Prediction of Molecular State of Therapeutic Agents in Biological Fluids from Theoretical Partition Coefficients Determined from Retention Factors

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ABSTRACT

Biological fluids (extracellular and intracellular) help maintain body homeostasis and therefore are very important for life. The n-octanol-water partition coefficients, (usually given as log P values), are used as a measure of lipophilicity. Lipophilicity of a therapeutic agent (drug) is one of the parameters, which influences its biological activity. The objective of this study was to investigate the ability of thin layer chromatography to provide reliable information on the lipophilic behaviors of drugs when exposed to ions in biological fluids. The chromatographic separation of the drugs was carried out on plain silica gel F_{254} plates as well as silica gel F_{254} plates impregnated with a sodium ion, potassium ion, and calcium ion respectively. The spots were visualized with UV_{254} light and iodine vapor respectively. All the ions decreased the retardation factor values (R_f values) of the fourteen drugs investigated. A linear relationship occurred when experimental retention factor values (R_m values) were plotted against known (literature) log P values of the drugs. In general, log P values of all the drugs studied increased as the concentration of the ions increased except for piroxicam (with Na^+ and Ca^{2+} ions respectively), loratadine (with Na^+ and K^+ ions respectively). The study suggests that retention factor values (R_m values) obtained from the thin-layer chromatographic analysis, could be used to predict the lipophilicity (invariably the cell membrane permeability) of the investigated drugs in biological fluids.

Key Words: *Biological fluids ions, retention factor, logarithm partition coefficient, chromatographic analysis.*

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INTRODUCTION

Water is a very significant portion of the human body. The total body water which is about 60 % of body weight is forty (40) liters. The body water is distributed into two main compartments namely extracellular and intracellular compartments [1]. These compartments are separated from each other by cell membranes. The intracellular compartment contains fluid whose capacity is about twenty-five (25) liters that is approximately 2/3 of body water. The extracellular fluid which is about 1/3 of the body water has a capacity of fifteen (15) liters. The extracellular fluid consists of plasma volume, interstitial fluid, and transcellular fluid. Both type fluids contain electrolytes necessary for fluid balance (sodium, chloride ions); osmotic pressure (sodium, chloride ions); acid-base balance (potassium, bicarbonate ions); energy (phosphate ions); blood clotting and bone integrity (calcium ions); enzymatic activities (magnesium ions). The distribution of the ions in intracellular fluid shows that potassium ion (100meq/liter), magnesium ion (123meq/liter) and phosphate ion (149 meq/liter) are the most abundant while

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chloride ion (2meq/liter) is the least. The extracellular fluid ions distribution indicates that sodium ion (142meq/liter), chloride ion (105meq/liter) are the most abundant, followed by potassium ion (5meq/liter), calcium ion (5meq/liter) while magnesium and phosphate ions (2meq/lite) respectively are the least.

The cell membrane consists of three main components namely lipids, proteins, and saccharides. The lipids constituting the boundary between the cell and its surroundings are the main skeleton of the cell membrane [2]. The proteins (embedded in lipids in different ways) regulate the exchange of substances between internal and external media and provide cellular signaling [3].

The saccharides are inside the cells and provide energy for cell activity while the polysaccharides on the cell surface are conjugated to proteins and lipids [4]. Cellular exchange of chemical compounds and signaling form the most important processes for biological activity and are strictly regulated by the plasma membrane. The exchange depends on two possible transport modes namely active and passive. Active transport requires a transport protein that uses energy (arising from ATP hydrolysis) to carry a molecule across a cell membrane while passive transport, (the most common mode of drug passage through membranes), entails diffusion of a drug molecule through the cell membrane with no outside assistance or energy input [5]. The rate of passive diffusion across a cell membrane is reported to be proportional to the partition coefficient of the drug between the lipophilic cell membrane and the external medium (aqueous environment); the diffusion coefficient of the drug through the membrane, and the drug's concentration gradient across the membrane [6]. The uptake and efflux of drugs in relevant compartments depends on membrane permeability. To predict passive membrane permeability, lipophilicity is the most critical parameter to be studied [7]. The parameter that determines the lipophilicity of a chemical compound is the logarithm partition coefficient (LogP) of the chemical compound between an aqueous and organic phase, usually water and octanol [8].

