A solubility of sulfacetamide using surfactants biology essay

Science, Biology

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Declaration

‘ I understand the nature of plagiarism and that it is a serious academic offence. I confirm that no material in this project has been plagiarised’Signed: Alwyn Chow

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Abstract

Background

Poorly soluble drugs can be a problem because they may not be able to dissolve in the body effectively. As a result, they stay in the body and can lead to toxicity. Surfactants can be used to increase the solubility of drugs by binding drugs into micelles. The use of surfactants on solubility of an antibacterial drug, sulfacetamide will be investigated in this project.

Aim

The aim of this product is to investigate the use of different types of surfactants on solubility of sulfacetamide. The surfactants being investigated are CHAPS, cetrimide, sodium dodecyl sulfate (SDS), Tween 20 and sodium taurocholate, and this project will also determine which type of surfactants are more effective.

Methods

A calibration curve of sulfacetamide was determined using a UV spectrophotometer and the critical micelle concentration (CMC) of SDS was determined using conductivity. Excess sulfacetamide were put into a range of concentration in each surfactant to see the effect on solubility and results were plotted on graphs.

Results

Anionic surfactants (SDS and sodium taurocholate) were found to be the most effective surfactants in improving solubility of sulfacetamide. The solubility of sulfacetamide remained the same in the presence of CHAPS, cetrimide and Tween 20.

Conclusion

It is possible for surfactants to increase the solubility of sulfacetamide around the CMC. Anionic surfactants are particular effective because the sodium ion present in sulfacetamide interacting with the anionic surfactants, therefore, enhance the solubility.

Significant and Impact of Study

The use of surfactants can be considered as one of the method for improving the solubility of drugs, so drugs can get to their site of action to exert their therapeutic effect. However, this research can be done more extensively using a wide range of surfactants and including different pH conditions.

Introduction

1. 1 Literature Review

There is little previous published work on the effect of surfactants on the solubility of sulfonamide in general, and none on sulfacetamide. The closest research undertaken was on sulfonamide, which involved the use of sulfacetamide sodium. However, this is slightly different from sulfacetamide alone because in the study, it was found that the reaction rate constants of sulfonamides with the reagent p-dimethylaminobenzaldehyde (DAB) is proportional to the amount of anionic surfactant sodium dodecyl sulphate (SDS) added except sulfacetamide sodium. This was thought to be due to the presence of sodium ion, which is cationic. The SDS will interact with the sodium ions instead of the ionised form of sulfacetamide. As a result, the rate constant decreased due to less binding of surfactant to the sulfacetamide.(1)Another study that was performed was on the effects of different types of surfactants on the dissolution of poorly soluble acidic drugs. The drugs involved were mefenamic acid, nimesulide and ibuprofen, they have similar pKa values (mefenamic acid: pKa 4. 2, ibuprofen: pKa 4. 4 and 5. 2) to sulfacetamide but slightly higher Log P values (mefenamic acid: Log P 5. 1, ibuprofen: Log P 4) (2). Three different types of surfactants:

cationic cetyltrimethylammonium bromide (CTAB), ionic sodium lauryl sulfate and non-ionic polysorbate 80 were used in the study. It was shown that the dissolution rates of these drugs are highly dependent upon the presence of surfactants. The dissolution rates of these drugs increase overall, with a more significant increase in the presence of CTAB. This was thought to be because the drugs are in anionic form at physiological pH, therefore, the cationic surfactant head group would interact electrostatically with the anions, making it easier to form soluble micelle complexes.(3)This experiment will be performed in a similar manner in the investigation of sulfacetamide, but will also cover any unstudied novel area of sulfacetamide. A similar approach was investigated with a beta-adrenoceptor blocking drug, carvedilol, which is a practically water insoluble drug. Two anionic surfactants, SDS and sodium taurocholate (STC), cationic CTAB and non-ionic tween 80 were used in the experiment. The results demonstrated that the solubility of carvedilol was increased with each of the surfactants used. However, the solubility of carvedilol is pH dependent, which means it exhibits different solubility at different pH. Not only was this study done at physiological pH, but it was also done in a variety of acidic conditions.(4)This experiment will be done at pH 7. 4 initially, solubility of sulfacetamide will be investigated at different pH values depending on the time available.

1. 2 Sulfonamide

1. 2. 1 History

Sulfonamide was the first antibacterial drug that was discovered in early 19th century. It is bacteriostatic which means that it inhibits the growth of

bacteria but it does not kill the bacteria (bactericide). (5) It was originally used in the dye industry. Gerhard Domagk, a German chemist who worked for Bayer at that time, discovered prontosil as the first sulfonamide drug to treat staphylococcal septicaemia.(6) Later, a French research team showed that prontosil is a pro-drug for sulfanilamide as it metabolised into sulfanilamide, which is a sulfonamide antibacterial. Sulfanilamide has a very low solubility; therefore, it can cause crystallisation in the kidneys, which is very harmful to the patients. However, sulfacetamide drugs made a big contribution during World War II, saving people’s lives including Winston Churchill when he suffered from pneumonia during that time. They also have an effect on preventing wound infections. Soldiers had powder and pills that contained " sulfa" in their first-aid-kit, and the powder could be spread onto open wounds. At first, sulfonamides were very effective in inhibiting the growth of a broad range of bacteria, however, resistance started to develop. Penicillin started to replace sulfonamides afterwards. Nowadays, sulfonamides are used orally mainly for urinary tract infection. Other forms are also available such as creams to be put onto burn wounds to prevent infection, drops and ointments to be used for eye infections. Some sulfonamide drugs can combine to other antibacterial for a greater effect (synergic effect). For example, co-trimoxaole is a combination of sulfamethoxazole and trimethoprim used to treat pneumocystis jirovecii pneumonia, toxoplasmosis and nocardiasis. Sulfamethoxazole acts on dihydropteroate synthase and trimethoprim acts on a later stage of the biochemical pathway, dihydrofolate reductase. This enzyme is responsible for the conversion of dihydrofolic acid to tetrahydrofolic acid. The

