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Quantitative structure–retention and retention–activity relationships of β-blocking agents by micellar liquid chromatography

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Abstract

Sixteen β-blocking agents (acebutolol, alprenolol, atenolol, bisoprolol, carteolol, celiprolol, esmolol, labetalol, metoprolol, nadolol, oxprenolol, pindolol, practolol, propranolol, sotalol and timolol) showing a large range of hydrophobicity (octanol–water partition coefficients, log *P* between −0.026 and 2.81) were subjected to micellar liquid chromatography with sodium dodecyl sulfate as micelle forming agent, and *n*-propanol as organic modifier. The correlation between log *P* and the retention factor extrapolated to a mobile phase free of micelles and organic modifier was investigated. The use of an interpolated retention factor or the retention factor for specific individual experimental mobile phases was however advantageous since the retention factors of all β-blocking agents were measurable in the selected mobile phases. Good correlations with log *P* and with in vitro biological parameters (cellular permeability coefficients in Caco-2 monolayers and apparent permeability coefficients in rat intestinal segments) were found. © 2001 Published by Elsevier Science B.V.

Keywords: Quantitative structure–retention relationship; Quantitative retention–activity relationship; Partition coefficient; β-Blockers

1. Introduction

When screening drug candidates it is essential to know to what extent a molecule passes biological membranes. The in vitro models of intestinal membranes (e.g., Caco-2 monolayers and rat intestinal segments) although good predictors [1], do not allow high-throughput screening. Therefore attention is paid to other methods, such as chromatographic ones.

The hydrophobicity (octanol–water partition coefficient, log *P*) of a substance can be determined with classical reversed-phase liquid chromatography (RPLC) systems. This results in quantitative structure–retention relationships (QSRRs) of the logarithms of the retention factors (log *k*) with log *P*. The retention factor for a 100% aqueous mobile phase (k_w) determined through extrapolation is often used [2–4]. The extrapolation requires elution with different mobile phases (MPs) and is therefore relatively time consuming. Interpolation can be done

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using gradient elution [4]. This requires less work, but is still time consuming, because between runs the column needs to be conditioned with the initial solvent, with which the gradient starts.

Since log *P* is considered to estimate the partitioning over a bio-membrane, it should be related to biological activity [5]. However, the C_{18} stationary phase does not have a structure similar to bio-membranes. Consequently, recently introduced stationary phase models of membranes such as immobilized artificial membrane (IAM) columns are regarded as better chromatographic screeners of the permeability of the bio-membranes [6,7]. Likewise micellar liquid chromatography (MLC) appears to be promising [8]. This technique utilizes an MP in which a surfactant is present above its critical micellar concentration (CMC). The formed micelles show a structural resemblance to bio-membranes [9,10]. Good correlations between log *P* and *k* or log *k* determined with MLC have been noted [11,12]. Since according to Woodrow and Dorsey [17] the thermodynamic signature of micelle–water partitioning is similar, as opposed to octanol–water, to biological partitioning, better correlations with in vitro biological parameters can be expected. A few studies revealed quantitative retention–activity relationships (QRARs) with MLC [13–16].

Based on previous work by Rapado-Martínez et al. [18], the elution behavior of β-blocking agents is re-examined. The number of β-blocking agents is extended to 16 and they are investigated with nine MPs differing in surfactant and organic modifier concentration. It is investigated what common parameter (cf. k_w values) can be determined for all MPs and its value in QSRRs is discussed. Correlations with in vitro biological parameters are also established.

2. Experimental

The β-blocking agents acebutolol, alprenolol, atenolol, bisoprolol, carteolol, celiprolol, esmolol, labetalol, metoprolol, nadolol, oxprenolol, pindolol, practolol, propranolol, sotalol and timolol were bought from Sigma or donated (Table 1). Stock solutions containing 100 μg/ml of these drugs were prepared in a 0.05 *M* sodium dodecyl sulfate (SDS, 99% purity; Merck, Darmstadt, Germany) solution.

The micellar MPs were made with the required amounts of SDS, *n*-propanol and 0.01 *M* sodium dihydrogenphosphate (for analysis, Scharlau, Barcelona, Spain). Before the addition of *n*-propanol, the pH was adjusted to 3.0 with HCl (for analysis, Panreac, Barcelona, Spain). This pH was selected to enhance the chromatographic efficiencies through the protonation of the silanol groups. The β-blocking agent solutions and MPs were prepared with nanopure water (Barnstead, Sybron, Boston, MA, USA).

