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Prediction of milk/plasma concentration ratios of drugs and environmental pollutants

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ABSTRACT

A large database of milk/plasma ratios, *M*/*P*, for 179 drugs and hydrophobic environmental pollutants has been constructed from literature data. Application of linear analyses shows that drugs preferentially partition into the aqueous and the protein phases of milk, but that the pollutants partition into the fat phase. No useful linear equation could be obtained for the entire 179 compound data set, but an artificial neural network with only five Abraham descriptors as input resulted in errors in $\log(1 + M/P)$ of only 0.0574, 0.116 and 0.093 log units for a training set of 135 compounds, an internal test set of 22 compounds and an external test set of 22 compounds respectively. These errors correspond to 0.203, 0.193 and 0.334 log units respectively when transformed into errors in $\log(M/P)$.

1. Introduction

For many years there has been concern over the presence of drug contaminants in human milk, as set out in recent reviews [1–4], but recently there has been additional concern over environmental pollutants in human milk, see for example [5–7]. Ito et al. [2,8] have proposed an 'Exposure Index' that relates the milk to plasma ratio, *M*/*P*, the milk intake, A, and the Infant Drug Clearance to a time averaged drug exposure level,

$$\text{Exposure Index}\,(\%) = (100 \times M/P \times A)/\text{Infant Drug clearance} \quad (1)$$

A key parameter, *M*/*P*, is the ratio of drug or pollutant concentration in milk to that in plasma. Hence any method that can be used to predict *M*/*P* ratios would be of very considerable value.

Not surprisingly, there have been numerous attempts to predict these ratios. However, it is quite difficult to compare results. Some authors use *M*/*P* itself, whereas other authors use $\log(M/P)$, many authors quote only the regression coefficient, *R*, or R^2, and do not give details of the standard error or the root mean square error (much more useful statistics), and some authors erroneously refer to fits of *M*/*P* or of $\log(M/P)$ as 'predictions'. In this work we make a distinction between statistics of fitting a set of values to some equation or algorithm, and statistics that refer to the prediction of values in some external test set that has not been used to set up the equation or algorithm used to obtain the predictive values.

Even aside from the way that results are presented, the actual system is very complicated. Fleishaker et al. [9] pointed out that a drug in plasma and in milk could exist either as the free drug or as protein-bound drug, and a drug in plasma and in milk could exist as a neutral species or as an ionised species depending on the pK_a of the drug and the pH. Furthermore, a drug could partition into the aqueous phase of milk or could partition into the separate fat phase of milk. Atkinson and Begg [10] published the first comprehensive analysis of *M*/*P* ratios, based on the suggestions of Fleishaker et al. [9]. They [9] set out plasma protein binding and milk protein binding for 14 drugs, and obtained an equation that related the two. Then milk protein binding could be estimated for any drug for which plasma protein binding was known; the two protein binding values were used as part of a fitting algorithm. Atkinson and Begg [10] also corrected for ionisation of acids or bases, using the Henderson–Hasselbach equation and taking the pH of plasma as 7.4 and that of milk as 7.2 (although other workers have used 7.0 as the pH of milk [11]). There are a number of assumptions in this ionisation correction. (a) There is an equilibrium between the unionised species in milk and the unionised species in plasma, but there is no equilibrium between the ionised species in milk and the ionised species in plasma; there seems to be no evidence for this assumption at all. (b) The pK_a of a drug in plasma is the same as the

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pK_a in milk (taken as the pK_a in water). As far as we know, no pK_a of any drug has been determined in plasma, and so there is no basis for taking pK_a in plasma as the same as that in water. The method of Atkinson and Begg [10,12,13] was examined by Larsen et al. [14] who concluded that it had little predictive power, but the conclusions of Larsen et al. have been challenged by Doogue et al. [15] and by Ilett [16].

Other workers have avoided the protein binding and the pK_a problems altogether. Agatonovic-Kustrin et al. [17] examined a set of 60 drug compounds, and calculated 61 descriptors for each drug. This was reduced to 26 descriptors for each drug in the final artificial neural network (ANN) which was applied to values of log(M/P). The set of 60 drugs was divided into a training set and an internal validation set (50 drugs) and an independent external test set of 10 drugs to test the predictive ability of the ANN. The root mean square errors (RMSE) in log(M/P) were 0.590 for the training set, 0.900 for the internal validation set and 0.425 for the external test set; the result is rather peculiar because errors in predictions for an external test set are invariably larger than errors in fitting a training set. In a later paper [18], the same workers examined a larger set of 123 drugs and applied an ANN to M/P ratios themselves. Nine calculated descriptors were used, but no statistics at all were given. Katritzky et al. [19] investigated M/P ratios of a set of 115 drugs, using log(M/P) as the dependent variable. They started with 850 descriptors for each drug and reduced this to the best 7 descriptors that were used in a multiple linear regression. After eliminating 15 drugs, a training set of 67 drugs could be fitted with an error of 0.324 log units, and an independent test set of 33 drugs could be predicted with an error of 0.332 log units. Zhao et al. [20] used a support vector machine, SVM, method to analyze M/P ratios for 126 drugs. The only statistic they gave was an 'accuracy' of 90.48%, which refers to classification into two sets, Class 1 with $0 > M/P > 0.1$ and Class 2 with $1 > M/P > 0.1$; however this classification appears to be at odds with data in their Table 1.