mechanism of this synergy effect is called sequential blockade as these two drugs act on different targets in the same biochemical pathway. Furthermore, sulfadiazine is used alone to treat toxoplasmosis and prevention of rheumatic fever recurrence.(7)All drugs that belong to the sulfonamide group have a common structure which is the sulfonamide group that contains a sulfonyl group which is attached to an amine group.(8) The structure is shown below. Sulfonamide. pngFigure Structure of SulfonamideIt can be used both topically and orally. A few examples of sulfonamide drugs are sulfadiazine, sulfamethoxazole, sulfadimidine and sulfacetamide, which is the drug that will be investigated in this project.

1. 2. 2 Pharmacology

Sulfonamide is active against both gram-positive and gram-negative bacteria and is effective in treating acute infections. The structure of sulfonamide is similar to the structure of para-aminobenzoic acid (PABA) which is a metabolite involved in folic acid biosynthesis in bacteria. Therefore, sulfonamide works by acting as a competitive antagonist of PABA(9), blocking the formation of dihydropteroic acid and inhibit the folate synthesis. As a result, the subsequent steps of folic acid biosynthesis pathway are blocked; thymidine, purines and methionine, which are essential for DNA and RNA synthesis and C1 metabolism, cannot be produced. There is a delayed period before sulfonamide begins to show an effect, because the bacteria consume the folic acid, thymidine, purines and methionine that they stored previously. When they have consumed all these stores, the bacteriostatic effect occurs.(10) Bacteria and humans require folic acid in order to grow;

bacteria make their own folic acid, which is then used to make DNA. Whereas in humans, we obtain folic acid through diet or supplements as we lack the enzymes that are responsible for making it. Therefore, it makes a good target for sulphonamides, which blocks the enzyme that human lack, dihydropteroate synthatase.(11) The structures of sulfacetamide and PABA are shown below: sulfacetmide + PABA. pngFigure Structure of Sulfacetamide and PABA

1. 2. 3 Antimicrobial Resistance

Antimicrobial resistance can occur due to the bacteria's physiology or inappropriate use of antibiotic treatments by healthcare professionals. Bacteria can alter their susceptibility to antibiotic by growing differently; they may grow slowly, use different metabolic pathways or grow in a biofilm. These are termed phenotypic resistance as the environment in which the bacteria grow causes the resistance. Therefore, the gene expression will change. Bacteria genes can also develop resistance by altering the genetic expression such as gene mutation or acquisition of resistance genes. These are termed genotypic resistance and this change in genetic expression will be permanent.(12)Antibiotic resistance should be avoided where possible because serious consequences may result. Bacteria are able to survive with the application of antibiotic treatment due to mutations. As a result, the antibiotic will not be effective to treat the infection. Moreover, the bacteria can continue to grow and the infection can spread. When the resistant microorganisms are spread further, the infection may last longer and become harder to treat. It is particular important for serious infections such

as pneumonia and tuberculosis where death can result. From the hospital point of view, prolonged and serious infection can increase the incidence and duration of hospital admissions. When the first-line antibiotic treatment fails, an alternative has to be used. This may be more expensive than the first-line antibiotic, and this would increase the cost of hospital treatment.(13)In 1940s, drug resistant occurred with streptomycin, the first antibiotic that was used for treating tuberculosis, and the drug was not effective anymore, causing many deaths. In 1950s, a Shigella strain which can cause dysentery was found to have resistance to sulfanilamides.(14) Resistance occurred due to the change in chromosomes and plasmids, so that the genetic codes for dihydropteroate synthase were altered. Therefore, the affinity of sulfonamide for the enzyme was reduced. Another cause of resistance is that there is an overproduction of PABA. As sulfonamide is a competitive inhibitor, overproduction of PABA will displace sulfonamide, rendering the sulfonamide ineffective. Cross-resistance between sulfonamides can also trigger drug resistance.(10)In order to minimise antibiotic resistance, antibiotics should be used wisely. This can be achieved by targeting the pathogen, so that a specific antibiotic is used solely for that particular pathogen. Some preventable diseases can be avoided by immunisation.

1. 2. 4 Sulfacetamide

Sulfacetamide is an anti-bacterial, which has various indications. It can be used for the treatment of acne and certain inflammatory conditions, such as seborrheic dermatitis, blepharitis and conjunctivitis.

1. 2. 5 Pharmacokinetics

Pharmacokinetics is how the body reacts to a drug when it is administered. Four processes are involved and they are absorption, distribution, metabolism and elimination (or excretion) (ADME). Absorption is the process of movement of a drug into the blood from the site of administration. Small, lipid soluble and non-ionised drugs are expected to be absorbed quicker. In general, most of the drug absorption takes place in the small intestine because it has a much greater surface area for absorption than the stomach. Distribution is the process of how drugs move throughout the body. Drugs will distribute into the body in order to have the desired effect. Lipophilic drugs are more likely to accumulate in the tissue. If the blood flow to the tissue is high, this will further increase the accumulation of drugs in the tissue. Some drugs that are insoluble can bind to proteins, and can be transported via the bloodstream. The hydrophobic part of the compounds, are attached to the hydrophobic group of the protein and the hydrophilic part of compounds attached to the protein so that the protein becomes water soluble. Metabolism is the chemical reaction where drugs are transformed to more hydrophilic species in the body, so that drugs can be excreted out. This is done by the liver and is an important process because drugs will stay in body without metabolism, and this may eventually lead to drug toxicity. Elimination is the excretion of drugs from the body. This process is interlinked with metabolism because people who have liver problem can reduce the metabolism of drugs, which will lead to toxicity. The same is also applied to people who have kidney problem. Therefore, the dose will need to be adjusted in patients who have these conditions.(15)It