As most drugs need to pass at least one cellular membrane to reach the site of action, it is vital to understand how some ions present in biological fluids compartments affect the drug molecules' lipophilicities. Therefore, the present study was aimed at predicting lipophilicities of some selected drugs (belonging to different pharmacological classes) in biological fluids using retention factors obtained from the thin-layer chromatography. A literature report [9] has shown that the retention factor (R_m) could be used to estimate the logarithm partition coefficient.

MATERIALS AND METHODS

Materials

Amoxicillin (GlaxoSmithKline, USA), Artesunate (Novartis, USA), Cimetidine (GlaxoSmithKline, USA), Diazepam (Roche Pharmaceuticals Inc., USA), Ibuprofen (Dexcel Inc., Israel), Levofloxacin (Ortho-McNeil Pharmaceuticals, Inc., USA), Loratadine (Cadila Pharmaceuticals Ltd, India), Metoclopramide (Pfizer Inc, USA), Metronidazole (DPT Laboratories, USA), Omeprazole (Mylan Pharmaceuticals Inc., USA), Piroxicam (Pfizer Inc., USA), Quinine (Cox Arthur H & Co Ltd, England), Ramipril (Bristol Laboratories, England) and Tramadol (Exlor SA, Belgium), silica gel F_{254} (BDH, England), TLC plates (20×10 cm) and TLC applicator (Toshniwal, India), developing chamber and syringe.

Method

Thin layer chromatographic plates were prepared by mixing silica gel F_{254} with double distilled water in 1:3 volume ratios with constant shaking for 5min until a homogeneous slurry was obtained. The resultant slurry was coated on the thin layer plate using a TLC applicator to give a 0.25-mm thick layer. Silica gel F_{254} impregnated plates were similarly prepared using salts of different concentrations as impregnating agents. The plates were air-dried and activated at a temperature of 105^0C for 30 minutes before being used in the analysis. The chromatographic separation of the drugs was carried out in the development chamber containing filter paper which enables the atmosphere to be saturated with vapor.

A mixture of chloroform/methanol/ethyl acetate (3:2:1) was used as the mobile phase. The mobile phase was about 1.5 cm in height in the development chamber. The 1% solutions (w/v) of studied drugs in methanol were applied about 2 cm above the bottom edge of the plate using the syringe. The developed chromatograms were dried and the spots were visualized with UV_{254} light and iodine vapor respectively.

The retardation (R_f) values were calculated and were used to calculate the retention factor (R_m) values.

RESULTS

The fourteen drugs investigated in the present study are amoxicillin (penicillin antibiotic), artesunate (antimalarial), cimetidine (antiulcer), diazepam (antianxiety/sedative), ibuprofen (antiinflammatory), levofloxacin (quinolone antibacterial), loratadine (antiallergic), metoclopramide (antiemetic), metronidazole (antibacterial), omeprazole (antiulcer), piroxicam (antiinflammatory), quinine (antimalarial), ramipril (antihypertensive) and tramadol (analgesic). The drugs were selected from prescription notes of patients and each prescription note contained at least three of the studied drugs.

The results in Table 1 indicate that all the ions investigated decreased the R_f values of the fourteen drugs. The decrease was observed as the concentration of each ion increases. At the highest concentration level (1.0 % w/v) investigated, Ca^{2+} seemed to have produced the greatest decrease in the R_f values of the drugs.

Table 1: Retardation factor (R_f) values at different electrolytes (salts) concentrations.