Due to the wide applicability of linear free energy relationships (LFERs) in a large number of areas, it seemed useful to start our investigations on the linear modeling and prediction of M/P value using LFER methods. Although the multiple linear regression analysis (MLRA) that is used in the implementation of LFERs is a very convenient method of analysis, it is limited (as the name implies) to linear processes. When nonlinear phenomena are significant to some extent within the data investigated, LFERs are no longer the appropriate method of analysis, and nonlinear modeling techniques such as artificial neural networks (ANNs) are necessary in order to build an accurate and reliable model. ANN has recently gained much popularity in dealing with nonlinear relationships [21]. A detailed description of the theory behind a neural network has been adequately described elsewhere [22]. An ANN is a biologically inspired computer program designed to learn from data in a manner of emulating the learning pattern in the brain. Most ANN systems are very complex high dimension processing systems. The relevant principle of supervised learning in an ANN is that it takes numerical inputs (the training data) and transfers them into desired outputs. The input and output nodes may be connected to the 'external world' and to other nodes within the network. The way in which each node transforms its input depends on the so-called 'connection weights' or 'connection strength' and bias of the node, which are modifiable. The output values of each node depend on both the weight strength and bias values. For the present purpose, the great power of ANNs stems from the fact that it is possible to train them. Training is done by continually presenting the networks with known inputs and outputs and modifying the connection weights and biases between the individual nodes. This process is continued until the output nodes of the network match the desired outputs to a stated degree of accuracy. Training of the ANN can be performed by using a back-propagation algorithm. In order to train the network using a back-propagation algorithm, the differences between the ANN output and its desired value are calculated after each training iteration and the values of weights and biases modified by using these error terms. In the MLR method, the analysis is limited to a certain number of possible interactions, but in the ANN method more terms can be examined for interactions between features. ANNs are capable of recognizing nonlinear relationships between inputs and outputs. In addition, the ANN can use qualitative as well as quantitative inputs, and does not require an explicit relationship between the inputs and the outputs.

It seems therefore that there is still scope for analyses of M/P or log(M/P) values for drugs, using both LFER and ANN methods, and for investigating if the same methods can be used for environmental pollutants; to date, the latter have not been studied at all.

2. Methods

The plasma/milk system is very complicated, and it is possible that simple linear equations for M/P or for log(M/P) might not be very successful. However, as a start we used the same method that we have previously employed [23–27] for partitions from blood or plasma to various biological systems. The method uses the linear free energy relationship, LFER, shown as Eq. (2).

$$\mathrm{SP} = c + eE + sS + aA + bB + vV \quad (2)$$

In Eq (2) SP is the dependent variable, for example M/P or log(M/P), and the independent variables are properties of drugs and environmental pollutants (solutes) as follows [28,29]. E is the solute excess molar refractivity in units of $(\mathrm{cm^3\,mol^{-1}})/10$, S is the solute dipolarity/polarizability, A and B are the overall or summation hydrogen bond acidity and basicity, and V is the McGowan volume in units of $(\mathrm{cm^3\,mol^{-1}})/100$. Even if Eq. (2) itself is not successful, the five descriptors might be useful.

In addition to Eq. (2) we used an ANN that can deal with nonlinear processes, as might be the plasma–milk process. As inputs we used the five solute descriptors shown in Eq. (2) as calculated by the PharmaAlgorithms software package 'Absolv' [30]. The values of M/P that we have used [12,18–20,31–101] both for drugs and environmental pollutants are given in Table 1, together with the calculated Absolv descriptors.

3. Results and discussion

3.1. LFER methods

The plasma to milk system is very complicated, even in terms of the separate phases of milk as shown in Fig. 1. Because the volumes of the phases are not the same, the relationship between the three equilibrium constants and the overall equilibrium constant for plasma to milk, M/P, is

$$M/P = K_{pf} \times V_f/V_m + K_{pp} \times V_p/V_m + K_{pw} \times V_w/V_m \quad (3)$$

In Eq. (3), V_f/V_m, V_p/V_m and V_w/V_m are the ratios of the volumes of the fat phase, the protein phase and the aqueous phase to the total milk phase (so that $V_f + V_p + V_w = V_m$). It is rather evident, just from Eq. (2) that linear equations are not likely to yield satisfactory results. When we applied Eq. (1) to the plasma to milk system, using M/P or log(M/P) or log($1 + M/P$) we obtained no useful equations. We therefore turned to the nonlinear method of artificial neural networks.

Table 1
Values of the plasma to milk ratio, *M*/*P*, for drugs and environmental pollutants, together with the calculated descriptors.