The MPs were filtered through nylon membranes of 0.45 μm pore size and 47 mm diameter (Micron Separations, Westboro, MA, USA). The drug solutions were also filtered before injection into the chromatographic column through PTFE membranes of 0.45 μm pore size and 13 mm diameter (Micron Separations). To avoid adsorption of the drugs the filters were conditioned by passing through at least 3 ml of the β-blocking agent solutions.

The chromatograph (HP 1050) was provided with an isocratic pump, an autosampler, a spectrophotometric detector and an integrator (HP 3396A) (Agilent Technologies, Palo Alto, CA, USA). A Kromasil analytical column (5 μm, 120×4.6 mm I.D.) from Scharlau was used. The flow-rate was 1 ml/min, the injection volume 20 μl and the detection wavelength 225 nm. The dead time was chosen to be the time at which the first solvent perturbation peak appears. Although this method is not preferred in the literature [19], it gave the most constant point throughout all the measurements.

Data acquisition was made with the Peak-96 software (Agilent Technologies, Avondale, PA, USA). Data were treated with MICHROM, a program developed by Torres-Lapasió [20]. The calculations of the regression coefficients and standard deviations (s_0) were performed with the SPSS for Windows 8.0.0 program (1997) of SPSS (Chicago, IL, USA).

The log *P* values were calculated from the structure by applying the freely available on-line interactive LOGKOW program (http://esc_plaza.syrres-.com/interkow/kowdemo.htm) of the Environmental Science Center of Syracuse Research Corporation (Syracuse, NY, USA). These data have been shown to be very correlated to experimental log *P* values.

Table 1
The β-blocking agents

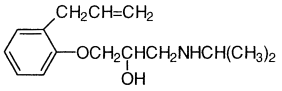
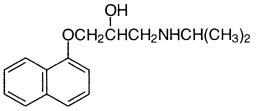
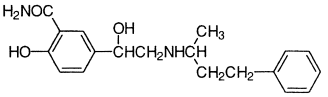
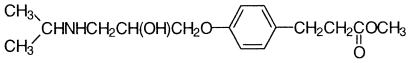
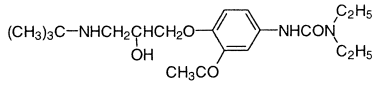
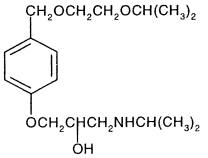
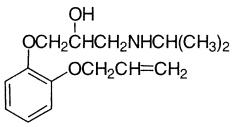
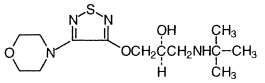
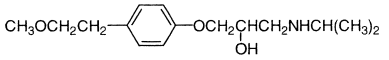
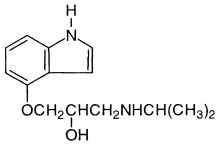
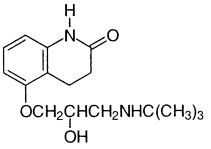
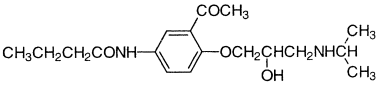
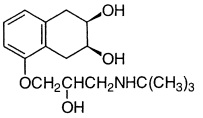
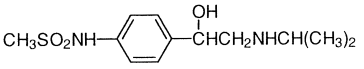
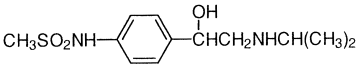
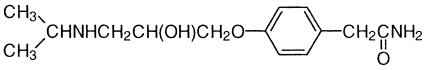
Compound[a]	Structure	Log P[b]	pK_a[c]
Alprenolol (Sigma, St. Louis, MO, USA)		2.81	9.19
Propranolol (Certa, Braine-l'Alleud, Belgium)		2.60	9.15
Labetolol (Glaxo, Tres Cantos, Madrid, Spain)		2.41	7.91
Esmolol (Du Pont–De Nemours, Le Grand Saconnex, Switzerland)		2.00	9.17
Celiprolol (Rhône-Poulenc Rorer, Alcorcón, Madrid, Spain)		1.93	9.12
Bisoprolol (Merck, Darmstadt, Germany)		1.84	9.16
Oxprenolol (Ciba-Geigy, Barcelona, Spain)		1.83	9.13
Timolol (Sigma, Steinheim, Germany)		1.75	8.86
Metoprolol (Sigma, St. Louis, MO, USA)		1.69	9.18
Pindolol (Sigma, St. Louis, MO, USA)		1.48	9.21

