Measuring partition coefficient

Abstract

This Laboratory study deals with the Analytical Procedure of the Measurement of Partition Coefficient. Partition Coefficient is a very important criterion for Organic substances. It finds use in Pharmaceutical Industry, Pollution abatement systems, Agro Chemicals, and Chemical Industry. There are many methods available for determining the Partition Coefficient, especially Instrument methods like, Chromatography, Electrophoresis etc. The method adopted here is a simple, reliable and versatile one, which utilizes basic principles of Chemical Analysis.

The method used was by measurement of pH and Colorimetric determination of the organic Ligand. The process used for partitioning was " Shaking Flask method". The given sample was diluted and buffered appropriately and an aliquot was partitioned with an equal quantity of the given oil. The pH of the aqueous phase was measured. The clear aqueous solution was further diluted appropriately and used for Colorimetric estimation using a Calibration graph prepared. These data were used for computation of apparent Partition coefficient and then true Partition coefficient.

Measuring Partition Coefficient

Chemistry is a material Science, dealing with the study of Physical and Chemical properties of the matter found in the universe. There are many disciplines in Chemistry dealing with different materials and properties, like Inorganic Chemistry, Organic Chemistry, Physical Chemistry, Pharmaceutical Chemistry, Analytical Chemistry etc. The Analytical Chemistry is a special branch of Chemistry dealing with the determination of Chemicals, quantity wise and quality wise. The Analytical Chemistry uses the knowledge

available in other branches of Chemistry, like Inorganic Chemistry, Organic Chemistry, Physical Chemistry, and many principles of Physics. The uses and applications of Analytical Chemistry are wide, and practically, in every aspects of human life, analytical Chemistry is involved in some way or other, say, in Clinical Chemistry, Pharmaceutical Chemistry, Forensic Chemistry, in commerce, in Customs Department and so on. The measurement of Partition Coefficient is a typical analytical procedure using many theoretical principles of various branches of Chemistry. It denotes the differential amounts of the substances found at equilibrium conditions in the organic phase and the aqueous phase for a set of conditions like Concentration, pH, Temperature etc. This Lab study aims at and involves, in addition to learning partitioning technique, Electro Chemical application – the pH measurement, colorimetric measurement, computational techniques, and Calculation procedures. The partition coefficient study assumes significance, because it finds use in Pharmaceutical Chemistry for drug design, development, and delivery, Pesticide design, soil Chemistry, designing of Chemical Plants by Chemical Engineers, and also for Chemists and Scientists working on Liquid – Liquid Equilibrium data.

METHODS

Sample preparation: Sodium salicylate solution of 0. 2 gram mol per liter (mol) was taken for this study. From this stock standard solution four Test samples, named A to D, were prepared. 10 ml of 0. 2 mol standard solution was pipetted into each of the four 100 ml volumetric flasks marked A to D and diluted to the mark with four buffer solutions of different pH, and mixed

thoroughly. So the concentration of the resultant diluted samples was 0. 02 mol each.

Partitioning: Aliquots of 25 ml of the above diluted samples, 4 Nos, were taken in 4 separating funnels. Then, 25 ml of the given oil was added in each of the 4 separating funnels, marked A to D, and mixed thoroughly and gently by inverting and rotating for 10 minutes. Then the mixture in the separating funnels were allowed to settle thoroughly. After the aqueous and organic layers became clear, the aqueous layers of the four separating funnels were drained into four glass beakers marked A to D.

pH measurement of aqueous phase : The pH of the four partitioned aqueous samples were measured using a pH meter.

Determination of Salicylate concentration in the Aqueous phase: For determining the Salicylate concentration, Colorimetric method was adopted where the absorbance of the Iron- Salicylate complex was measured. The procedure adopted for developing the standard and test samples is given below.

Preparation of Standard color Solutions: Four different Standard solutions of Sodium Salicylate, namely, 0. 00125mol, 0. 0025 mol, 0. 00375 mol and 0. 005mol were prepared along with a blank.

Five test tubes were taken. The first one was marked as 1 (Blank), and the others as 2, 3, 4, and 5.

To the blank, 1 ml of water was added, and in the others, one ml each of the prepared standards were added. Then 2 ml of the given Ferric Nitrate was

added to all the test tubes. Then 5 ml of water was added into all the five test tubes. All the test tubes were shaken gently to mix the contents thoroughly and waited for some time for the complete development of the color. The five solutions represented 0. 0000 mol, 0. 00125mol, 0. 0025 mol, and 0. 00375 mol and 0. 005mol Salicylic acid concentration respectively.