was known that the carboxylate anion form of PABA is active, therefore, the ionised form of sulfacetamide is active as it mimics the structure of PABA. (16)The degree of ionisation of sulfacetamide by using the equation: The pKa for sulfacetamide is 1. 8 and 5. 4. The experiment will also be carried out in universal buffer pH 7. 4, therefore: From the answer above, sulfacetamide will be 99% ionised at physiological pH, therefore, it should have a high anti-bacterial activity. In general, ionised drugs are thought to be soluble in water but not soluble in lipid membranes whereas uncharged drugs are soluble in lipid membranes but not in water. Therefore, the log P value will need to be taken into account in order to determine the lipophilicity of the drug. Log P measures the lipophilicity of a drug, where P is defined as the partition coefficient. The higher the P value, the higher the lipid solubility of the drug. This value can have an effect on the drug's biological activities.(17) It can be shown by the equation below: Sulfacetamide has a log P value of -1. 0. It means that sulfacetamide will be hydrophilic. In the discussion earlier, small, lipid soluble and non-ionised drugs are expected to be absorbed quicker. However, sulfacetamide is hydrophilic and it is only active in its ionised form. Therefore, sulfacetamide is unlikely to go into brain and cause side effects in the central nervous system and the solubility of sulfacetamide is expected to be quite low.

1. 3 Drug Absorption/Solubility

Drug development is a very long and expensive process as it can take decades and millions of dollars from the beginning, final approval and the marketing of new drugs.(18) High throughput screening (HTS) is a method

that is used commonly and was thought to increase the drug discovery process. However, this method still takes years and remains a costly approach. Structure-based and ligand-based modelling have become more widely used as they utilise the knowledge of three-dimensional structures of the targets and use the chemistry of the known molecules to bind to the target, respectively.(19) An alternative method, virtual screening has been used to provide quicker and more accurate searches from large libraries to identify the compounds that can bind to a biological drug target and become active. It involves molecular docking and scoring which is a quick process in identifying lead compounds.(20)Drugs need to be dissolved in order to be effective. If they don’t dissolve, the drugs tend to stay and accumulate in the body. As a result, it would be toxic to the body. A recent research was studied using in silico approaches to predict the solubility of drugs in aqueous media. This is an important issue for drug development due to the toxic effects of the drug in the body and the effects on ADME.(21) However, there are more than 40% new chemical entities (NCEs) developed in the drug industry that are practically insoluble in water, therefore, there are a range of methods used to improve the solubility of drugs. The use of surfactants is one of the methods used, and it is an old but basic method used to improve the dissolution of poorly soluble drugs. The use of surfactants to increase solubility of drugs is also one of the approaches to improve patients’ safety.(22) This project will also use the surfactants to investigate the solubility of sulfacetamide. The table below is the Biopharmaceutical Classification System. It is provided by the U. S. Food and Drug Administration (FDA), and is used to predict the intestinal drug

absorption. Ideally, it would be perfect if all drugs are in class 1 as it has high solubility and permeability, therefore, high bioavailability. However, more than 70% of new drugs are not in class 1. Solubility and permeability are two of the several factors that determine the bioavailability of drugs. Therefore, those 70% of drugs do not have high bioavailability. In addition, class 4 drugs will have poor bioavailability because they are low in both solubility and permeability, and may lead to gastrointestinal mucosal toxicity. For class 2 drugs, increase the solubility can increase the bioavailability. (23)BiopharmaceuticalClassificationSystem. jpgFigure Biopharmaceutical Classification System (BCS)

1. 4 Surfactants

Surfactants are compounds that can reduce the surface tension in a solution. They have hydrophobic tails (hydrocarbon chains) and hydrophilic heads (polar), and this is called amphiphilic compounds. There are four main classes of surfactants, non-ionic, anionic, cationic and zwitterionic, and they all differ in their hydrophilic head groups. Non-ionic surfactants have no charged head groups and usually contain alcohols. Both anionic and cationic surfactants carry net charges, negative charge and positive charge respectively. Examples of anionic surfactants include sulfate and carboxylates whereas cationic surfactants include cetyl trimethylammonium bromide (CTAB). Zwitterionic surfactants have head groups with an anionic region and a cationic region attached to the same molecules. In other words, they can act as an ionic or a cationic surfactant depending on the pH of the solution. CHAPS is an example of this where the sulfonate is anionic and the

quaternary ammonium cation is cationic. The structures of these surfactants can be shown in the diagram below. Figure Chemical structure of a) Tween 20(24), b) SDS(25), c) Cetrimide(26, 27) and d) CHAPS(27)The pH of the aqueous media can affect the solubility of drugs. For example, a weak base drug has the highest solubility at pH 4. 3 due to the ionised form of the drug. Conversely, a weak acidic has the highest solubility at higher pH because of the ionised form of the drug.