Drug	NaCl	KNO_3	$CaCl_2$
	R_f value	R_f value	R_f value
	0.0 0.1 0.5 1.0 (% w/v)	0.0 0.1 0.5 1.0 (% w/v)	0.0 0.1 0.5 1.0 (% w/v)
Amoxacillin	0.79 0.78 0.67 0.58	0.79 0.70 0.66 0.60	0.79 0.64 0.61 0.55
Artesunate	0.69 0.57 0.50 0.47	0.69 0.53 0.49 0.44	0.69 0.49 0.42 0.38
Cimetidine	0.81 0.73 0.58 0.45	0.81 0.64 0.59 0.54	0.81 0.57 0.53 0.49
Diazepam	0.73 0.71 0.68 0.64	0.73 0.66 0.58 0.53	0.73 0.62 0.56 0.51
Ibuprofen	0.58 0.46 0.40 0.32	0.58 0.43 0.39 0.35	0.58 0.43 0.38 0.35
Levofloxacin	0.84 0.82 0.73 0.66	0.84 0.71 0.64 0.58	0.84 0.64 0.57 0.51
Loratadine	0.54 0.51 0.45 0.40	0.54 0.49 0.45 0.41	0.54 0.45 0.41 0.37
Metoclopramide	0.67 0.60 0.52 0.48	0.67 0.56 0.52 0.48	0.67 0.49 0.45 0.41
Metronidazole	0.80 0.71 0.61 0.58	0.80 0.65 0.57 0.53	0.80 0.63 0.52 0.46
Omeprazole	0.68 0.57 0.51 0.45	0.68 0.48 0.47 0.41	0.68 0.43 0.40 0.35
Piroxicam	0.72 0.63 0.56 0.48	0.72 0.44 0.40 0.37	0.72 0.55 0.54 0.49
Quinine	0.60 0.52 0.49 0.43	0.60 0.47 0.42 0.39	0.60 0.42 0.35 0.32
Ramipril	0.77 0.55 0.47 0.42	0.77 0.52 0.49 0.42	0.77 0.50 0.46 0.41
Tramadol	0.74 0.54 0.46 0.42	0.74 0.48 0.44 0.39	0.74 0.45 0.40 0.35

In Figure 1, a linear relationship was observed when experimental R_m values were plotted against known (literature) log P values of the drugs. The regression equation fitting the calibration curve is log P = 7.321R_m + 4.9603.

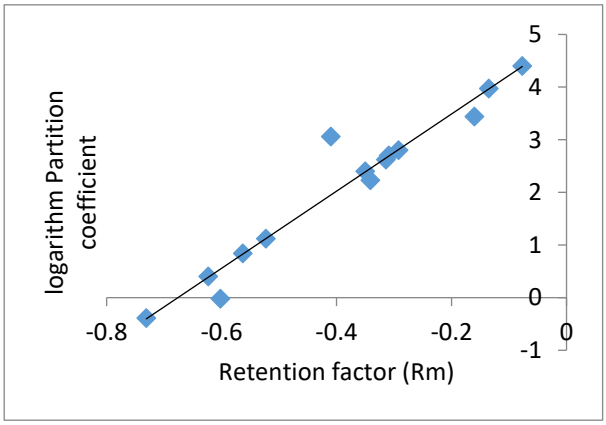

Figure 1: Plot of retention factor (R_m) versus logarithm partition coefficient

The results in Table 2 show that when compared to literature octanol/water logarithm partition coefficient (log P) of the drugs, sodium ion (Na^+) at a concentration level of 0.1 % w/v decreased log P of quinine, piroxicam, diazepam, loratadine, and levofloxacin respectively. At the same concentration level of the ion, an increase in log P was observed for amoxicillin, artesunate, cimetidine, ibuprofen, omeprazole, metoclopramide, metronidazole, ramipril, and tramadol respectively. However, at the highest concentration (1.0 % w/v) studied, an increase in log P of quinine, diazepam, levofloxacin was observed. The results also indicated that Na^+ ion decreased log P of loratadine and piroxicam at all concentration levels (0.1% w/v, 0.5 % w/v, 1.0 % w/v respectively) of the ion.

Table 2: Theoretical logarithm Partition Coefficient as calculated from the Regression Equation

Drug	NaCl	KNO_3	$CaCl_2$
	Log P	Log P	Log P
	0.0 0.1 0.5 1.0 (% w/v)	0.0 0.1 0.5 1.0 (% w/v)	0.0 0.1 0.5 1.0 (% w/v)
Amoxacillin	0.84 0.846 2.71 3.96	0.84 2.21 2.93 3.63	0.84 3.20 3.53 4.29
Artesunate	2.4 4.92 5.29 5.88	2.4 4.65 5.03 5.75	2.4 5.24 5.99 6.48
Cimetidine	0.4 2.22 3.89 5.56	0.4 3.09 3.87 4.46	0.4 4.04 4.62 5.14
Diazepam	2.82 -0.245 1.83 3.14	2.8 0.897 2.40 3.01	2.8 1.60 2.78 3.64
Ibuprofen	3.97 5.49 6.31 7.41	3.97 5.80 6.40 6.87	3.97 5.91 6.48 6.90
Levofloxacin	-0.39 -3.31 -.040 1.42	-0.39 2.14 3.06 3.99	-0.39 3.09 3.95 4.78