Preparation of Calibration Graph: The Colorimeter (Spectrophotometer) was set at the wave length of 624 nm. Placed the blank in the cuvete in the colorimeter and adjusted the absorbance as zero. Then the other standard solutions were placed one by one and noted the absorbance readings. Calibration Graph was plotted, plotting concentration of salicylic acid in X axis and Absorbance at the y axis.

Preparation of Test samples: 10 ml of each of the four Partitioned aqueous layers were diluted with water to 50 ml, thus making a diluted sample. From these, 1 ml solution each were placed in four test tubes, marked A, B, C, & D. Then, 2 ml ferric Nitrate and 5 ml water were added in all the four test tubes and treated similar to the Standard tubes.

Measurement of Salicylic acid concentration of the test samples: The absorbance of all the four test samples were measured similar to the standards. The Salicylic acid concentration of the test samples were arrived from the Calibration graph. The concentration arrived was of the diluted samples. So the concentration of the partitioned aqueous phase was multiplied 5 times to get the concentration of the salicylic acid. This gives the Cw , i. e., the concentration of the salicylic acid in the partitioned aqueous solution.

Determination of CO: The Cw was subtracted from the concentration of the buffered solution, i. e., 0. 02 mol, to get the CO.

Determination of Hydrogen Ion concentration: From the pH of the four partitioned aqueous solutions, Hydrogen Ion concentrations were calculated.

CALCULATIONS

Step 1. Calculation of H+ and 1/H+ from the pH

Model calculation for Experiment A; pH = 2. 35.

pH is the negative logarithm to base 10 of Hydrogen ion concentration.

So Hydrogen ion concentration is the antilog of – 2. 35= 0. 00447

Reciprocal of Hydrogen ion concentration = 1/H+ = 1/0. 00447 = 223. 9

Similarly H+ and 1/H+ are calculated for other experiments and tabulated below.

Experiment

pH of the buffer added

Resultant pH

[H+]

1 / [H+]

A

2. 0

2. 35

0. 00447

223. 9

B

2. 8

3. 22

0. 000603

1660

C

3. 3

3. 85

0. 000141

7079

D

4. 0

4. 02

0. 0000955

10471

Step 2a. Calculation of the concentration of salicylate added to each separating funnel: The salicylic acid concentration of the sample taken = 0. 2 gm. mol/Liter. 10 ml of this solution was diluted with buffer to 100 ml. So the concentration of the diluted solutions, added to each

separating funnel, taken for the Partition experiment were 0. 2×10/100 = 0. 02 gm mol/L each.

Step 2b. Calculation of Cw and Co: The partitioned concentration of salicylic acid in water and oil, denoted by [S (aq)] and [S (org)] are Cw and Co in the formula respectively.

25 ml of the Solution A (0. 02 gm mol Sodium Salicylate, buffered with buffer of 2. 0 pH) was partitioned with 25 ml of oil. After separation of the phases, the pH was measured in the aqueous phase. Then the aqueous phase was diluted five fold for colorimetric estimation. The absorbance obtained for experiment A was 0. 065 and the corresponding concentration obtained for experiment A from the calibration graph = 0. 00054. So the concentration of this undiluted Partitioned aqueous solution, [S(aq)] , is five times of the value determined calorimetrically = 0. 00054x 5 = 0. 0027. This is CW

The concentration of salicylic acid in the organic phase is the concentration of the diluted solution taken for the Partition experiment, minus concentration of the undiluted Partitioned aqueous solution, i. e. CO = (0. 02-CW) = (0. 02 -0. 0027) = 0. 0173gm mol/L.

Apparent partition coefficient P’ = CO / CW = 0. 0173/0. 0027 = 6. 4

1/P’ = 1/6. 4= 0. 156

CW, CO, P’ and 1/P’ for other experiments were also calculated like wise and tabulated below.

The calibration graph of this study is attached separately.

Exp

Absorbance

Salicylate Concentration in aqueous phase of the diluted aliquot, from calibration Graph

Salicylate Concentration in aqueous phase the undiluted aliquot, CW i. e. ([S(aq)])

Salicylate Concentration in Organic phase

CO i. e. ([S(org)]) =

0. 02- S(aq)

A

0. 065

0. 00054

0. 0027

0. 0173

B

0. 138

0. 00116

0. 0058

0. 0142

C

0. 221

0. 00218

0. 0109

0. 0091

D

0. 267

0. 0025

0. 0125

0. 0075

Calculation of P’ and 1/P’

Exp

[S(aq)] i. e. CW

[S(org)] i. e. CO

P’ = (CO / CW)

1/P’

A

0. 0027

0. 0173

6. 4

0. 156

B

0. 0058

0. 0142

2. 45

0. 408

C

0. 0109

0. 0091

0. 83

1. 200

D

0. 0125

0. 0075

0. 6

1. 667

Step 3. Preparation of 1/H+ vs. 1/P’ Graph and Calculation of P and Ka: A graph was plotted with 1/H+ in X axis and 1/P’ in Y axis. The slope, Ka/P was estimated from the graph = 0. 0001518. The intercept, 1/P, was at 0. 13, and hence, P = 1/0. 13 = 7. 69.