Name	*E*	*S*	*A*	*B*	*V*	CAS number	Ref.	*M*/*P*
Acetazolamide	1.64	2.55	0.85	1.50	1.3369	59-66-5	31	0.30
Acyclovir	1.90	2.27	0.82	2.19	1.5217	59277-89-3	18	2.35
Alprazolam	2.59	1.95	0.00	0.84	2.2041	28981-97-7	20	0.001
Amitriptyline	1.71	1.31	0.00	0.77	2.3996	50-48-6	12	0.83
Amoxycillin	2.70	3.22	1.55	2.90	2.5356	26787-78-0	18	0.028
Amphetamine	0.79	0.95	0.21	0.69	1.2389	300-62-9	32	4.98
Ampicillin	2.48	3.01	1.06	2.62	2.4769	69-53-4	18	0.295
Aspirin	0.84	1.42	0.57	0.77	1.2879	50-78-2	18	1.63
Astemizole	3.10	2.70	0.13	1.64	3.5563	68844-77-9	33	4.40
Atenolol	1.48	1.97	0.78	1.85	2.1763	29122-68-7	18	2.10
Aztreonam	2.73	4.15	1.38	3.14	2.7607	78110-38-0	12	0.007
Baclofen	1.10	1.47	0.78	1.02	1.5766	1134-47-0	19	0.82
Bupivacaine	1.32	1.59	0.26	1.19	2.5139	2180-92-9	34	0.34
Bupropion[a]	1.07	1.32	0.13	0.94	1.9406	34841-39-9	35	5.545
Caffeine	1.48	1.90	0.00	1.27	1.3632	58-08-2	12	0.51
Captopril	1.17	1.77	0.57	1.13	1.6215	62571-86-2	19	0.031
Carbamazepine	2.12	2.06	0.39	0.92	1.8106	298-46-4	18	0.465
Carbamazepine 10,11-epoxide	2.24	2.17	0.39	1.10	1.8037	36507-30-9	36	0.79
Carbenicillin	2.44	3.14	1.42	2.41	2.5924	4697-36-3	18	0.02
Cefotaxime	2.97	3.91	1.07	3.07	2.9301	63527-52-6	18	0.16
Cefoxitin	2.64	3.74	1.29	2.65	2.7735	35607-66-0	18	0.00
Ceftriaxone	4.32	5.18	1.33	4.36	3.4802	73384-59-5	18	0.045
Cephalexin	2.53	3.27	1.06	2.54	2.4339	15686-71-2	18	0.012
Cephalothin	2.32	3.29	0.84	2.25	2.6150	153-61-7	19	0.14
Chloramphenicol	1.84	2.66	0.87	1.65	2.0728	56-75-7	18	0.655
Chlormethiazole	0.79	0.91	0.00	0.30	1.1451	533-45-9	37	0.73
Chloroquine	1.85	1.63	0.13	1.29	2.6344	54-05-7	37	1.40
Chlorprothixene	2.21	1.57	0.00	0.88	2.4037	113-59-7	38	1.48
Cimetidine	1.66	1.87	0.74	1.86	1.9563	51481-61-9	39	1.70
Ciprofloxacin	2.20	2.50	0.73	1.85	2.3046	85721-33-1	18	1.495
Citalopram	1.66	1.87	0.00	1.08	2.5327	59729-33-8	40	1.80
Clemastine	1.70	1.55	0.00	0.97	2.7646	15686-51-8	18	0.375
Clomipramine	1.94	1.66	0.00	0.89	2.5239	303-49-1	18	1.03
Clonazepam	2.36	2.25	0.47	1.09	2.1072	1622-61-3	41	0.33
Clozapine	2.46	1.82	0.18	1.44	2.4310	5786-21-0	42	2.79
Codeine	2.16	1.92	0.23	1.58	2.2057	76-57-3	43	2.16
Cotinine	1.24	1.54	0.00	1.14	1.3867	486-56-6	44	0.78
Dapsone	1.87	2.84	0.45	1.35	1.8047	80-08-0	45	0.34
Descarboethoxy-loratadine	2.07	1.55	0.13	0.99	2.3723	100643-71-8	46	0.80
Desipramine	1.80	1.58	0.13	0.90	2.2606	50-47-5	18	0.915
Desmethyldoxepin	1.73	1.45	0.13	0.92	2.1765	1225-56-5	47	1.275
Diazepam	2.11	1.72	0.00	1.04	2.0739	439-14-5	12	0.16
Dieldrin	2.12	1.31	0.00	0.61	2.0065	60-57-1	48	6.00
Digoxin	3.67	4.46	1.58	4.32	5.7525	20830-75-5	12	0.55
Diltiazem	2.42	2.55	0.00	2.12	3.1365	42399-41-7	18	0.98
Disopyramide	1.77	2.26	0.49	1.64	2.9074	3737-09-5	49	0.90
Dothiepin	2.05	1.46	0.00	0.89	2.4222	113-53-1	50	1.59
Dothiepsulfoxide	2.23	2.50	0.00	1.45	2.4809		50	1.18
Doxepin	1.75	1.46	0.00	0.98	2.3174	1668-19-5	47	1.37
Doxorubicin	3.75	3.69	1.17	3.34	3.7284	23214-92-8	19	1.19
Doxycycline	3.37	3.69	1.73	3.15	3.0992	564-25-0	33	0.34
Erythromycin	2.51	3.04	1.05	4.63	5.7730	114-07-8	12,33	0.455
Ethanol	0.21	0.45	0.31	0.31	0.4491	64-17-5	51	0.90
Ethosuximide	0.74	0.94	0.34	0.93	1.1175	77-67-8	52	0.80
Fentanyl	1.86	2.18	0.00	1.33	2.8399	437-38-7	19	2.45
Flecainide	0.63	1.68	0.41	1.32	2.5960	54143-55-4	19	2.54
Fluconazole	2.16	2.45	0.31	1.42	2.0062	86386-73-4	53	0.74
Flunitrazepam	2.14	2.15	0.00	1.15	2.1433	1622-62-4	33	0.54
Fluoxetine	1.01	1.19	0.13	0.78	2.2400	54910-89-3	54	0.68
Flurbiprofen	1.50	1.51	0.57	0.58	1.8389	5104-49-4	19	0.019
Fluvoxamine	0.66	0.95	0.23	1.14	2.3113	54739-18-3	55	1.32
Haloperidol	2.00	2.08	0.31	1.45	2.7979	52-86-8	18	0.64
Heptachlor epoxide	2.03	1.38	0.00	0.60	1.9557	1024-57-3	56	3.92
Hexachlorobenzene	1.33	1.23	0.00	0.00	1.4508	118-74-1	57	20.0
Hydromorphone	2.04	1.79	0.27	1.32	2.0648	466-99-9	58	2.57
Ibuprofen	0.78	1.01	0.57	0.51	1.7771	15687-27-1	18	0.00
Imipramine	1.81	1.59	0.00	0.95	2.4015	50-49-7	18	0.76
Indomethacin	2.44	2.49	0.57	1.24	2.5299	53-86-1	18	0.19
Labetalol	2.15	2.30	1.00	1.72	2.6432	36894-69-6	18	1.70
Lamotrigine	2.40	2.13	0.45	0.93	1.6453	84057-84-1	18	0.425
Levodopa	1.33	1.77	1.56	1.44	1.4307	59-92-7	59	0.30

