

**School of Pharmacy
Department of Health Sciences**

**Solubility Studies of Prilocaine and Lignocaine with Hydroxypropyl Beta
Cyclodextrin**

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**This thesis is presented for the Degree of
Master of Pharmacy
of
Curtin University of Technology**

June 2007

ACKNOWLEDGMENTS

I wish to express sincere appreciation to Professors Bruce Sunderland and John Parkin for their assistance in the preparation of this manuscript. In addition, special thanks to IT officer John Hess for his timely help.

I also wish to say my special gratitude to Laboratory Manager Mr. Michael Boddy whose familiarity with the needs and ideas of the laboratory researching and his depth of knowledge in instruments and basic chemistry and his way of calculation all were very helpful during the early researching phase of this undertaking and whose good natured fun and lively personality created an environment for good work in the laboratory.

Also I would like to take this opportunity to show my sincere thanks to my father Shri Babulal C Munot and mother Mrs. Ratnamala B Munot with special affection towards my younger sister Pratibha Aphale and my brother in law Rahul Aphale for boosting me to look up and for all their financial help to allow me to complete the given task sincerely. I also thank my brother Vivek B Munot, and my sister in law Vaishali V Munot for there kind words and support throughout this pursuit.

I would not be able to pursue my efforts so far without the constant support of my very close friends Mr. Mark Rank, Mr. Rahul Patankar, Mr. Mandar Joshi, Mr. Kedar Mandalkar and Mr. Dhananjay Mandalkar. This task again wouldn't have been possible and easier with the support of my very good friends Mr. Milind Acharya, Mr. Pranab Sarmah, Mr. Kausal Bagade, Mr. Chandrashekar Didi and Mr. Guruprasad.

In the later phase of my thesis I highly counted and relied on the support of my friend Ram who did more than enough to help me to lift and built optimism through my personal errors. My special thanks are also extended towards Ms Neha Gandhi and Mr. Sebastian for their guidance in formatting my thesis.

My very special thanks goes to Prof Michael Garlepp for being so wonderfully helpful and Professor Bruce Sunderland again for his valuable insights and proper guidance throughout my masters and for those extra hours that I was so in need of to put myself up the mark.

Thanks also to the members of the faculty and administrative staff that includes Jeffery Hughes, Leanne Stafford, Cornelia Locher, Shelley Kinsella, Eric Helmerhorst, Ricardo Mancera, Leanne Haggart, Michael Stack, Charmaine D'costa, Daphane Dsouza, Jennfer Ramsay, Jorge Martinez and Robert Cvetkovski and Angela Samec for their support and valuable input.

My Dedication

My Life Is Full Of Odds and I Live By It

Yet there is more to learn for me from
Supervisor, Professor Bruce Sunderland
Head of Department, Professor Michael Garlepp
Laboratory Manager Michael Boddy
And I.T. Officer John Hess

This manuscript is personally contributed to
my Parents and my sister Pratibha, my supervisor
Professor Bruce Sunderland
Laboratory Manager Michael Boddy
And to my friend Ram

ABSTRACT

Formulation of local anaesthetics in different dosage forms, including those for oral, parenteral, and topical application have being widely investigated. All of these formulations include local anaesthetics in their salt forms. The lipophilic nature of the bases of local anaesthetics may influence the rate of the pharmacological effect. There has been very little research done towards this aspect of local anaesthetics.

Prilocaine base and lignocaine base possess greater lipophilicity than their salts. The salt forms undergo dissociation in the body. To maximise the absorption rate lipophilicity plays an important role. The aim of the present study is to evaluate the potential of using prilocaine and lignocaine individually and in combination as bases for parenteral formulations using cyclodextrins as complexing agents. Cyclodextrins are widely used as complexing agents to increase the solubility of poorly soluble drugs. Hydroxypropyl- β -cyclodextrin (HP β CD) was the first choice amongst the different cyclodextrins to be evaluated as a solubility enhancer as it does not show nephrotoxicity and is more bio-available compared to other cyclodextrins.

Method: Prilocaine base was prepared from its salt and lignocaine base was obtained from Sigma Pharmaceuticals. Solubilities were examined individually and in combination by the phase solubility method and complex formation investigated. The mobile phase used was methanol:water (55:45) with phosphate buffer at pH 5.5. An A_L type solubility isotherm was obtained for the influence of HP β CD on the solubilities of prilocaine and lignocaine. Complexation was investigated for both prilocaine and lignocaine to HP β CD by NMR.

Results: The measured solubilities of prilocaine and lignocaine individually at 30% HP β CD from 25°C to 42°C were 1.96-7.91 moles/L and 1.69-4.55 moles/L respectively. The solubilities in combination were 0.91-3.68 moles/L for prilocaine and 1.03-8.35 moles/L for lignocaine respectively. The NMR data suggested that complexation involves the aromatic ring for both prilocaine and lignocaine apart from methene and methyl groups for prilocaine and ethyl amide and aromatic methyl groups for lignocaine.

TABLE OF CONTENTS

1	INTRODUCTION.....	1
1.1	CLASSIFICATION OF PAIN.....	1
1.2	MEDICATIONS USED IN PAIN	2
1.3	MECHANISM OF ACTION OF PRILOCAINE HYDROCHLORIDE AND LIGNOCAINE HYDROCHLORIDE.....	2
1.4	FORMULATIONS OF LIGNOCAINE AND PRILOCAINE HYDROCHLORIDES	4
1.5	DRUG SOLUBILITY	7
1.5.1	<i>SOLUBILITY AND PARENTERAL FORMULATIONS.....</i>	<i>11</i>
1.6	SOLUBILITY IMPROVEMENT BY THE COSOLVENCY METHOD.....	11
1.6.1	<i>COSOLVENTS AND ADDED MATERIALS.....</i>	<i>13</i>
1.6.2	<i>THEORIES FOR COSOLVENCY.....</i>	<i>14</i>
1.7	SOLUBILISATION OF DRUGS USING MICROEMULSIONS	15
1.7.1	<i>SURFACTANTS FOR MICROEMULSIONS.....</i>	<i>16</i>
1.7.2	<i>TYPES OF MICROEMULSIONS.....</i>	<i>17</i>
1.7.3	<i>PHASE BEHAVIOUR OF MICROEMULSIONS IN IMPROVING SOLUBILITY.....</i>	<i>17</i>
1.7.4	PROBLEMS ENCOUNTERED WITH MICROEMULSIONS.....	18
1.8	SELF-EMULSIFYING DRUG DELIVERY SYSTEM (SEDDS).....	21
1.9	SOLUBILISATION OF DRUGS BY USE OF COMPLEXING AGENTS	22
1.9.1	<i>CYCLODEXTRINS AND SOLUBILISATION OF DRUGS.....</i>	<i>23</i>
1.9.2	<i>ASSOCIATION CONSTANT AND CYCLODEXTRINS.....</i>	<i>25</i>
1.9.3	<i>PHYSICOCHEMICAL ASPECTS OF DRUG AND COMPLEXES.....</i>	<i>27</i>
1.9.4	<i>FACTORS INFLUENCING INCLUSION COMPLEX FORMATION.....</i>	<i>27</i>
1.9.5	<i>APPLICATIONS OF CYCLODEXTRINS IN THE PHARMACEUTICAL INDUSTRY.....</i>	<i>29</i>
1.10	NEED FOR PARENTERAL FORMULATION OF PRILOCAINE AND LIGNOCAINE	32

