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Chromatographic approach for determining the relative membrane permeability of drugs

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Abstract

With the aid of the experimental dependence of the theoretical plate height (H) on the flow-rate (U), values of diffusion coefficients as the permeation rate, of the compounds in a polymeric stationary phase were calculated from solute mass transfer. This approach is proposed for modeling the relative diffusion rate of a drug within the membrane. After the successful separation of opioid compounds using a C_{18} derivatized polystyrene–divinylbenzene polymer HPLC column, the slopes of H–U plots increase quantitatively in the order of meperidine$<$alfentanil$<$fentanyl$<$sufentanil, indicating that the large mass transfer resistance slows down the penetration of molecules. A constant intercept for the experimental plate height supports the proposal interpretation. A good correlation between the diffusion coefficients and hydrophobicity (log P_{oct}) from the traditional shake-flask method was obtained for the opioid compounds, demonstrating that the more lipophilic molecules penetrate deeper into the stationary phase leading to a lower migration rate under the specified conditions. Plot of the diffusion coefficients versus potency ratio for different opioids after intravenous administration reflect the values of the dynamic process in drug studies. The work herein differs from existing studies by measuring the permeability of drugs into the stationary phase rather than providing membrane partition coefficients for a series of analogues. Thus, the study of drug permeability combined with other physico-chemical properties, such as hydrophobicity, may provide additional information on drug–membrane interactions. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Relative membrane permeability; Meperidine; Alfentanil; Fentanyl; Sufentanil

1. Introduction

The study of the interactions of biomolecules with cell membranes is a well-developed field. This fundamental biological process is commonly characterized by the membrane partition coefficient (K_m), which represents the equilibrium solute distribution between the aqueous phase and the membrane. Measurements of drug membrane partitioning have been the subject of many studies over the last several decades. Three models of systems have been developed to measure solute–membrane partitioning: (i) organic solvent–aqueous partitioning systems, such as octanol–water [1–3], (ii) chromatographic partitioning systems using either octadecyl silica (ODS) or immobilized artificial membranes (IAMs), including immobilized liposome chromatography (4–9), as a stationary phase [10–13], and (iii) liposome partitioning systems [14–16].

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It has been suggested that these models, despite their obvious advantages, also have some drawbacks for the measurement of the membrane partitioning of drugs. First, the permeation of solute through a lipid membrane cannot be described adequately by a simple solubility/diffusion model, because both the diffusion and the solubility of the solute molecule are position dependent according to the ''Fick's Law of diffusion''. Collander [17] found that *sec*-butanol crossed the membrane of nitella five times more rapidly than its more spherical isomer *tert*.-butanol, whereas their partition coefficients in standard model systems varied by at most a factor of two. The same conclusion has been drawn for other biomembranes [18,19]. Furthermore, a recent study by Tomlin et al. [20] demonstrates a large (~15-fold) potency difference between the optical isomers of the potent intravenous anesthetic etomidate in tadpoles. Such stereospecificity obviously cannot be accounted for by ''classical'' lipid theories. Second, liquid chromatographic approaches offer a number of advantages over the static methods. These include speed of determination, better reproducibility, ease of automation, the requirement of a very small amount of the sample to be determined and the capability of separating impurities in the sample simultaneously with the determination. However, the partition coefficient is difficult to measure precisely using either conventional HPLC or IAM chromatography, and there is little direct quantitative information regarding the partition coefficient because the ratio of the volumes of the stationary and mobile phases are typically not mentioned [21]. Third, although liposomes can model solute partitioning into cell membranes [22], the liposome method is time-consuming, and there are many experimental obstacles that limit the wide-scale development of liposomes as an in vitro model for predicting the drug–membrane interaction [16]. Apparently, it is almost impossible to measure the rate of permeation process of drug through phospholipid bilayers.

