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Steady-State Concentration Distribution of Artificial Receptor and Target Analyte in Plasticized PVC Membrane between Solutions Differing in Target Analyte Concentration

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A barbiturate receptor has proven effective in improving selectivity in solid-phase microextraction of barbiturates when doped into plasticized poly(vinyl chloride) (PVC). It would be beneficial to have selective extractions for any given organic species; however, the receptors do not exist. They will be found by screening of libraries of potential receptors; thus, a screening method is needed. It is important to screen the receptors in the medium in which they will work: plasticized PVC. We hypothesize that we can make receptors move in solution in response to the presence of a solute to which they bind. This work examines whether we can establish a sufficient free energy gradient for a good receptor to move to a predetermined place in space. A difference in the barbiturate solute (substrate or guest) concentration in solutions bathing the two sides of a plasticized PVC membrane containing the barbiturate receptor (or host) creates a spatial concentration gradient of the substrate in the membrane. This causes the receptor's chemical potential to vary across the membrane. Upon binding to the analyte, the receptor undergoes a local activity drop, which decreases its free energy. This process produces a flux of receptor to accumulate at place where there is a high substrate concentration. A concentration gradient of substrate can be maintained across the membrane at steady state. In membranes for which the formation of the complex is favored, the receptor responds to the gradient of substrate. In membranes for which binding is not favored, a gradient of substrate is completely ignored by the receptor. Thus, the receptor does respond to the gradient but only if the concentration gradient of guest corresponds to a chemical potential gradient.

In the field of molecular recognition applied to analytical chemistry, a major effort has focused on the selective detection of ions. In comparison, the applications for neutral organic substrates are fewer. Since the early 1990s, we have established the advantages and limitations of using artificial receptors to facilitate the extraction of barbiturates.[1−5] A synthetic molecular receptor[6] increases the extraction efficiency of phenobarbital into chloroform up to 40-fold.[1] Being nonvolatile organic solvents, plasticizers[7] permit extraction with minimal environmental burden. Plasticizers affect the formation constant for phenobarbital−artificial receptor complex formation.[2] High formation constants were obtained in plasticizers that are poor hydrogen bond donors and acceptors. This is not unexpected because the artificial receptor **1** binds to barbiturates by forming six hydrogen bonds within a cavity (Figure 1). Solvatochromic parameters of plasticizers were determined, and the thermodynamic parameters responsible for solute distribution were correlated with them.[3] We can therefore predict the extraction selectivity in plasticizers.[4] These ideas have been applied in a powerful analytical technique, solid-phase microextraction (SPME).[5,8]

Techniques such as SPME with an artificial receptor are very promising for decreasing detection limits and eliminating laboratory solvent usage. The major weakness is a lack of suitable receptors. A very sensible approach to generating new receptors is to use combinatorial chemistry or mixture synthesis[9,10] and then separate the mixture with a suitable screening method. We and others[11] have shown that plasticizers can have a significant effect on molecular recognition. Therefore, the screening must take place in plasticizers or, if the PVC itself influences the chemistry, in the membrane.

We hypothesize that a screening method can be effected by using the most basic precept of separation science.[12] That is, molecules (e.g., good and bad receptors) can be separated on the basis of a gradient of free energy. If a gradient of concentration of the target solute could be maintained within a receptor-containing solution, then each receptor would "feel" a free energy gradient according to its affinity to the target solute.

