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Computational prediction of solubilizers' effect on partitioning

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Abstract

A computational model for the prediction of solubilizers' effect on drug partitioning has been developed. Membrane/water partitioning was evaluated by means of immobilized artificial membrane (IAM) chromatography. Four solubilizers were used to alter the partitioning in the IAM column. Two types of molecular descriptors were calculated: 2D descriptors using the MOE software and 3D descriptors using the Volsurf software. Structure–property relationships between each of the two types of descriptors and partitioning were established using partial least squares, projection to latent structures (PLS) statistics. Statistically significant relationships between the molecular descriptors and the IAM data were identified. Based on the 2D descriptors structure–property relationships $R^2Y = 0.99$ and $Q^2 = 0.82$–0.83 were obtained for some of the solubilizers. The most important descriptor was related to log *P*. For the Volsurf 3D descriptors models with $R^2Y = 0.53$–0.64 and $Q^2 = 0.40$–0.54 were obtained using five descriptors. The present study showed that it is possible to predict partitioning of substances in an artificial phospholipid membrane, with or without the use of solubilizers.

Keywords: IAM chromatography; In silico prediction; Solubilizers; Molecular descriptors; Structure–property relationship

1. Introduction

In drug discovery the introduction of combinatorial chemistry and high throughput screening methods has led to changes. Focusing on *in vitro* activity in high throughput screening generates high-potency compounds, but often it is at the expense of traditional biopharmaceutical properties such as lipophilicity or solubility (Lipinski et al., 1997). Computational methods based on simple descriptors are a promising alternative to early drug discovery experimental methods (Matsson et al., 2004). There is a demand for fast prediction of absorption, distribution, metabolism and excretion properties (ADME). The use of computational methods for estimating ADME properties based on molecular parameters have emerged to meet this demand (van de Waterbeemd and Gifford, 2003).

As most drugs are absorbed into the systemic circulation by ways of passive transcellular transport, screening for the ability to cross-membranes is an important step in the drug development process. A key element in membrane permeation is the partitioning of the compound between the membrane and the surrounding aqueous phase. Immobilized artificial membrane (IAM) chromatography, a technique which uses phospholipids as the stationary phase, is in general designated to evaluate partitioning between an aqueous compartment and phospholipids (Pidgeon and Venkataram, 1989; Pidgeon et al., 1995; Yang et al., 1997).

The purpose of the present study was to develop a computational method for the prediction of drug partitioning in membranes influenced by solubilizers. This was done by examining the partitioning in an IAM system in the presence of a number of different solubilizers. We selected eighteen structurally diverse test compounds. For each compound two types of molecular descriptors were calculated, 2D descriptors with the MOE software (Chemical Computing Group Inc., 2006) and 3D descriptors with the Volsurf software (Cruciani et al., 2000b). The results from the IAM study were analyzed in relation to the

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molecular descriptors using partial least squares projection to latent structures (PLS).

2. Materials and methods

An overview of the experimental strategy is given in Fig. 1.

2.1. Selection of test compounds

Eighteen structurally diverse compounds were selected based on Volsurf descriptors. The compound database used in the experimental design consisted of 1869 compounds. About 1831 of the structures were derived from Taub et al. (1997). The remaining structures were derived from an in-house database consisting mostly of drug-like compounds.

2.2. Chemicals

The test compounds used in the study were: 3-chlorophenylacetic acid and 3-hydroxyphenylacetic acid (Sigma–Aldrich Chemie, Germany); L-phenylalanine, *trans*-cinnamic acid, hypoxanthine, timolol, maleate salt and *cis*-1,3-pentadiene (Sigma Chemical Co., USA); 4-(methylsulfonyl)benzoic acid (Aldrich Chem. Co., USA); pyrrole and sulfanilamide (Fluka Chemie AG, Switzerland); 4-aminophenol, hydrocortisone, indigo carmine, nicotinic acid methyl ester, prednisolone phosphate, amaranth, phenobarbital and carbamazepine. The solubilizers used were: dodecyl sulfate sodium salt (SDS) (Merck, Germany); Tween 20 (Unikem, Denmark); glycocholic acid (Sigma Chemical Co., USA) and ethanol. Phosphate buffered saline (PBS) (Life Technologies, UK) was used as blind. The structures of the tested compounds are shown in Fig. 2.