In this paper, we used a different dynamic HPLC approach to predict the membrane permeability of drugs. With the aid of the experimental dependence of the theoretical plate height on the mobile phase flow-rate and the band broadening equation, the retention behavior and band broadening of opioid compounds on a C_{18} derivatized polystyrene–divinylbenzene polymer column (PRPLC) was determined. Experimental plate-height data were obtained, and diffusion coefficients (D_s) of solute into the stationary phase were calculated, which reflects the solute transfer rates within a given medium under specific conditions. A constant intercept and linear correlation between the calculated theoretical plate height and the experimental flow rates of opioids provide further proof for this proposal. In the application of this approach to study the membrane permeability of drugs, PRPLC was selected to mimic the lipid membrane, because PRPLC has been recognized as the gold-standard for thermodynamic studies of biological partitioning processes [23] and has been used successfully for alkane–water partition coefficient determination for compounds containing dramatically varying functionalities [24]. Recently, lipophilicity measurement by means of an alternative PRPL in this laboratory [25] showed that there appears to be no significant specific interaction between the solutes and the polymeric stationary phase, which is beneficial to the measurement of permeability of a solute in the stationary phase. The opioid compounds were selected as drug probes, because it has been postulated that opioid anesthesia may involve a general membrane effect because of the much closer correlation between electroencephalograph or anesthetic effects and membrane lipid content as opposed to serum opioid levels [26]. Nevertheless, it is not the purpose of this report to discuss extensively the concepts of membrane mimetism, their merits and shortcomings, instead the focus is on a new approach that will determine the permeation rate of drugs within the HPLC stationary phase.

The interaction between drugs and biological membranes has been studied previously by the chromatographic approach [10,11,23,24]. However, our present work differs from these earlier studies by measuring the relative diffusion rate of the drug within the hydrocarbon environment rather than providing a new scale of hydrophobicities or partition coefficients. To our knowledge, this is the first study to reveal the relationship between potency and the dynamic behavior of drugs during the chromatography process, suggesting that permeability might be a better model for the assessment of the QSAR of drugs in biological systems. In addition, the diffusion coefficient is, in general, directly proportional to the product of its partition coefficient and diffusion

constant in bulk liquid hydrocarbon [27]. Thus, the measurement of diffusion coefficients could be extended to the study of the interaction between drugs and cell membranes. Many different chromatographic stationary phases have been developed to mimic the fluid cell membrane for the study of drug–membrane interactions. This simple and sensitive approach may serve as a valuable tool for quantitatively describing the drug–membrane interaction.

2. Materials

The HPLC system consisted of an LC-600 pump, a variable wavelength UV detector SPD-6AV set at 254 nm from Shimadzu (Kyoto, Japan), a SIL-10A Autoinjector, a one-channel recorder Kipp and Zonen BD 40, a column heater from Alltech Associates (Deerfield, IL, USA), and a polymeric analytical C_{18} column (150×4.6 mm I.D., 5 μm particle size) from Advanced Separation Technologies (Whippany, NJ, USA).

Fentanyl citrate and meperidine were purchased from Sigma (St. Louis, MO, USA). Sufentanyl citrate and alfentanil chloride were supplied by Taylor Pharmaceuticals (Decatur, IL, USA).

3. Method

HPLC was performed at 35 °C using a basic mobile phase consisting of 50% methanol in 0.01 *M* K_2HPO_4 (pH=10.50). On the basis of a plot of height equivalent to H versus U, the diffusion coefficient (D_s) of the solute in the stationary phase was determined by the injection volume of 10 μl samples with a solute concentration of 30 μg/ml. The plate height was calculated from an experimental chromatogram by the width at half-height method. The data of chromatography were directly collected by a record.

4. Results

In HPLC, band-broadening effects can be expressed as follows:

$$H = B/U + C_s U + (1/2\ \lambda d_p + D_m/d_p^2 U)^{-1} \quad (1)$$

The term B/U represents molecular diffusion in the longitudinal direction in the mobile phase. The $C_s U$ term represents mass transfer resistance caused by solute diffusion in the stationary phase. The last term (where D_m is the solute diffusion coefficient in the bulk mobile phase and U is the flow rate of the mobile phase) is the coupling of eddy diffusion and mobile phase terms for solute dispersion in the mobile phase. The diffusion coefficient of solute (such as phenol in methanol–water mixtures) is ca. 10^{-9} m^2 s^{-1} [28], and usually the flow-rate used is of the order of mm/s and the particle size d_p is ca. 5 μm, so the contribution of the last term is mainly due to $2\lambda d_p$. In HPLC, this longitudinal term does not make an important contribution to band broadening if the flow-rate is sufficiently high [29]. In the current experiments, the flow-rate is above 1.5 mm/s, and consequently the eddy diffusion and diffusion in the mobile phase in Eq. (1) may be omitted. This approximation is consistent with the findings that broadening due to eddy diffusion and flow distribution is little affected by the mobile phase flow velocity [30]. Thus, Eq.1 can be simplified as follows:

$$H = C_s U + 2\lambda d_p \quad (2)$$

In general, C_s is a function of the diffusion coefficient (D_s) of the solute in the stationary phase, the relative migration rate of the solute in the mobile phase, and the particle size and pore structure parameters. C_s can be computed from the theoretical treatment of Gidding [31]:

$$C_s = R(1-R)d_p^2/30D_s \quad (3)$$

where R is the retention ratio, defined here for each solute by V_o/V_R, and V_o is the void volume of the column. On the basis of plot of a plate-height equivalent to H versus U, the diffusion coefficients (D_s) of solutes in the stationary phase were determined. The flow-rate was defined here by L/t_o, where L is the length of the HPLC column and t_o is the retention time of methanol.

From HPLC, the value of V_o is estimated to be 1.5 cm^3, which was used for calculating the R-value for each opioid. Curves showing the dependence of H on eluent flow-rate for opioids are given in Fig. 1, in which the slopes increase quantitatively in the order of meperidine<alfentanil<fentanyl<sufentanil. D_s

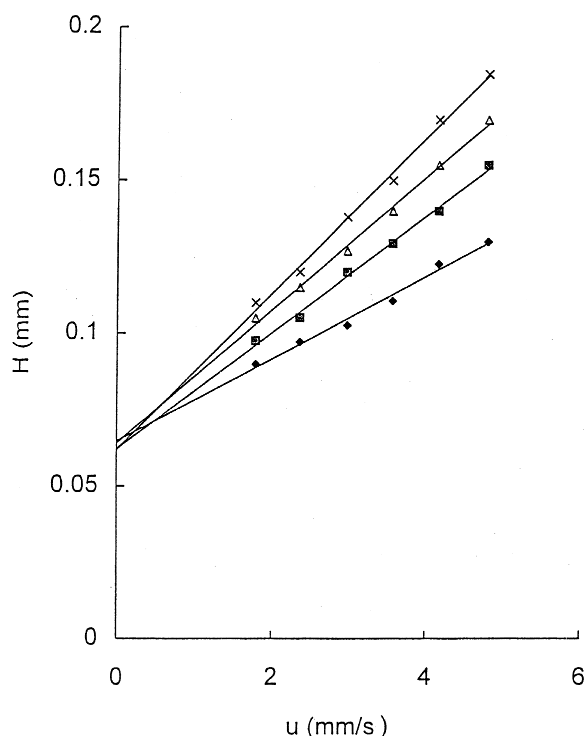

Fig. 1. H–U dependence of opioids on polymer ODS column. Eluent: 50% methanol in 0.01 M K_2HPO_4 (pH=10.50). Key to symbols: ♦ Meperidine; ▩ Alfentanil; △ Fentanyl; × Sufentanil;.

of opioids can be computed from Eq. (3) and the values of the mass transfer resistances (C_s), as listed in Table 1. Linear correlations between the calculated theoretical plate-height and the experimental flow-rates were obtained with correlation coefficients that exceeded 0.99. The intercept for the linear regression of each opioid compound was similar (see Fig. 1). A high correlation between D_s and either hydrophobicity (log P_{oct}) from the traditional shake-flask method (Fig. 2) [32] or potency ratio of different opioids after intravenous administration ln P (Fig. 3) [33] was obtained for the opioids.

Table 1
Slope of H–U curve (C_s), Diffusion coefficients (D_s), Potency ratio (P)[a] and Octanol/H_2O partition coefficient (P_{oct})[b]

Compounds	C_s	D_s ($\times 10^{-16}$ cm^2 s^{-1})	P	P_{oct}
Meperidine	0.0135	5.4	1	39
Alfentanil	0.0206	3.2	137	145
Fentanyl	0.0223	0.98	550	813
Sufentanil	0.0304	0.41	8500	1778

[a] P from Ref. [21], p. 293.
[b] P_{oct} from Ref. [21], p. 354.