Table 1 contd.
The β-blocking agents

Compound[a]	Structure	Log P[b]	pK_a[c]
Carteolol (Madaus, Köln, Germany)		1.42	9.13
Acebutolol (Sigma, St. Louis, MO, USA)		1.19	9.12
Nadolol (Squib, Esplugues de Llobregat, Barcelona, Spain)		1.17	9.17
Practolol (ICI-Farma, Madrid, Spain)		0.53	9.17
Sotalol (Sigma, St. Louis, MO, USA)		0.37	9.19
Atenolol (Zeneca Farma, Madrid, Spain)		−0.026	9.17

[a] The compounds are ordered according to the hydrophobicity.
[b] Calculated from the structure by applying the on-line interactive LOGKOW program of the Environmental Science Centre of Syracuse Research Corporation.
[c] Calculated with the ACD/pK_a database 4.06 of the Advanced Chemistry Development Corporation.

The acid–base dissociation constants, pK_a, were obtained with the ACD/pK_a database 4.06 (1999) of the Advanced Chemistry Development Corporation (Toronto, Canada).

3. Results and discussion

3.1. Retention behavior of the β-blocking agents

The concentrations of surfactant (SDS) and organic modifier (*n*-propanol) in the MP were changed according to an experimental design (Fig. 1). The concentration limits were based on previous experience, in accordance with the practical possibilities of the technique [18]. The retention factors of the β-blocking agents are given in Table 2. Consistent with findings by Rapado-Martínez et al. [18]

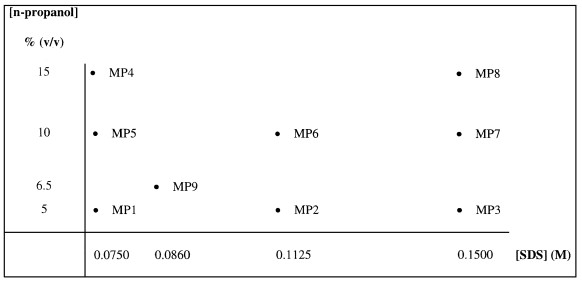


Fig. 1. The experimental design.

Table 2
Retention factors for the β-blocking agents on each MP of the experimental design

Compound	Mobile phase composition[a]								
	MP1, 0.0750 *M*–5%	MP2, 0.1125 *M*–5%	MP3, 0.1500 *M*–5%	MP4, 0.0750 *M*–15%	MP5, 0.0750 *M*–10%	MP6, 0.1125 *M*–10%	MP7, 0.1500 *M*–10%	MP8, 0.1500 *M*–15%	MP9, 0.0860 *M*–6.5%
Alprenolol	112.1	71.1	52.4	45.9	64.2	40.1	29.1	21.0	78.1
Propranolol	87.3	54.6	40.2	37.9	51.2	31.6	23.0	17.2	60.8
Labetalol	61.6	38.9	29.1	29.3	39.9	25.1	18.4	13.7	45.7
Esmolol	34.0	22.2	16.6	15.6	21.7	13.8	10.4	7.77	24.8
Celiprolol	20.5	13.8	9.80	10.9	14.8	9.29	7.00	5.44	15.2
Bisoprolol	39.4	26.0	19.4	20.1	26.6	17.0	12.7	9.85	28.7
Oxprenolol	61.1	39.4	29.4	26.9	37.3	23.5	17.4	12.9	43.4
Timolol	40.9	26.8	20.1	16.5	24.0	15.3	11.5	8.26	28.7
Metoprolol	34.7	22.8	17.1	16.3	22.8	14.7	11.0	8.22	25.8
Pindolol	15.6	10.2	7.61	8.14	10.8	6.93	5.21	4.15	11.8
Carteolol	11.6	7.68	5.75	5.75	7.82	5.07	3.82	3.04	8.68
Acebutolol	16.5	11.0	8.11	9.79	12.6	8.03	6.04	4.88	12.8
Nadolol	15.8	10.4	7.79	7.43	10.3	6.64	5.00	3.85	11.7
Practolol	7.16	4.79	3.52	4.03	5.32	3.46	2.64	2.19	5.57
Sotalol	8.71	5.75	4.29	4.41	6.00	4.00	3.04	2.44	6.71
Atenolol	8.29	5.46	4.11	3.82	5.41	3.57	2.75	2.15	6.23

[a] SDS–propanol (v/v).

split peaks for nadolol occurred. In this case the first peak was used for retention measurements.