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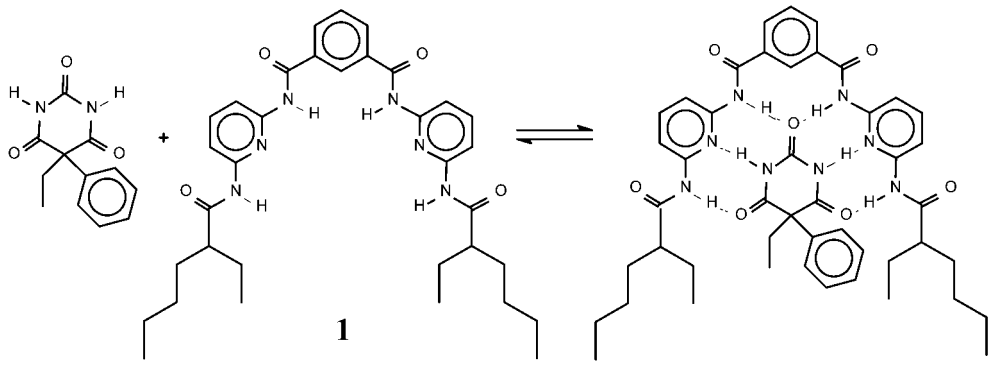

Figure 1. Structures of phenobarbital, receptor **1**, and the complex between them.

The mechanisms of carrier-mediated transport of cations through ISE membranes[13−18] have been studied. In the modeling[13] and analysis[14,19,20] of ion selective electrode membranes, concentration profiles of carriers (receptors) have been observed. Slices of PVC membranes, when stacked, yield a functioning membrane that can be later dissected and analyzed. More recently, microscopic observation of a ring-shaped membrane[17] has been used to deduce carrier concentration profiles. We have adopted the former method for this work. We are unaware of any work of this kind not related to ion sensors. It needs to be stressed that the driving force in ion sensors can be many orders of magnitude larger than we anticipated in this work. For example, Bakker et al.[21] reported on a series of ionophores including valinomycin and BME-44 et al. for alkali-metal and alkaline- earth-metal ions in which the complex formation constants were on the order of $10^{5.5}$–$10^{9.3}$ for 1:1 stoichiometry. In the current system, we anticipate an overall equilibrium constant on the order of 10^3 or less. As the driving force is very small, we need to test the idea that we can control the receptor concentration profile by the establishment of a solute concentration profile in a membrane. This is the objective of the work described in this paper. We first outline the theory and then describe the experiments.

If a target molecule (solute) has a concentration of [S](0) just inside the membrane at the source phase/membrane phase interface and the solute concentration is zero just inside the membrane at the membrane phase/receiving phase interface, then its concentration at any point in the membrane phase at steady state, [S]*(x)*, is

$$[\mathrm{S}](x) = [\mathrm{S}](0)\left(1 - \frac{x}{l}\right) \tag{1}$$

in which l is the total thickness of membrane and x is the distance from the source phase/membrane interface. Our hypothesis is based on the idea that there is local chemical equilibrium in the membrane but not overall, as in the case of chromatography. At equilibrium, the concentration of *free* receptor must be constant across the membrane. From the binding equilibrium, we have

$$[\mathrm{RS}](x) = K_\mathrm{f}[\mathrm{S}](x)[\mathrm{R}] \tag{2}$$

where [RS](x) is the concentration of complex at x, [R] is the concentration of free receptor, and K_f is the equilibrium constant for the formation of the complex. From mass balance,

$$[\mathrm{R}]l + \frac{1}{2}[\mathrm{RS}](0)l = [\mathrm{R}]'l \tag{3}$$

where [R]′ is the receptor concentration at $t = 0$. Combining eqs 2 and 3, we have

$$[\mathrm{R}] = \frac{[\mathrm{R}]'}{1 + \dfrac{[\mathrm{S}](0)K_\mathrm{f}}{2}} \tag{4}$$

$$[\mathrm{RS}](x) = \frac{2[\mathrm{S}](0)[\mathrm{R}]'K_\mathrm{f}}{2 + [\mathrm{S}](0)K_\mathrm{f}}\left(1 - \frac{x}{l}\right) \tag{5}$$

Equation 5 shows that the complex concentration decreases as solute phenobarbital concentration decreases. Because of the local chemical equilibrium, at any point, x, the higher the concentration of phenobarbital and/or the larger the formation constant the higher the complex concentration will be.