Ka = (Ka/P) x P = 0. 0001518 x 7. 69 = 0. 001167; pKa = – log of 0. 001167 = 2. 93

DISCUSSION

The study results show a definite trend of higher ingress of the organic acid, i. e., Salicylic acid, into the organic layer at a lower pH and vice versa. This is in accordance with the theory, which implies, at a lower pH, the H+ ion concentration will be higher, which will in effect enhance association of the ions, R- + H+ â†’ RH, to form unionized molecule that can enter the organic phase. So the unionized acid will be predominantly in the organic layer. At

higher pH, the H+ ions will be low and there will be the tendency of the acid to ionize in the aqueous phase, RH â†’ R- + H+ , thus preventing the acid to enter the organic phase. So the ionized acid ion will be predominantly in the aqueous layer. This is established in this experiment; CO, the concentration of salicylic acid in organic phase is highest, 0. 0173mol, in Experiment A, where the pH is the lowest, 2. 35 ; and lowest, 0. 0075 mol, in Experiment D where the pH is the highest , 4. 02. Consequently the Apparent Partition coefficient P' which is the ratio of CO / CW is highest in Experiment A and lowest in Experiment D. This shows, the pH of the solutions affect the partitioning.

The accuracy of the study depends both on the accuracy of pH measurement and the measurement of absorbance. The linearity of the graph- 1/H+ vs. 1/P' depends on both the measurements. But the curve was not perfectly linear as expected.

The potential sources of errors . While carrying out the Chemical Analysis, one has to be aware of the potential sources of errors. Alexeyev (p 48) classifies the errors in " Quantitative Analysis" as Systematic errors, random errors and mistakes. The systematic errors are: errors of the method, errors of apparatus & reagents, and Operative errors. Random errors do happen during

any analysis and one has to be vigilant and careful to avoid them. Mistakes are crude errors caused by careless noting of the readings in the instruments, parallax error, improper labeling of the various test samples ending with confusion while tabulating the readings etc.

The possible systematic errors in this study are: errors of the method, say non uniform pH among the four test in the colorimetric estimation. Lyalikov. Y (p 40) warns, “ many colored compounds are sensitive to Hydrogen Ion concentration”. “ Changes in pH not only affects extinction, but change spectrophotometer curve of the substance as well”, Lyalikov. Y (41). The calibration curve obtained is not straight as expected, showing the colored complex did not obey Beer Lambert Law, which states, absorbance is proportional to molar extinction coefficient, Îµ, depth of the solution layer, L, and concentration, C. (A= Îµ x L x C). It was expected at least to be a smooth curve of a definite pattern . But the curve is not very smooth indicating some error, may be varying final pH of the colored solutions.

Possible errors of apparatus: leaking separating funnel. Possible errors of reagents: accuracy of the buffers.

Operative errors: Possible non uniform mixing during partitioning, incorrect and non uniform draining from pipettes. It was expected that the curves of Calibration graph and that of 1/H+ vs. 1/P’ to be straight lines. But they are not straight as expected. The reason may be due to some or a combination of the above cited errors.

Comparison of the result with Literature : The literature value for Ka of salicylic acid as given by Harris, Daniel. C. (p 183) is Ka = 1. 07 x 10¯3. The result obtained in this Lab study is 0. 001167. This is higher than the value reported in “ Quantitative Chemical Analysis” by about 9%

The phenomenon of partitioning: The chemical substances exhibit different solubility in different solvents. Solvents may be classified into two groups,

Aqueous and Non aqueous, in other words polar and non polar. Similarly the chemicals may be classified as Hydrophilic and Hydrophobic. Hydrophobic substances can also be termed as Lipophilic. A hydrophilic substance will easily dissolve in an aqueous solvent and a hydrophobic (Lipophilic) substance will easily dissolve in non aqueous (Organic) solvent. If a substance is in contact with both the Hydrophilic and Hydrophobic Solvents, the substance will get distributed in both the solvents and the proportion of distribution will be according to the nature of the substance with respect to its Hydrophilic or hydrophobic nature and this property is termed as the Partition coefficient.