Table 1 (*continued*)

Name	*E*	*S*	*A*	*B*	*V*	CAS number	Ref.	*M*/*P*
Lidocaine	1.10	1.50	0.26	1.17	2.0589	137-58-6	34	1.07
Loratadine	2.19	2.09	0.00	1.14	2.8694	79794-75-5	46	1.20
Lorazepam	2.37	1.83	0.64	1.29	2.1141	846-49-1	60	0.205
Medroxyprogesterone	1.67	2.49	0.17	1.35	2.8211	520-85-4	18	0.72
Mefloquine	1.28	1.04	0.38	1.22	2.3270	53230-10-7	61	0.145
Mepindolol	1.73	1.47	0.60	1.51	2.1499	23694-81-7	62	2.60
Methadone	1.51	1.72	0.00	1.09	2.7078	76-99-3	12	0.83
Methimazole	1.11	1.10	0.00	0.60	0.8407	60-56-0	31	1.03
Methotrexate	3.51	4.23	1.85	2.82	3.2197	59-05-2	18	0.04
Methyldopa	1.30	1.73	1.56	1.45	1.5716	555-30-6	63	0.265
Methylphenidate	1.01	1.29	0.13	0.94	1.9092	113-45-1	64	1.10
Metoclopramide	1.50	2.31	0.50	1.63	2.3398	364-62-5	19	0.915
Metoprolol	1.10	1.22	0.29	1.52	2.2604	37350-58-6	65	2.55
Metronidazole	1.12	1.75	0.31	0.86	1.1919	443-48-1	12	0.98
Mexiletine	0.85	1.00	0.23	0.90	1.5794	31828-71-4	12	1.48
Mianserin	2.01	1.61	0.00	1.03	2.1520	24219-97-4	33	2.20
Midazolam	2.41	1.76	0.00	0.80	2.2628	59467-70-8	66	0.09
Minoxidil	1.07	0.85	0.42	1.62	1.6957	38304-91-5	18	0.76
Mirtazapine	2.08	1.67	0.00	1.22	2.1109	61337-67-5	67	1.10
Moclobemide	1.43	2.04	0.27	1.36	1.9905	71320-77-9	68	0.72
Morphine	2.23	1.59	0.50	1.47	2.0648	57-27-2	43	2.46
Nadolol	1.68	1.56	0.83	1.90	2.4923	42200-33-9	69	4.60
Naproxen	1.54	1.49	0.57	0.75	1.7821	22204-53-1	19	0.16
N-Desmethylsertraline	1.88	1.59	0.21	0.77	2.1238	87857-41-8	70	1.64
Nefopam	1.60	1.46	0.00	0.92	2.0786	13669-70-0	71	1.20
Nicotine	1.01	1.03	0.00	0.91	1.3710	54-11-5	18	2.25
Nitrazepam	2.21	2.17	0.47	1.10	1.9848	146-22-5	66	0.27
Nitrendipine	1.56	2.26	0.13	1.54	2.6354	39562-70-4	18	0.35
Nitrofuranthoin	1.65	2.03	0.24	1.34	1.4533	67-20-9	72	2.25
Norethindron	1.81	2.44	0.40	1.07	2.4376	68-22-4	12	0.19
Norfluoxetine	1.06	1.34	0.21	0.88	2.0991	56161-73-0	54	0.56
Nortriptyline	1.69	1.30	0.13	0.72	2.2587	72-69-5	12	0.65
Noscapine	2.39	3.09	0.00	2.09	2.8751	128-62-1	73	0.29
o,*p*′-DDE	1.80	1.53	0.00	0.30	2.0526	3424-82-6	56	3.00
o,*p*′-DDT	1.76	1.51	0.00	0.22	2.2180	789-02-6	56	15.2
Ofloxacin	2.26	2.58	0.57	2.05	2.5042	82419-36-1	19	1.19
Olanzapine	2.30	1.59	0.13	1.45	2.3742	132539-06-1	74	0.38
Oxazepam	2.23	1.75	0.64	1.29	1.9917	604-75-1	75	0.10
Oxprenolol	1.23	1.69	0.29	1.61	2.2174	6452-71-7	18	0.37
p,*p*′-DDD	1.67	1.44	0.09	0.22	2.0956	72-54-8	56	23.5
p,*p*′-DDE	1.80	1.53	0.00	0.30	2.0526	72-55-9	56	7.37
p,*p*′-DDT	1.76	1.51	0.00	0.22	2.2180	50-29-3	56	11.58
Paracetamol	1.12	1.66	0.91	0.93	1.1724	103-90-2	18	0.88
Paroxetine	1.79	1.77	0.13	1.23	2.3868	61869-08-7	18	0.75
PCB 114	2.05	1.57	0.00	0.05	1.9362	74472-37-0	76	4.350
PCB 118	2.07	1.59	0.00	0.11	1.9362	31508-00-6	77	1.816
PCB 138	2.18	1.65	0.00	0.05	2.0586	35065-28-2	77	1.910
PCB 146	2.20	1.68	0.00	0.04	2.0586	51908-16-8	77	2.009
PCB 153	2.20	1.68	0.00	0.04	2.0586	35065-27-1	77	1.901
PCB 156	2.17	1.65	0.00	0.04	2.0586	38380-08-4	77	1.866
PCB 170	2.29	1.72	0.00	0.00	2.1810	35065-30-6	77	1.618
PCB 180	2.30	1.75	0.00	0.00	2.1810	35065-29-3	77	1.549
PCB 194	2.41	1.82	0.00	0.00	2.3034	35694-08-7	77	1.199
PCB 199	2.41	1.85	0.00	0.00	2.3034	52663-75-9	77	1.371
PCB 202	2.42	1.88	0.00	0.00	2.3034	2136-99-4	76	6.08
PCB 206	2.54	1.93	0.00	0.00	2.4258	40186-72-9	77	0.729
PCB 209	2.67	2.03	0.00	0.00	2.5482	2051-24-3	77	0.505
PCB 33	1.81	1.41	0.00	0.19	1.6914	38444-86-9	76	0.64
PCB 52	1.98	1.54	0.00	0.18	1.8138	35693-99-3	76	2.11
PCB 74	1.94	1.50	0.00	0.12	1.8138	32690-93-0	77	1.963
PCB 99	2.07	1.57	0.00	0.06	1.9362	38380-01-7	77	2.023
Pefloxacin	2.03	2.42	0.57	1.91	2.4132	70458-92-3	78	0.96
Penicillin G	2.27	2.71	0.84	2.05	2.3771	61-33-6	33	0.315
Penicillin V	2.31	2.86	0.84	2.27	2.4358	87-08-1	18	0.37
Perphenazine	2.87	2.33	0.23	1.84	3.0191	58-39-9	79	0.90
Pethidine	0.99	1.26	0.00	0.97	2.0501	57-42-1	19	1.25
Phenacetine	0.96	1.55	0.41	0.87	1.4542	62-44-2	12	0.67
Phenobarbitone	1.56	1.81	0.52	1.29	1.6999	50-06-6	18	0.50
Phenytoin	1.94	2.04	0.44	1.14	1.8693	57-41-0	80	0.18
Piroxicam	2.56	3.12	0.72	2.12	2.2500	36322-90-4	81	0.02
Prednisolone	2.19	3.02	0.73	1.97	2.7546	50-24-8	18	0.13
Procainamide	1.33	2.11	0.50	1.49	2.0178	51-06-9	18	3.20
Propranolol	1.76	1.44	0.29	1.36	2.1480	525-66-6	12	0.36
Propylthiouracil	1.35	1.74	0.39	1.03	1.2791	51-52-5	31	0.23
Pseudoephedrine	0.98	0.94	0.38	1.12	1.4385	90-82-4	12	2.50

