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Micellar liquid chromatography for prediction of drug transport

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Abstract

The vast majority of well absorbed drugs are transported passively across the cell membranes. Physicochemical descriptors of drug molecules that are believed to influence transcellular transport are routinely used to predict drug absorption by means of complex mathematical models. In this paper, a new in vitro method, based on the retention data in micellar liquid chromatography (MLC), is validated for the prediction of passive drug absorption. The retention of a heterogeneous drugs set in MLC using Brij 35 as surfactant in the mobile phase is compared with the retention data reported in literature obtained in red cell membrane lipid liposomes, human red cell membranes vesicles (vesicles), native membranes of adsorbed red cells (ghosts) and egg phospholipids liposomes [Beigi et al., Int. J. Pharm., 164 (1998) 129–137]. Finally, the correlation between the logarithm of retention factors in MLC and reported oral drug absorption values for barbiturates and β-blockers is studied. Predictive regression models for estimating oral drug absorption using the logarithm of the retention values as independent variable are proposed.

Keywords: Micellar liquid chromatography; Drug adsorption; Pharmaceutical analysis; Barbiturates; Beta-blockers

1. Introduction

The search for new pharmacologically active compounds in drug discovery programmes often neglects biopharmaceutical properties such as drug absorption. As a result, poor biopharmaceutical characteristics constitute a major reason for the low success rate for candidates in clinical development. Since the cost of drug development is many times larger than the cost of drug discovery, predictive methodologies aiding the selection of orally bioavailable drug candidates are of profound significance [1].

Drug absorption is influenced by many physiological factors, but it also depends on the solubility, particle size, chemical form, and other physicochemical characteristics of the drug itself. The dissociation constant and hydrophobicity of a drug, as well as the pH at the absorption site, dictate the absorption characteristics of a drug from solution. The critical role of hydrophobicity in drug absorption is a guiding principle in drug development [2]. Hydrophobic drugs with favorable partition coefficients are usually well absorbed after oral administration. The selection of a more hydrophobic compound from a series of research compounds often results in improved pharmacological activity.

Chromatography is a powerful technique for the measurement of physicochemical parameters. The application of chromatographic parameters in structure–activity relationships gives rise to a new field, quantitative retention–activity relationships (QRARs) [3,4]. In order to emulate the biological barriers different reversed stationary phases have been developed [5–9]. Pidgeon and co-workers [5–

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7] developed the so-called immobilized artificial membranes (IAMs), they are chromatographic surfaces synthesized by covalently immobilizing phospholipids to silica propyl amide particles. Lundahl and co-workers [8,9] proposed the use of immobilized liposomes in capillary continuous bed with covalently linked C_4 or C_8 alkyl ligands.

A simpler and reproducible approach consists of the use of micellar liquid chromatography (MLC). In this chromatographic modality reversed stationary phases and surfactant solution above the critical micellar concentration (CMC) as mobile phases are used [10–14]. Under these conditions, the stationary phase is modified by hydrophobic and/or silanophilic adsorption of surfactant monomers [12] and it structurally resembles the ordered array of the membranous hydrocarbon chains. The hydrophilic/hydrophobic character of surfactants adsorbed resembles the polar membrane regions. In consequence, the stationary phase provides both hydrophobic and electronic sites of interaction emulating biological barriers. In addition, the retention of compounds in MLC which depends on hydrophobic, electronic and steric properties of compounds, is obtained in flow conditions in similar way as the phenomena of absorption, transport and excretion of drugs in the body.

Successful applications of MLC to construct QRAR models which describe the substituted phenols bioactivity [15], the anesthetic potency of local anesthetics [16], the hypnotic activity of barbiturates [17], the α- and β-adrenergic activity of catecholamines [18], the toxicity and anxiolitic activity of benzodiazepines [19], the antypsychotic activity of phenotiazines [20] and the biological responses of tricyclic anti-depressants [21] have been reported.

In this paper, the possibility of using retention in MLC with Brij 35 as surfactant as an in vitro system to predict passive drug adsorption is studied. The retention in micellar liquid chromatography with Brij 35 of β-blockers, benzodiazepines and phenothiazines is compared with the retention data reported in Ref. [22] on egg phospholipids liposomes (EPLs), on lipids extracted from human red cell membrane vesicles (MLs), on cytoskeleton-depleted human red cell membranes (vesicles), and on human red cell membranes (ghosts). These correlations are compared with those obtained by correlating the retention in IAMs with the retention data for the above systems. Finally, quantitative relationships between the MLC retention data using Brij 35 of two homologous series, β-blockers and barbiturates and oral drug absorption (literature values, [23–25]) are studied and the predictive ability of models is evaluated.

