

$$\Delta G^0_{RS(I\rightarrow II)} = \Delta G^0_{R(I\rightarrow II)} + \Delta G^0_{S(I\rightarrow II)} + \Delta G^0_{assoc(II)} - \Delta G^0_{assoc(I)} \quad (8)$$

where $\Delta G^0_{R(I\rightarrow II)}$ is the free energy of receptor transfer ($\Delta G^0_{R(II)} - \Delta G^0_{R(I)}$), $\Delta G^0_{S(I\rightarrow II)}$ is the free

Table 3
Partition coefficients (K_p) for phenobarbital at 22.0 ± 1°C

Solvent	K_p (20% solutions)	K_p (100%)
Chloroparaffin	4.4 ± 0.21	0.064 ± 0.052
Chloroform	6.1 ± 1.5[a]	6.1 ± 1.5
Epoxidized soya oil	7.9 ± 0.47	24 ± 0.071
Dioctyl phthalate	8.0 ± 0.95	1.7 ± 0.18
Dioctyl sebacate	8.4[b]	4.6 ± 0.12
Dioctyl phthalate–N-ethyl-*p*-toluene sulfonamide (9:1, w/w)	9.5 ± 0.35	4.5 ± 0.48
Methylene chloride	11 ± 0.071	7.2 ± 0.25
Dioctyl phthalate–chloroparaffin (3:1, w/w)	12 ± 0.35	0.94 ± 0.35
Tributyl phosphate–chloroparaffin (3:1, w/w)	22 ± 5.0	130 ± 11
Tributyl phosphate	63 ± 0.28	170 ± 30
Decanol	77 ± 4.6	20 ± 0.71

[a] Pure chloroform.
[b] Single-point measurement.

energy of phenobarbital transfer ($\Delta G^0_{S(II)} - \Delta G^0_{S(I)}$), $\Delta G^0_{assoc(II)}$ is the free energy of association in phase II, and $\Delta G^0_{assoc(I)}$ is the free energy of association in phase I (chloroform). The quantities ΔG^0_R, ΔG^0_S, and ΔG^0_{assoc} were calculated using the equation

$$\Delta G^0 = -RT\ln(P) \quad (9)$$

where R is the universal gas constant in kJ mol^{-1}, T is the temperature in Kelvin, and P is the experimentally determined property (e.g. receptor solubility, K_p, and K_f). These systems are assumed to be at equilibrium. The calculated $\Delta G^0_{RS(I\rightarrow II)}$ values are summarized in Table 4.

3.5. Choice of solvent: enhancement of extraction

The first stage of development is to determine which plasticizer is most ideal for device fabrication. Recall, that the extraction enhancement factor is $1 + K_f[R]_{t,org}$. Therefore, plasticizers that promote binding and contain a high concentration of receptor will be most suitable for device development. Fig. 5 shows a log–log plot of receptor solubility versus formation constant for 20% solutions. In Fig. 5, the solid lines represent the results when the enhancement factors are 20 (upper line) and 5 (lower line). The most efficient and selective extractions are obtained when K_f and $[R]_t$ are large. Originally, we had hoped to find a plasticizer with equal or superior performance to that of chloroform. Fig. 5 clearly illustrates that chloroform is the most ideal solvent for enhanced extractions; however, one cannot make a plasticized PVC extraction medium with chloroform. Nonetheless, there is still sufficient data to determine which of the plasticizers offers the greatest potential in the area of device development.

In Fig. 5, the points lying on a line parallel to those drawn have equal values of $K_f[R]_t$. Plasticizers in the lower right-hand portion of Fig. 5 (dioctyl sebacate and chloroparaffin) are not very good candidates because they have low receptor solubility and therefore offer no enhancement in extraction efficiency. In the upper left-hand corner of the plot are found decanol, tributyl phosphate, and epoxidized soya oil which dissolve receptor, but support complex formation only poorly. The plot shows that dioctyl phtha-