Table 1 (*continued*)

Name	*E*	*S*	*A*	*B*	*V*	CAS number	Ref.	*M*/*P*
Pyrimethamine	2.26	2.01	0.45	0.99	1.8458	58-14-0	45	0.56
Quazepam	2.03	1.57	0.00	0.49	2.3900	36735-22-5	18	4.13
Quinapril	2.11	3.01	0.71	2.00	3.4114	85441-61-8	82	0.12
Risperidone	2.59	2.23	0.00	1.70	3.0395	106266-06-2	83, 88	0.26
Rofecoxib	1.66	2.43	0.00	1.15	2.2324	162011-90-7	85	0.25
Rosaramicin	2.13	2.89	0.43	3.29	4.6068	35834-26-5	86	0.12
Roxithromycin	2.58	2.90	1.05	5.12	6.5538	80214-83-1	18	0.035
Sotalol	1.52	1.86	0.74	1.75	2.1010	3930-20-9	37	3.74
Sertraline	1.83	1.44	0.13	0.67	2.2647	79617-96-2	87	1.76
Sulfamethoxazole	1.99	2.43	0.59	1.21	1.7244	723-46-6	33	0.10
Sumatriptan	1.90	2.05	0.68	1.61	2.2723	103628-46-2	88	4.90
Suprofen	1.51	1.89	0.57	0.81	1.9026	40828-46-4	89	0.014
Temazepam	2.24	1.76	0.17	1.34	2.1326	846-50-4	18	0.14
Terbutaline	1.39	1.31	1.38	1.63	1.8377	23031-25-6	31	1.04
Tetrachloroethylene	0.60	0.73	0.00	0.12	0.8370	127-18-4	48	3.00
Tetracycline	3.36	3.59	1.73	3.27	3.0992	60-54-8	33	0.95
Theobromine	1.46	1.89	0.24	1.22	1.2223	83-67-0	60	0.82
Theophylline	1.46	1.99	0.35	1.29	1.2223	58-55-9	91	0.70
Tiapamil	1.98	4.37	0.00	2.73	4.0846	57010-31-8	92	0.44
Timolol	1.74	1.68	0.29	1.91	2.3759	91524-16-2	93	0.80
Tinidazole	1.02	2.40	0.00	1.13	1.6959	19387-91-8	94	1.005
Tolmetin	1.54	1.93	0.57	0.97	1.9798	26171-23-3	18	0.005
Tramadol	1.23	1.15	0.31	1.30	2.2340	27203-92-5	95	2.20
Trazodone	2.64	2.47	0.00	1.92	2.7304	19794-93-5	12	0.53
Triprolidine	1.73	1.50	0.00	1.06	2.3585	486-12-4	96	0.53
Valproic acid	0.17	0.62	0.57	0.40	1.3102	99-66-1	97	0.053
Venlafaxine	1.20	1.23	0.31	1.16	2.3749	93413-44-6	98	2.50
Verapamil	1.76	3.00	0.00	1.89	3.7861	52-53-9	99	0.60
Vigabatrin	0.50	0.99	0.78	0.99	1.0852	60643-86-9	18	1.00
Zidovudine	1.62	1.77	0.47	1.70	1.8192	30516-87-1	100	1.45
Zolpidem	2.35	2.39	0.00	1.33	2.4740	82626-48-0	37	0.16
Zonisamide	1.84	1.95	0.44	1.11	1.3858	68291-97-4	101	0.93
Zopiclone	2.66	3.20	0.00	2.43	2.6228	43200-80-2	18	0.555