Loratadine	4.4 2.62 3.12 3.89	4.4 3.04 3.71 4.33	4.4 3.82 4.46 5.10
Metoclopramide	2.62 3.74 4.76 5.19	2.62 4.14 5.24 5.26	2.62 5.08 5.66 6.12
Metronidazole	-0.02 2.07 3.53 4.13	-0.02 2.96 4.10 4.62	-0.02 3.36 4.67 5.42
Omeprazole	2.23 4.02 4.79 5.55	2.23 5.20 5.34 6.13	2.23 5.88 6.18 6.96
Piroxicam	3.06 -.684 1.43 2.72	3.06 3.61 4.31 4.72	3.06 1.24 1.47 2.62
Quinine	3.44 2.14 3.48 3.95	3.44 3.21 4.18 4.59	3.44 4.12 4.65 5.83
Ramipril	1.12 4.29 5.34 6.03	1.12 4.70 4.97 5.93	1.12 4.89 5.41 6.17
Tramadol	1.35 4.50 5.39 5.96	1.35 5.17 5.78 6.31	1.35 5.60 6.18 6.90

With potassium ion (K^+), log P of quinine, diazepam, and loratadine decreased at a concentration level of 0.1 % w/v, while an increase in log P values of other studied drugs at the same concentration was observed.

In the Table, Ca^{2+} ion decreased the log P of piroxicam at all concentration levels investigated.

At the maximum concentration of salts investigated (1.0 % w/v), Ca^{2+} ion showed the most increase in log P for all the drugs except for cimetidine and ibuprofen (Na+ gave the most increase).

In general, it was observed that log P values of all the drugs studied increased as the concentration of the ions increased except for piroxicam (with Na^+ and Ca^{2+} ions respectively), loratadine (with Na^+ and K^+ ions respectively).

DISCUSSION

The logarithm partition coefficient of each drug utilized in the present investigation was obtained from the literature [10-12]. Although Na^+ and K^+ ions were the ions of interest, due to their physiological activities, Ca^{2+} ion was investigated to enable the study to postulate the probable mechanism of interactions between the ions and the drugs. Furthermore, concentrations of the ions significantly higher than their concentrations in biological fluids were studied to avoid ambiguity in predicting the probable molecular state (lipophilicity) of the investigated drugs in extracellular and intracellular fluids respectively.

The chromatographic analysis revealed that calcium ion exhibited the most increase in the adsorptivity of all the drugs investigated except for piroxicam. The effect could be explained in terms of ionic charge and size. Calcium ion when compared to sodium and potassium ion, has the greatest charge and largest ionic size (invariably largest ionic volume) and these properties must have contributed to its strongest interaction with the investigated drugs. The same explanation could also be used to interpret the effects of sodium and potassium ions on the adsorptivity of the drugs on the stationary phase.

In the analysis, each drug was defined by its retardation factor (R_f) value that corresponds to its relative migration compared to the solvent. Equation 1 defines the R_f value as:

$$R_f = \frac{distance\ moved\ by\ the\ drug}{distance\ moved\ by\ the\ solvent\ front} \qquad 1$$

The retention factor (R_m) for each drug was also calculated using equation 2

$$R_m = log(\frac{1-R_f}{R_f}) \qquad 2$$

The efficiency (N) of the plate for each drug was calculated using migration distance (x) of each drug and its spot diameter (d) according to equation 3:

$$N = 16(\frac{x}{d})^2 \qquad 3$$

The low values (less than 2 %) of the relative standard deviation suggested the suitability of the silica gel plates used in the analysis.

The linearity of the calibration curve (Figure 1) permitted the calculation of log P values of the drugs analyzed by salts impregnated thin layer chromatography using the experimental retention factor (R_m) values. A linear relationship has been reported to exist when the separation mechanism is the partitioning of an analyte between mobile and stationary phases [13]. Furthermore, the retention factor has also been reported to be a useful parameter to determine the lipophilicity of a chemical substance [9].