It should be remarked that with each MP, the molecules, though covering a big range of hydrophobicity, can be measured within a reasonable timespan. This is a definite advantage as opposed to classical aqueous–organic RPLC, where different MPs would be needed to obtain measurable k values for the more polar and non-polar compounds. The retention order of the β-blocking agents is essentially the same for the different MPs. Thus, the partitioning principle appears to stay the same and correlations with, e.g., log P are not expected to change very much from one MP to another.

García-Alvarez-Coque et al. [21] indicated that the simplest acceptable model to describe the retention measurements in MLC is:

$$1/k = b_0 + b_1[\mathrm{M}] + b_2\varphi + b_{12}[\mathrm{M}]\varphi \text{ (model 1)}$$

where [M] represents the concentration of surfactant forming micelles, φ the concentration of organic modifier in the MP, and b_0, b_1, b_2, b_{12} are the regression coefficients. [M] is the total concentration of SDS minus its CMC. In practice the maximal CMC of the surfactant, being that in a 100% aqueous MP, is often used. However, the CMC decreases with increasing concentrations of organic modifier and thus the [M] values were calculated using the corresponding CMCs ($5.7\cdot10^{-3}$ *M*, $5.5\cdot10^{-3}$ *M*, $4.7\cdot10^{-3}$ *M*, and $3.8\cdot10^{-3}$ *M*, for SDS solutions containing 5, 6.5, 10 and 15%, v/v, *n*-propanol, respectively [22]).

The relative fitting error is given by:

$$\epsilon = \frac{\sum_{i=1}^{n} |k_i^{\mathrm{e}} - k_i^{\mathrm{c}}|}{\sum_{i=1}^{n} k_i^{\mathrm{e}}} \quad (1)$$

where k_i^{c} represents the retention factor calculated from the model and k_i^{e} the measured value in mobile phase i, while n is the number of mobile phases tested for the number of experiments performed. Errors of about 1% with a minimum of 0.6% and a maximum of 2% were obtained. Consequently this model fits well for all β-blocking agents, at least within the experimental domain.

3.2. Extrapolation to k_m

As for classical RPLC, a parameter independent of the value of the two main variables in MLC (i.e.,

concentrations of surfactant and organic modifier) might also be useful in correlation studies. Similarly to k_w in RPLC a parameter k_m can be defined as the retention factor extrapolated to an MP with zero micelle concentration and zero organic modifier [12].

The confidence intervals for k_m are calculated from the 95% confidence interval for the intercept, b_0:

$$b_0 \pm t_{0.025;\, n-p} s_{b0} \quad (2)$$

where $t_{0.025;\, n-p}$ is the t-value for a two-sided distribution at the 95% confidence level with n the number of experiments (here 9) and p the number of coefficients in the model; s_{b0} is the standard deviation of the estimated regression coefficient b_0.

With model 1 the β-blocking agents have b_0 coefficients with a confidence interval containing zero and therefore they are not significantly different from zero. Consequently extreme positive and negative k_m values (i.e., $1/b_0$) are obtained, which are both physically meaningless and statistically insignificant. These k_m values are therefore not suited for correlation studies with log P or other biological data. It can be concluded that the model satisfactorily fits the data in the experimental domain, but it is not suited for extrapolation.

Consequently a model proposed earlier by Strasters et al. [23] (model 2) and an extended model were considered:

$$\log k = b_0 + b_1[\mathrm{M}] + b_2\varphi \text{ (model 2)}$$

$$\log k = b_0 + b_1[\mathrm{M}] + b_2\varphi + b_{12}[\mathrm{M}]\varphi \text{ (model 3)}$$

Compared to model 1 the fitting errors (Eq. (1)) of these models are somewhat higher. They range from 3 to 6% for the different β-blocking agents, with the exception of bisoprolol whose retention behavior they do not model at all.