2.3. Immobilized artificial membrane experiments

An IAM.PC.DD2 10 cm × 4.6 mm, 12 μm, 300 Ang. column (Regis Technologies, USA) was employed in the experiments. 0.01 M PBS, pH 6.50, aqueous solution with 0.10% ethanol, 0.01% SDS, 0.01% Tween 20 or 0.001% glycocholic acid were used as the mobile phase in the experiments. The test compounds were dissolved in the mobile phase. The column was equilibrated 1 h prior to the studies. Flow rate was 1.0 ml/min, injection was 10 μl 0.01 M and UV absorbance was measured for detection.

2.4. Computational methods

Molecular structures were built in extended conformations using the Sybyl molecular modeling system (Version 6.6) (Tripos Assosiates Inc., 2000) and the energy was minimized using the Tripos Force Field (Clark et al., 1989). The dielectric constant was four. Partial atomic charges were calculated in Spartan (Version 5.0) (Wavefunction Inc., 1997) before the 2D molecular descriptors were calculated with the MOE-program (Chemical Computing Group Inc., 2006) and the 3D descriptors were calculated with Volsurf (Version 2.0.2) (Cruciani et al., 1998; Discovery Ltd., 2000; Cruciani et al., 2000b).

A total of 146 2D descriptors were generated representing the following types of descriptors: physical property descriptors, surface area descriptors, atom and bond count descriptors, connectivity and shape descriptors, adjacency and distance descriptors, pharmacophore feature descriptors, and partial charge descriptors (Xue et al., 2003).

Fifty-six 3D descriptors were computed with Volsurf and they describe molecular properties like shape and size, hydrophilic/hydrophobic areas, interactions and balance and a

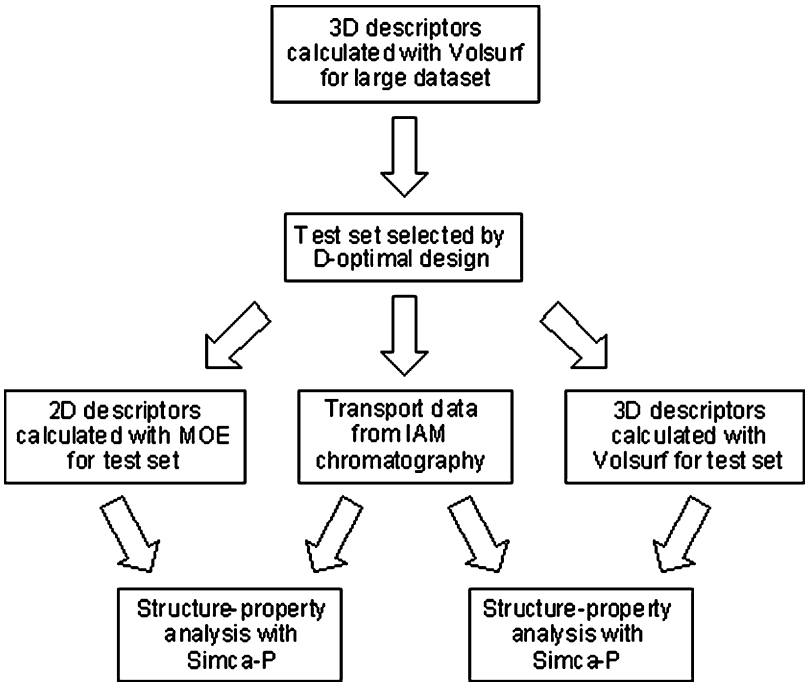

Fig. 1. Experimental strategy.