2. Experimental

2.1. Instrumental and measurement

A Hewlett-Packard HP 1100 chromatograph with an isocratic pump, an UV–visible detector and an HP Vectra computer (Amsterdam, The Netherlands) equipped with HP-Chemstation software (A0402, 1996) was used. The solutions were injected into the chromatograph through a Rheodyne valve (Cotati, CA, USA) with a 20-μl loop. Spherisorb octadecyl-silane ODS-2 C_{18} (5 μm, 120×4 mm), Kromasil octadecyl-silane ODS-2 C_{18} (5 μm, 50×4.6 mm I.D.), Kromasil octadecyl-silane ODS-2 C_{18} (5 μm, 150×4 mm) columns and the corresponding guard columns of similar characteristics (35×4 mm) (Scharlau, Barcelona, Spain) were used to obtain the retention data of barbiturates, phenothiazines, benzodiazepines and β-blockers, respectively. In all cases the mobile phase flow-rate was 1 ml min^{-1} except for phenothiazines where the flow-rate used was 1.5 ml min^{-1}. Detection of compounds was performed at 254 nm for barbiturates and 240 nm for benzodiazepines and β-blockers. All the assays were carried out at room temperature. The log k_{MLC} values determined in this study were averages of at least triplicate determinations.

2.2. Reagents and standard

Mobile phases were prepared by aqueous solutions of polyoxyethylene (23) lauryl ether (Brij 35, Acros, Geel, Belgium). The pH of the micellar eluent was adjusted to 7.4 with 0.05 *M* phosphate buffer, prepared with disodium hydrogenphosphate and potassium dihydrogenphosphate (analytical reagent, Panreac, Barcelona, Spain). In order to reproduce the osmotic pressure of biological fluids, NaCl (purism,

Panreac) was added to the micellar mobile phase (9.20 g l^{-1}).

Barbiturates: amobarbital, aprobarbital, barbital, butalbital, butabarbital, butethal and allobarbital (Sigma, St. Louis, MO, USA) were included in the study. Several Spanish pharmaceutical laboratories kindly donated: phenobarbital (Bayer, Barcelona, Spain), secobarbital (UCB, Barcelona, Spain), pentobarbital (B. Braun Medical), butalbital (Sandoz, Barcelona, Spain). Benzodiazepines were obtained from different Spanish pharmaceutical laboratories: flunitrazepam and diazepam (Rohipnol, Valium, Productos Roche, Madrid, Spain), nitrazepam (Serenade, Alter, Madrid, Spain) and oxacepam (Adumbran, Boeringer Ingelheim, Barcelona Spain). Desmethyldiazepam (Sigma). Phenothiazines: chlorpromazine from the pharmaceutical Nemactil (Rhone-Poulenc, Madrid, Spain), fluphenazine (Guinama, Valencia, Spain), Promethazine (Sigma–Aldrich, Madrid, Spain). β-Blockers: atenolol, acebutolol, nadolol, metoprolol, pindolol, oxprenolol, alprenolol, propranolol from Sigma–Aldrich.

Stock standard solutions were prepared by dissolving 10 mg of the compound in 10 ml of 0.05 *M* phosphate buffer, pH 7.4. Working solutions of 10 mg l^{-1} were prepared by dilution of the stock standard solutions using the mobile phase solution.

Barnstead E-pure, deionized water (Sybron, Boston, MA, USA) was used throughout. The mobile phase and the solutions injected into the chromatograph were vacuum-filtered through 0.45-μm and 0.22-μm nylon membranes, respectively (Micron Separations, Westboro, MA, USA).

2.3. Software and data processing

Excell 7.0 from Microsoft Office and SPSS 8.0 software were used to perform the statistical analysis of the linear regression.

2.4. Predictive ability of the models

To evaluate the predictive ability of the models, the comparison between the fit error (e.g., the root mean square error of calibration, RMSEC), the prediction error based on cross-validation (e.g., root mean square error of cross-validation, RMSECV) parameter that includes both interpolation and extrapolation information [26] and the RMSECVi parameter [19] for measuring only the interpolation information was used.

From a qualitative point of view, the more differences between RMSEC and RMSECV or RMSECVi exist, the lower QRAR models obtained robustness is and then, more cautions must be taken in future predictions.

3. Results and discussion

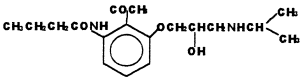
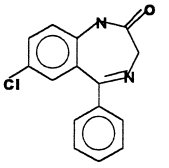
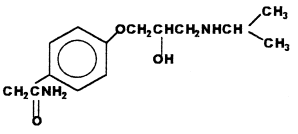
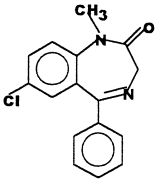
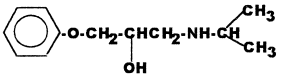
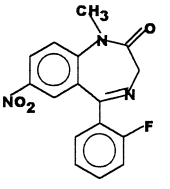
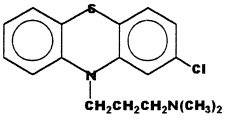
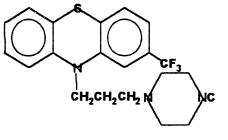
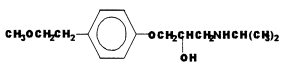
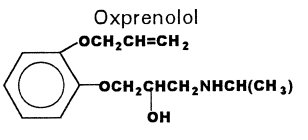
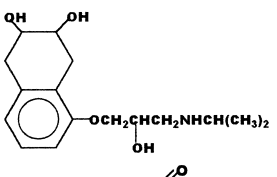
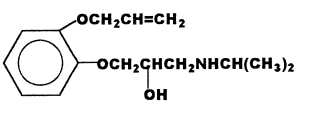
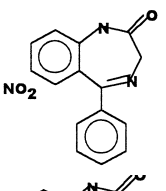
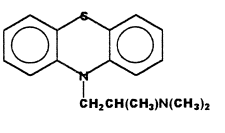
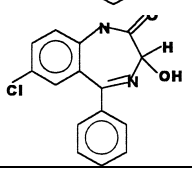
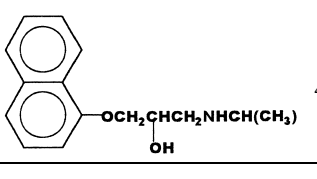
3.1. Comparison between retention data in MLC, biomembranes and EPLs