In this paper, we report relevant diffusion coefficients to estimate the equilibration time in the system; we show to what degree PVC influences solute partitioning and binding to receptor. We test the hypothesis that the concentration of receptor will respond to a solute gradient, even with a driving force only on the order of a few *RT*.

EXPERIMENTAL SECTION

Reagents. Tributyl phosphate (TBP), poly(vinyl chloride) (PVC), and HPLC grade tetrahydrofuran were purchased from Aldrich (Milwaukee, WI). Phenobarbital was purchased from Sigma (St. Louis, MO). Dioctyl sebacate (DOS) (Fluka Chemical Co., Ronkonkoma, NY) was used as received. Santicizer 141 (isodecyl diphenyl phosphate) was a gift from Monsanto (St. Louis,

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MI). pH 9.0 buffer was prepared with 0.025 M sodium borate aqueous solution. All other compounds were AR grade or better and purchased from commercial sources. The details of the barbiturate receptor synthesis have been described elsewhere.[5]

Preparation and Determination of the Density of Membranes. Membranes that contain **1** were made with mass ratio PVC:plasticizer:**1** = 33%:66%:1% or 21%:78%:1%. Membranes that do not contain **1** were made with mass ratio PVC:plasticizer = 33%:67%. The mixture (1 g total in mass) was dissolved in 20 mL of THF and then transferred into a specially made dish (diameter 7.5 cm) with an optical flat glass as a bottom surface. The dish was covered with a tissue, and the solvent was left to evaporate overnight. The formed membrane was then carefully taken off from the glass surface and used in the experiment. Several membranes were weighed, and their volumes were determined by measuring the thickness, h, and radius, r, of the membrane ($V = \pi r^2 h$). The density of the membrane is 1.15 ± 0.018 kg/L (error is standard error of the mean, $n = 4$).

Determination of D_C, K_p, and K_f. Capillary electrophoresis was used to determine phenobarbital concentration in distribution experiments. These data were used to determine the distribution ratio (D_C) and partition ratio (K_p) for phenobarbital and the formation constants (K_f) for the phenobarbtial−receptor complex in plasticized PVC and certain plasticizers.

Partioning Studies (D_C and K_p). Define q as the fraction of phenobarbital of all forms remaining in the aqueous phase at equilibrium and Φ (V^o/V^w) as the phase ratio. The distribution ratio is the ratio of the total analytical concentration of a solute in the extract to its total analytical concentration in the other phase. The partition ratio is the ratio of the concentration of a substance in a single definite form in the extract to its concentration in the same form in the other phase at equilibrium. The expressions for D_C and K_p are

$$D_C(\mathrm{R}) = \frac{[\mathrm{S}]_o + [\mathrm{SR}]_o + \cdots}{[\mathrm{S}]_w + [\mathrm{S}^-]_w + \cdots} = \frac{1-q}{q\Phi} \qquad (6)$$

$$K_p = \frac{[\mathrm{S}]_o}{[\mathrm{S}]_w} = D_C(0)\left(1 + \frac{K_a}{(\mathrm{H}^+)}\right) \qquad (7)$$

in which $D_C(0)$ is the distribution ratio when receptor is not present, while $D_C(\mathrm{R})$ is the distribution ratio when receptor is present. The latter is a function of the total receptor concentration. K_a is the first acid dissociation constant of phenobarbital (5.01×10^{-8} M), and (H^+) is the proton activity. The extraction was carried out in a pH 5.0 buffer. Therefore, in the case when receptor is absent (no complex form in the organic phase), the distribution ratio and the partition ratio are different by only about 1%.

For D_C and K_p determinations, plasticized PVC membranes made of different plasticizers were prepared. The membranes were cut into small pieces to facilitate partitioning and then weighed on an analytical balance (AT262, Mettler Toledo, Inc., Columbus, OH). The membranes were then transferred into scintillation vials containing a known volume of 500 μM phenobarbital aqueous solution (pH 5.0 acetic acid−sodium acetate buffer). The volume phase ratios were chosen so that quantitative analysis could be achieved on a CE instrument. The contents were stirred with a 15-position synchronized stirrer (speed control 200 rpm; Cole-Parmer, Chicago, IL). A 30 μL volume of the contents was sampled at 15, 30, 60, and 120 min.