Partition coefficient finds application in Pharmaceutical industry, agrochemical Industry, Pollution studies and for designing of Chemical Process by Chemical Engineers.

Drugs are meant to be ingressed into human body. The partition coefficient finds use in drug design, as it is a measure of the hydrophobicity of the drug concerned. If the partition coefficient is high, it denotes high hydrophobicity (high lipophilicity) and such a drug will easily enter the lipid regions of the organs and stay for longer time and hence may prove toxic. On the other hand a low Partition coefficient denotes a hydrophilic nature and hence the drug will stay longer in the aqueous regions – blood stream and will not readily ingress into the tissues. So the absorption, excretion and penetration of the drugs into the body organs are related to the Log P value of a drug. An intermediate Partition coefficient is preferred while designing the drugs by the Pharmacologists. Earll. Mark enumerates the optimum Partition Coefficient, as Log P, for different types of drug applications.

Optimum CNS penetration around Log P = 2 +/- 0. 7 (Hansch)

Optimum Oral absorption around Log P = 1. 8

Optimum Intestinal absorption Log P = 1. 35

Optimum Colonic absorption Log P = 1. 32

Optimum Sub lingual absorption Log P = 5. 5

Optimum Percutaneous Log P = 2. 6 (& low mw)

The drug has to be designed accordingly for each of the application. The Formulation and dosing forms, as given by Earll. Mark:

Low Log P (below 0) Injectable

Medium (0-3) Oral

High (3-4) Transdermal

Very High (4-7) Toxic build up in fatty tissues

The drug has to go into human body through different routes, say, mouth, skin, Blood etc all having different pH. So the drug has to be designed taking into consideration of the effect of pH. Mark Earll gives the pH of the various parts of the body: Stomach 2, Kidneys 4. 2 (variable), Small Intestine: Fed 5. 0 &Fasted 6. 8, Duodenal Mucus 5. 5, Plasma 7. 4.

According to Chemie. DE information service GMBH, The Hydrophobic drugs are preferentially distributed to hydrophobic compartments such as lipid bilayers of cells while hydrophilic drugs preferentially are found in

hydrophilic compartments such as blood serum. The Partition coefficient of the drug determines the Absorption. Distribution, Metabolism and excretion of the drugs. When a drug is admitted orally, it passes through the alimentary canal and has to be absorbed through the lipid layers of the epithelial membrane of the small intestine. So the drug should be sufficiently Lipophilic as to pass through the lipid layers. At the same time it should not be too lipophilic, otherwise, it will stay permanently in the epithelial calls and will not enter the blood stream for transport to the required location. Similarly the drug has to be metabolized and excreted after its function is over. This also depends on the Hydrophobicity. Similarly the other forms of drug administration are also need to be studied in this aspect. So control of the Hydrophobicity (lipophilicity) while developing the drug is important. Here is the use of Partition coefficient measurement, which is a measure of the hydrophobicity.

Partition coefficients find use in designing pesticides. One has to design the insecticide in such a way it has got a very high partition coefficient , i. e. , having high hydrophobicity, rather

high lipophilic tendency, so that the insecticide easily penetrates into the organisms and stay permanently causing high toxicity, thus proving its efficacy in killing the pests. But, the adverse consequence is, the pollution aspect, vide Chemie. DE information service GMBH.

In partition studies, Octanol/ water system is normally used. Earll. Mark states, “ Octanol was chosen as a simple model of a phospholipid

membrane; however it has shown serious shortcomings in predicting Blood-brain barrier or skin penetration".

Berthold says,

" The most needed liquid- liquid partition coefficient is the octanol-water partition coefficient. Ko/w is accepted as a good reference parameter for solute hydrophobicity. Indeed, Ko/w can be rapidly estimated using capillary electrophoresis with a micellar or micro emulsion solution and/or RPLC"

Leahy, Taylor and Wait of ICI have proposed, in addition to octanol, chloroform, cyclohexane and propylene glycol dipelargonate (PGDP) for modeling biological membranes, notes Earll. Mark.

For determining the Partition Coefficient, there are many other Instrumental methods, like, HPLC. Paper Chromatography, Thin layer chromatography and Electrophoresis. Berthod. A and Carda-Broch. S. enumerates the various analytical Techniques. They are: Shake-flask method, HPLC method, Micro emulsion electro kinetic capillary electrophoresis, Counter-current chromatography (CCC), Co current CCC, Micellar electro kinetic capillary chromatography (MEKC), Micro emulsion electro kinetic capillary chromatography (MEEKC)

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