The confidence intervals of the k_m values (i.e., 10^{b_0}) for model 3 embrace those for model 2; their k_m values are comparable (Table 3). As expected for bisoprolol no model yields physically relevant results. Thus model 2 seems to be most suited for extrapolation. Its confidence intervals are the narrowest. The log k_m obtained with this model correlates best with log P ($r=0.911$) (see also Fig. 2). The differences among the models' correlation coefficients are, as can be expected, small.

3.3. Interpolation

Extrapolation always leads to a decreased precision of the estimates. For this reason, interpolation to the middle of the design (0.1125 *M* SDS and 10% *n*-propanol) was also studied. The calculated k values for these conditions are compared with the measured values (Table 4) and it seems that model 1 yields the best predictions. This could be anticipated, as the fitting error from this model is the smallest.

The confidence intervals for the calculated $1/k$ or log k ($\hat{y}_0$ in Eq. (3)) at given values of SDS and *n*-propanol (x_0) were determined by:

$$\hat{y}_0 \pm t_{0.25,n-p} s_e \cdot \sqrt{x_0^T (X^T X)^{-1} x_0}$$

$$s_e = \sqrt{\frac{\sum e_i}{n-p}}$$

$$e_i = (y_i - \hat{y}_i) \quad (3)$$

Table 3
Examples of extrapolation of models 2 and 3

Compound	Model 2			Model 3		
	k_m	Confidence interval k_m		k_m	Confidence interval k_m	
		Min	Max		Min	Max
Alprenolol	327	248	432	312	154	634
Oxprenolol	166	129	212	162	86.3	305
Metoprolol	89.0	74.2	107	90.0	56.4	144
Pindolol	37.7	31.3	45.4	39.3	24.5	63.2
Practolol	16.1	13.8	18.8	18.0	12.5	26.1
Atenolol	19.7	16.2	24.0	23.1	14.7	36.5

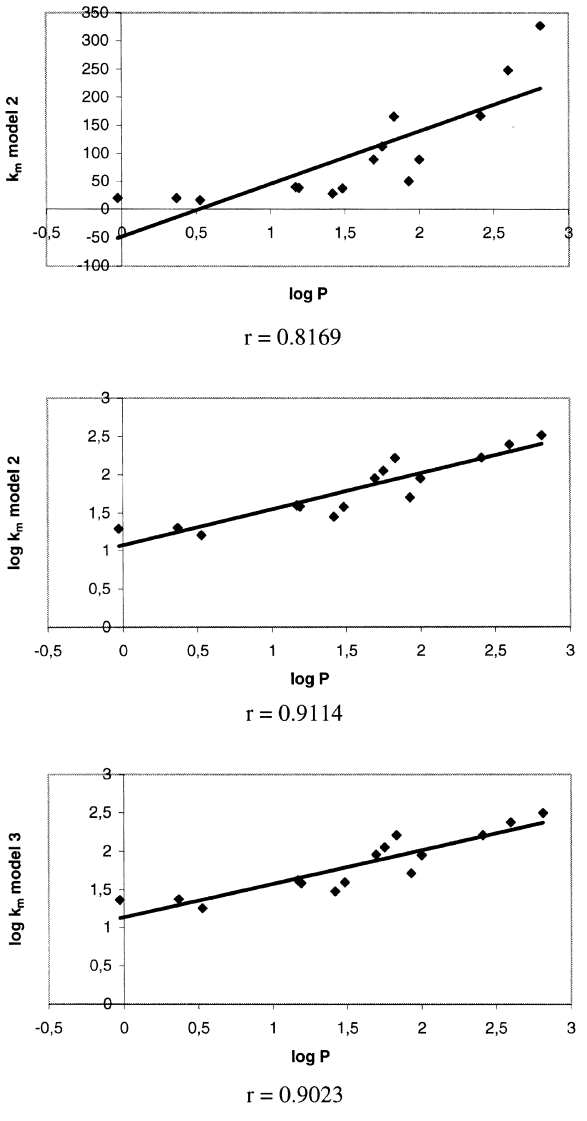


Fig. 2. QSRR between log P and k_m or log k_m for model 2 and model 3. The results from bisoprolol were not included.

where X is the independent variables matrix, s_e the residual variance and e_i a residual, y_i represents the ith measured value of $1/k$ or log k and $\hat{y}_i$ the calculated values ($1/k$ or log k) from the model.