For the determination of D_C and K_p of phenobarbital in Santicizer 141, 15 mL of 500 μM phenobarbital solution (pH = 5.0 buffer) was transferred into a scintillation vial containing 1 mL of Santicizer 141. The contents were gently mixed for a few seconds using a Vortex-Genie (speed control 2; Scientific Industries, Bohemia, NY) and placed on a magnetic stirrer (stirring speed 200 rpm) for 30 min. The samples were then removed from the stirrer and centrifuged for 10 min using an IEC clinical centrifuge (speed control 7; International Equipment Co., Needham Height, MA). The aqueous layer was then transferred to a vial and examined by CE. All of the liquid−liquid extractions involving plasticizers were carried out at 32 ± 1 °C (heating effect comes from the stirrer), and duplicate samples were tested. Phenobarbital standards were prepared daily, and a calibration curve was plotted for each set of runs. Separations were carried out on an ISCO 3850 capillary electropherograph (ISCO Inc., Lincoln, NE). A detection window was opened 40 cm from the injection end on a 65 cm fused silica capillary (50 μm i.d, Polymicro Technologies, Inc., Phoenix, AZ). The separation buffer was 0.11 M tris solution adjusted to pH 8.0 with TAPSO (3-{[tris(hydroxymethyl)methyl]-amino}-2-hydroxypropanesulfonic acid). Standards and samples were injected by applying 0.5 psi at the end of capillary for 5 or 30 s depending on the substrate concentration. During separation, a potential of 26 kV was applied. The current was about 25 μA. The detection wavelength was set to 240 nm. Data acquisition and integration were controlled by EZ Chrom (Scientific Software, Inc., San Ramon, CA).

Formation Constant (K_f) Determinations. Complex formation between phenobarbital and **1** happens inside the receptor-containing membrane. The formation constant can be calculated from experimental results as shown in eq 8,

$$K_f = \frac{1 - q - D_C(0)\Phi q}{D_C(0)([\mathrm{R}]_T \Phi q - [\mathrm{S}]_w(1 - q - D_C(0)\Phi q))} \qquad (8)$$

where $[\mathrm{S}]_w$ is the concentration of substrate in the aqueous phase at equilibrium. For the determination of K_f in plasticized PVC, the general procedures described above were followed. For the determination of phenobarbital and receptor complex in Santicizer 141, 10 mg of **1** was dissolved in 5 mL of Santicizer 141 and mixed with 15 mL of 500 μM phenobarbital aqueous solution in a scintillation vial. A 30 min mixing time with a Vortex-Genie was proven to be sufficient to reach the extraction equilibrium. After mixing, the contents were then centrifuged for 10 min (speed control 7). The aqueous layer was then analyzed by CE. All of the aqueous−membrane partitioning experiments were conducted at room temperature (23.0 ± 1 °C) and examined in duplicate.

Transport Experiments. Either two standard polystyrene cuvettes (capacity 4.5 mL) or locally manufactured Plexiglas chambers were used in each setup. For the setup made of polystyrene cuvettes, an opening (diameter 0.55 cm) was drilled in each cuvette. The distance between the centers of the opening to the bottom of the cuvette was 0.6 cm. With the openings facing each other, the two cuvettes were held together with a plasticized

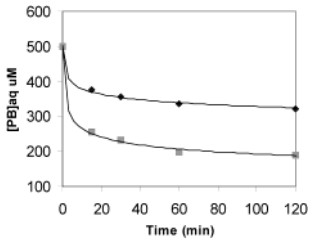

Figure 2. Time-dependent phenobarbital concentration during extraction from pH = 5.0 buffered phenobarbital aqueous solution (500 μM) to DOS/PVC membrane (black) and DOS/PVC/**1** membrane (gray) (Lines are best fits to eq 9.)