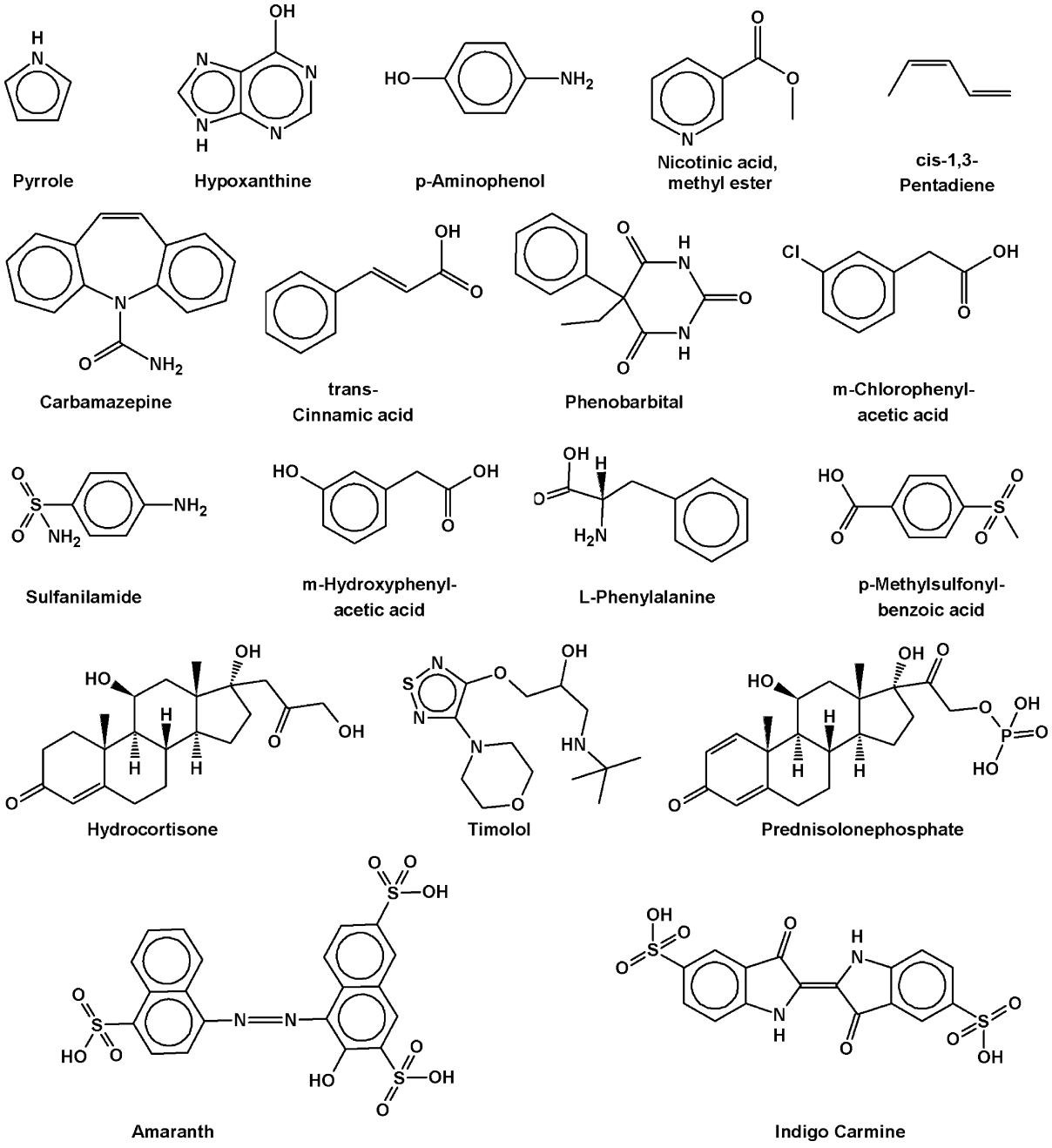

Fig. 2. Structures of the 18 compounds used in the study.

complex parameter describing the ability to form micelles. A thorough explanation of the descriptors is reported in the literature (Cruciani et al., 1998, 2000a,b).