[a] Alternative CAS number is 34911-55-2.

3.2. *Nonlinear modeling of* M/P

Since the results of linear modeling using the LFER method were not successful we decided to use ANN as a nonlinear feature mapping technique. Preliminary work showed that $\log(M/P)$ was a better dependent variable than M/P. However, several values of M/P approach zero, or are actually given as zero, see Table 1, so that the value of $\log(M/P)$ then approaches $-\infty$. In order to include all the drugs in Table 1 in our analysis we used the function $\log(1 + M/P)$. An ANN program was written in FORTRAN 77. This network was feed-forward fully connected using three layers with sigmoidal transfer function. The Abraham LFER descriptors, *E*, *S*, *A*, *B*, and *V*, were used as inputs of the network and the signal of the output node represents the $\log(1 + M/P)$ value of the compounds. Thus, this network has five nodes in the input layer and one node in the output layer. The value of each input was divided into its mean value to bring them into dynamic range of the sigmoid transfer function of the network. The initial values of weights were randomly selected from a uniform distribution that ranged between −0.3 and +0.3 and the initial values of biases were set to be one. These values were optimized during the network training.

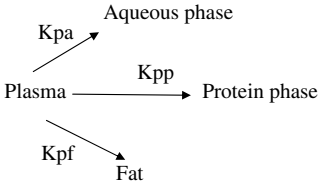

Fig. 1. Possible processes for the distribution of compounds between plasma and milk.

The back-propagation algorithm was used for the training of the network. Before training, the network parameters have to be optimized. These parameters are number of nodes in the hidden layer, weights and biases learning rates, and the momentum. Then the optimized network was trained using a training set for the adjustment of weights and biases values. It is known that a neural network can become over-trained. An over-trained network has usually learned the stimulus pattern it has seen perfectly, but cannot give an accurate prediction for unseen stimuli, and it is no longer able to generalize. There are several methods for overcoming this problem. One method is to use an internal test set, often denoted as a validation set, to evaluate the predictive power of the network during its training. In this method, after each 1000 iterations, the network was used to calculate the M/P value of molecules included in the internal test set. To maintain the predictive power of the network at a desirable level, training was stopped when the value of the error for the internal test set started to increase. Since the test error is not a good estimate of the generalization error, the prediction potential of the model was evaluated on a third set of data, named the external test set. The compounds in the external test set were not used during the training process and were reserved to evaluate the predictive power of the generated ANN.

The training set, the internal test set and the external independent test set were assigned as follows. Compounds were arranged in order of descending M/P values, and the training, internal and external test set compounds were selected from this list at desired intervals. In an initial study we used the LFER parameters, obtained by the method of Abraham, that are based on experimental data. These LFER parameters were available for 129 compounds in the data set, and were used in the training and test sets. The ANN calculated root mean square error, RMSE, in log units for the training set of 97 compounds, the internal test set of 16 compounds and the external test set of 16 compounds was 0.040, 0.155 and 0.145 respectively.