PVC-supported membrane in between. Membranes were cut into a size that covered the opening. Pieces of membrane were stacked together and secured between the two cuvettes, serving as the organic phase. For the Plexiglas setup, two chambers (capacity 100 mL) were placed facing each other lengthwise. Four holes were drilled on the side of each chamber with membranes stacked in between. As in the polystyrene cuvette, the distance between the center of the opening and the bottom of the cuvette was 0.6 cm. The thickness of the membrane was measured by a Starrett electronic digital micrometer (resolution 0.001 mm; L.S. Starrett Co., Athol, MA). One cuvette contained saturated phenobarbital aqueous solution (source phase); in the other was pH 9.0 basic buffer solution (receiving phase). Steady-state time was estimated according to the Einstein equation: $t = l^2/2D$. The device was kept on a synchronized magnetic stirrer until steady state was reached.

After steady state was reached, the system was opened up and the membranes were separated from one another. After rinsing with basic buffer and deionized water, the membranes were left to dry in a desiccator overnight. Each piece of membrane was then weighed and transferred into a scintillation vial containing a known volume of basic buffer (0.1 M $Na_4B_2O_9$ adjusted to pH 10.0 with 0.1 M NaOH). The volume phase ratio differed for various membranes so as to maintain the UV absorbance in a range that quantitative analysis could be performed. The scintillation vials were placed on a synchronized stirrer. A 24 h period was found to be sufficient for a thorough back-extraction. All the back-extractions were conducted at 32 ± 1 °C. The aqueous phase was then examined by UV absorption spectrophotometry. Quartz cells with path lengths of 1.0 and 0.1 cm were used depending on the phenobarbital concentration in the buffer solution. After the UV absorbance measurement, each piece of membrane was rinsed with deionized water and kept in a desiccator overnight, and then the membrane was dissolved in 2.5 mL of THF (HPLC grade). The receptor concentration was obtained by measuring absorbance at 302 nm.

RESULTS AND DISCUSSION

Diffusion Coefficient of Phenobarbital in Plasticized PVC-Supported Membranes. Figure 2 shows an example of phenobarbital concentration in the aqueous phase versus time of exposure to membranes 125 ± 5 μm thick. Using a model of a slab suspended in a stirred solution of limited volume,[22] we determined the diffusion coefficient of phenobarbital in each membrane. The solution of the diffusion equation is

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=1}^{\infty} \frac{2\alpha(1+\alpha)}{1+\alpha+\alpha^2 q_n^{\,2}} \exp(-Dq_n^{\,2}t/l^2) \qquad (9)$$

where

$$\alpha = \frac{a}{Kl} = \frac{V_{aq}}{KV_{slab}} \qquad (10)$$

in which M_t is the total amount of solute in the slab at time t and M_∞ is the corresponding quantity after infinite time. In the model, the slab and the volume of solution have unit area, so their thicknesses represent their volumes. The volume of the solution is a, l is the volume of the slab, and K is the partition factor. The partition factor is the partition ratio in the cases where receptor is absent, and the distribution ratio in the case where receptor is present.

The values of q_n are the nonzero positive roots of[23]

$$\tan q_n = -\alpha q_n \qquad (11)$$

Four terms ($n = 1-4$) are sufficient to attain convergence. The sum of squared differences between the data and the calculation was minimized to obtain D.