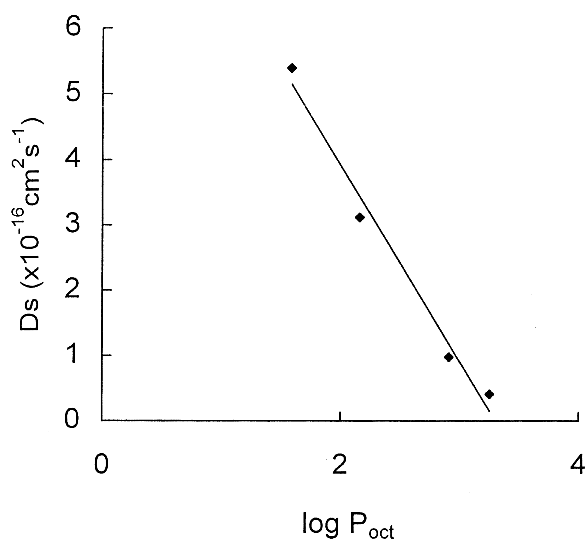

Fig. 2. Plot of diffusion coefficient (D_s) in the stationary phase of polymer ODS HPLC versus log P_{oct} from traditional method [26] for opioids.

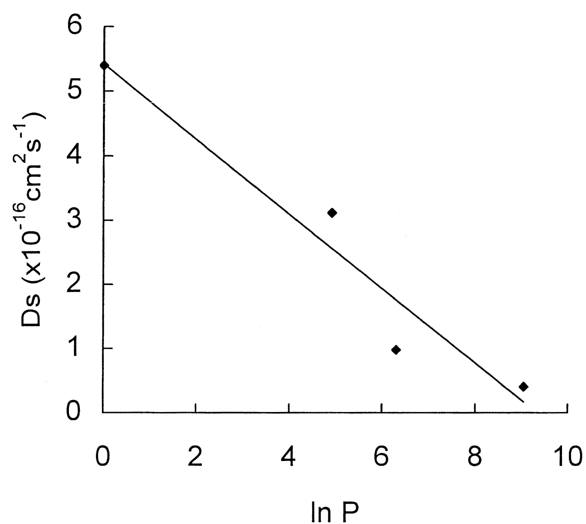

Fig. 3. Plot of diffusion coefficient (D_s) in the stationary phase of polymer ODS HPLC versus potency ratio (ln P) of different opioids after intravenous administration (Ref. [21]).

5. Discussion

The membrane permeability of drugs is of great importance to many biological processes, representing a measure of how rapidly drugs are transferred within biological membranes. Unlike the membrane partition coefficient, little attention has been paid to this critical step owing to technical difficulties. In the present chromatographic study, with the aid of the experimental dependence of theoretical plate height on the flow-rate, values of diffusion coefficients, as the permeation rate, of the compounds in a polymeric stationary phase were calculated from the solute mass transfer contribution. The correlation coefficients (0.99) obtained for linear regression for the opioids and the constant intercept of the H–U plots provides evidence in support of this simplified expression of HPLC, which is consistent with the previous description of mass transfer between mobile, ''stagnant mobile'', and stationary phases [34]. Meanwhile, it had been found that the slope of the H–U line is greater for 50 μm than it is 5 μm particles, suggesting that the large mass transfer resistance (due to the large particle) resulted in the large chromatographic band-broadening, which also indicates that the study of dynamic processes for this purpose is possible. Calculation of diffusion coefficients of opioids in the stationary phase from the mass-transfer dispersion term suggests that small molecules diffuse in the stationary phase of reversed-phase HPLC with different diffusion rates, which reveals more detailed dynamic information regarding solute–membrane interactions.