2.5. Data analysis and statistics

The data analysis was performed by means of PLS using the software Simca-P (Version 8.0 and 10.5) (Wold, 1995). The IAM data was changed to a logarithmic scale prior to data analyses, submitted to unit variance scaling and mean centering. Different analyses were performed for each of the experimental data using the descriptors as *Y*-data. PLS is very useful for analyzing multivariate data that are correlated, coliniar or have missing observations. The method transforms data, in this study the 56 Volsurf descriptors and 146 MOE descriptors, respectively, into a few new *X*-values. The correlation between these new *X*-data and the *Y*-data (the experimental data) is then identified. R^2Y is the portion of variance explained by the PLS model, and Q^2 is a measure of the predictive power of the model.

3. Results

3.1. Diversity of data set

The 18 compounds studied experimentally were selected from the 1869 compounds based on the Volsurf generated 3D

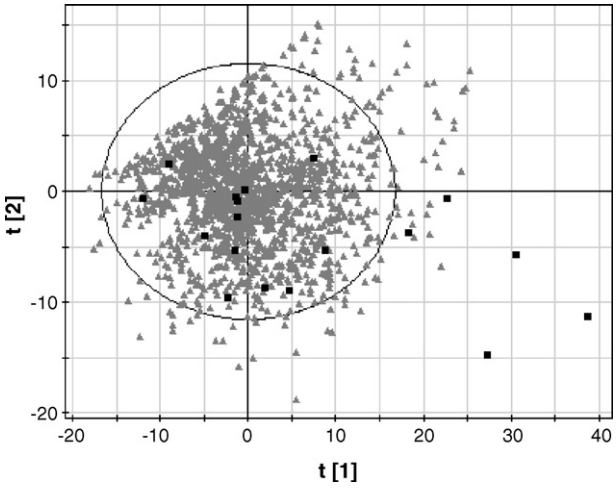

Fig. 3. PC 1 + 2 from PCA (MOE-descriptors) of 1869 compounds used for selection. The 18 compounds selected for the test set are highlighted.

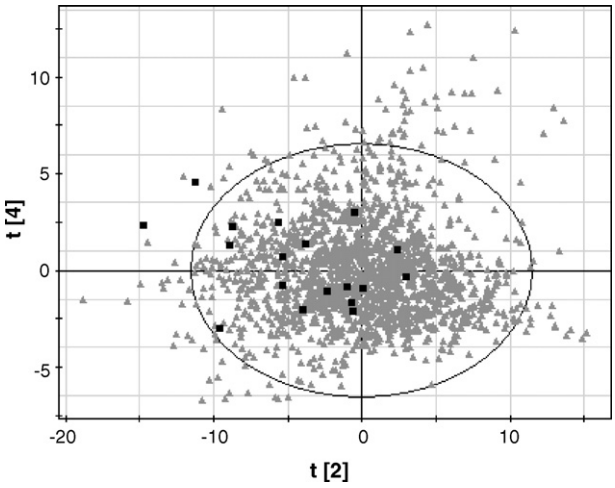

Fig. 4. PC 3 + 4 from PCA (MOE-descriptors) of 1869 compounds used for selection. The 18 compounds selected for the test set are highlighted.

descriptors. Since the test set has been selected solely on the Volsurf 3D descriptors, it is interesting to see if the compounds are representative if considering the MOE 2D descriptors, too.

The full data set (1869 compounds) was analyzed for the MOE-descriptors. Figs. 3 and 4 show the first four components of the principal component analysis (PCA). The 18 compounds comprising the test set have been highlighted in the plot, which confirms that these compounds represent the full data set well.