Table 4
Calculated free energies of transfer for the RS complex

Solvent	$\Delta G^0_{RS(I\rightarrow II)}$ (20%) (kJ mol^{-1})	$\Delta G^0_{RS(I\rightarrow II)}$ (100%) (kJ mol^{-1})
Methylene chloride	−2.5	5.0
Chloroform	0[a]	0
Decanol	0.28	7.4
Dioctyl phthalate–chloroparaffin (3:1, w/w)	0.42	11
Dioctyl phthalate	1.3	15
Tributyl phosphate–chloroparaffin (3:1, w/w)	1.6	[b]
Dioctyl phthalate–N-ethyl-*p*-toluene sulfonamide (9:1, w/w)	1.9	14
Epoxidized soya oil	3.4	23
Chloroparaffin	3.6	21
Dioctyl sebacate	4.3	14
Tributyl phosphate	4.5	0.32

[a] Pure chloroform.
[b] No value obtained.

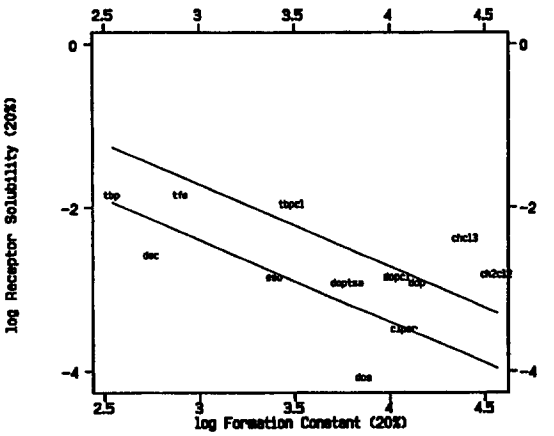

Fig. 5. Plot of log Receptor Solubility (20%) versus log K_f (20% plasticizer level in chloroform): chloroform (chcl3), methylene chloride (ch2cl2), dioctyl phthalate (dop), dioctyl phthalate–chloroparaffin (3:1, w/w) (dopcl), dioctyl phthalate–N-ethyl-*p*-toluene sulfonamide (9:1, w/w) (doptsa), chloroparaffin (clpar), dioctyl sebacate (dos), epoxidized soya oil (eso), decanol (dec), tributyl phosphate (tbp), tributyl phosphate–chloroparaffin (3:1, w/w) (tbpcl), and 2,2,2-trifluoroethanol (tfe). The solid lines represent enhancement factors of 20 (upper) and 5 (lower).

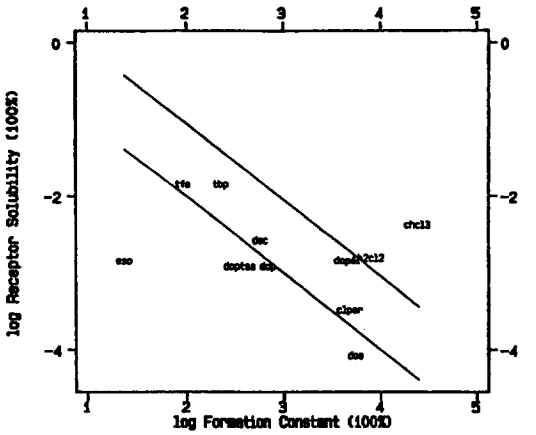

Fig. 6. Plot of log Receptor Solubility (100%) versus log K_f (100% plasticizer level): chloroform (chcl3), methylene chloride (ch2cl2), dioctyl phthalate (dop), dioctyl phthalate–chloroparaffin (3:1, w/w) (dopcl), dioctyl phthalate–N-ethyl-*p*-toluene sulfonamide (9:1, w/w) (doptsa), chloroparaffin (clpar), dioctyl sebacate (dos), epoxidized soya oil (eso), decanol (dec), tributyl phosphate (tbp), and 2,2,2-trifluoroethanol (tfe). The solid lines represent enhancement factors of 20 (upper) and 5 (lower).

late, dioctyl phthalate–chloroparaffin mixture and the tributyl phosphate–chloroparaffin mixture have nearly equivalent extraction efficiencies.