1.10.1	<i>OBJECTIVES OF THIS STUDY</i>	33
2	MATERIALS AND METHODS	34
2.1	MATERIALS	34
2.2	METHODS	34
2.2.1	<i>ULTRAVIOLET (UV) SPECTROPHOTOMETRY FOR PRILOCAINE HYDROCHLORIDE, LIGNOCAINE HYDROCHLORIDE, AND TETRACAINE HYDROCHLORIDE</i>	35
2.2.2	<i>METHOD DEVELOPMENT FOR PRILOCAINE HYDROCHLORIDE, LIGNOCAINE HYDROCHLORIDE, AND TETRACAINE HYDROCHLORIDE</i>	35
2.2.3	<i>PREPARATION OF PRILOCAINE AND LIGNOCAINE BASES</i>	36
2.2.4	<i>PREPARATION OF HPβCD SOLUTION</i>	37
2.2.5	<i>MAINTAINING THE pH OF PRILOCAINE BASE AND LIGNOCAINE BASE SOLUTION</i>	38
2.2.6	<i>PREPARATION OF 0.5M SODIUM CARBONATE SOLUTION</i>	38
2.2.7	<i>PREPARATION OF 5M SODIUM HYDROXIDE SOLUTION</i>	38
2.3	SOLUBILITY STUDIES OF PRILOCAINE AND LIGNOCAINE ALONE AND IN COMBINATION AT 25° C.....	39
2.3.1	<i>SOLUBILITY OF PRILOCAINE WITH HPβCD</i>	39
2.3.2	<i>SOLUBILITY OF LIGNOCAINE WITH HPβCD</i>	39
2.3.3	<i>SOLUBILITY OF PRILOCAINE AND LIGNOCAINE WITH HPβCD</i>	40
2.3.4	<i>SOLUBILITY STUDIES OF PRILOCAINE AND LIGNOCAINE ALONE AND IN COMBINATION AT ELEVATED TEMPERATURES</i>	40
2.4	NUCLEAR MAGNETIC RESONANCE (NMR).....	41
3	RESULTS	42
3.1	DETERMINATION OF UV SPECTRA FOR PRILOCAINE HYDROCHLORIDE, LIGNOCAINE HYDROCHLORIDE, AND TETRACAINE HYDROCHLORIDE	42
3.1.1	<i>STANDARD CURVES OF PRILOCAINE HYDROCHLORIDE, LIGNOCAINE HYDROCHLORIDE, AND TETRACAINE HYDROCHLORIDE</i>	43

3.1.2	<i>CALIBRATION ASSAYS FOR A COMBINATION OF PRILOCAINE HYDROCHLORIDE AND LIGNOCAINE HYDROCHLORIDE</i>	47
3.2	SOLUBILITY STUDIES.....	49
3.2.1	<i>SOLUBILITY STUDIES OF PRILOCAINE WITH HPβCD AT 25°C, 35°C, AND 42°C</i>	49
3.2.2	<i>SOLUBILITY DATA OF LIGNOCAINE WITH HPβCD AT 25°C, 35°C, AND 42°C</i>	49
3.2.3	<i>SOLUBILITY DETERMINATION OF A COMBINATION OF PRILOCAINE AND LIGNOCAINE WITH HPβCD AT 25°C, 35°C, AND 42°C</i>	50
3.2.4	<i>SOLUBILITIES AND ASSOCIATION CONSTANTS OF PRILOCAINE AND LIGNOCAINE WITH HPβCD INDIVIDUALLY AND IN COMBINATION AT 25°C, 35°C AND 42°C</i>	50
3.2.5	<i>EFFECT OF ENTHALPY, ENTROPY AND FREE ENERGY ON SOLUBILITIES STUDIES OF PRILOCAINE AND LIGNOCAINE ALONE AND IN COMBINATION WITH HPβCD.</i>	63
3.3	INVESTIGATION OF HP β CD COMPLEX FORMATION WITH PRILOCAINE AND LIGNOCAINE BY NMR.....	69
3.4	GRAPHICAL COMPARISON OF CHEMICAL SHIFTS OF PROTONS OF PRILOCAINE AND LIGNOCAINE IN THE PRESENCE AND ABSENCE OF HP β CD.....	77
4	DISCUSSION	79
5	CONCLUSIONS	88

TABLE OF FIGURES

Figure 1: Pain Classification	1
Figure 2: Chemical Structure of prilocaine hydrochloride.	3
Figure 3: Chemical structure of lignocaine hydrochloride.	3
Figure 4: Mechanism of action for lignocaine hydrochloride and prilocaine hydrochloride (RNH ⁺).	4
Figure 5: Schematic representation of the most commonly encountered self-association structures in water, oil or a combination thereof.....	17
Figure 6: A hypothetical pseudo-ternary phase diagram of an oil/surfactant/water system with emphasis on microemulsion and emulsion phases. Within the phase diagram, existences fields are shown where conventional micelles, reverse micelles or water-in-oil (w/o) microemulsions and oil in water o/w microemulsions are formed.	18
Figure 7: Schematic representation of the A-type phase diagrams.....	25
Figure 8: UV spectra of prilocaine hydrochloride, lignocaine hydrochloride, and tetracaine hydrochloride.....	42
Figure 9: Calibration graph of prilocaine hydrochloride at 230 nm by HPLC	44
Figure 10: Calibration graph of lignocaine hydrochloride at 230 nm by HPLC	45
Figure 11: Calibration graph of tetracaine hydrochloride at 230 nm by HPLC.....	46
Figure 12: Calibration graphs of prilocaine hydrochloride and lignocaine hydrochloride in combination at 230 nm by HPLC.....	48
Figure 13: Solubility data for prilocaine at 25°C	52
Figure 14: Solubility data for prilocaine at 35°C	53
Figure 15: Solubility data for prilocaine at 42°C	54
Figure 16: Solubility data for lignocaine at 25°C	55
Figure 17: Solubility data for lignocaine at 35°C	56
Figure 18: Solubility data for lignocaine at 42°C	57
Figure 19: Solubility data for prilocaine at 25°C	59
Figure 20: Solubility data for lignocaine at 25°C	59
Figure 21: Solubility data for prilocaine at 35°C	60
Figure 22: Solubility data for lignocaine at 35°C	60
Figure 23: Solubility data for prilocaine at 42°C	61

Figure 24: Solubility data for lignocaine at 42°C	62
Figure 25: Linear fit graph showing R values for Prilocaine.....	64
Figure 26: Linear fit graph showing R values for lignocaine.	65
Figure 27: Linear fit graph showing R values for Prilocaine in presence of Lignocaine.....	66
Figure 28: Linear fit graph showing R values of Lignocaine in presence of Prilocaine.	67
Figure 29: Chemical structure of prilocaine.....	69
Figure 30: Chemical structure of lignocaine.....	70
Figure 31: 1H NMR spectra of (a) HPβCD, (b) prilocaine, (c) lignocaine, (d) prilocaine-HPβCD complex and (e) lignocaine-HPβCD complex.	76
Figure 32: Chemical shift of prilocaine in the presence and absence of HPβCD	77
Figure 33: Chemical shift of lignocaine protons in the presence and absence of HPβCD	78

1 INTRODUCTION

1.1 CLASSIFICATION OF PAIN

Happiness is what a man seeks throughout his life. Tranquility and rationality are the cornerstones of happiness¹. Happy feelings and harmony is what every heart desires, but sometimes unknowingly body pain is associated with it and to produce tranquility and rationality we need medications like narcotics, non-steroidal anti-inflammatory drugs (NSAID'S) and anaesthetics.

