

Apparent partition coefficient of quinalbarbitone



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The effect of ionization on the apparent partition coefficient of quinalbarbitone was investigated using the shake-flask method. High partition coefficient reflected the high lipophilicity of the drug. In the shake-flask experiment, calibration curve was constructed for the determination of concentration of quinalbarbitone in solutions of different pH values. Low ionization of quinalbarbitone in acidic environment will result in high apparent partition coefficient as it is extracted more into the n-octanol layer. This is the case when quinalbarbitone was absorbed through GI tract and crossed the blood-brain-barrier easily.

Quinalbarbitone is weak acid with pKa 7.9 and hence is significantly ionized at pH values over 6. Chemically, it is known as 5-allyl-5-(1-methylbutyl) barbiturate.[1] For all the drug formulations, it is converted to sodium salt, which is more water-soluble for administration to the patients.

Quinalbarbitone sodium is very soluble in water, soluble in alcohol, and practically insoluble in ether.[1] Quinalbarbitone is a short-acting barbiturate and it is used for severe intractable insomnia only in patients already taking barbiturates (hypnotic).[5]

In this experiment, we are investigating the apparent partition coefficient of quinalbarbitone. The general principle of the shake-flask method is based on the drug partitioning. Drug is allowed to equilibrate between two immiscible liquids, then the concentration in both layers are determined after they have been separated.

An understanding of partition coefficient and the effect of pH on partition coefficient is useful in relation to the extraction and chromatography of

drugs. The partition coefficient for a compound (P) can be simply defined as,
[4]

where C_o = concentration of the substance in an organic phase

C_w = concentration of the substance in water

In other words, partition coefficient reflects the lipophilicity relative to the hydrophilicity. The greater the P, the more a substance has an affinity for the organic media. P is often quoted as a log-P value. N-octanol is more commonly used as an organic phase experimentally because, to some extent, it resembles the biological membrane in our body.

P_{app} is the apparent partition coefficient that varies with pH. From the Henderson-Hasselbalch equation:[4]

For acids: $P_{app} =$

Experimental (Materials and Methods):

200mL of Solution A and 100mL of Solution B with 50 $\mu\text{g mL}^{-1}$

quinalbarbitone solution in 0.5M NaOH and in water respectively were prepared from the 0.02%w/v stock solution. For example, 50mL of stock solution was pipetted out and mixed with 150mL of 0.5M NaOH in order to make 200mL of Solution A.

A range of 50mL calibration standards containing 5, 10, 15, 20, 25 and 30 $\mu\text{g mL}^{-1}$ of quinalbarbitone in 0.5M NaOH was prepared using the Solution A made. For example, 5mL of Solution A was pipetted out and mixed with

45mL of 0.5M NaOH in order to make 50mL of 5 $\mu\text{g mL}^{-1}$ solution in a round-bottomed flask.

Using the 30 $\mu\text{g mL}^{-1}$ standard, absorbance of different wavelengths near the expected λ_{max} (254nm) were recorded and the one which gave maximum absorbance was confirmed as the λ_{max} . The UV spectrophotometer was set to 254nm for the absorbance measurement of each standard using 0.5M NaOH as the blank. All the data gathered was used to plot a calibration curve of absorbance against concentration for the quinalbarbitone.

10mL of Solution B, 10mL of 0.1M HCl or buffer solutions with pH 6.6, 7.0, 7.4, 8.0 or 9.0 and 20mL of n-octanol were added into six separating funnels. This provided a system with the drug, aqueous phase and organic phase. The funnels were shaken at frequent intervals for 30 minutes to allow the layers to separate fully. Vigorous shaking should be avoided. After that, the aqueous layer was run off and left with the organic layer. 20mL of 0.5M NaOH was added and it was further shaken for 5 minutes. Finally, the absorbance of the aqueous (bottom) layer was measured for the six partitioning samples. The concentration of quinalbarbitone in the 0.5M NaOH (which was extracted into n-octanol) can be calculated based on the absorbance reading.