Drug partitioning into liposomes and biomembranes has been demonstrated to show good correlations with drug diffusional permeability across lipid bilayers and with drug efficacy [22]. The characteristics of MLC systems using Brij 35 as surfactant make to expect that it would be an adequate system for modeling drug partitioning in biological membranes.

In order to study the similarity between MLC systems and other well-recognized mimics natural systems of biomembranes, the retention data for a heterogeneous set of compounds (benzodiacepines, β-blockers and phenothiazines) on MLC (k_{MLC}) were compared with the retention factors obtained in natural membranes [red cell membrane lipid liposomes (MLs), human red cell membranes vesicles containing transmembrane proteins (vesicles), native membranes of adsorbed red cells, (ghosts)] and in EPLs [22].

The retention data in MLC were calculated as retention factors, $k=(t_r-t_0)/t_0$, where t_r is the retention time of the test compound and t_0 corresponds to column dead time. For MLs, vesicles, ghosts and EPLs, the retention data were defined as $K_s=(V_r-V_0)/A$; where V_r is the retention volume of the drug, V_0 the retention volume of a small and hydrophilic reference molecule and A is the molar concentration of immobilized phospholipids. The K_s values of the compounds were taken from Ref. [22]. Table 1 shows the structure, the partition coefficient logarithm (log P) values in the biphasic octanol–water solvent system for the non-ionic forms and their corresponding retention data obtained using

Table 1
Structure, log *P* values for the non ionic forms and retention factor at 0.02 *M* Brij 35 mobile phase for 16 compounds

Compound	$k_{MLC} \pm s$	Log *P*	Compound	$k_{MLC} \pm s$	Log *P*
Acebutolol 	2.95±0.03	1.77	Desmethyldiazepam 	100.0±0.2	3.1
Alprenolol 	38.90±0.05	2.59	Diazepam 	63.09±0.02	2.96
Atenolol 	0.60±0.03	−0.11	Flunitrazepam 	50.12±0.07	2.12
Chlorpromazine 	251.2±0.4	5.20	Fluphenazine 	56.23±0.08	5.90
Metoprolol 	3.79±0.03	1.20	Oxprenolol 	10.47±0.05	1.62
Nadolol 	1.09±0.02	0.23	Pindolol 	4.89±0.04	1.65
Nitrazepam 	63.1±0.1	2.84	Promethazine 	199.5±0.7	4.65
Oxazepam 	50.12±0.09	2.31	Propranolol 	44.67±0.05	3.56