Compared to extrapolation, the confidence intervals are, as could be expected, much narrower (Tables 3 and 4). For model 1 the intervals are narrowest. Models 2 and 3 have larger intervals except for bisoprolol, which however they do not model. The QSRRs show the same linear correlation between the log k interpolated and the log P of the β-blocking agents for all models ($r=0.921$) (Fig. 3). It can be concluded model 1 gives the best interpolation results (which confirms previous findings [21]). Model 2, which was best when extrapolating, now yields worse results. However this does not noticeably influence the correlation coefficient between the estimated k values and log P (when bisoprolol is excluded). The reason is that the three models describe the retention rather well.

3.4. Correlating k or log k of individual mobile phases with log P

A further question is whether one needs extrapolation or interpolation at all. Is it acceptable to use a specific individual MP or does one need the more elaborate procedure consisting of obtaining results with different MPs? When correlating log P with log k_w obtained by classical RPLC, extrapolation is necessary because it is often not possible to select an MP in which all compounds have acceptable k values. This is not the case here: on all MPs selected measurable k values were obtained for all compounds investigated.

The correlation coefficients between log P and the experimental log k of MPs 1–9 are found to be reasonably good ($r=0.913$ to 0.928) and comparable to the previous situations. The correlations between the log k of the MPs themselves are all high (0.995–0.999), which means they all contain the same information. When the experimental log k of the nine MPs are plotted against the log P of the 16 β-blocking agents the regression lines are parallel. The parallelism of the regression lines log $k=a_0+a_1$ log P (Table 5) was confirmed with a t-test on the equivalence of the highest and lowest a_1 values [24]. The a_0 coefficients of the regression lines decrease with increasing amounts of SDS and n-propanol (Table 5). Consequently, adding SDS and/or n-propanol to the MP decreases the difference in hydrophobicity between the mobile and the stationary phase and lowers the overall k. The correlations confirm that electrostatic interaction between the negatively charged SDS and the positively charged β-blocking agents does not interfere with the main governing force, namely hydrophobicity [18].

Table 4
Examples of interpolation to the middle of the design with models 1, 2 and 3

Compound	Model 1				Model 2				Model 3			
	k interpolated	Confidence interval *k* interpolated		%[a]	*k* interpolated	Confidence interval *k* interpolated		%[a]	*k* interpolated	Confidence interval *k* interpolated		%[a]
		Min	Max			Min	Max			Min	Max	
Alprenolol	40.8	40.2	41.5	1.7	45.6	42.4	48.9	13	45.6	42.0	49.5	14
Oxprenolol	24.0	23.6	24.4	2.3	26.4	24.8	28.2	13	26.4	24.6	28.5	13
Metoprolol	14.8	14.7	14.9	0.92	16.1	15.3	16.9	9.6	16.1	15.2	17.0	9.5
Pindolol	7.09	6.97	7.22	2.4	7.62	7.26	7.99	10	7.62	7.21	8.04	9.9
Practolol	3.53	3.48	3.59	2.0	3.75	3.61	3.90	8.3	3.75	3.59	3.91	8.2
Atenolol	3.65	3.59	3.71	2.2	3.93	3.73	4.13	9.9	3.92	3.72	4.13	9.7

[a] % Absolute difference calculated using the measured *k* in Table 2: (|measured *k* − interpolated *k*|/measured *k*)×100.

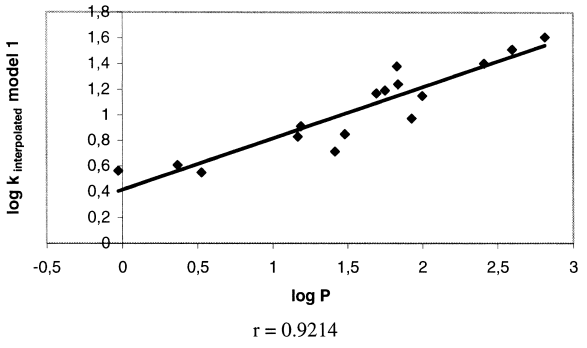


Fig. 3. QSRR between log *P* and the interpolated log *k* of model 1.