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. Pain can be broadly classified as acute pain and chronic pain. Acute pain is the normal predicted physiological response to an adverse chemical, thermal, or mechanical stimulus associated with surgery, trauma or acute illness². Chronic pain is a state in which pain is persistent and the cause of the pain cannot always be removed or is difficult to treat. Chronic pain may be associated with a long term incurable or intractable medical conditions or disease. Different types of pain can be summarised as shown in Figure 1 below.

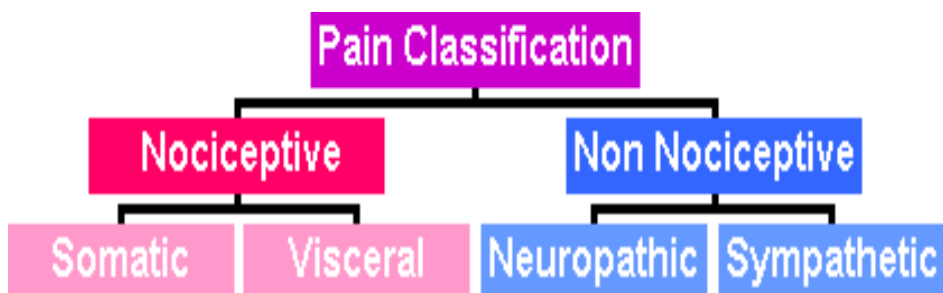


Figure 1: Pain Classification

Nociceptive pain arises from the stimulation of specific pain receptors that respond to heat, cold, vibration, stretch and chemical stimuli released from damaged cells. Non-nociceptive pain arises from within the peripheral and central nervous system where the pain is being generated by nerve cell dysfunction³.

1.2 MEDICATIONS USED IN PAIN

A variety of medications is available to control pain. They fall into the categories of analgesics, narcotics, and NSAID'S. These medications are generally suitable for giving relief of pain not associated with a surgical procedure. Pain associated with surgery is generally reduced by the intervention of general anaesthesia or by the use of local anaesthetics. Commonly used general anaesthetics are halothane, isoflurane, desflurane and a new compound sevoflurane with low solubility in the blood which expedites "wash-out" and rapid recovery from anaesthesia³.

General anaesthetics are systemic in action and produce loss of consciousness, whereas local anaesthetics are defined as agents that cause loss of sensation with or without the loss of consciousness. Local anaesthetics produce anaesthesia at the site of application such as lignocaine, prilocaine, tetracaine/amethocaine, ropivacaine, bupivacaine, and mepivacaine. Local anaesthetics are used for the control of preoperative and postoperative pain. They fall into two categories, the aminoamides (amide ethers) and aminoesters (amine esters). Aminoamides include lignocaine, bupivacaine, mepivacaine, and prilocaine and aminoesters include procaine, chlorprocaine, and tetracaine. Prilocaine hydrochloride and lignocaine hydrochloride are the oldest and most widely used local anaesthetics. They are used as antiarrhythmic agents, for chronic pain relief in cases of neuralgia, and in dental surgeries.

1.3 MECHANISM OF ACTION OF PRILOCAINE HYDROCHLORIDE AND LIGNOCAINE HYDROCHLORIDE

A drug is often formulated into a dosage form in which it is more stable and the drug then undergoes transformation in the biological system to show a biological action in its desired form. A lipophilic drug is often more stable as its salt which is hydrophilic and it then dissociates in the biological system as lipophilic and hydrophilic components and the lipophilic component is absorbed by the cell membrane to produce a desired action. Local anaesthetics such as prilocaine hydrochloride

($C_{13}H_{20}N_2O.HCl$) and lignocaine hydrochloride ($C_{14}H_{22}N_2O.HCl$) show similar mechanisms of action in the body.

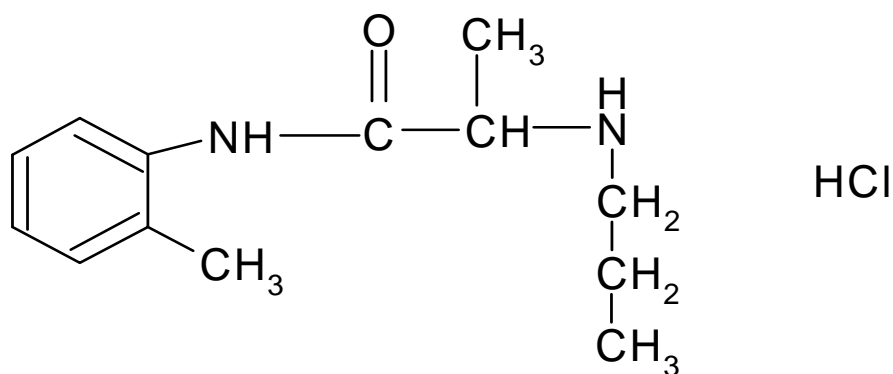


Figure 2: Chemical Structure of prilocaine hydrochloride.

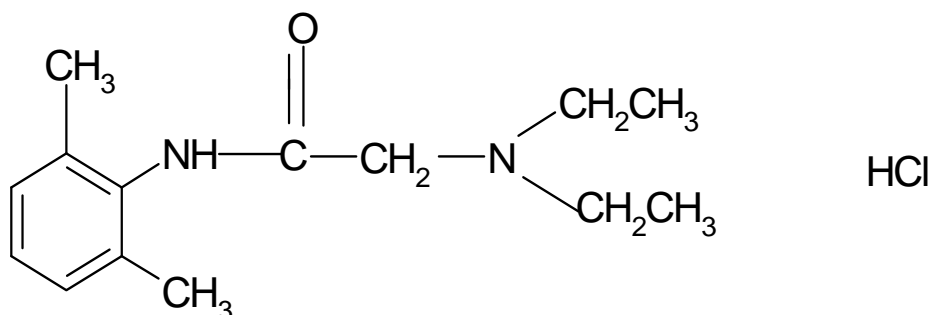


Figure 3: Chemical structure of lignocaine hydrochloride.

They act by blocking the initiation and propagation of action potentials by blocking the Na^+ channels. The activity of lignocaine hydrochloride and prilocaine hydrochloride is predominantly pH dependent and is increased in alkaline pH when the proportion of ionised molecule is low. This is because the drugs have to penetrate the nerve sheath and the axon membrane to reach the inner end of the Na^+ channel. Unionised molecules of the drug show greater penetration through cell membranes because of the greater lipophilicity. The ionised form is not membrane permeable, and therefore the penetration is very poor in acidic pH. This pH dependence can be clinically important, since inflamed tissues are often acidic, and are thus resistant to local anaesthetics⁴.

The mechanism of action for lignocaine hydrochloride and prilocaine hydrochloride (RNH^+) is well explained in Figure 4.

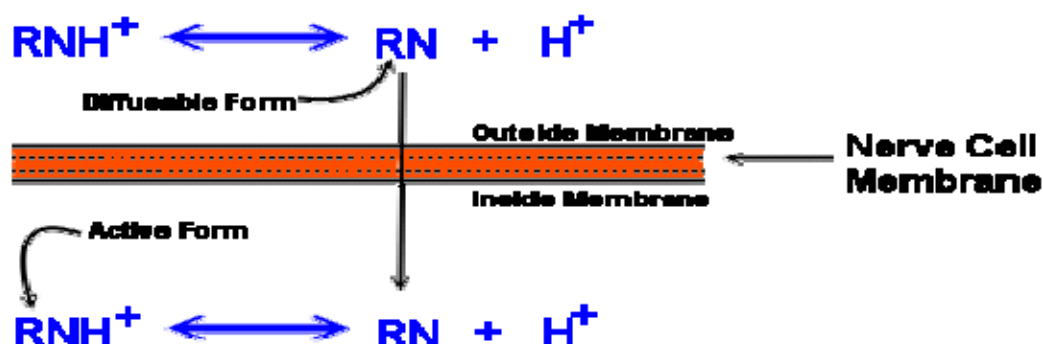


Figure 4: Mechanism of action for lignocaine hydrochloride and prilocaine hydrochloride (RNH^+).