1. 5 Critical Micelle Concentration

As the concentration of surfactants increases, the surface tension decreases until a point where the surface layer is saturated with surfactants. As a result, there will be an aggregation of surfactant molecules. This is called micellisation. Micelles are formed by aggregation of the amphililic molecules, so that the hydrophilic heads faces towards the surrounding solvent (environment) and hydrophobic tails facing the micelle centre, having only little or no contact with water. Adding more surfactants will have no effect on surface tension. At this point, a sharp change occurs on the graph of surface tension against surfactant concentration, and this is the critical micelle concentration (CMC)(17).. CMCFigure Graph shows the change in surface tension versus concentration of surfactant before and after micelle formation(28)There are two theories describing the formation of micelle. The first one is called phase separation model, where micelles separate surfactant molecules at the point of saturation concentration. Another theory is that the micelles can constantly be associated and dissociated, so that the CMC can be given as a range of concentration rather than an exact

value(17). In micellar solubilisation, there are several sites in a micelle for the drug to be solubilised, which are represented in the diagram below. Hydrophilic drugs are expected to be adsorbed onto the outer surface of the micelle (1), or they can be located in between hydrophilic head groups (2). Drugs which are less soluble are expected to situate between the hydrophilic head groups and the first half of the hydrophobic tails (3). Lastly, insoluble drugs which are hydrophobic may sit on the core of the micelle, that is the hydrophobic region of the micelle (4)(29). In this experiment, sulfacetamide will be in the first scenario as it has a log P of -1, which is considered to be hydrophilic. Therefore, it will be expected to adsorb onto the micelle surface. Figure Diagram presentation of possible locations of drugs which can be solubilised in micelles.

1. 6 Lipinski's Rule of Five

This rule was analysed by a chemist Christopher Lipinski on the pharmacological and biological activity of several drugs. He concluded that there are a few criteria that have to be met in order to have a drug that can be easily absorbed by the body and be permeable to a membrane. It is called Lipinski's rule of five because all the numbers involved are the multiples of five.(30) The criteria are: The molecular weight is less than 500 – sulfacetamide has a molecular weight of 214. 2, so it matches this criteria. Log P, which represents the drug's lipophilicity, is less than 5 – sulfacetamide has a Log P of -1. 0. Again, sulfacetamide is satisfied with this rule. Number of hydrogen bond donor is less than 5 – there are 2 amine groups in sulfacetamide. Number of hydrogen bond acceptor is less than 10 – there are

3 oxygen atoms and 2 nitrogen atoms. However, these rules are only used for prediction in drug compounds that are absorbed by passive diffusion. In addition, these rules do not give a certain answer on whether a drug is soluble in vivo. Therefore, this experiment will be carried out in vitro to predict the solubility of sulfacetamide in vivo.

Aim and objectives

2. 1 Aims

Sulfacetamide is a poorly soluble acidic drug; this project will investigate whether the addition of different types of surfactants would increase drug solubility. The extent of dissolution of sulfacetamide will be assessed in the presence of anionic, zwitterionic, neutral and cationic surfactants.

2. 2 Objectives

Be able to use various equipment in the lab such as pipette, UV absorbance spectrophotometer, centrifuge and weighing balance. Construct a calibration curve for sulfacetamide by dissolving into universal buffer pH7. 4. Be able to calculate the weight of surfactants required at a particular volume, so that the appropriate amount of stock solutions can be prepared. Investigation of influence of different types of surfactants on solubility within a range of concentration. Be able to plot and compare the results on graphs, so that the influence of solubility can be analysed.

2. 3 Hypothesis

Sulfacetamide is an acidic drug, therefore, at physiological pH 7. 4, the drug will be negatively charged as it loses a proton. Experiments will be

performed using saturated sulfacetamide solutions in the presence of the four different types of surfactants: anionic, cationic, zwitterionic and neutral surfactants, initially in a physiological buffering solution of pH 7. 4. The addition of cationic surfactant should show the greatest increase on solubility compared to other surfactants. This is thought to be because they are acidic drugs in an anionic form at physiological pH. Cationic surfactant head group would then interact electrostatically with the drug anions to form soluble micelle complexes more easily and the CMC value decreases.

3. Method

3. 1 UV Absorbance Spectrophotometer of Sulfacetamide

According to the Clarke's Analysis of Drugs and Poisons, sulfacetamide has an absorbance peak between 271nm and 256nm in acid and alkali condition, respectively.(2) The average is 263. 5 and after discussion with the supervisor, 263nm was used throughout the whole experiment to measure the absorbance of sulfacetamide. FigureFigure UV spectrum of Sulfacetamide

3. 2 Calibration Curves

A calibration curve is a graphical presentation between absorbance versus concentration of drug i. e. sulfacetamide. By measuring the absorbance of a known concentration of solution containing drug and surfactants at a later stage of this experiment, an unknown concentration of the drug can be determined to see at which the concentration of the drug was required to produce the corresponding effect. According to Beer Lambert Law, the absorbance is proportional to the concentration of sample i. e. drug and the

light path length. The equation is shown below: A is absorbance, a is absorptivity coefficient, c is concentration of sample, l is light path length.

3. 3 Calibration Curve for Sulfacetamide Dissolved in Buffer

Firstly, approximately 10mg of sulfacetamide was weighed using a mass balance. The drug was transferred into a scintillation vial. Then same relative amount of buffer (10mM citric acid, 10mM sodium dihydrogen orthophosphate and 10mM boric acid) was added. For example, the weight of drug in the first time was 13. 2mg, therefore, 13. 2ml of buffer was added. Since a scintillation vial only has approximately 20ml of capacity, a mass of approximately 10mg of drug is preferred so that the vial can have enough capacity for the buffer. By doing this, a 1mg/ml solution was produced. The wavelength in the spectrophotometer was set to 263nm, and a solution, which contained only buffer was zeroed to be used as a reference solution. The vial was then put in the sonicator for dissolving. Once the drug had dissolved, a small amount of solution was removed, placed in a spectrophotometer and a reading taken. A reading of 9999 means that the solution was too concentrated for the spectrophotometer to read, and the sample needed to be diluted. Ideally, a reading of 1 or below has to be achieved in order to be accurate. Since the solution was too concentrated, a 1 in 10 dilution was made and read again. If the reading was between 1 and 3, a 1 in 2 dilution had to be made. This dilution was continued until a reading below 3 was given. Once a reading of 1 or below was achieved, 1 in 2 serial dilutions were made and read in the spectrophotometer until a low reading (below 0. 1) was obtained. A minimum of 4 readings below a value of

0. 1 were required. This procedure was repeated three times to ensure accuracy. The average of these three sets of readings were plotted on a graph.