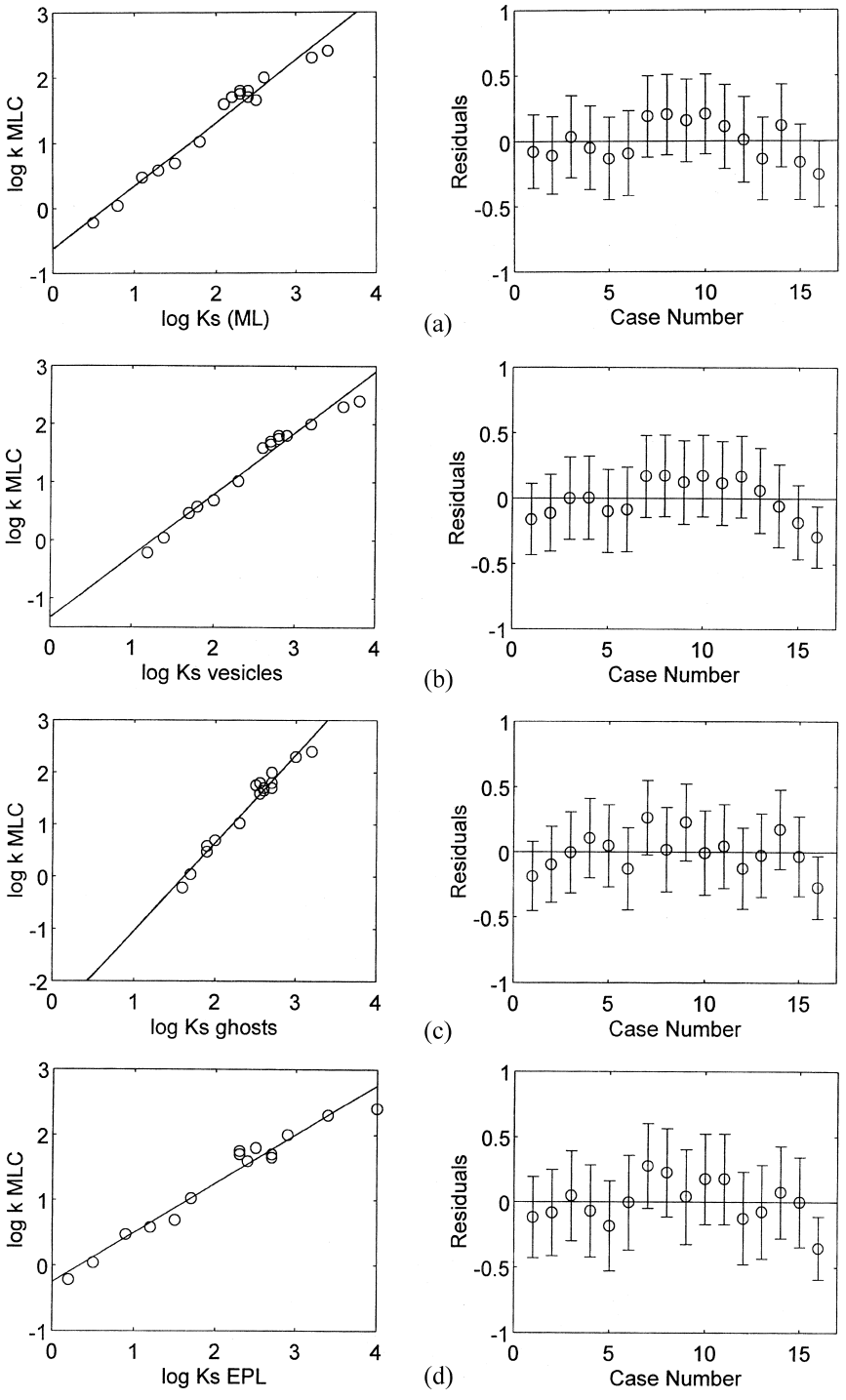


Fig. 1. Comparison between the retention data in MLC (log k_{MLC}) for a heterogeneous set of compounds at 0.02 *M* Brij 35 mobile phase and retention data, log K_s, on (a) red cell membrane lipid liposomes, (b) human red cell membranes vesicles, (c) native membranes of adsorbed red cells, ghosts and (d) egg phospholipids liposomes, EPLs; together their residuals plots.

0.02 *M* Brij 35 as mobile phase (k_{MLC}) for the studied compounds.

Fig. 1 shows the plots retention data in MLC of compounds versus their retention values on biomembranes and EPLs, log K_s, together the corresponding residuals plots. As can be observed excellent correlations were obtained in all cases. As shown figures there is a random distribution of the residuals and practically they all were statistically equal to zero which suggests, from a qualitative point of view, the adequacy of the linear model to data.

Table 2 shows the statistical analysis of the linear regressions obtained by correlating the log k_{MLC} values for 16 drugs with the corresponding log K_s obtained with the different systems assayed. As can be observed, high R^2 values were obtained in all cases; $R^2=0.97$ for MLs, vesicles and ghosts and $R^2=0.96$ for EPLs. In addition, the *P*-values of the models obtained were less than 0.05, that indicates these relationships were statistically significant at the 95% confidence level. Linear relationships were also achieved from MLC retention data obtained using other Brij 35 concentrations (0.04 and 0.06 *M*) as mobile phases.

The linear relationships between retention in MLC and MLs and vesicles show slope values statistically equal to the unity, at a 95% confidence level. It probably means the MLC system shows the same sensitivity that the other systems to the compounds properties variations which determine their retention. The slope for the log k_{MLC}–log $K_{s,ghosts}$ linear relationship was higher than unity, that indicates that the ghosts system is less sensible than MLC to the compounds properties variations which determine the retention. On the contrary, for the log k_{MLC}–log $K_{s,EPLs}$ linear relationship the slope was lower than unity, indicating that the EPL system is more sensible than MLC system for the compounds properties variations.

The differences between the slopes probably reflect the extent of polar and non-polar interactions. In this sense the systems MLC, MLs and vesicles show similar hydrophilic/hydrophobic character. In ghost, the membrane with highest hydrophilic character, the hydrophilic interactions are more important. Consequently the compounds with higher hydrophilic character are relatively more retained and the compounds with higher hydrophobic character are less retained. On the contrary, in EPLs, probably the most hydrophobic system mainly constituted by phosphatidylcholine and phosphatidylethanolamine, the hydrophobic interactions are more important than the hydrophilic [22].

The results shown indicate that the MLC systems with Brij 35 reflect adequately the relative importance of the hydrophobic and hydrophilic interactions of drugs that occurs in natural biomembranes.