RNH^+ in the tissue is in equilibrium with RN and H^+ . The unionised form (RN) then passes through the cell membrane as it possesses sufficient lipophilicity and again is associated with H^+ to form RNH^+ which is an active form that binds to Na^+ channel to produce its action⁴. Thus if lipid solubility has to be increased the drugs should remain in their unionised form. Thus a rapid onset of action after parenteral administration is postulated as the dissociation step is eliminated and alkaline pH is maintained, which facilitates penetration through cell membranes.

1.4 FORMULATIONS OF LIGNOCAINE AND PRILOCAINE HYDROCHLORIDES

Lignocaine has been formulated as a microemulsion. In a recent research study a pseudoternary phase diagram of the prepared lignocaine microemulsion with different surfactants and cosurfactants was reported, the diameters of droplets were determined and in addition viscosity, electric conductivity and refractivity. In addition its appearance and system type was studied by electron microscopy. A stable lignocaine microemulsion of the o/w type was formulated⁵. In another invitro study, potential of the application of short term iontophoresis for the topical delivery of lignocaine hydrochloride microemulsion was found to significantly increase the influx of the

lignocaine in a microemulsion compared to an aqueous drug solution under the same iontophoresis protocol⁶.

A trial to determine the effectiveness of a lignocaine transdermal patch for chronic pain was performed and proven effective for chronic pain⁷. Another study performed on only two patients, with postherpetic neuralgia suggested that application of lignocaine patches to the painful area helped to reduce the allodynic component of the pain syndrome⁸ i.e. occurrence of pain other than the area stimulated. There are also several anecdotal reports of the use of lignocaine patches in osteoarthritic knee pain and myofascial trigger points which are not well documented⁸. A 4% liposomal preparation of lignocaine called ELA max[®] is another topical preparation reported to be as effective as EMLA[®] cream - a eutectic mixture of prilocaine and lignocaine, for venipuncture in children, but with faster onset^{9,10}.

Prilocaine an amide local anaesthetic, is a secondary amino derivative of toluidine. It is somewhat less potent than lignocaine and considerably less toxic in peripheral tissues. Clinically it produces less vasodilation and is similar to other amide local anaesthetics in relative freedom from allergic reactions. Prilocaine's primary limiting factor clinically is the production of methemoglobinemia, a side effect caused by its metabolite o-toluidine¹¹. Prilocaine hydrochloride has been formulated mainly as a solution for intravenous use in regional anaesthesia¹². Encapsulation of prilocaine in liposomes facilitated the controlled release of prilocaine increasing time duration of the sensory nervous blockade and constituted a good choice to replace local anaesthetic formulations¹³. Prilocaine has also been tried as a topical formulation in comparison with lignocaine in terms of efficacy and safety for fiberoptic bronchoscopy¹⁴. Pharmacokinetic studies of dermal penetration of prilocaine using a microemulsion formulation found eight fold increase in dermal penetration rates by compared to a conventional o/w type emulsion. Transdermal drug permeation from the microemulsion, is related to the molecular mobility of the drugs in the vehicle, which depends on the internal structure of the microemulsion. The study also demonstrated a significant decrease in the lag time and indicated a mean increase in dermal penetration rate of almost 2-fold that of a commercially available hydrogel¹⁵.

Both lignocaine and prilocaine have been formulated in combination and with other local anaesthetics for improved efficacy. Some of the formulations can be summarised as below. EMLA[®] cream is a successful formulation used topically in many surgical procedures¹⁶. It is found to be effective as a cream in both paediatric surgeries and in adults^{8, 17}.

A lipid depot formulation of 1:1 mixture of lignocaine and prilocaine has been studied in different concentrations and its use found favourable efficacy for sciatic nerve block as the duration of sensory sciatic block was prolonged by the lipid depot formulation compared to the aqueous solution¹⁸. Lignocaine with epinephrine and prilocaine with felypressin are the most widely used solutions by dental practitioners in comparison to other local anaesthetics in the UK¹⁹.

Other new formulations of lignocaine based local anaesthetics include a novel lignocaine/tetracaine-based peel (a cream base that forms a flexible film on drying and comes off easily) that has recently been developed. It is applied to the skin as a cream and, once air dried, is removed as a flexible film. It may prove useful in providing adequate dermal anaesthesia for dermatologic laser surgery²⁰.

Lignocaine as such is widely formulated as parenteral and topical preparations as well as an oral formulation in the form of Mexiletine[®]. The widely formulated preparations of lignocaine include solutions, infusions²¹, microemulsions²², gels²³, creams, sprays and patches⁷.

Mexiletine[®], has been used to reduce chronic pain episodes caused by erythromelalgia²¹. Lignocaine infusion has also been found to give relief of pain caused by erythromelalgia²¹. Lignocaine as a 1% and 2% solution with epinephrine is available as a local anaesthetic and is given to produce local anaesthesia during surgery, while a 5% hypertonic solution has been tried for spinal anaesthesia without much success²⁴. Lignocaine as a 4% gel has been used for laser assisted anaesthesia prior to intravenous cannulation. Intravenous cannulation causes pain therefore, the procedure is assisted with topical anaesthetic agents, but the absorption of topical

anaesthetics is limited by the stratum corneum, the outermost layer of the epidermis. Laser irradiation helps remove the stratum corneum, leading to enhancement in uptake of topical agents like lignocaine gel²³.

1.5 DRUG SOLUBILITY

Though there are different dosage forms such as solid, liquid, and semisolid in which a drug can be formulated, solutions are a desired dosage form for pharmaceuticals and in biological systems because drugs in solution give more rapid action compared to solid or semisolid dosage forms. Advantages of an oral formulation are convenience and ease of handling. Potential for bioavailability to large patient populations is achieved in solutions but faces the limitation of cost²⁵. Lack of drug solubility is the main hindrance if the drug, in its original state, has to be formulated in solution or a parenteral form. Different methods have been tried to improve the solubility of drugs.

Solubility and solubility studies thus play an important role in pharmaceuticals because they define the rate of dissolution of the drug. The higher the solubility of the drug the more rapid is its rate of solution when other factors are equal, in the absence of chemical reaction²⁶. Aqueous solubility is the most desirable characteristic for both oral and parenteral formulations.

Many factors govern the rate of drug dissolution in solution and hence the solubility. The factors that are important for the discussion are the nature of the drug substance, its hydrophobicity, its shape, its surface area, its state of ionisation, the influence of pH, and drug pKa²⁶.

The influence of the nature of the drug substance can be explained with the example of novobiocin an aminocoumarin antibiotic used against *Staphylococcus* species. The acid salt of novobiocin is absorbed, but its ionised state as the monosodium salt shows an increase in solubility by about 300 times. The solution of ionised novobiocin was found to be unstable. Knowledge about its shape and structure shed some light on how to obtain a stable and more soluble form, thus the amorphous form of novobiocin was found to give higher solubility than its crystalline form or its sodium salt²⁶.

The ionisation state of a drug also plays an important role and is governed by two major factors. A) The entropy of mixing which favours complete miscibility of all components. B) The difference between the sum of the drug-drug (DD) and water-water (WW) interactions on the one hand and the drug-water (DW) interactions on the other. This difference is related to the activity coefficient of the drug in water γ_w by

$$RT \ln \gamma_w = DD + WW - 2DW \quad \text{Eq (1)}$$

If,

$$DD + WW - 2DW > 0 \quad \text{Eq (2)}$$

as is the case with non electrolytes in water, there will be less than complete mixing and the drug will have a finite solubility in water. The greater the difference between (the adhesive) and (cohesive) interactions, the lower the solubility will be.