The main drawback of this method is that the LFER parameters are obtained from experimental data, and this limits the generality of the method. The PharmaAlgorithms Absolv software package [30] calculates the Abraham LFER descriptors using fragmentation methods, and so can be applied more generally – for example to candidate drugs that have not even been synthesised. To evaluate the credibility of the PharmaAlgorithm Absolv calculated LFER descriptors, we used ANN for the calculation of $\log(1 + M/P)$ for the identical 129 compounds as used above, with the LFER parameters obtained through the Absolv software [30]. The obtained RMSE in log units for training, internal and external test sets is 0.039, 0.158 and 0.133 respectively. As can be seen, there are no significant differences between the model that uses Abraham descriptors obtained from experimental data, and the model that uses calculated Abraham descriptors. We conclude that the descriptors calculated by the PharmaAlgorithm Absolv software are adequate for the calculation and prediction of M/P ratios, and so we applied ANN to the entire 179 compound data set, using the Absolv calculated descriptors, *E*, *S*, *A*, *B*, and *V*. The compounds in the data set were split into a training set of 135 compounds, an internal test set of 22 compounds and an external test set of 22 compounds. The optimized ANN has five inputs (equal to the number of LFER parameters), 14 nodes in the hidden layer and one node in the output layer. The calculated standard errors for $\log(1 + M/P)$ are 0.057, 0.116 and 0.093 for the training set, the internal test set and the external test set respectively. In Fig. 2 the plot of the calculated versus the experimental values of $\log(1 + M/P)$ for the external test set is shown. The differences between calculated and experimental values of $\log(1 + M/P)$ are plotted against the experimental values in Fig. 3; the random propagation of errors shows that there are no systematic errors in the constructed ANN model. A disadvantage of

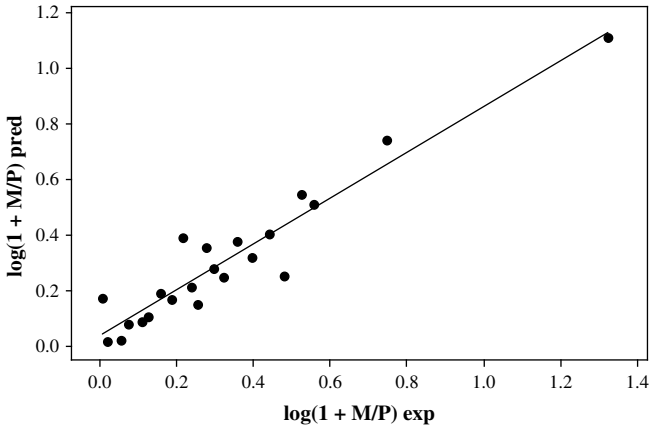

Fig. 2. The plot of ANN predicted versus experimental value of $\log(1 + M/P)$ for the external test set.

the ANN method is that it is difficult to make a quantitative assessment of the relative importance of the various input parameters. However, we find that the qualitative importance is $B > E > S > V > A$.

The RMSE values obtained using $\log(1 + M/P)$ as input are not at all the same as those when $\log(M/P)$ is used as an input. In order to compare our work with previous studies, we transformed the errors for the individual compounds into errors in $\log(M/P)$ and obtained for the three data sets, values of 0.203, 0.193, and 0.334 log units. A comparison of present results with previous results is in Table 2. When transformed into errors in $\log(M/P)$, the present results are rather better than those from previous studies. The method of Agatonovic-Kustrin et al. [17] uses a very large number of descriptors, but yields poorer values of RMSE for the smaller data set of 60 compounds. Katritzky et al. [19] obtain RMSE values very close to those we find, but only by removing a substantial proportion of data points as outliers. Fifteen compounds were omitted from an initial set of 115 compounds, to leave a data set of 100 compounds, very much smaller than our data set of 180 compounds, in which no compounds at all were left out. We note also that the RMSE obtained by Katritzky et al. [19] for their external test set was obtained as the value for the plot of observed and predicted $\log(M/P)$ ratios. Such an error is always smaller than the error obtained from the observed and predicted values themselves.

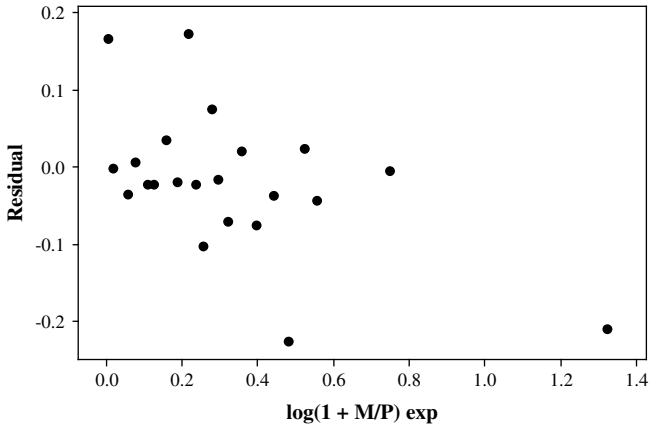

Fig. 3. A plot of residuals of ANN predicted versus experimental values of $\log(1 + M/P)$ for the external test set.

Table 2
Statistics for the correlation and prediction of M/P ratios.

Reference	Total no.	Variable	No. of descriptors		RMSE for training and test sets		
			Start[a]	Final[b]	Training	Internal test	External test
This work	179	$\log(1 + M/P)$	5	5	0.056	0.109	0.090
This work	179	$\log(M/P)$	5	5	0.203	0.193	0.334
[17]	60	$\log(M/P)$	61	26	0.590		0.425
[19]	100[c]	$\log(M/P)$	850	7	0.324		0.332[d]

[a] Original number of descriptors calculated.
[b] Number of descriptors used in the model.
[c] Fifteen of the original 115 drugs were excluded.
[d] Error of the plot of observed and predicted values.