IAMs have been also proposed as an in vitro system to predict bioactivity and permeability of drugs [27,28]. Beigi et al. [22] compared the retention factors in IAMs, for compounds included in this study, with those obtained in MLs, vesicles,

Table 2
Statistical analysis and predictive features of the models log $k_{MLC}=a+b$ log K_s corresponding to the retention data obtained using a 0.02 *M* Brij 35 mobile phase[a]

Relationship (*n*)	$a\pm$ts (*P*-value)	$b\pm$ts (*P*-value)	R^2	S.E.	*F* (*P*-value)	RMSEC	RMSECV1	RMSECV1i
MLs (16)	−0.6±0.2 (<0.0001)	0.96± 0.10 (<0.0001)	0.97	0.1527	392.3973 (<0.0001)	0.1428	0.1665	0.1496
Vesicles (16)	−1.3±0.3 (<0.0001)	1.06±0.11 (<0.0001)	0.97	0.1519	396.4041 (<0.0001)	0.1421	0.1717	0.1378
Ghosts (16)	−2.7±0.4 (<0.0001)	1.69± 0.18 (<0.0001)	0.97	0.1508	402.6441 (<0.0001)	0.1410	0.1676	0.1340
EPLs (16)	−0.3± 0.2 (<0.0001)	0.75±0.09 (<0.0001)	0.96	0.1693	316.3830 (<0.0001)	0.1584	0.1904	0.1491

[a] Definitions: *n*, number of available data; ts, 95% confidence interval for coefficient estimates; R^2, *R*-squared adjusted for degrees of freedom; S.E., standard error of the estimate; *F*, *F* ratio; RMSEC, root mean square error of calibration; RMSECV, root mean square error of cross-validation (leave-one-out); RMSECVi, root mean square error of cross-validation (leave-one-out) for interpolated data.

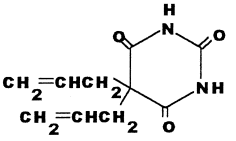
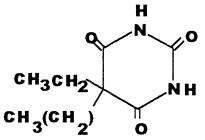
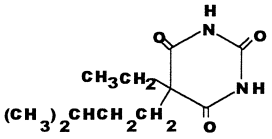
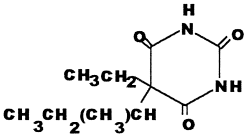
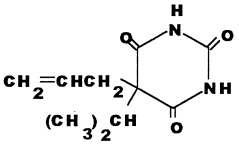
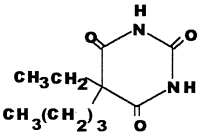
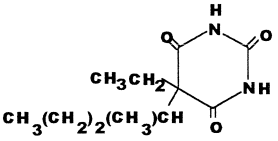
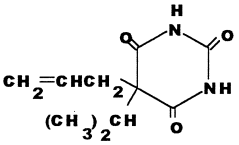
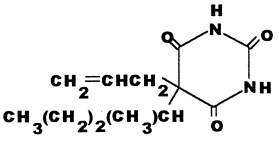
ghosts and EPLs. The slope values obtained were similar to those obtained in MLC, however the correlation coefficients were lower (R^2=0.79, 0.81, 0.76 and 0.83, for the systems cited above, respectively).

3.2. Correlations between drug retention on MLC and oral drug absorption

Drugs are most commonly given orally and the gastrointestinal tract plays a major role in determining the rate extent of drug absorption. The gastrointestinal barrier, that separates the lumen of the stomach and intestines from the systemic circulation and the sites of drug action; is a complex structure composed of lipids, proteins, lipoproteins and polysaccharides. The barrier has the characteristics of a semipermeable membrane, permitting the rapid passage of some chemicals while retarding the passage of others [23,24].

Most drugs used in clinical practice are administered orally and they cross this barrier before reaching the systemic circulation. Thus the characteristics of the gastrointestinal barrier are of considerable importance in biopharmaceutics [1]. The vast majority of well-absorbed drugs are transported passively

Table 3
Structure, log *P* values and retention factor at 0.02 *M* Brij 35 mobile phase for barbiturates

Compound	$k_{MLC}\pm s$	Log *P*	Compound	$k_{MLC}\pm s$	Log *P*
Allobarbital 	8.2±0.6	1.17	Barbital 	3.37±0.13	0.68
Amobarbital 	29.8±.0.9	2.07	Butabarbital 	18.16±0.02	1.56
Aprobarbital 	12.9±0.1	1.27	Buthetal 	19.3±0.4	1.65
Pentobarbital 	40±6	2.07			
Phenobarbital 	13.2±0.9	1.42			
Secobarbital 	53±6	1.97			