3. 4 Determination of Critical Micelle Concentration

The CMC value of SDS was determined using Jenway 4510 conductivity meter. First of all, SDS was dissolved in universal buffer pH 7. 4 in a 100ml volumetric flask to produce 10mM of stock solution. The stock solution was diluted further to produce a range of concentration: 0mM, 0. 5mM, 1. 5mM, 2. 5mM, 3. 5mM, 6mM, 7mM and 10mM. 10mM of the original stock solution was made because the theoretical CMC value of SDS is 6 – 10mM. The conductivity meter was calibrated with 0. 01M of potassium chloride and rinsed with distilled water before each measurement. The conductivity was then measured at the concentration produced above.

3. 5 Preparation of Stock Solution of Surfactants

In all these experiments, the same method was applied to different types of surfactants. First of all, a stock solution of surfactant was made.

3. 5. 1 CHAPS

CHAPS was the first surfactants used in this investigation and the following method will be used to demonstrate the general procedure for dissolving. CHAPS has a molecular weight of 614. 88 and 10mM 200ml of stock solution was made so that a graph of absorbance against surfactant concentration can be produced when all the results are collected. Used the equation: 10mM = 10mol/L = 0. 01 mol/L0. 01 mol in 1000L, but 200ml was required,

therefore, 0. 01 ÷ 1000 × 200 = 0. 002mol in 200ml. Substituted 0. 002mol into the equation above →= 1. 230g of CHAPS was needed to make 200ml of stock solution. 1. 230g was measured and was poured into the 200ml volumetric flask. Universal buffer pH 7. 4 was added into the volumetric flask. The volumetric flask was put into the sonicator for 5 minutes. When CHAPS had fully dissolved, add more buffer into the volumetric flask and made up to mark. A range of concentrations of surfactant were produced. The range was determined by the CMC of the surfactant. CHAPS has a CMC of 6 – 10mM. In collaboration with the supervisors, a concentration range from 0mM to 10mM were compared. As the concentration of the stock solution is 10mM, it is easier to produce the required concentration. For example, if 1mM of stock solution is to be prepared, 1ml of stock solution is added and 9ml of buffer is added so that there are 10ml of 1mM CHAPS in the scintillation vial. Similarly, if 10mM of solution is to be prepared, 10ml of stock solution is added to scintillation vial and no buffer needed. The table shows volume of stock solution and buffer needed for each concentration. Therefore, 11 scintillation vials were needed. Concentration of CHAPS/mMVolume of stock solution/mlVolume of buffer/ml00101192283374465556647738829911010 0Table Volume of stock solution and buffer needed to make up the required concentration of CHAPS

3. 5. 2 Cetrimide

The same method was applied to make the stock solution of cetrimide. In this occasion, 500ml of stock solution were made so that the excess of solution could be shared with other students. The same equation was used

again: Cetrimide has a molecular weight of 364. 45 and 10mM was made. Therefore, mass = 0. 01 ÷ 1000 × 500 × 364. 45 = 1. 822g of cetrimide was needed to make 500ml of stock solution. Step 3 to 10 from above is repeated. The CMC of cetrimide is 1mM. Although this is much lower than CHAPS, the same range of concentrations were compared. In addition, more precise concentration were measure around the CMC, so that the table below was produced. Concentration of Cetrimide/mMVolume of stock solution/mlVolume of buffer/ml00100. 20. 29. 80. 40. 49. 60. 60. 69. 40. 80. 89. 21191. 21. 28. 822833744655566477388299110100Table Volume of stock solution and buffer needed to make up the required concentration of cetrimide

3. 5. 3 SDS

A concentration of 10mM of SDS stock solution was prepared using the same method so that it was diluted to 0mM, 1mM, 2mM, 3mM, 4mM, 5mM, 6mM, 7mM, 8mM, 9mM and 10mM. The CMC is 7 – 10mM from the literature value, therefore, this concentration range was produced. Concentration of SDS/mMVolume of stock solution/mlVolume of buffer/ml0010119228337446555664773882991101 00Table Volume of stock solution and buffer needed to make up the required concentration of SDS

3. 5. 4 Tween 20

Same method was applied in the preparation of Tween 20, however, the CMC is 0. 6mM from the literature value. Therefore, a lower concentration, 0. 1mM of Tween 20 was produced. Concentration of Tween 20/mMVolume of stock solution/mlVolume of buffer/ml00100. 01190. 02280. 03370. 04460. 05550.