4. General discussion

As shown in Fig. 1, the plasma–milk distribution system is very complicated, and it is no surprise that a simple linear equation is not adequate to fit the data. In addition to the five Abraham descriptors, we also used a variety of other descriptors, either alone, or in combination with the Abraham descriptors, but could obtain no useful linear equation. Katritzky et al. [19] did indeed use a linear equation to analyze M/P ratios, but had to exclude 15 out of the original 115 drugs, that is no less than 13%. Unless there is some specific reason for these 15 drugs to be outliers, then statistically 13% of all predictions of M/P ratios are expected to be outliers – an unsatisfactory result. It is only by using a nonlinear method that we can include 179 drugs and environmental pollutants, with no outliers at all. An advantage of the present method is that the five descriptors we use are all calculated ones [30], and hence can be obtained for further drugs and pollutants. We explored the use of other descriptors, such as the water to octanol partition coefficient, as $\log P_{oct}$, but found no advantage at all.

One very considerable advantage of the present method is that, for the first time, environmental pollutants are included with drug molecules, and this paves the way for predictions to be made of M/P ratios for such pollutants. The M/P ratios themselves are not exceptional, except that a few pollutants have rather large M/P ratios, for example *p*,*p*′-DDD; *p*,*p*′-DDT; *p*,*p*′-DDE; *o*,*p*′-DDT. It is instructive to carry out a principal component analysis on the five descriptors, *E*, *S*, *A*, *B* and *V*. The scores of the first two principal components, PC1 and PC2 contain 84% of the total information in the five descriptors, and a plot of PC2 against PC1 is shown in Fig. 4. The pollutants lie in a different area to most of the drugs, and hence occupy a different chemical space. This does not matter for the present analysis, because our database includes both the pollutants

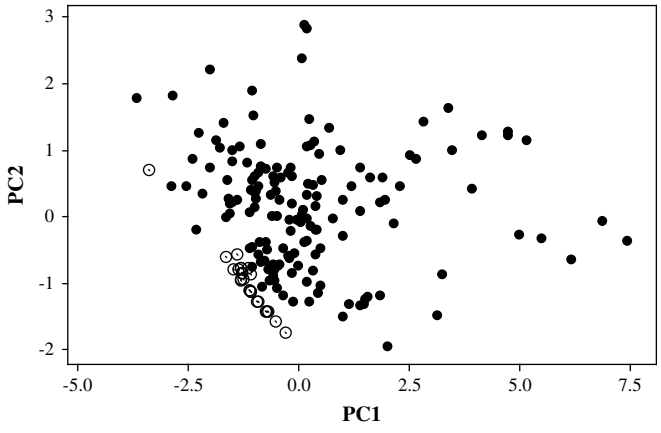

Fig. 4. A plot of the scores for PC2 against PC1: ○ environmental pollutants, ● drugs.

and drugs. However, for analyses that use only data on drugs, predictions for pollutants will be subject to extra uncertainty.

One difficulty over amassing a collection of M/P ratios from a variety of sources is that the method used to obtain M/P ratios may vary. However, many sources do not describe the technical method in any detail, and in any case there are more important variables that can influence the M/P ratios. These include the number of subjects used, differences between mothers who smoke and those who do not, the age of the mother, the gestational age, and the time after medication was taken (in the case of mothers on medication). Taking into account all these variables, it is remarkable that we can fit the training set with RMSD = 0.20 and predict an external test set with RMSD = 0.30 in $\log(M/P)$. Indeed, with such a collection of data we doubt that fitting the data to much less than 0.20 log units in $\log(M/D)$ is possible, without technically overfitting the data.

It is interesting that Agatonovic-Kustrin et al. [17] and Katritzky et al. [19] made no pH–pK_a correction in their analysis. We also have made no such correction. In view of our interpretation of the lack of any sound basis for the correction, and the results obtained without any correction, we doubt if any pH–pK_a correction is justified. In a similar vein, Agatonovic-Kustrin et al. [17], Katritzky et al. [19], and ourselves made no correction for protein binding in plasma and protein binding in milk. Whatever protein binding occurs must be accounted for within the ANN. This has the decided advantage that M/P ratios can be predicted for drugs and pollutants for which no protein binding data exists, without having to predict protein binding in some separate algorithm.

Although the artificial neural network we have constructed allows little interpretation of the data in any physicochemical way, it is a remarkably simple way to predict M/P ratios through the prediction of $\log(1 + M/P)$. All that is required is the SMILES notation for any drug or environmental pollutant. The Absolve descriptors can be calculated [30], and the ANN used to obtain $\log(1 + M/P)$. SMILES notations can be sent to the corresponding author who will calculate the Absolv descriptors using the PharmaAlgorithms method [30], and then Dr Fatemi will provide the predicted $\log(1 + M/P)$ values.

5. Conclusions

A linear analysis of M/P or $\log(M/P)$ or $\log(1 + M/P)$ for drugs yields no satisfactory equation, but an artificial neural network applied to 179 drugs and pollutants can fit the data for 135 compounds with a standard error of 0.06 log units in $\log(1 + M/P)$ and can predict an external test set of 22 compounds with a standard error of 0.09 log units. These errors correspond to 0.20 and 0.33 log units in $\log(M/P)$. Only five descriptors are used as input for the ANN. This result, using a much larger data set than previously, with no compounds omitted, is the best quantitative analysis of M/P ratios yet obtained.

The key strength of the neural networks in QSPR studies is their ability to allow for flexible mapping of the selected features by manipulating their functional dependence implicitly, unlike regression analysis. Neural networks can handle both linear and nonlinear relationships, of particular importance when dealing with such a complicated system as the plasma–milk equilibrium. This capability offsets the large computing time required and the complexity of the ANN method with respect to MLR.

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