As a hypnotic, quinalbarbitone must be a weak acid in order to ionize in blood plasma and lipophilic enough to cross the blood-brain-barrier in order to exert its therapeutic effect. Quinalbarbitone is significantly ionized at pH value greater than 6. This is shown in Table 8 where the percentage of

ionization increases from 0% to 92.64% as the pH values of aqueous phase increases from 1 to 9. At low pH, quinalbarbitone is less ionized and therefore, the large amount of unionised species which are much more lipid soluble, will cross the biological membranes much more rapidly than the ionised species. This suggests that the quinalbarbitone will get absorbed more efficiently across a membrane especially in the stomach with pH 1 to 2. Degree of ionization is not the only factor that influences the drug's absorption. It basically explains the behaviour of a drug only. The other factor is the partition coefficient.[2]

Partition coefficient defines the equilibrium of the drug between the organic and the aqueous phases, depending on the relative affinity for each phase. [2] Greater lipid solubility is reflected as a larger partition coefficient. There is an optimum balance between aqueous and lipid solubility for maximum biological activity at maximum value of partition coefficient.[3] The coefficient is determined only for drugs at less than their saturation concentration in both phases.[3] However, partition coefficient applies only to unionised drugs and it assumes equilibrium state.

Hansch et al. showed that a range of non-specific hypnotic drugs with widely different types of structure were found to have a log P values around 2, provided they were not rapidly metabolized or eliminated.[2] As a rule of thumb, drugs that are targeted for central nervous system (CNS) should have a log P value of approximately 2 so that they can be transported across the CNS quicker. Besides, the therapeutic use of different barbiturates reflects the importance of partition coefficients. For example, quinalbarbitone with pKa 7.9 and Papp 2.0, is used as a short-acting

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hypnotic because it rapidly enters brain tissues (CNS), whereas phenobarbital with similar pK_a 7.4 and P_{app} 1.4, is used for the chronic treatment of epilepsy, rather than treating insomnia. Furthermore, the two R groups on the barbiturates will also confer to their lipophilicity.

Quinalbarbitone with long alkyl side chains is more lipid-soluble, hence it has high percentage of extraction and higher P_{app} than phenobarbitone.

The literature $\log P$ value for quinalbarbitone is 2.0. The experimental $\log P$ value is 1.29, which is lower than the literature value. The factor that is most likely to contribute to the difference in both values is due to the inefficiency in extraction. In this experiment, only one extraction is done which limits the efficiency in extraction of the drug into the organic phase. Other than that, we did not perform the shaking with constant magnitude and frequency which will limit the extraction of drug. We can achieve the most efficient extraction by performing large number of extractions with small portions of extracting liquid and using a machine with set intensity to perform the shaking.[7] Besides, the aqueous layer might not be completely run off which causes inaccuracies in determination of concentration of quinalbarbitone in n-octanol.

Another reason might be due to the presence of impurities (other solutes, normally salts) as they might affect the results (% of extraction) by forming complex with solute or by salting out one of the phase.[7] This definitely lowers the percentage of extraction and thus, lowering the P value. Other factor influencing the P value (and $\log P$ value) is the choice of partition solvent in which the solvent (n-octanol) used in this experiment donate and accept hydrogen bonds and is known as amphiprotic.[8] This is because the <https://assignbuster.com/apparent-partition-coefficient-of-quinalbarbitone/>

H-bonding reduces P as water solubility of the drug increases. The drug activity and its partition coefficient are strongly correlated and the accuracy of the correlation depends upon the solvent system used as the model.[2] In this experiment, *n*-octanol gives consistent results for drugs absorbed in the GI tract though *n*-octanol is not the same as our biological membranes.

From Table 7 and Table 8, the percentage of ionization is closely related to the percentage of extraction into the organic solvent. The lower the pH of the aqueous solvent, the lower the percentage of ionization, the larger the amount of unionized species in the mixture, the more lipid soluble the solute or the drug is. This means more of the drug will get extracted from the aqueous phase into the organic phase resulting in higher percentage of extraction as compared to those where the drugs are dissolved in solution of higher pH values. Higher pH will result in higher degree of ionization and hence, more drugs will remain in the aqueous phase and will not get extracted into the organic solvent.

The method used in this experiment is known as “shake-flask” method. There are some limitations associated with this method. First, it is time consuming (> 30 minutes per sample). Next, complete solubility must be attained, and it could be difficult to detect small amounts of undissolved material. If the compound is extremely lipophilic or hydrophilic, the concentration in one of the phases will be exceedingly small, and thus difficult to quantify. Lastly, relative to chromatographic methods, large amounts of material are required.