There are two main reasons for selecting reversed-phase PRPLC. First the chromatographic retention on long-chain hydrocarbon stationary phases such as C_{18}-bonded phases is dominated by a partitioning mechanism [35]. The distinction is that ''partitioning'' implies that the solute is fully embedded within the stationary phase, which approximates the situation when water or non-electrolytes cross the lipid bilayer membrane by a solubility-diffusion mechanism [27]. Second, it is likely that ODS chromatography can model the hydrophobic contribution of the drug–membrane interaction, and also the interaction between solutes and the polar lipid head groups. The bilayer interior is more like oil [27], and although biological membranes possess polar and charged groupings on their surfaces, these groupings will have no effect on the equilibrium coefficient [36]. Diamond and Katz [37] suggest that the head-group region of bilayers may present the most resistance to the permeation of lipophilic solutes, while the alkane-like interior of the bilayer may limit the permeation of polar solutes. Because most compounds of pharmaceutical interest contain at least one polar functionality, the diffusion coefficient obtained in C_{18} stationary phase may represent the rate determined step. Meanwhile, Hansch and Fujita [38] have explained the general applicability of relating log P (membrane–water partition coefficient) to biological activity in terms of the necessity of a compound to travel from some extracellular phase to some site of action in the cellular phase. They realized that the nature of most membranes are ''more or less organic phases'', and that the key process is partitioning. Therefore, a method to determine oil–water partition coefficients by HPLC is to load the stationary phase with the oil of interest, and measure the capacity factor with the oil-saturated mobile phase. On the other hand, when mixtures of methanol and water are employed as the mobile phase, Scott and Simpson [39] have shown that the solvent is adsorbed on the surface of the reversed-phase material, and this changes the nature of the surface of the reversed-phase matrix [39]. Because methanol can act as both a proton donor and acceptor, the surface layer of the stationary phase is modified by adsorbed methanol [40]. Also, Slaats et al. [41] have shown that the adsorption of methanol from methanol–water eluents reaches a maximum at about 20% (v/v) methanol and then remains approximately constant. These data have been interpreted as indicating that a monolayer of methanol is formed on the surface of the bonded phase [10]. The alkaline pH region was carried out in this approach for the measurement of hydrophobic parameters of unionized forms of basic compounds. Consequently, it is likely that ODS chromatography can model the hydrophobic contribution to the drug–membrane interaction, as well as the interaction between solutes and the polar lipid head group. Although exactly mimicking a cell membrane may not be possible using HPLC, the commercial possibility, reliable composition, and reproducibility for the stationary phase make it an attractive approach.

Compared with the diffusion coefficient of solute in the mobile phase (10^{-9} m^2 s^{-1}) [28], a much smaller diffusion coefficient of solute in the stationary phase was achieved (Table 1), demonstrating that the solute diffusion in the stationary phase of RPLC is restricted. The same results of diffusion of polystyrene in the stationary phase of gel permeation chromatography [44] and proteins in the stationary phase of TSK SW column [45] reflected the reliability of this approach. Fig. 1 shows that the slopes increase with the aforementioned order indicating the larger mass transfer resistance of solute in the stationary phase following this order which, of course, led to the smaller D_s. Fig. 2 shows that the D_s are inversely proportional to the drug's octanol/H_2O partition coefficient [32], showing that the more hydrophobic the opioid, the smaller the migration rate in the stationary phase will be. Coupling these data and the same retention behavior order of opioids, it is evident that the relative deeper migration in the stationary phase with the more hydrophobic opioid caused the large mass transfer resistance and the smaller D_s. This finding is consistent with a previous study on biological membrane properties, indicating that the addition of an alkyl carbon group will have a large retarding effect on the rate of internal membrane diffusion for a small molecule [36]. This result demonstrates that the C_{18} stationary phase is a good model for studying biomembrane transport since the interior region of phospholipid membranes presents an alkane barrier to permeation [42,43], and provides information about ascertaining the correlation between drug hydrophobicity and its diffusion rate. From the high correlation between the D_s and ln P, we postulate that the drug membrane permeability (or diffusion rate) plays a role in drug potency as well as its hydrophobicity. These data, would suggest that the potency of a drug is partly controlled by the depth the compound penetrates into a lipid membrane.

6. Nomenclature

HPLC	High-performance liquid chromatography
P_{oct}	*n*-Octanol–water partition coefficient
RPLC	C_{18} Reversed-phase liquid chromatography
H	Theoretical plate height
U	Flow-rate
ODS	Octadecyl silica
IAMs	Immobilized artificial membranes
K_m	Partition coefficient
D_s	Diffusion coefficients in the stationary phase
D_m	Diffusion coefficient in the mobile phase
C_s	Mass transfer resistances
QSAR	Quantitative structure-relationship

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