Mathematically, the observed solubility of a solute X_w is given by the ideal solubility and the activity coefficient as

$$\log X_w = \log X_i - \log \gamma_w \quad \text{Eq (3)}$$

Where X_w = observed solubility of a solute in water.

X_i = ideal solubility of a solute in water.

Both crystalline structure effects as reflected by X_i and solution interactions as reflected by γ_w can contribute to the insolubility of a solute and both these factors can be modified as a means of solubilising the drug²⁷.

Many drugs are either weak organic acids or weak organic bases or their salts and the degree to which these drugs would ionise in solution is dependent upon the pH. pH thus is one of the primary influential properties that has an effect on the solubility of most drugs that contain ionisable groups. The degree of ionisation is one of the most important parameters considered for improving the solubility of acidic and basic drugs. This can be illustrated by the example of tetracycline hydrochloride solution. The hydrochloride in tetracycline lowers the pH of tetracycline hydrochloride increasing its solubility. Similarly, erythromycin is labile at pH 4 and hence unstable in the stomach contents. Erythromycin is an active form which shows antimicrobial action. Thus conversion of erythromycin to erythromycin stearate makes it less soluble in the stomach and is not as susceptible to degradation and dissociates in the intestine yielding the free base. Thus, making erythromycin less soluble in the stomach shows enhanced activity²⁶.

To improve the solubility of certain drugs pH control and inclusion complex formation methods can be used as solubilisation techniques. An example of this is naringenin, a weakly acidic compound with low water solubility. A study evaluated that the combined use of ionization and complexation increased the solubility of the unionized and ionized naringenin. This study provides evidence of the role of pH, pK_a and complexation in increasing the total aqueous solubility. The study was carried out at pH 4 and 8 with inclusion complex formation with parent beta cyclodextrin (β CD), and its derivatives of 2-hydroxypropyl- β CD (HP β CD) and methyl β CD. An A_L profile obtained suggested that naringenin, both in the uncharged and charged state formed soluble complexes in a 1:1 ratio, to different extents. The study also proposed that the affinity of the unionized naringenin for the hydrophobic cavity of cyclodextrin is higher than that of the ionized form, due to the more hydrophilic character of this form²⁸.

In another study performed on thiazolobenzimidazole similar results were obtained. Thiazolobenzimidazole is an experimental drug for the treatment of AIDS and exhibits low water solubility (11 μ g/mL) and is almost impossible to administer in an injectable solution at a target concentration of 10 mg/mL. Thiazolobenzimidazole has

a single ionisable functional group which exhibits an increase in solubility with decreasing pH consistent with a pKa of 3.55 and the maximum solubility attainable by pH adjustment was only 0.4mg/mL (at pH 2). The inclusion complex of thiazolobenzimidazole in either its neutral or protonated form with HP β CD was found to improve solubility by forming 1:1 complexes. The equilibrium constants obtained were at 81 and 1033 M⁻¹ respectively, this giving a 3 fold greater solubility. Although the formation of a protonated complex was less favoured in comparison to the neutral complex, contribution of this species to the overall solubility of thiazolobenzimidazole predominated at low pH. Thus, using a combined approach of pH enhancement and complexation with HP β CD, gave a solubility enhancement of three orders of magnitude²⁹.

Factors such as polymorphism or nonsolvated crystals and anhydrate solutes also affect drug solubility or dissolution and are related to drug absorption. In a recent study the general trends of solubility ratios of polymorphs for 55 compounds (81 solubility ratios due to existence of multiple forms for some compounds) and the ratios of anhydrate/hydrate for 17 compounds (924 ratios due to existence of multiple forms) were evaluated. The study for polymorphs was based on both aqueous and nonaqueous solubility data because polymorph solubility ratio is independent of the solvent used, whereas for the anhydrate/hydrate solubility ratios, only aqueous solubility ratios were used. The study was carried out in the temperature range of 20-40°C. The study revealed that polymorph solubility ratio and anhydrate/hydrate solubility ratios were typically less than 2, but the anhydrate/hydrate solubility ratios appeared to be more spread and higher than the typical ratio for nonsolvated polymorphs³⁰.

Like polymorphs the amorphous form of pharmacologically active materials has also received considerable attention because it represents the most energetic solid state of a material and thus provides the biggest advantage in terms of solubility and bioavailability. For polymorphs the improved solubility can be estimated from the knowledge of different thermodynamic properties of the different forms such as the melting point, heat of fusion, and heat capacity of each form. The measurement and

estimation of the solubility and bioavailability improvements of the amorphous form of a drug present a significant challenge because of the non equilibrium nature of the amorphous state. The two major limitations that affect correct measurements are the difficulty involving the accurate characterisation of the thermodynamic properties and its tendency to rapidly revert to the crystalline state upon exposure to small quantities of solvents³¹.

1.5.1 SOLUBILITY AND PARENTERAL FORMULATIONS

Solubility plays an important role when the drug is to be formulated for parenteral purposes. Although insoluble drugs can be given in oral formulations in the form of suspensions or emulsions, parenteral formulations are preferred to use solutions. Parenteral administration of sparingly soluble substances, especially by the intravenous (IV) route, is a major challenge in the pharmaceutical industry, and several techniques have been used to increase the solubility of a drug. Additives, such as salts, cosolvents, surfactants, and complexing agents are all means to improve the solubility of an insoluble drug to an extent that can be predictable²⁶.

Converting a drug to its salt form is one way of improving the solubility, but not all drugs show biological activity or desired action in their salt form, and thus other approaches for increasing the solubility include cosolvents, surfactants, complexation ligands, and lipids³². Solvent modification is achieved by use of cosolvents such as ethanol, propylene glycol, polyethylene glycol 400 and glycofurol. Surfactants used to improve solubility fall into the categories of ionic and non ionic surfactants in microemulsions and self-emulsifying drug delivery systems (SEDDS). Complexation techniques include modification of solubility with complexing ligands like cyclodextrin³³. A new technique of forming an isotropic system with mono and diglyceride agents such as Capmul[®] is also suggested as a method to improve the solubility of an insoluble drug for parenteral formulation.

1.6 SOLUBILITY IMPROVEMENT BY THE COSOLVENCY METHOD

The most widely used method in the pharmaceutical sector so far for solubilisation of an insoluble drug is cosolvency. It is a technique, in which the drug is more soluble in a mixture of solvents than in one solvent alone, cosolvency has been employed for wide range of dosage forms for the improvement of solubility³⁴.

Cosolvents reduce the hydrogen bond density of aqueous systems that reduce the cohesive force interactions of water and make water less effective in squeezing out nonpolar solutes from the solution. The combination of the hydrogen group makes the cosolvent part polar and part nonpolar thus reducing surface tension, dielectric constant, and solubility parameters which result in increased solubility of nonpolar solutes³⁴. Thus cosolvents act by reducing the polarity by interfering with water-water hydrogen bonds³⁴.

Cosolvency has been utilised in different formulations including solids and liquids. For example various concentrations (5-40%) of the solid binary systems with polyethylene glycol 6000 were employed to increase solubility and dissolution of meloxicam³⁵. Cosolvency techniques have also found use in spray freezing of liquid like in Danazol with polyvinyl alcohol, poloxamer 407, and polyvinylpyrrolidone K-15 in a micronised powder formulation³⁶.