Table 1. Diffusion Coefficients of Phenobarbital in Various Membranes Obtained from Time-Dependent Extraction Experiment of Phenobarbital from Aqueous Solution to Membrane Phases

membrane	diffusion coeff (cm^2/s)	membrane	diffusion coeff (cm^2/s)
DOS/PVC	1.2×10^{-8}	Santicizer 141/PVC/**1**	1.8×10^{-8}
DOS/PVC/**1**	1.2×10^{-8}	TBP/PVC	1.5×10^{-8}
Santicizer 141/PVC	2.4×10^{-8}	TBP/PVC/**1**	1.3×10^{-8}

The diffusion coefficients obtained (Table 1) confirmed that these membranes behave like a viscous liquid in partitioning experiments. The magnitude of the diffusion coefficients obtained in this work matches well with earlier works conducted on diffusion coefficient determinations in polymeric membranes. By a microscopic method, Schneider et al. determined the diffusion coefficient of chromoionophore ETH 2439 in an ion-selective DOS/PVC membrane to be $(2.8 \pm 0.4) \times 10^{-8}$ cm^2 s^{-1}.[17] By using other methods, Iglehart et al. have determined the diffusion coefficient of valinomycin in bis(1-butylpentyl) adipate, bis(2-ethylhexyl) sebacate, and 4-nitrophenyl octyl ether (*o*-NPOE)-based PVC membranes as 1.5×10^{-8}, 1.7×10^{-8}, and 3.0×10^{-8} cm^2 s^{-1}, respectively.[24] Armstrong and Horvai have determined the diffusion coefficient for the Ca^{2+}-selective ionophore (−)-(*R*,*R*)-*N*,*N*′-bis[11-ethoxycarbonyl)undecyl]-4,5-tetramethyl-3,6-dioxaoctanediamide (ETH 1001) in *o*-NPOE/PVC as 1.1×10^{-8} cm^2 s^{-1}.[24]

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Table 2. K_p and K_f Values of Phenobarbital in Receptor-Doped Plasticized PVC Membrane and Plasticizers Obtained in Extraction Experiment of Phenobarbital from Aqueous Solution to Membrane Phases

plasticizers/membrane	K_p	K_f (M^{-1})
dioctyl sebacate	4.60 ± 0.12^b	$(5.6 \pm 2.4) \times 10^{3\,b}$
dioctyl sebacate/PVC	6.19 ± 0.21^a	176.9 ± 13.6^a
Santicizer 141	161.6 ± 2.3^a	41.6 ± 2.1^a
Santicizer 141/PVC	36.4 ± 0.59^a	13.9 ± 1.6^a
tributyl phosphate	170 ± 30^b	$(2.3 \pm 0.057) \times 10^{2\,b}$
tributyl phosphate/PVC	207.8 ± 2.7^a	6.0 ± 1.0^a
dioctyl phthalate	1.7 ± 0.18^b	$(7.1 \pm 0.19) \times 10^{2\,b}$
chloroparaffin	0.064 ± 0.052^b	$(2.5 \pm 1.0) \times 10^{4\,b}$

[a] Errors are standard error of the mean, $n = 4$. [b] Data from citation.[2,5] Errors are standard errors of the mean; n varies.

Knowing the diffusion coefficients is useful for estimating the time needed for transportation in steady-state experiments.

Influence of Solvent on Phenobarbital Partition Ratio and Complex Formation Constant. The distribution ratio was measured by CE analysis. On the basis of eq 7, the partition ratio can be calculated from D_C in the absence of receptor and the first dissociation constant. Table 2 lists the partition ratios for phenobarbital in each of the membranes compared with the partition ratios in corresponding pure plasticizers.[2]

The formation constant of the phenobarbital−receptor complex has been studied in $CDCl_3$ by H^1 NMR ($K_f = 3.3 \times 10^4\ M^{-1}$)[5] and in various plasticizers. Compared with the K_f in pure plasticizers,[2] all the K_f values are lower in the membrane phase (Table 2). It is obvious that the introduction of PVC plays an unrecognized but important role. We have analyzed earlier published data to see if this was the case in Li and Weber's work.[5] We found that K_f = 141 and 195 M^{-1} in DOP/PVC/**1** and chloroparaffin/PVC/**1** membrane, respectively. That is, the formation constants were lower in plasticized PVC membranes than in plasticizer itself. Although this current study used a different methodology from their experiment, the formation constants are very comparable.