3.2. IAM chromatography

The capacity factors (K_{IAM}) shown in Table 1 were calculated on the basis of the retention times (Rt) and are proportional to the partitioning (Eq. (1)). The capacity factor is the retention time relative to the void volume. If the capacity factor is high, the test compound prefers the stationary membrane-like phase to the mobile phase. Citric acid was used for determination of the void volume.

$$K_{IAM} = \frac{Rt_{compound} - Rt_{citric\ acid}}{Rt_{citric\ acid}} \quad (1)$$

The rank order of the test compounds were not significantly influenced by the type of solubilizer. Void volume and retention times (not shown) changed with the type of solubilizer used, but K_{IAM} remained unchanged.

3.3. Models for structure–property relationships

Statistically significant models for prediction of the IAM capacity factors were identified based on the 2D and 3D descriptors, respectively. The statistically significant models explaining the relationships between molecular structures and the IAM data are illustrated in Table 2.

Table 1
Capacity factors (K_{IAM}) of the IAM-study

Compound	Control (PBS)	SDS	Glycocholate	Ethanol	Tween 20
Amaranth	25.0 ± 0.3	7.685 ± 0.169	13.5 ± 3.3	20.0 ± 0.6	7.113 ± 1.581
Carbamazepine	47.5 ± 0.1	48.3 ± 0.2	37.5 ± 0.4	43.4 ± 0.2	25.8 ± 1.3
Cis-1.3-pentadiene	5.79 ± 0.01	7.77 ± 0.04	5.77 ± 0.02	5.55 ± 0.01	7.84 ± 0.26
Hydrocortisone	40.0 ± 1.5	31.2 ± 1.2	27.6 ± 0.1	34.5 ± 0.7	12.1 ± 0.6
Hypoxanthine	0.244 ± 0.000	0.188 ± 0.006	0.230 ± 0.003	0.213 ± 0.000	0.255 ± 0.034
Indigo carmine	13.2 ± 0.1	10.1 ± 0.1	5.42 ± 0.05	10.2 ± 0.1	6.944 ± 1.151
m-Chlorophenylacetic acid	1.07 ± 0.00	0.170 ± 0.004	0.853 ± 0.012	1.01 ± 0.00	0.890 ± 0.101
m-Hydroxyphenylacetic acid	0.296 ± 0.071	1.78 ± 0.01	0.222 ± 0.003	0.235 ± 0.000	0.245 ± 0.034
Nicotinic acid, methylester	1.88 ± 0.00	1.97 ± 0.01	1.61 ± 0.01	1.69 ± 0.00	1.13 ± 0.06
p-Aminophenol	0.630 ± 0.000	1.02 ± 0.01	0.622 ± 0.043	0.566 ± 0.000	0.671 ± 0.039
Phenobarbital	6.24 ± 0.02	4.78 ± 0.02	5.25 ± 0.09	5.79 ± 0.00	5.79 ± 0.19
Phenylalanine	0.212 ± 0.009	0.253 ± 0.004	0.215 ± 0.003	0.199 ± 0.000	0.235 ± 0.034
p-Methylsulfonylbenzoic acid	0.163 ± 0.000	0.688 ± 0.005	0.148 ± 0.003	0.140 ± 0.000	0.194 ± 0.033
Prednisolonephosphoric acid	7.83 ± 0.10	1.40 ± 0.01	4.82 ± 0.02	7.04 ± 0.01	4.91 ± 0.35
Pyrrole	0.844 ± 0.000	0.935 ± 0.006	0.913 ± 0.016	0.806 ± 0.004	1.15 ± 0.07
Sulfanilamide	0.733 ± 0.000	0.493 ± 0.005	0.645 ± 0.009	0.654 ± 0.000	0.777 ± 0.041
Timolol	8.08 ± 0.01	n.d.	7.02 ± 0.02	6.98 ± 0.02	2.89 ± 0.11
Trans-cinnamic acid	1.09 ± 0.00	0.253 ± 0.004	0.878 ± 0.008	0.995 ± 0.004	0.795 ± 0.041

n.d.; not determined due to laboratory problems.