In summary, correlations with log *P* of the interpolated log *k* as well as the log *k* obtained with individual MPs have similar correlation coefficients. The extrapolated log k_m values correlate slightly less well with log *P* (slightly lower *r*). Thus it seems that using an MP within the experimental area is to be preferred, at least for the β-blocking agents.

3.5. QRARs with biological in vitro results

A limited correlation study of six β-blocking agents (alprenolol, atenolol, metoprolol, oxprenolol, pindolol and practolol) with some biological in vitro results was carried out (Table 6 and Fig. 4). The cellular permeability coefficients (P_c) of Caco-2 monolayers and apparent permeability coefficients (P_{app}) of rat intestinal segments were taken from Ref. [25]. The relationships between log *P* and both series of in vitro results seem curvilinear (Fig. 4a). The correlation of the Caco-2 monolayer permeability with log *k* and even *k* from MP 6 (center point of the design) appear to be better than with log *P* (Table 6; Fig. 4a and c). The correlation of log *k* from MP 6 with the permeability of rat intestinal segments gives

Table 5
Log *k* of MPs 1–9 as a function of log *P*

MP (% *n*-propanol, *M* SDS)	a_0 (S.E.)	a_1 (S.E.)	*r*
MP1 (5%, 0.075 *M*)	0.7253 (0.0900)	0.4376 (0.0518)	0.9143
MP2 (5%, 0.1125 *M*)	0.5525 (0.0879)	0.4300 (0.0506)	0.9152
MP3 (5%, 0.150 *M*)	0.4263 (0.0891)	0.4282 (0.0513)	0.9125
MP4 (15%, 0.075 *M*)	0.4508 (0.0767)	0.4102 (0.0442)	0.9275
MP5 (10%, 0.075 *M*)	0.5842 (0.0791)	0.4118 (0.0456)	0.9240
MP6 (10%, 0.1125 *M*)	0.4058 (0.0787)	0.4017 (0.0453)	0.9212
MP7 (10%, 0.150 *M*)	0.2926 (0.0779)	0.3936 (0.0448)	0.9200
MP8 (15%, 0.150 *M*)	0.2043 (0.0732)	0.3770 (0.0422)	0.9224
MP9 (6.5%, 0.086 *M*)	0.6163 (0.0862)	0.4227 (0.0496)	0.9156

Table 6
Correlation coefficients for QRAR with in vivo results

	P_c of Caco-2	P_{app} of rat intestinal segments
P_c of Caco-2		0.9736
Log P	0.9438	0.9660
Log k of MP 6 MLC	0.9624	0.9970
k of MP 6 MLC	0.9918	0.9595
Log k on IAM	0.8900	0.9244
k on IAM	0.9503	0.9020