Though cosolvency has been highly utilised in the design of many different formulations, it has found its main use in parenteral dosage forms because of the irritating effects of most surfactants and the low toxicity of many cosolvents, and because of the relatively greater ability of cosolvents to solubilise nonpolar drugs. The most frequently used low toxicity cosolvents for parenteral use are propylene glycol, ethanol, glycerin, and polyethylene glycol²⁷. For example drugs like ketoprofen for aqueous injection have increased the aqueous solubility by using hydrotropes and the cosolvency method³⁷.

The solubility of Septrin[®] infusion has been improved by the cosolvency method. Septrin[®] is an admixture of a poorly soluble weakly acidic drug sulphamethoxazole and the weakly basic drug trimethoprim. When mixed together the admixture is

incompatible and precipitates. Addition of 40% propylene glycol allows both substances to coexist in solution form²⁶.

For parenteral formulations such as an intravenous infusion of biphenyl-dimethyl dicarboxylate (BDD) concentrate, cosolvency proved to be the method of choice. BDD is a synthetic analogue of schizandrin C, one of the lignoid-type components isolated from Fructus schizandrae, and has been widely prescribed for improvement of liver function and symptoms of patients with liver disease. Its oral preparations have limited bioavailability due to its extremely low solubility in water. A study using a ternary solvent system of N,N-dimethylacetamide (DMA)/alcohol/water and Cremophor EL/DMA/alcohol were found to effectively improve the solubility of BDD in these cosolvents and surfactants, and the results showed that the cosolvent systems were effective for solubilizing BDD up to the concentration that might be employed for preparation of parenteral dosage forms³⁸.

1.6.1 COSOLVENTS AND ADDED MATERIALS

Solubilisation of a drug with cosolvency alone can be a difficult task, due to the ability of the drug to precipitate upon dilution in the blood stream. The addition of surfactants or complexing agents can successfully overcome this problem. For example the solubility of two poorly soluble drugs indomethacin and phenytoin was studied using a mixture of DMA and dimethylsulfoxide (DMSO) and Gelucire[®] 44/14 as a surface active agent. Cosolvents DMA and DMSO affected the micellar morphology. DMA helped form large structures by being entrapped in the hydrophobic region of the micelles and DMSO reduced the interfacial layer. It was found that Gelucire[®] as a surfactant did not significantly improve the solubility profile of indomethacin and phenytoin³⁴.

In another study flavopiridol [5,7-dihydroxy-8-(4-N-methyl-2-hydroxypyridyl)-6' -chloroflavone hydrochloride] a flavanoid with weak electrolyte properties and an intrinsic aqueous solubility of 0.024 mg/mL was combined with a buffer and cyclodextrin and a buffer and cosolvent. It was known that cosolvency, complexation, or pH control alone cannot produce an acceptable 10 mg/mL formulation that will not

precipitate when diluted in the blood stream. Therefore, a combination study was undertaken to analyse if an acceptable 10 mg/mL formulation could be produced. This study found that flavopiridol shows good stability for at least one year in 30% HP β CD/0.1 M citrate buffer (pH 4.52) and does not precipitate for at least one hour upon dilution with Sorensen's phosphate buffer pH 7.4³⁹.

Yet another study has combined the effects of cosolvency and cyclodextrin for a nonpolar drug. Fluasterone, was studied by employing a mathematical model. Fluasterone is a structural analogue of dehydroepiandrosterone used for cancer treatment. This study determined the total drug solubility of fluasterone by the summation of three drug species present in the solution: free drug [D], drug-ligand binary complex [DL], and drug-ligand-cosolvent ternary complex [DLC]. The model established the dependencies of the three species. The intrinsic drug solubility, [D(u)], the cosolvent solubilizing power, δ , the binary and ternary intrinsic complexation constants, $K(b)(int)$ and $K(t)(int)$, and the cosolvent destabilizing powers for the binary and the ternary complexes, $\rho(b)$ and $\rho(t)$. The model explained the decline in drug solubility produced by low cosolvent concentrations as well as the increased solubility produced by high cosolvent concentrations that were observed at all cyclodextrin concentrations⁴⁰.

1.6.2 THEORIES FOR COSOLVENCY

Methods to determine cosolvency fall into log-linear models and non log-linear models. The log-linear model is the simplest proposed model, which suggests that the solute is not altered by the solvent, the crystal structure of the solute remains unchanged, and the solvent does not dissolve in the solute and remains pure. The model also suggests that the composition of a mixed solvent is a linear combination of its components and that the free energy of mixing a solute with a mixed solvent, G_{mix} , is a linear combination of its free energy of mixing with the component solvents.

$$\Delta G_{mix} = f_w \Delta G_w + f_c \Delta G_c \quad \text{Eq (4)}$$

Where ΔG_w is pure water, ΔG_c is pure solvent, and f_w and f_c are the volume fractions of water and cosolvent in the mixture, respectively. Replacing the free energy terms with their corresponding solubilities gives:

$$\log S_{\text{mix}} = f_w \log G_w + f_c \log G_c \quad \text{Eq (5)}$$

where S is the molar solubility of the solute. Solubilities of methyl, propyl, and butyl esters of *p*-hydroxy- and *p*-aminobenzoates have been determined in propylene glycol:water mixtures and positive deviations were observed at high volume fractions and negative deviations were observed at low volume fractions³⁷. Log-linear cosolvency models have been studied for solubilisation in the most common solvents such as propylene glycol, ethanol, polyethylene glycol 400, and glycerin⁴¹.

Other linear models used to determine cosolvency are an extended Hildebrand solubility approach, excess free energy equations, combined nearly ideal binary solvent/Redlich-Kister equation and Margule equations. These can be converted into a general single model which expresses the logarithm of mole fraction solubility of a solute as a power series of volume fraction of the cosolvent. The non linear models include the mixture response surface methods, two step solvation model and modified Wilson model which can be converted to a nonlinear general form. It has also been shown that the general single model and a non-linear general model are mathematically identical⁴².

1.7 SOLUBILISATION OF DRUGS USING MICROEMULSIONS

Microemulsions, are a branch of emulsion technology and a concept that was introduced in the 1940s by Hoar and Schulman when they successfully generated a single phase dispersion by using a milky emulsion with hexanol⁴³. Though the concept is old it was found that these formulations attracted particular attention in regards to improved solubility. For example, an improved solubility was obtained for transdermal delivery of poorly water-soluble Vinca alkaloid derivative, vinpocetine by use of oleic acid, Labrasol[®] (C8 and C10 polyglycolysed glycerides), Transcutol P[®], and double-distilled water. Vinpocetin showed about 3160-fold increased

solubility compared to that in water and the apparent permeation rate across excised rat skin was improved⁴⁴.

In yet another study, acyclovir, a poorly soluble drug, displayed higher solubility in microemulsion formulations using Labrafac[®] (10%), Labrasol[®] (32%), Plurol Oleique[®] (8%), and water (50%). The in vitro intraduodenal diffusion and in vivo study revealed an increase of bioavailability by 12.78 times after oral administration of the microemulsion formulation as compared with the commercially available tablets⁴⁵.

Apart from improving the solubility of many known drugs, microemulsions also act as drug delivery vehicles by incorporating a wide range of drug molecules. Microemulsions have been shown to be able to protect labile drugs, control drug release, increase bioavailability and reduce patient variability⁴⁶. Furthermore, it is also possible to formulate microemulsions for most of the routes suitable for administration such as oral⁴⁷ ocular, pulmonary, topical⁴⁸ and intravenous^{46, 49}.