The final device will contain a significantly higher level of plasticizer (ca. 70% by weight) and, therefore, we have constructed a second log–log plot (Fig. 6) to understand plasticizer effects at higher concentrations (100% level of plasticizer). In Fig. 6, the solid lines represent enhancement factors of 10 (upper line) and 2 (lower line). With these data, the mixture containing dioctyl phthalate and chloroparaffin offers the greatest potential for device fabrication. A comparison of Figs. 5 and 6 shows that as the concentration of plasticizer increases in solution, the enhancement factor will decrease due to a decrease in either receptor solubility, binding, or both. It is anticipated that the enhancement factor of a device based on dioctyl phthalate and its chlorinated mixture will fall between 10 and 20.

3.6. Choice of solvent: Selectivity

The selectivity of the device depends on the chemical properties of the solvent. The ability of a plasticizer to extract phenobarbital from aqueous solution is a good indicator of solvent selectivity. Phenobarbital is relatively polar, and as discussed earlier [1], serum contains a number of polar interferences. Therefore, we have made the assumption that the more difficult the extraction of phenobarbital becomes in the absence of receptor, the fewer will be the quantity of interfering species present in the extract. In such a medium, then, extraction selectivity would be maximized for the analyte if the receptor is effective in that medium. To illustrate the differences in selectivity among solvents, a log–log plot of the partition coefficients for phenobarbital (K_p) versus the quantity $1 + K_f[R]_t$ is given in Fig. 7. Fig. 7 has been divided into quadrants which have been given the following general classifications: A (low selectivity, low binding), B (high selectivity, low binding), C (low selectivity,

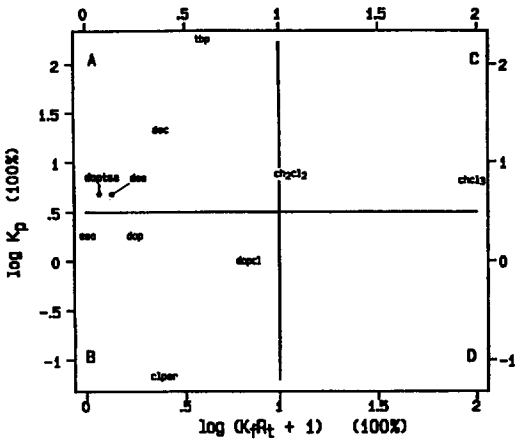

Fig. 7. A log–log plot of K_p (100%) versus $1 + K_f[R]_t$ (100%): chloroform (chcl3), methylene chloride (ch2cl2), dioctyl phthalate (dop), dioctyl phthalate–chloroparaffin (3:1, w/w) (dopcl), dioctyl phthalate–N-ethyl-*p*-toluene sulfonamide (9:1, w/w) (doptsa), chloroparaffin (clpar), dioctyl sebacate (dos), epoxidized soya oil (eso), decanol (dec), tributyl phosphate (tbp), and 2,2,2-trifluoroethanol (tfe). The solid lines represent enhancement factors of 10 (upper) and 2 (lower).

high binding), and D (high selectivity, high binding). In quadrant A, these plasticizers (e.g. decanol and tributyl phosphate) have high K_p values and therefore have poor selectivity, though they will extract phenobarbital, and by assumption other polar solutes, effectively. The selectivity for phenobarbital is better in B with chloroparaffin. Chloroparaffin is a good solvent for selectivity (low K_p) but, unfortunately, a poor solvent for enhancement. Chloroform and methylene chloride are intermediate solvents for selectivity but are fairly good solvents for enhancement, as illustrated by quadrant C. Interestingly, quadrant D remains vacant. Solvents that fall in this quadrant will not only have a higher degree of selectivity than chloroform but will have reasonable enhancement factors as well. Of the plasticizers examined, dioctyl phthalate and its chlorinated mixture offer the greatest potential for device development.

4. Conclusions

In this work, we have shown that a broad group of PVC plasticizers possesses equally broad characteristics of relevance to the selective extraction of phenobarbital. For example, receptor solubility and phenobarbital partition coefficients vary over more than two and three orders of magnitude, respectively. An analysis in two regards, extraction efficiency and extraction selectivity, has shown that, while no solvent is ideal, dioctyl phthalate, of the investigated solvents, offers the best hope for device development.

Acknowledgement

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