06640. 07730. 08820. 09910. 1100Table Volume of stock solution and buffer needed to make up the required concentration of Tween 20

3. 5. 5 Sodium Taurocholate

A concentration of 10mM of sodium taurocholate was prepared and was diluted to make 0mM, 1mM, 2mM, 3mM, 4mM, 5mM, 6mM, 7mM, 8mM, 9mM and 10mM. Concentration of Sodium Taurocholate/mMVolume of stock solution/mlVolume of buffer/ml00101192283374465556647738829911010 0Table Volume of stock solution and buffer needed to make up the required concentration of sodium taurocholate

3. 5. 6 SDS with tris(hydroxymethyl)aminomethane (tris) pH 7. 4

A stock solution of 20mM SDS was made with 30mM of tris buffer pH7. 4 which was then diluted to 0mM, 1mM, 2mM, 3mM, 4mM, 5mM, 6mM, 7mM, 8mM, 9mM, 10mM, 12mM, 14mM, 16mM, 18mM and 20mM. Approximately 1. 15g of SDS powder was needed and diluted with tris to make 200ml stock solution (, therefore, (0. 02 ÷ 1000) × 200 × 288. 38 = 1. 5352g.) The range of concentrations of SDS can be shown in the table below. Concentration of SDS/mMVolume of stock solution/mlVolume of tris/ml001010. 59. 521931. 58. 542852. 57. 563773. 56. 584694. 55. 5105512641473168218912010 0Table Volume of stock solution and buffer needed to make up the required concentration of SDS with tris pH 7. 4

3. 6 Dissolving Sulfacetamide in Surfactants

When all the surfactants were prepared, add an excess amount of sulfacetamide into each vial until it has saturated. The amount added to each vial should be approximately the same. Dissolve the drug in the sonicator for 5minutes, then use the Clifton Cyclone mixer for 1 min and finally, use the Stirling Mixer for 5 minutes. When a saturated solution is made, use the Gilson pipette to take out 3ml of the saturated solution from each vial and transfer it to the eppendorfs. 1. 5ml of saturated solution was not enough for the absorbance as the solution did not reach the height for UV to transmit through the cuvette. Therefore, two 1. 5ml of eppendorfs are needed. When each concentration of saturated solution was transferred to the eppendorfs, they were put into the centrifuge and were centrifuged for 5 minutes. Before the UV absorbance was read, the wavelength in the spectrophotometer was set to 263nm, and a solution which contained only buffer was zeroed to be used as a reference solution. After centrifugation, transfer 2ml of saturated solution of the same concentration to the cuvette so that the UV light could pass through the cuvette and the solution. This step was repeated for other concentrations. The absorbance was read on the UV spectrophotometer. If the reading is 9999, this means the solution is too concentrated for the spectrophotometer to read. Therefore, dilution will be needed until a reading below 0. 1 is obtained. (1 in 10, 1 in 100, 1 in 1000)Repeat step 5 for three times so that an average can be obtained. Plot the average readings for each concentration of saturated solution on a graph and produce an error bar to confirm accuracy.

3. 7 Statistical Analysis

The results were analysed through Microsoft Excel 2010. For every surfactants used, the absorbance were repeated three times so that three sets of results were produced. An average was calculated and standard deviation was also determined to see the degree of accuracy within those three sets of results. The solubility of sulfacetamide was determined using the equation from the calibration curve. The error bars were also produced so that any anonymous result or human error could be seen, and therefore, could be analysed. Student's t-test was used as a statistical test so that any difference between the solubility of sulfacetamide at pH 7. 4 before and after the CMC in the presence of surfactants can be seen. Student's t-test requires a null hypothesis, in this case, there is no difference in solubility of sulfacetamide before and after the CMC of surfactant present at 5% significant level. P value will be generate to allow both sets of data to be compared and give a confidence that those two sets of data are different. If P <0. 05, there is a 5% chance that there is no difference. Therefore, the null hypothesis is rejected. In other words, the hypothesis is not true as there is 95% chance that the solubility of sulfacetamide before and after the CMC are different. Conversely, if P> 0. 05, the null hypothesis can be accepted as there is confidence of 95% that there is no significant difference between both sets of data.(31)

5. Discussion

5. 1 Critical Micelle Concentration

The results of the determination of CMC for SDS are shown in section 4. Initially, the conductivity increases as the concentration of SDS increases. This can be explained by the existence of surfactant monomers. As the concentration of SDS increases, the charge groups increase. As a result, the conductivity increases. Above the CMC, the monomers assemble together and form micelles. Increasing the SDS concentration only causes an increase in micelle concentration. This reduces the space for SDS monomers in the solution, so the charged groups do not increase as much as that before the CMC. Therefore, a less increase in conductivity is observed after the CMC. (33)

5. 2 Solubility of Sulfacetamide in Surfactants

5. 2. 1 CHAPS

The results for the effect on the solubility of sulfacetamide are summarised in section 4. At pH 7. 4, addition of CHAPS does not increase the solubility of sulfacetamide. The CMC is 6mM from the literature value and there is no significant increase in solubility after the CMC. Sulfacetamide is presented as an anionic form, therefore, CHAPS was first used to see the solubility of sulfacetamide. The solubility of sulfacetamide is very unstable as it fluctuates throughout the concentration range. It may be due to the nature of the surfactant, CHAPS is a zwitterionic surfactant which carries a positively charged and negatively charged head groups. As a result, there may be electrostatic interaction between the surfactant head groups and the anionic

form of sulfacetamide. No previous study on the solubilisation of sulfacetamide with CHAPS can be found in the literature, therefore, no certain explanation can be drawn. However, the head groups of a zwitterionic surfactant have polar head groups, which contain both negative and positive charges. Surfactant of this type can be pH dependent where it may behave from cationic to anionic surfactant as pH increases, so that the zwitterionic characteristics may only be present at a particular range of pH. For CHAPS, it behaves as zwitterionic between pH 2-12.(34) Zwitterionic surfactants can also behave as non-ionic surfactants at isoelectric point, where both positive and negative head groups are completely ionised. Nevertheless, CHAPS contains a quaternary ammonium groups which will be permanently charged and this will behave as zwitterionic over a wide range of pH environment. What can be assumed here is that, CHAPS do not behave as cationic, anionic or non-ionic surfactant in this experiment.(35)The reading at 4mM is only once measurement because the solution was spilled from the vial. Therefore, the average reading could not be measured. Care should be taken next time in order to avoid lost of any sample.