Phenobarbital and Receptor Concentration Profiles in Membrane Phase. In the transport experiment setup, the dimension of membrane phase can be optimized through some approximate calculations. According to the Einstein equation ($t = l^2/2D$), the distance over which the separation happens should not be too long since the time needed to reach steady state increases with the square of this distance. However, if the separation distance is too short, the process of recovering the good receptor will be more difficult. Therefore, the smallest number of membrane layers that could convincingly show a receptor concentration gradient was used. Most experiments used six layers of membranes for transport.

The receptor's concentration was measured in portions of a cast membrane. The receptor concentration was consistent within the membrane prior to the transport experiment. The pooled standard deviation, s, of the receptor concentration in 4 pieces of membranes from each of 4 separate preparations was 1.17%.

The critical factor for **1** to form a concentration profile (eq 5) is a spatial phenobarbital concentration gradient. The phenobarbital concentration profile in a membrane without **1** present is shown in Figure 3 for DOS and Santicizer 141 membranes,

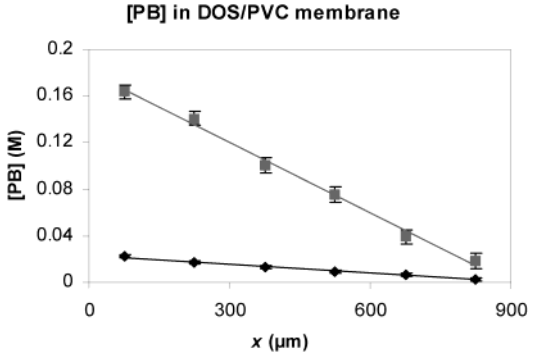

Figure 3. Phenobarbital concentration profile at steady state in PVC/DOS (black) and PVC/Santicizer 141 (gray) membrane. (Lines are best fit.)

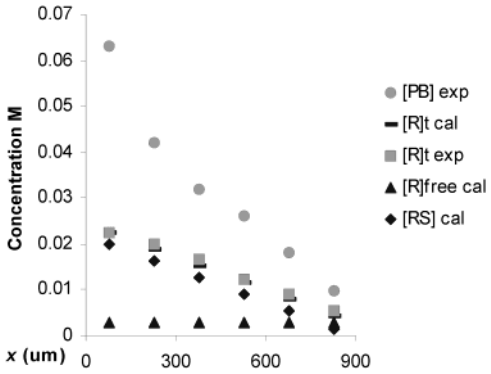

Figure 4. Phenobarbital and **1** concentration profile at steady state in PVC/DOS/**1** membrane.

respectively. The 95% confidence interval for each duplicate determination was calculated from a pooled standard deviation on the basis of multiple parallel experiments. It can be seen that phenobarbital concentration in the membrane phase decreases linearly from source phase to receiving phase, as expected.

When there was no phenobarbital concentration gradient, the receptor concentration remains approximately constant across the membrane. Regression of the concentration of receptor on the distance across the membrane leads to a slope (95% confidence interval) of -7.5×10^{-8} (-2.8×10^{-7}−1.3×10^{-7}) M/μm and an adjusted r^2 of −0.002. In other words, there is no correlation of concentration with distance. The average concentration of receptor **1** in the membrane was 0.0128 M.

The concentration profile of phenobarbital and **1** in a PVC/DOS/**1** membrane phase is shown in Figure 4 along with calculated results of free receptor concentration $[R]_{free}$ and phenobarbital−receptor complex concentration [RS]. (This plot represents a single 6-membrane stack; thus, there are no error bars. These data are typical.) The phenobarbital concentration gradient is maintained in the presence of receptor. The receptor concentration profile has changed from its constant value across the membrane to a linear profile that decreases from source phase side to receiving phase side. This matches our expectation. On the side of the membrane next to the phenobarbital source phase, a high receptor concentration was observed, whereas, on the side next to the basic buffer receiving phase, the receptor shows the minimum concentration. The data indicate that the phenobarbital concentration gradient in the membrane phase is the driving force for the receptor to accumulate near the source phase.