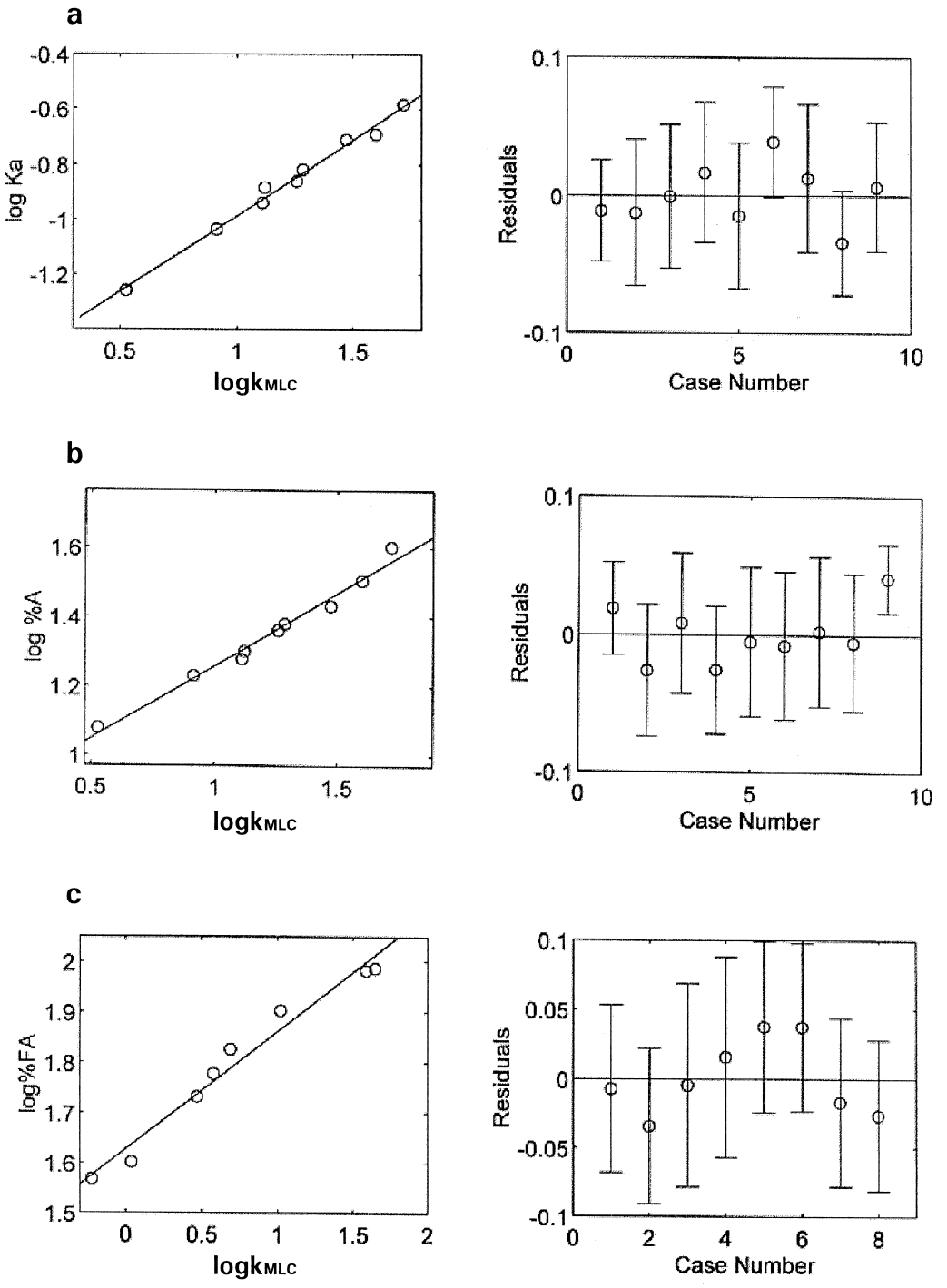


Fig. 2. Oral drug absorption–retention data in MLC at 0.02 *M* Brij 35 mobile phase relationships for barbiturates (a and b) and β-blockers (c) with their residuals plots.

across the lipophilic cell membranes. Physicochemical descriptors of drug molecules (log *P*, ionization constants, molecular volume, dynamic polar surface area, parachor, etc.) that are believed to influence transcellular transport are routinely used to predict drug absorption by means of complex mathematical models. Single physicochemical descriptors such as partition coefficients are not reliably predictive of drug absorption.

In this paper, a new in vitro method, based on the retention data in MLC, is validated for the prediction of passive drug absorption. The compounds included in this study were a group of barbiturates, which structures, log *P* values and the retention data in MLC (log k_{MLC}) obtained using 0.02 *M* Brij 35 as surfactant in the mobile phase are shown in Table 3, and a group of β-blockers (Table 1).

Fig. 2 shows the relationships between the log k_{MLC} data and the values K_a (absorption constant in rat intestine) [23] for barbiturates,% *A* (% absorption in rat intestine in 1 h) [24] for barbiturates, and % F_a (% the absorbed fraction after oral administration in humans) [25] for β-blockers, together the corresponding residuals plots. As can be observed there is a random distribution of the residuals and practically they all were statistically equal to zero which suggests, from a qualitative point of view, the adequacy of the model to data.