The pharmacokinetic phase of drug action includes the Absorption, Distribution, Metabolism and Excretion (ADME). Absorption is defined as the passage of drug from its site of administration into the bloodstream after enteral administration.[3] Distribution is the transport of the drug from the area of absorption to its site of action.[3] Metabolism is the biotransformation of the drug into a more water-soluble form to be excreted in urine.[3] Excretion is the elimination of unwanted substances from the body.[3]

In term of clinical significance of partitioning with regard to absorption of quinalbarbitone, the extremely acidic environment in the stomach (pH of 1-2) cause less quinalbarbitone to get ionized and behaves much more lipid soluble as the P value is higher as compared to environment with higher pH values. Table 9 shows the Papp values for each six mixtures. The higher the Papp (log Papp) values imply a highly lipophilic drug, where they are more likely to move through the lipid bilayer of the biological membranes. This eventually leads to more quinalbarbitone molecules absorbed from the stomach lining into the bloodstream by passive diffusion. This also suggests that the quinalbarbitone molecules easily and rapidly get across the blood-brain-barrier and reach the target site to exert their therapeutic effect.

Although the theory states that weak acids like quinalbarbitone will preferentially be absorbed from the stomach, these drugs are basically absorbed quite effectively from the small intestine even if they exist in a predominantly ionized form.[6] Gastric emptying will accelerate and hasten the passage of the drugs into the upper intestine with its higher pH environment and much larger surface area which are designed for drug

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absorption. Small amount of unionised drugs is absorbed and continually being carried away by the rich blood supply of the gut. This leads to equilibrium established and more unionised drugs get absorbed.[6]

Lipophilicity is the dominant factor that affects its distribution in the body.[2]

The higher partition coefficient of the drug which means it is much more lipid soluble, the more rapid it passes through the membrane of the tissue and therefore it is more distributed throughout the body. The degree of protein binding is also a function of lipophilicity. Quinalbarbitone has 70% protein binding in plasma.[1] Even though it has a reasonably high P values, its distribution is limited by the plasma protein binding. Thus, it has a low volume of distribution 1.5L/kg as most of quinalbarbitone are bounded to the plasma protein and do not get distributed widely throughout the whole body system. It gets absorbed in the stomach due to extremely acidic environment and thus, it is distributed more widely in the stomach. Due to the fact that small intestine is the organ designed for drug absorption,[6]the resulting volume of distribution is fairly reasonable to say that quinalbarbitone is fairly distributed in the body. In short, quinalbarbitone has the highest lipophilicity, highest plasma and brain protein binding, and the shortest duration of action as compared to other barbiturates.

Most drugs are metabolized first in liver into a more water-soluble form before being eliminated from the body. Oxidation is the most common phase I metabolic pathway. During drug oxidation, a hydroxyl group is added to the lipophilic part of the drug or a short alkyl group is removed, usually is the methyl group.[2] This process is catalyzed by cytochrome P450.[3] The major metabolic reactions involved in quinalbarbitone are hydroxylation of

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both R groups with further oxidation of the β -position on the butyl side-chain.[2]

Quinalbarbitone is excreted via renal excretion. Renal excretion involves both filtration at the glomerulus and secretion along the nephron.[3] By virtue of the relationship between pKa and pH, the slightly basic urine will increase the percentage of ionization of the quinalbarbitone, thereby reducing the amount of filtered drug reabsorbed through luminal surface of nephron and therefore increasing its renal elimination.[2] The percentage of extraction is reduced as the degree of ionization increases. The resulting P values become small indicating low lipophilicity which means quinalbarbitone hardly to get through the membrane and back into the circulation. This is the basis of the management of barbiturate overdose. As urine is acidic, quinalbarbitone will be excreted unchanged in it as the percentage ionized is small or even negligible. In fact, it is less than 5% of an oral dose is excreted unchanged in the urine as quinalbarbitone alone is lipid-soluble.[1] It is extensively metabolised to the more water-soluble form of metabolites for excretion.

Conclusions:

Partition coefficient defines the equilibrium of the drug between the organic and the aqueous phases, depending on the relative affinity for each phase. Highly unionized (low % ionization) quinalbarbitone means that it is more lipid-soluble which is reflected in high partition coefficient (high % extraction) can rapidly cross the blood-brain-barrier and exert its hypnotic effect. Log P determined is 1.29 which is slightly lower than the literature log P value, 2.0.

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In terms of ADME, quinalbarbitone is absorbed in GI tract, highly distributed in our body, mostly hydroxylated and excreted via urine. Since the concentration of quinalbarbitone is determined using UV-visible spectroscopy, therefore the analysis should be done at maximum wavelength so that the absorbance will be high and constant around the chosen wavelength.