Table 2
PLS models for prediction of solubilizers' effect on drug partitioning

Enhancer	Volsurf 3D descriptors		MOE 2D descriptors	
	R^2Y	Q^2	R^2Y	Q^2
Control (PBS)	0.64	0.54	0.99	0.83
SDS	n.s.	n.s.	0.32	0.13
Glycocholate	0.49	0.49	0.99	0.82
Ethanol	0.53	0.53	0.99	0.83
Tween 20	0.40	0.40	0.99	0.83

For each solubilizer R^2 and Q^2 values of models with variable selection have been listed. The descriptors making only minor contributions to the models (typically VIP < 0.8 and 1.0) were eliminated. n.s.: no significance.

The PLS analysis of the MOE 2D descriptors revealed R^2Y and Q^2 values on 0.99 and 0.82–0.83, for the control, glycocholic acid, ethanol and Tween 20 data. Some of the descriptors were insignificant in the model and therefore disposed.

The loadings plots (not shown) indicate the relative importance of the descriptors. Although the loadings plots for the three enhancers, glycocholate, ethanol and Tween 20, and the control are different, they all show that $S\log P$ is the most important descriptor. The $S\log P$ descriptor is the log values of the octanol/water partition coefficient calculated by an atomic contribution model (Wildman, 1999).

Analysis using the Volsurf descriptors gave a different picture. R^2Y values range from 0.40 to 0.64 and Q^2 was 0.40–0.54. The Volsurf descriptors have previously been very successful in predicting intestinal lymphatic transfer (Holm and Hoest, 2004), where passive diffusion across a phospholipids membrane also is the primary process. Though there was a significant relationship between descriptors and experimental results, in this study the relationship was less pronounced.

The loading plot (not shown) revealed that surface area (S), volume (V), surface/volume ratio (R) and two hydrophobic surface area descriptors (D1 and D2) were useful in the model. An illustration of the variable importance on projection (VIP) is shown in Fig. 5. The figure shows D1 and D2 were not as crucial to the model as the size-related descriptors (V, S and R).

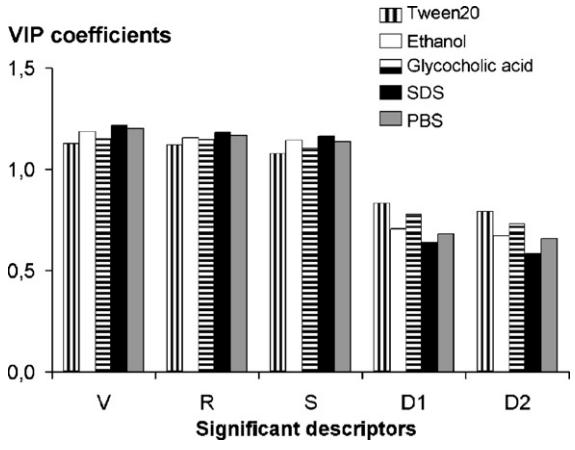

Fig. 5. VIP-coefficients for the Volsurf descriptors; volume (V), volume/surface area ratio (R), surface area (S), hydrophobic area at two different interaction energy levels (D1 and D2) for the four solubilizers and the PBS reference.

It is important to note that the patterns of the VIP plots were similar regardless of the type of solubilizer used. These similarities imply an analogous mechanism in the retention of compounds.

4. Discussion

The solubilizers (or transport enhancers) used in the study are well known to affect the transcellular transport of compounds (Aungst et al., 1996; LeCluyse and Sutton, 1997; Xia and Onyuksel, 2000) in different ways (Ganem et al., 1997). SDS, Tween 20 and glycocholate are surfactants and their amphiphilic characteristics enable them to perturb phospholipid membranes. Tween 20 affects the phosphoric part of the membrane, but at relatively higher concentrations than SDS (Anderberg et al., 1992). In high concentrations, SDS is in fact strong enough to dissolve biological membranes and is characterized as harmful (Muranishi, 1990). SDS is considered difficult to use in pharmaceutics, however, it is found to be a safe permeation enhancer at 0.35–0.70 mM (rat jejunum) (Legen et al., 2006). Glycocholate primarily acts by altering the structure of the membrane (Schubert et al., 1983). Glycocholate is a solubilizer with relatively low toxicity and good transport enhancing properties (Lindhardt and Bechgaard, 2003).