Table 4 shows the statistical analysis and the predictive features of the retention–oral absorption models obtained. Strong correlations were obtained (R^2=0.99, 0.98, 0.97, for K_a, % *A*, % F_a, respectively), in all cases. For all models, the *P*-value obtained was less than 0.05, which indicates the relationship between oral drug absorption values and the log *k* values was statistically significant at the 95% confidence level. In this case coefficients were also significant ($P<0.05$) at the same confidence level. The standard error (S.E.) of the models can be used to construct prediction limits for new observations.

These results indicates that log k_{MLC} can be used to identify drugs which may be poorly absorbed at an early stage of the drug discovery process. The MLC system could also be applicable in the prediction of passive drug transport across other epithelial barriers, such as the blood–brain barrier.

In order to compare the predictive ability of the models in terms of cross-validated data, the RMSEC, RMSECV and RMSECVi values were obtained (see Table 4). As can be observed for all models the RMSEC, RMSECV and RMSECVi values were similar. This suggests that predictions based on interpolations and extrapolations should be reasonably adequate. Fig. 3 shows the predicted (fitted and cross-validated) vs. actual oral absorption values for the available data. As can be observed in general, the ability of log k_{MLC} values in describing and predicting oral drug absorption is adequate.

4. Conclusions

Most drugs used in clinical practice are administered orally and they cross the gastrointestinal barrier before reaching the systemic circulation. Drug absorption is influenced by many physiological factors but it also depends on the physicochemical characteristics of the drug itself. Different systems have

Table 4
Statistical analysis and predictive features of the models log(absorption)=$a+b$ log k_{MLC} corresponding to the retention data obtained using a 0.02 *M* Brij 35 mobile phase[a]

Relationship (*n*)	$a\pm$ts (*P*-value)	$b\pm$ts (*P*-value)	R^2	S.E.	*F* (*P*-value)	RMSEC	RMSECV1	RMSECV1i
K_a (9)	−1.54±0.07 (<0.0001)	0.55±0.05 (<0.0001)	0.99	0.02	618.61 (<0.0001)	0.0202	0.0255	0.0270
% *A* (9)	0.84±0.07 (<0.0001)	0.42±0.05 (<0.0001)	0.98	0.02	366.88 (<0.0001)	0.0199	0.0297	0.0174
% F_a (8)	1.63±0.04 (<0.0001)	0.23±0.04 (<0.0001)	0.97	0.03	195.28 (<0.0001)	0.0257	0.0339	0.0344

[a] For abbreviations see Table 2.

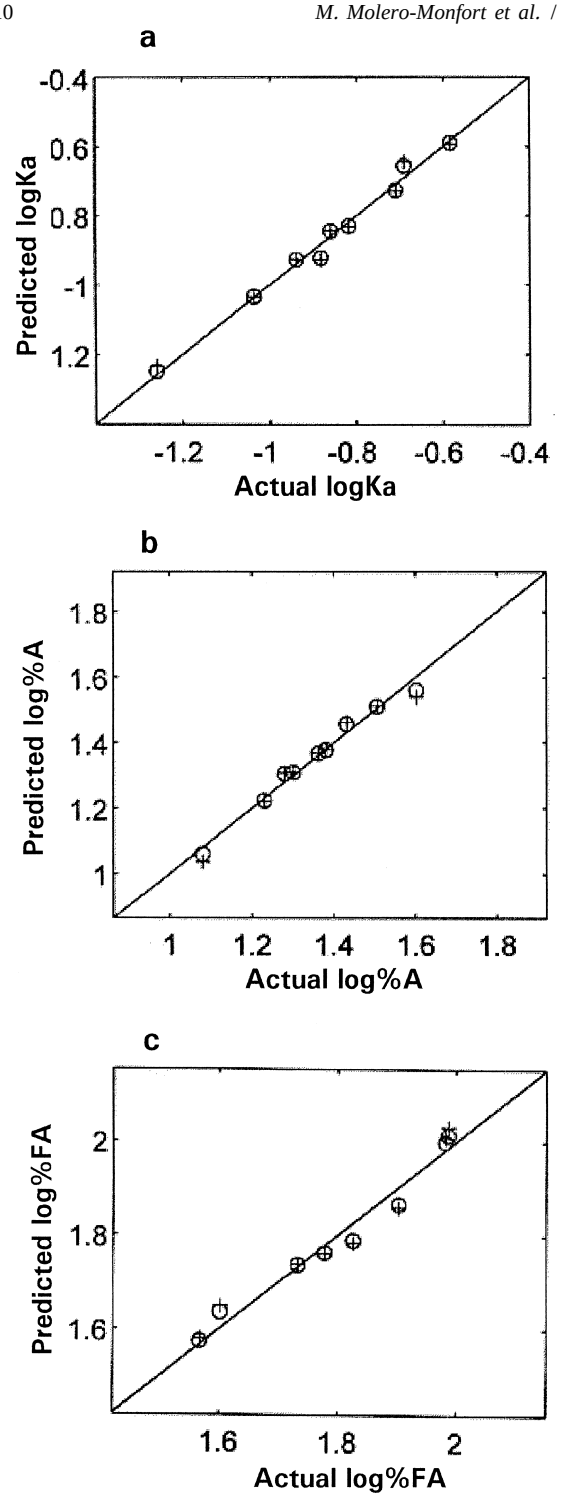


Fig. 3. Validation plots for retention–drug absorption models: predicted versus actual values. Fitted (○) and cross-validated (+) results are shown.

been proposed in order to emulate the absorption of drugs in gastrointestinal barrier, that include the use of natural biomembranes as MLs, vesicles and ghosts. The results shown in this paper indicate that the MLC systems with Brij 35 reflect adequately the relative importance of the hydrophobic and hydrophilic interactions of drugs that occurs in the naturals biomembranes. Therefore, a single chromatographic parameter, log k_{MLC}, can be used to predict drug absorption at an early stage of the drug discovery process.