Table 3. Comparison of Distribution Ratios of Phenobarbital Obtained from Gradient Experiments and the Partition Experiments[a]

plasticizers	D_C(gradient expt)	D_C(partition expt)
dioctyl sebacate/PVC	5.73 ± 0.1	6.03 ± 0.20
dioctyl sebacate/PVC/**1**	14.0 ± 0.55	18.1 ± 0.31
Santicizer 141/PVC	40.9 ± 1.5	36.4 ± 0.59
Santicizer 141/PVC/**1**	43.0 ± 0.8	41.5 ± 0.78

[a] Errors are the standard errors of the mean; $n = 4$.

As shown in the introduction, we can calculate free receptor and complex concentration profiles in the membrane. The sum of these two will yield the total receptor concentration. Figure 4 also shows the comparison of calculated and experimental data. It shows a fair fit between the theoretical calculation result and the experimental result.

Distribution ratios obtained from transport experiments (D_C = phenobarbital's total concentration in the membrane next to the source phase divided by its concentration in the source phase) were compared with the D_C obtained from the partition experiments obtained from CE analysis. Results are shown in Table 3. A fairly good match was found in all the cases. This indicates that local thermodynamic equilibrium was achieved at the source phase/membrane phase interface.

At this point, we do not yet have a mixture of candidate receptors; however, we can further test our hypothesis with a weakly binding receptor. With a weakly binding receptor, the influence of the substrate concentration gradient on the receptor concentration gradient should be nil. The plasticizer controls the binding constant between substrate and receptor. Unlike DOS, Santicizer 141 is polar and has a good solubility toward phenobarbital. At steady state, both phenobarbital and the receptor concentration profiles were measured in the membrane phase using Santicizer 141 as plasticizer. Figure 5 shows that phenobarbital still maintains a linear concentration gradient and in comparison to the case without **1** the slope is the same within experimental error. On the other hand, the receptor's concentration remains constant across the membrane, as prior to the transport. Within experimental error, its concentration profile did not change. The receptor's linear concentration profile is "switched on" when there is a strong binding between receptor **1** and phenobarbital; it can be easily "switched off" by decreasing the formation constant with a polar plasticizer solvent.

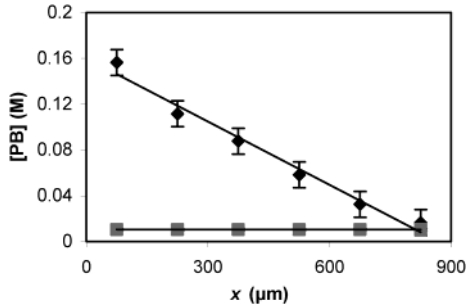

Figure 5. Phenobarbital (black) and **1** (gray) concentration profile at steady state in PVC/santicizer 141/**1** membrane. (Lines are best fit.)

Finally, it is appropriate to describe how this procedure will be applied in a screen for novel receptors. The products of synthesis, ideally from a solid-phase synthesis to avoid laborious cleanup procedures, can be combined and put into a membrane preparation. As the pure receptor candidates exist, any straightforward analytical technique such as CE or HPLC, including mass spectrometric detection, can be developed to separate and quantitate the individual components of the library. This clearly limits the size of the library to a few tens of compounds, perhaps up to 100. If the library is sufficiently large, many such membranes, each with a different set of receptors, may be prepared. Once membranes are prepared, they can be subjected to the procedure demonstrated in this paper. Analysis of the proximal and distal membranes in the membrane stack will demonstrate clearly which are concentrated in the proximal membrane. Many such membrane stacks may be equilibrated in parallel. Although the screening procedure seems slow, one must bear in mind at least two facts. Once the receptor-library-containing membranes are made, they can be used for screening for any number of receptors in parallel or they can be stored and used in the future. Also, once an effective receptor is identified, it can be used for analytical extractions for a long time; i.e., the screening for a particular solute or guest only needs to be done once.

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