1.7.1 SURFACTANTS FOR MICROEMULSIONS

Surfactants/or surfactant mixtures/and or cosurfactants in microemulsions play an important role in improving the solubility of drugs formulated as microemulsions, but pose the greatest challenge in the design of a thermodynamically stable microemulsion formulations⁴⁷. The surfactant/mixture of surfactant/and/or cosurfactant form a microstructure at the interface of a two phase system, forming a one phase isotropic system. The surfactant can be non-ionic like polyoxyethylene surfactants eg Brij 35 or sugar esters like sorbitan monooleate (Span 80)⁵⁰, cationic, or anionic like alkyltrimethylammonium bromide⁵⁰ and sodium dodecyl sulphate, or zwitterionic such as phospholipids like lecithin (phosphatidylcholine) commercially available from soybean and eggs. Lecithin is very popular because it exhibits excellent biocompatibility⁴⁶. Combinations of ionic and non-ionic surfactants are also found to be effective at increasing the extent of the microemulsion region.

Dependent on the polar head and a nonpolar tail and the self association of a surfactant molecule, a number of different structures are formed giving an optically

isotropic microemulsion phase. This is represented in Figure 5, which shows different phases that are formed by self-association of the surfactant⁴⁶.

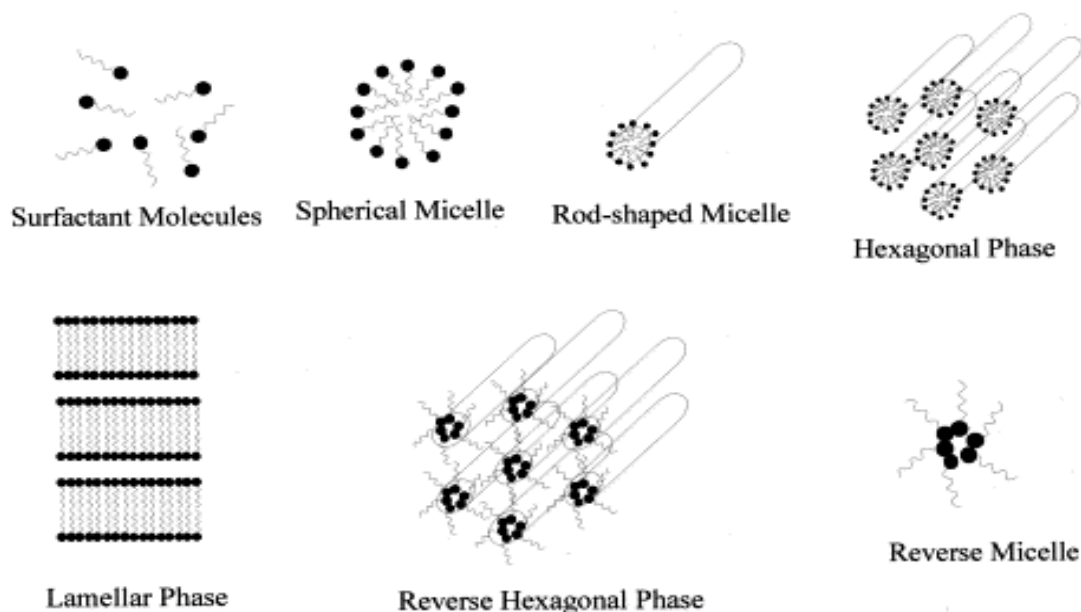


Figure 5: Schematic representation of the most commonly encountered self-association structures in water, oil or a combination thereof.

1.7.2 TYPES OF MICROEMULSIONS

Three types of microemulsions that are formed are oil in water, (o/w emulsion), where the volume of the oil fraction is less, or water in oil emulsion, (w/o emulsion), where the volume of the water is less and a bi-continuous microemulsion where amounts of water and oil are similar.

1.7.3 PHASE BEHAVIOUR OF MICROEMULSIONS IN IMPROVING SOLUBILITY

The improvement or enhancement of solubility using microemulsions and the surfactant properties can well be explained by the ternary phase behaviour diagram. The phase behaviour of simple microemulsion systems is represented by oil, water and surfactant. In the case of microemulsions for pharmaceutical applications, the microemulsion will contain additional components such as a cosurfactant and/or drug. In this case pseudo-ternary phase diagrams are used, where a corner will typically

represent a binary mixture of two components such as surfactant/cosurfactant, water/drug or oil/drug⁴⁶. The typical hypothetical pseudo-ternary phase diagram is shown in Figure 6.

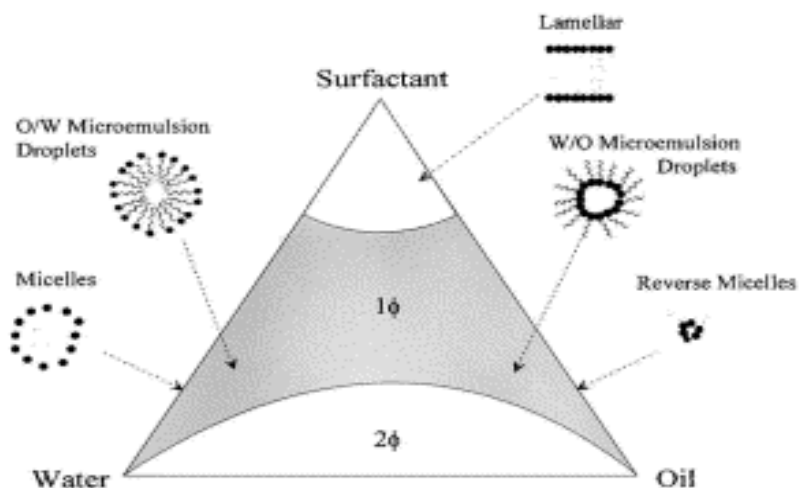


Figure 6: A hypothetical pseudo-ternary phase diagram of an oil/surfactant/water system with emphasis on microemulsion and emulsion phases. Within the phase diagram, existences fields are shown where conventional micelles, reverse micelles or water-in-oil (w/o) microemulsions and oil in water o/w microemulsions are formed.

1.7.4 PROBLEMS ENCOUNTERED WITH MICROEMULSIONS

Microemulsions have been employed to increase the solubility of many drugs that are practically insoluble in water, along with incorporation of proteins for oral, parenteral, as well as percutaneous/transdermal use.

Although, microemulsions are considered to be thermodynamically stable systems with low viscosity, using the correct surfactant and/or surfactant mixture and/or cosurfactant in a correct concentration poses the greatest challenge in the design of a thermodynamically stable microemulsion formulation⁴⁷. To overcome this problem much research has been reported. A recent study has shown that microemulsions formulated using medium chain triglycerides as a nonpolar component and lecithin

and short chain alcohol/C₃-C₄ as a surfactant improves the stability of microemulsions⁴⁷. Another study suggested strategies to choose surfactants/cosurfactants for the formation of a stable and dilutable microemulsion⁴⁷.

Microemulsions have been used mainly in topical, oral, ocular, pulmonary, and parenteral formulations. In topical formulations, microemulsions are mainly employed to enhance the percutaneous, dermal, or epidermal absorption of the drug. For example o/w and w/o microemulsions based on oleic acid as oil phase and mixtures of Labrasol[®] and Plurol Oleique CC 497[®] as surfactant were employed in the delivery of prostaglandin E₁⁵¹.