The results of this work make to expect the MLC system could also be applicable in the prediction of passive drug transport across other barriers, such as the blood–brain barrier.

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References

[1] M.E. Winter's Basic Clinical Pharmacokinetics, Applied Therapeutics, Washington, DC, 1994.
[2] W.H. Oldendorf, W.G. Dewhurst, in: W.G. Clark, J. del Giudice (Eds.), Principles of Psychopharmacology, 2nd ed., Academic Press, New York, 1971, pp. 183–191.
[3] J.G. Dorsey, M.G. Khaledi, J. Chromatogr. A 656 (1993) 485–499.
[4] W.J. Lambert, J. Chromatogr. A 656 (1993) 469–484.
[5] S. Ong, H. Liu, X. Qui, G. Bhat, C. Pidgeon, Anal. Chem. 67 (1995) 755–762.
[6] H. Liu, S. Ong, L. Glunz, C. Pidgeon, Anal. Chem. 67 (1995) 3550–3557.
[7] S. Ong, H. Liu, C. Pidgeon, J. Chromatogr. A 728 (1996) 113–128.
[8] Y. Zhang, C.-M. Zeng, Y.-M. Li, S. Hjertén, P. Lundahl, J. Chromatogr. A 749 (1996) 13–18.
[9] A. Lundqvist, P. Lundahl, J. Chromatogr. B 699 (1997) 209–220.
[10] D.W. Armstrong, F. Nome, Anal. Chem. 53 (1981) 1662–1666.
[11] M. Arunyanart, L. Cline-Love, Anal. Chem. 56 (1984) 1557–1561.
[12] A. Berthod, I. Girard, C. Gonnet, Anal. Chem. 58 (1986) 1356–1358.

[13] M.J. Medina-Hernández, M.C. García Alvarez-Coque, Analyst 117 (1992) 831–837.
[14] J.P. Foley, Anal. Chim. Acta 231 (1990) 237–247.
[15] E.D. Breyer, J.K. Strasters, M.G. Khaledi, Anal. Chem. 63 (1991) 828–833.
[16] L. Escuder Gilabert, S. Sagrado, R.M. Villanueva Camañas, M.J. Medina-Hernández, Anal. Chem. 70 (1998) 28–34.
[17] M. Cuenca-Benito, S. Sagrado, R.M. Villanueva Camañas, M.J. Medina-Hernández, J. Chromatogr. A 814 (1998) 121–132.
[18] J.M. Sanchis Mallols, R.M. Villanueva Camañas, S. Sagrado, M.J. Medina-Hernández, Chromatographia 46 (1997) 605–612.
[19] M. Molero-Monfort, S. Sagrado, R.M. Villanueva Camañas, M.J. Medina-Hernández, Biomed. Chromatogr. 13 (1999) 394–400.
[20] Y. Martín Biosca, M. Molero-Monfort, S. Sagrado, R.M. Villanueva Camañas, M.J. Medina-Hernández, Biomed. Chromatogr., (1999) in press.
[21] C. Quiñones Torrelo, S. Sagrado, R.M. Villanueva Camañas, M.J. Medina-Hernández, J. Med. Chem. 42 (1999) 3154–3162.
[22] F. Beigi, I. Gottschalk, C.L. Hägglund, L. Haneskog, E. Brekkan, Y. Zhang, T. Österberg, P. Lundahl, Int. J. Pharm. 164 (1998) 129–137.
[23] J. Wagner, Fundamental of Clinical Pharmakokinetics, Drug Intelligence Publications, IL, 1983.
[24] L.S. Schanker, J. Med. Pharm. Chem. 2 (1960) 343.
[25] C. Lindsay De Vane, C. Rick Jarecke, in: W.E. Evans, J.J. Schentag, W.J. Jusko (Eds.), Applied Pharmacokinetics, Applied Therapeutics, Vancouver, 1992.
[26] H. Martens, T. Naes, Multivariate Calibration, Wiley, Chichester, 1989.
[27] C. Pidgeon, S. Ong, H. Lin, X. Qiu, M. Pidgeon, A.H. Dantzig, J. Munroe, W.J. Hornback, J.S. Kasher, L. Glunz, T. Szczerba, J. Med. Chem. 38 (1995) 590–594.
[28] A. Nasal, M. Sznitowska, A. Bucinski, R. Kaliszan, J. Chromatogr. A 692 (1995) 83–89.