5. 2. 2 Cetrimide

The results for the effect of cetrimide on the solubility of sulfacetamide are summarised in section 4. It was expected that cetrimide would have the greatest effect on solubility amongst other type of surfactants. It is because at pH 7. 4, sulfacetamide is 99% ionised and presented as anionic form. The cationic cetrimide can interact with anionic sulfacetamide so that it enhances the solubilisation into micelles and therefore increases the solubility.

However, the results do not support this statement. It may be due to the presence of sodium ions in sulfacetamide. A study was done before on the investigation of rate of reaction of sulfonamide drugs, it was found that the sodium ions in sulfacetamide may contribute to the interaction between the surfactant and the drug itself. It may suggest that the positively charged sodium ions can interact with the cationic cetrimide, which increases the electrostatic repulsion between the two species of the same charge.(1) As a result, the solubility remains unchanged as the concentration of cetrimide increases. In appendix 2, the first absorbance of sulfacetamide at the presence of cetrimide at 2mM is 0. 364. This is thought to be the most likely cause of error which affected the average absorbance overall. A lower concentration is obtained as a result of this. This may be due to dilution error. Since the dilution factor for sulfacetamide was 1000, serial dilution had to be made before the absorbance was read. The volume of buffer and the drug solution might not be measured accurately.

5. 2. 3 SDS

The effect of solubility of sulfacetamide with the addition of SDS is shown in section 4. It was expected that SDS would have the least effect in solubility due to the electrostatic repulsion between the anionic state of sulfacetamide and the anionic SDS. However, it did not happen as there is a slight increase in solubility with the addition of SDS compared to that in the addition of cetrimide. In previous study on weak acidic drugs, the solubility of drugs were found to have the least increase when an anionic surfactant was added because of the repulsive effect between the ionised form of the weakly acidic

drug and the anionic form of surfactant.(3)Two poorly soluble drugs, trimethoprim and sulfamethoxazole were investigated on the effect of solubility with the addition of SDS. The results showed that trimethoprim has a greater increase in solubility that that with sulfamethoxazole in the presence of SDS. It was because that trimethoprim is a cationic drug whereas sulfamethoxazole is an anionic drug. Therefore, trimethoprim reduces the electrostatic repulsion with the anionic SDS. As a consequence, trimethoprim showed a greater solubility compared to sulfamethoxazole. (36)In this experiment, the solubility only increases slightly which agrees with the study above. The solubility of sulfacetamide also increases continuously which means that the solubility of sulfacetamide does not depend on the formation of CMC (micelle formation).

5. 2. 4 Tween 20

The effect of solubility of sulfacetamide with the addition of non-ionic surfactant, Tween 20 is shown in section 4. There is barely any increase in solubility despite the presence of Tween 20. As the CMC for Tween 20 is 0. 6mM, a wider data range was done around the CMC to see a more detailed effect on solubility. The solubility of sulfacetamide only increases by 1. 5mM from 0mM of tween to 0. 6mM. However, the overall solubility remains constant. This can be explained by the hydrophilicity of sulfacetamide, which has a log P of -1. 0, which means that it is hydrophilic. Hydrophobic interaction of drugs with non-ionic surfactants is thought to be important in incorporating into non-ionic micelle.(36) A negative log P means that sulfacetamide is too hydrophilic to get into the non-ionic micelle Tween 20.

Furthermore, sulfacetamide is 99% ionised at pH 7. 4. As mentioned in section 1. 2. 5, ionised drugs are thought to be more water soluble. Tween 20 is non-ionic, therefore the ionic state of sulfacetamide may hinder the binding into the non-ionic Tween 20 micelle. A research was done by Saveyn et al. on solubilisation of flurbiprofen with Tween 20. The results showed that there is an increase in solubility when Tween 20 was added at both pH 4 and pH 5. 4. Flurbiprofen is also a weak acidic drug with pKa of 4. 22 similar to sulfacetamide. However, the log P of flurbiprofen is 4. 2, which is highly hydrophobic unlike sulfacetamide. The research was performed in lower pH environment, therefore, the degree of ionisation is less than that in pH 7. 4. (37) As a results, a higher solubility in flurbiprofen than sulfacetamide has been observed. The increase in solubility of sulfacetamide observed at 0. 6mM may not due to micelle formation because the solubility at declines again at 0. 07mM. The P value suggests that the difference in solubility of sulfacetamide before and after CMC is small, therefore, the rise in concentration at 0. 06mM may be due to experimental error. The cuvette used was scratched, affecting the light passing through it. This may be the possible reason of a high absorbance.