CONCLUSION

Biological fluids could variably affect the partition coefficient (the ability of the drug to dissolve in the cell membrane) and the diffusion coefficient (the rate at which the drug moves within the cell membrane) of the studied drugs. At concentration levels within the biological fluid, sodium ion (Na^+) decreased lipophilicity (log P) of quinine, piroxicam, diazepam, loratadine, and levofloxacin respectively. However, the same ion was observed to increase the lipophilicity of amoxicillin, artesunate, cimetidine, ibuprofen, metoclopramide, metronidazole, omeprazole, ramipril, and tramadol respectively. Similarly, potassium ion (K^+) decreased lipophilicity of quinine, diazepam, and loratadine respectively, while the lipophilicity of amoxicillin, artesunate, cimetidine, ibuprofen, levofloxacin, metoclopramide, metronidazole, omeprazole, piroxicam, ramipril, and tramadol respectively was increased.

Finally, the study suggests that retention factor values (R_m values) obtained from the thin-layer chromatographic analysis, could be used to predict the lipophilicity (invariably the cell membrane permeability potential) of the investigated drugs in biological fluids since there was good linear correlation between retention factor (R_m) and log partition coefficient (log P) of the studied drugs.

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Conflict of Interest

The authors declare that they have no conflict of interest.

REFERENCES

[1] Clinical Chemistry: Theory, Analysis, Correlation, 5th Edition. Edited by Lawrence A. Kaplan, Amadeo J. Pesce and Steven Kazmierczak, 2009.

[2] Van Meer G, Voelker DR, Feigenson GW. Membrane lipids: where they are and how they behave. Nature reviews Molecular cell biology. 2008 Feb;9(2):112-24.

[3] Filomeni G, Rotilio G, Ciriolo MR. Glutathione disulfide induces apoptosis in U937 cells by a redox-mediated p38 MAP kinase pathway. The FASEB Journal. 2003 Jan;17(1):64-6.

[4] Hart GW, Copeland RJ. Glycomics hits the big time. Cell. 2010 Nov 24;143(5):672-6.

[5] Seddon AM, Casey D, Law RV, Gee A, Templer RH, Ces O. Drug interactions with lipid membranes. Chemical Society Reviews. 2009;38(9):2509-19.

[6] Wolak DJ, Thorne RG. Diffusion of macromolecules in the brain: implications for drug delivery. Molecular pharmaceutics. 2013 May 6;10(5):1492-504.

[7] Liu X, Testa B, Fahr A. Lipophilicity and its relationship with passive drug permeation. Pharmaceutical research. 2011 May 1;28(5):962-77.

[8] Shuo, S., Shu-zhi, L., Mao-bo, D., Ke-ya, G., Li-hua, S., Zu-guang, Y, Determination of equilibrium solubility and apparent oil/water partition coefficient of artesunate. Chinese Journal of Experimental Traditional Medical Formulae. 2013; 19: 9-12.

[9] Czyrski A, Kupczyk B. The determination of partition coefficient of 6-mercaptopurine derivatives by thin layer chromatography. Journal of Chemistry. 2012 Sep 3;2013.

[10] 10. Hansch, C., Leo, A., Hoekman, D., Exploring QSAR-hydrophobic-electron and steric constants, Washington DC, Am Chem Soc. 1995; 168.

[11] Pyka AL, Babuska M, Zachariasz MA. A comparison of theoretical methods of calculation of partition coefficients for selected drugs. Acta Pol Pharm. 2006;63(3):159-67.

[12] Pallicer JM, Rosés M, Ràfols C, Bosch E, Pascual R, Port A. Evaluation of log Po/w values of drugs from some molecular structure calculation software. ADMET and DMPK. 2014 Jul 18;2(2):107-14.

[13] Czyrski A, Hermann T, Rubis B, Rybczyńska M, Sledz D. Biopharmaceutical characterization of some new papaverine decomposition products. Die Pharmazie-An International Journal of Pharmaceutical Sciences. 2011 Mar 10;66(3):165-7.