Ethanol is a small polar molecule, which works by penetrating the polar head groups of the phospholipids, thereby altering the barrier function of the membrane. Ethanol also changes the physiochemical properties of both the membrane phase and the surrounding water layer. These actions occur even at low concentrations, and at higher concentrations of ethanol irreversible damage to membranes occurs.

The solubilizers have an effect on the transport across membranes, and the effect on the IAM column is obvious from the retention times. The effect is, however, less pronounced when considering capacity factors. Though solubilizers allegedly affect the membrane phase, the void volume, which is strongly influenced by the aqueous phase, varied depending on the solubilizer used.

It is well known that molecules are generally flexible and may adopt different conformations, which each contribute to the physicochemical properties. Previously, a dynamic approach which uses different methods of generating conformations and the Boltzmann weighted average to predict properties from molecular structures have been used (Norinder et al., 1997; Krarup et al., 1998; Kelder et al., 1999; Alifrangis et al., 2000). Of course the use of static molecular structures is debatable, but, generally, studies using the Boltzmann weighted average have not led to a significant improvement in the prediction power and, consequently, a static approach is used in this study.

It is not surprising that volume and surface area is important in this prediction model. Small molecules penetrate membranes easier than large molecules (Xiang and Anderson, 1994; Camenisch et al., 1996). The volume/surface ratio (R), which also holds predictive value, relates to the globularity of the compounds because spherical molecules have a larger volume/surface ratio than elongated molecules. Additionally, R relates to size, since smaller molecules generally have a smaller volume/surface ratio. This is in accordance with "the rule of

five" regarding prediction of permeability and drug absorption suggested by Lipinski et al. (1997), where the size is described by molecular weight.

The rule of five also uses hydrogen donors and acceptors in the inclusion criteria, which corresponds nicely with the use of descriptors D1 and D2. These descriptors are associated with the difference in surroundings between the aqueous phase and membrane phase. Nitrogen, oxygen and connected hydrogen atoms have the ability to form intermolecular hydrogen bonds in contrast to atoms possessing more hydrophobic characteristics. The hydrogen bonds must be broken in order for molecules to be able to enter the membrane phase, and therefore hydrophobic molecules without intermolecular hydrogen bonds penetrate membranes more easily. The hydrophilic (polar) surface area possesses a scientifically accepted predictor of permeability (Palm et al., 1998; Winiwarter et al., 1998; Krarup et al., 1998).

Log P is a predictor of membrane permeability. It is additionally used in the rule of five mentioned earlier. The fact that the S log P turned out to be the most important MOE descriptor is therefore of no surprise. Though it seems obvious, the use of log P as a predictor in systems using solubilizers/transport enhancers, is a novel approach. Statistically significant prediction models were obtained, though the SDS model was very weak.

A set of 18 compounds was used for deriving these models and significance was identified. The prediction models are statistically significant, but they cannot be characterized as powerful. The models should not be applied to larger data sets without caution.

Future investigations should aim to identify the solubilizers' effect on permeability/partitioning in other *in vitro* systems. Cultivated cell monolayers, like MDCK cells or Caco-2 cells, are simple systems, but they are complex compared to the IAM system. Transport enhancers functioning through different mechanisms may be expected to show dissimilar effects in more complex systems. The IAM system is a relatively simple system, perhaps too simple to identify differences between the solubilizers. A more complicated experimental model should be used for future discriminations between solubilizers.

Acknowledgements

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