5. 2. 5 Sodium Taurocholate

Another anionic surfactant but with a small aggregation number of 4 was used to see the effect on solubility of sulfacetamide. The results are summarised in section 4. Sodium taurocholate is a micelle which can form bile salt.(38) As the concentration of sodium taurocholate increases, there are more micelles available to solubilise the drug. Therefore, a slight

increase in solubility of sulfacetamide can be seen. A study was done by Chakraborty et al. on the solubilisation of carvedilol with sodium taurocholate as one of the surfactants used in the assessment. It was shown that the solubility decreases at 3mM of sodium taurocholate but it increases again at 10mM. This is because the insoluble salt has formed at this low concentration. From 10mM onwards, more micelles are available so that the solubility of carvedilol increases.(4)In this experiment, the concentration of sodium taurocholate was done up to 10mM so a further effect on solubility cannot be seen. However, assuming the pattern shown in section 4 is the behaviour of solubility of sulfacetamide with sodium taurocholate, the line can be extrapolated. Therefore, when a higher concentration is reached, the solubility of sulfacetamide will increase further. A sudden rise of concentration of sulfacetamide at 4mM of sodium taurocholate may possibly due to micelle formation. This may suggest that the CMC of sodium taurocholate is 4mM, which helps to solubilise sulfacetamide.(4)

5. 2. 6 SDS in tris pH 7. 4

The results are shown in section 4. A wider range of SDS concentration was tested but the solubility of sulfacetamide does not increase to a greater extent. It follows the same pattern to that with SDS in universal buffer pH 7. 4. In the discussion earlier, anionic SDS are expected to have the least effect on solubility. Regardless the buffer, solubility of sulfacetamide in both universal buffer pH7. 4 and tris pH 7. 4 does not increase dramatically. However, P value is 0. 048, which confirms that there is significant difference in solubility of sulfacetamide before and after CMC. Therefore, the idea about

the interaction between the sodium in the universal buffer and sulfacetamide can be eliminated and does not affect the solubility of sulfacetamide.

Comparing Surfactants

The graphs and the statistical analysis results are discussed in section 4. The solubility of sulfacetamide only seems to have effect on anionic surfactants. The Student's t-tests have also confirmed that there are significant improvement on solubility of sulfacetamide only in the presence of SDS and sodium taurocholate. The pattern of the results obtained in this experiment may imply that the sodium ion might be present in sulfacetamide, so it favours the attractive interaction between positively charged sodium and negatively charged anionic surfactants. Therefore, this interaction helps micelles and surfactants to solubilise the drug. Khalil and Al-khiro found that sulfacetamide sodium has the least effect on solubility compared to other sulfonamide drugs after the addition of SDS.(1)CHAPS, cetrimide and Tween 20 do not have significant effect on solubility of sulfacetamide. CHAPS is a zwitterionic surfactant and it can only be assumed that the zwitterionic charge in CHAPS did not solubilise sulfacetamide. Cetrimide shows no increase in solubility because it is cationic surfactant and is thought to have repulsive interaction with the sodium ion in sulfacetamide. Tween 20 is a non-ionic surfactant and is hydrophobic, sulfacetamide is too hydrophilic to get into the Tween 20 micelles.

Limitation

There are some limitations in this experiment. Some anomalies are identified in some of the observations above. Some points on the graph are slightly out

of range. This is due to experimental errors and some human errors. Experimental errors could be due to the scratched cuvettes mentioned previously, therefore, affecting the absorbance readings. Human errors could be due to the number of dilutions made in this experiment. The drug being investigated in this experiment requires a dilution factor of 1000. The accuracy may be affected by the number of dilution made. This drug had to be diluted three times from the original solution to 1 in 1000 solution, therefore, a small error in each dilution could lead to a large inaccurate absorbance readings. Spillage of solution also contributed to one of the anomaly in this experiment, so the second and third readings could not be repeated. In future studies, care must be taken when handling solutions. More concentration range could have been done in sodium taurocholate as the CMC from the literature value is 3 – 11mM and it was only done up to 10mM. The solubility of sulfacetamide seems to carry on increasing. Therefore, a further concentration range can be done to see the effect of solubility of sulfacetamide after the micelle formation.

Conclusion

The solubility of sulfacetamide in the presence of different types of surfactants was investigated in this project. Only anionic surfactants were found to have any improvement on solubility of sulfacetamide. The solubility starts to increase roughly around the CMC of surfactants. Therefore, it can be concluded that only anionic surfactants can help to solubilise sulfacetamide. Although this project has demonstrated that anionic surfactants improve the solubility of sulfacetamide, it should be noted that this experiment was done

solely at pH 7. 4 due to the time available. If this experiment were to repeat, a wider range of pH should be performed as it can mimic different conditions in the body. For example, lower pH mimics the acidic environment in gastrointestinal tract; similarly, higher pH mimics the basic environment in the intestine. By doing this, different degree of ionisation of sulfacetamide will occur and the most effective surfactants on improving the solubility of this drug will be different. More surfactants could be used to help to explore new trends and the effectiveness of surfactants for this particular drug, especially zwitterionic surfactants, which have insufficient explanation and evidence on improving solubility of sulfacetamide. The CMC of the zwitterionic surfactants could be established by other techniques, to clarify the point at which solubilisation would be expected to occur. As zwitterionic surfactants do not conduct electricity, conductivity cannot be used to determined the CMC. Instead, pyrene fluorescence probing has been widely used to determine CMC for zwitterionic surfactants. This is a method where pyrene is solubilised surfactants medium, and intensity of vibrational brands of pyrene are observed so that the CMC value can be observed.(39)With much more time, a much wider range of anionic surfactants with differing properties such as aggregation number, hydrophilicity or hydrophobicity and size should be investigated, so that the solubility of sulfacetamide can be understood more in depth. Furthermore, solubilisation of drugs could also be examined with other techniques such as F19 NMR, H1 NMR(37), lipid-based systems(40), drug dispersion(41) and so on, to see if the effect on solubility of drugs varies between methods. The results and the statistical analysis have proved that it is possible to improve the solubility of drugs using

different types of surfactants, depending the characteristics of the drugs. Although there are some anomalies and human errors during the experiment, these results can still give confidence for prediction of solubilisation drugs by surfactants.