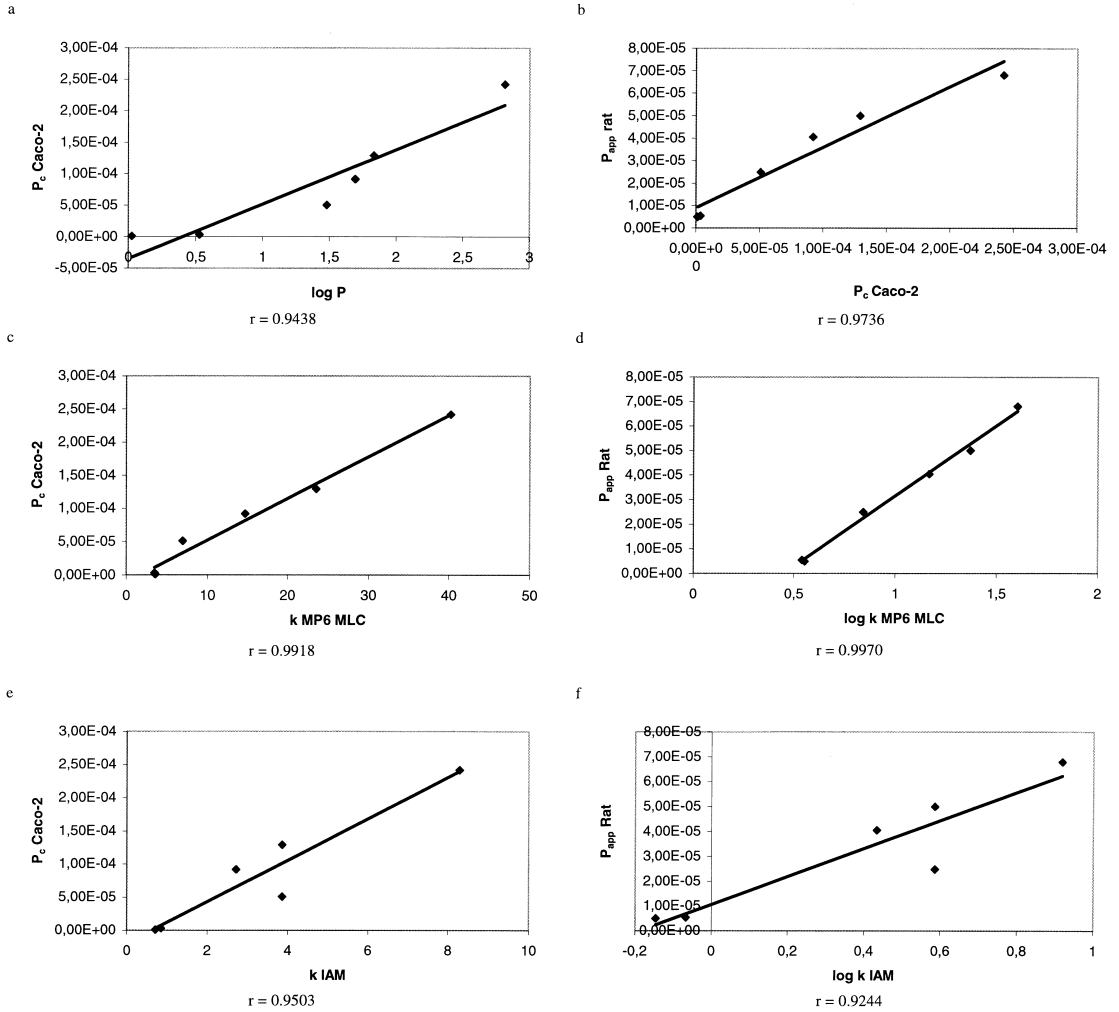


Fig. 4. QRAR with in vivo results; (a) log P versus P_c Caco-2, (b) P_c Caco-2 versus P_{app} rat, (c) k MP6 MLC versus P_c Caco-2, (d) log k MP6 MLC versus P_{app} rat, (e) k IAM versus P_c Caco-2, (f) log k IAM versus P_{app} rat.

an even better result (Fig. 4d). Since IAM is mentioned as a good predictor of the permeability [6,7], a comparison between IAM results from Nasal et al. [26] with the biological in vitro results was also made. Table 6 and Fig. 4e and f, show the IAM results do not correlate as well as those from MLC.

4. Conclusions

If one needs to extrapolate, model 2 proposed by Strasters et al. [23] is to be recommended. Interpolation is however to be preferred for the fundamental reason that it is more precise than extrapolation. As model for the interpolation we propose model 1 by García-Alvarez-Coque et al. [21], because it is the most precise. However, extrapolation or interpolation to a common factor is not necessary since a wide range of molecules, very different in hydrophobicity, can be determined with one MP. Alternatively, one could decide to use one of the MPs proposed here, which would speed up considerably the screening procedures. The quality of the QSRR between log *P* and different MPs is comparable so that any MP in the examined domain can be used and the choice depends on practicalities (the retention times). We suggest 0.10 *M* SDS–10% *n*-propanol, which is situated close to MP 6 in the middle of the design. Our main conclusion is therefore that MLC with a single point works well for the purposes studied.

MLC can be used for two types of correlation. On the one hand MLC can be used to predict log *P* values. The correlations seem to be quite good. It should be remarked that the correlation quality does not only depend on the MLC results but also on the reliability of the log *P* values. It is known that this reliability is limited both for calculated and published measured values [27,28]. Furthermore, MLC seems to be a promising technique for the prediction of membrane permeability. Although the data set with biological in vitro results is too small to draw final conclusions. These findings however encourage further QSSR–QSAR studies of MLC in comparison or in combination with other methods like LC with IAM and micellar electrokinetic capillary chromatography (MECC).

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