Another study evaluated absorption of oral nanocapsules of insulin dispersed in a microemulsion for intragastric administration to diabetic rats using poly (iso-butyl cyanoacrylate) (PBCA) for nanoencapsulation. The microemulsion consisted of a mixture of medium-chain mono-, di- and tri-glycerides as the oil component, polysorbate 80 and sorbitan mono-oleate as surfactants and an aqueous solution of insulin. The intragastric administration of insulin-loaded nanocapsules dispersed in the biocompatible microemulsion resulted in a significantly greater reduction in blood glucose levels of diabetic rats than an aqueous insulin solution or insulin formulated in the same microemulsion. This study demonstrated that the formulation of peptides within PBCA nanocapsules that are administered dispersed in a microemulsion can facilitate the oral absorption of encapsulated peptides⁵².

Microemulsions can be employed for IV use⁴⁷, however, IV administration imposes rigorous demands on the non-toxicity of the formulations³³. Aqueous parenteral formulations containing propofol using o/w microemulsion systems were developed in a recent study. Propofol is an oily liquid and therefore was used as the oil phase and its content fixed to 1%, w/w. Pseudoternary phase diagrams reflected the concentration range of surfactant and cosurfactant and the optimum ratio between them for microemulsion formation. The suitability of the microemulsion as a parenteral formulation was evaluated from the stability and haemolysis tests. Among the surfactants and cosurfactants screened, a mixture of Solutol HS 15-ethyl alcohol

(5/1) showed the largest o/w microemulsion region in the phase diagram. It was found that 1% (w/w) of propofol was solubilised with 8% (w/w) of Solutol HS[®] 15-ethyl alcohol (5/1) to obtain an average droplet size (150 nm). The content of propofol in the systems was not significantly changed at 40°C for 8 weeks. The haemolysis test also showed that this formulation was non-toxic to red blood cells. Thus, this study proposed that propofol can successfully be solubilised with an o/w microemulsion system⁵³.

In another study an attempt was made to develop a poorly water-soluble lipophilic drug ibuprofen eugenol ester using a phospholipid-based microemulsion. Ibuprofen eugenol ester (IEE), a highly lipophilic compound, was synthesized from ibuprofen and eugenol. A microemulsion system was formulated consisting of Miglyol 812[®], soybean lecithin (SbL) and poly (ethylene glycol), (660)-12-hydroxystearate (Solutol HS-15[®]), and PEG 400 and ethanol as oil phase, along with surfactants and cosurfactants, to form a stable parenteral microemulsion. The ibuprofen blood concentration after intravenous administration of the microemulsion was determined and compared with that of an ibuprofen solution. The solubility of IEE obtained in this form was about 21,000 times higher than that in water. It was concluded that the microemulsion system might be a promising intravenous dosage form of poorly water-soluble lipophilic drugs⁵⁴.

The solubility of flurbiprofen, a poorly water-soluble drug, was improved by formulating it in an oil-in-water (o/w) microemulsion suitable for parenteral administration. Varying ratios of oil to surfactant were prepared with ethyl oleate, Tween 20 and an isotonic solution. The effect of the particle size of the microemulsion and solubility of flurbiprofen in the microemulsion were studied. The mean droplet diameter of microemulsions containing less than 1% (w/w) of flurbiprofen was below 100 nm. However, the mean droplet diameters tended to increase at room temperature. When the different systems were compared it was found that the pharmacokinetic parameters of flurbiprofen after intravenous administration of a flurbiprofen-loaded microemulsion to rats were not significantly different from those of flurbiprofen in phosphate-buffered saline solution. The

maximum solubility of flurbiprofen in the microemulsion system was found to be 10 mg/ml. It was concluded that microemulsions of flurbiprofen prepared with ethyl oleate and Tween 20 can be used as a parenteral drug carrier for this and other poorly water-soluble drugs, provided that physical stability can be properly addressed^{55, 52}.

1.8 SELF-EMULSIFYING DRUG DELIVERY SYSTEM (SEDDS)

This method is a new approach to emulsions for improving the solubility of incorporated drugs. Self-emulsifying drug delivery systems (SEDDS) are closely related to but a different branch of emulsion systems. The two new approaches that are applied to make the insoluble drugs soluble by this system are self-microemulsifying drug delivery system (SMEDDS) and self-nanoemulsifying drug delivery system (SNEDDS).

SMEDDS typically comprises a mixture of surfactant, oil and drug (known as the concentrate) which when introduced into the body is rapidly dispersed to form droplets of approximately the same size range as those observed in microemulsion systems. Once dispersed such systems would be expected to behave *in vivo* in much the same way as oil-in-water (o/w) microemulsions.

Self-nanoemulsifying systems (SNEDDS) are isotropic mixtures of oil, surfactants, and cosurfactants along with the drug. When these systems come in contact with gastrointestinal fluids, they disperse as very fine droplets in the nanometer size range. The droplet size, turbidity, and drug release characteristics depend on formulation variables, such as the nature and concentrations of the oil, surfactant, or cosurfactant⁵⁶.

SEDDS systems are mainly used to improve the solubility of water insoluble drugs for oral absorption. These systems have not been employed in parenteral formulations. For example, a recent *in vitro* study of SEDDS and SMEDDS systems using carvedilol, a poorly water soluble drug, showed an increase in the solubility, dissolution rate, and, ultimately, oral bioavailability. SEDDS and SMEDDS showed that the dissolution rate for carvedilol was more than double when compared with that from tablets. Also the SEDDS formulation significantly improved the oral

bioavailability of carvedilol by 413% when compared with commercially available tablets⁵⁷.

1.9 SOLUBILISATION OF DRUGS BY USE OF COMPLEXING AGENTS

Complexation is defined as the reversible association of m molecules of a substrate S with n molecules of a ligand species L to form a new species S_mL_n ²⁷. This can be shown in the following Equations (6 and 7)



The equilibrium constant $K_{m:n}$ for the interaction may be defined as

$$K_{m:n} = \frac{[S_mL_n]}{[S]^m [L]^n} \quad \text{Eq (7)}$$

There are different types of complexes that are formed and these can be defined in terms of association or complexation constants and equilibria²⁷.

Types of complex	Examples
Coordination	Cis-Dichlorodiamineplatinum (II)
Chelates	Calcium EDTA
Metal-olefin	Ferrocene
Inclusion	Digitonin-cholesterol
Molecular complexes	Phenol-PEG; benzoic acid-caffeine

Cyclodextrins(CD's) are heterogeneous, amorphous, hygroscopic substances, produced in large quantities by a hydrolytic process and obtained as the primary product from the splitting of the glycosidic linkage with one molecule of water⁵⁸.

CD's have a hydrophilic outer surface and a lipophilic central cavity. CD molecules are relatively large with a number of hydrogen donors and acceptors and, thus, in general they do not permeate lipophilic membranes. In the pharmaceutical industry CD's have mainly been used as complexing agents to increase aqueous solubility of

poorly soluble drugs, and to increase their bioavailability and stability. Studies in both humans and animals have shown that CD's can be used to improve drug delivery from almost any type of drug formulation⁵⁹.

The three major CD's are crystalline, homogeneous, non-hygroscopic substances, which are of a torus-like macro ring shape, built up from glucopyranose units. α -CD or cyclomaltohexose comprises 6 glucopyranose units. The β -CD or cyclomaltoheptose comprises 7 glucopyranose units. The γ -CD or cyclooctaamylose comprises of 8 such units⁵⁸.

1.9.1 CYCLODEXTRINS AND SOLUBILISATION OF DRUGS

To determine whether CD's are the right choice as solubilisation enhancers for poorly water soluble drug the CD utility number is used. The solubilisation of a poorly soluble drug can be explained as follows:

The fundamental property that describes the strength of interaction between a drug and a CD is the binding constant (or stability constant) K , which is related to the thermodynamic property ΔG^0 , standard free energy change during complexation.



The total aqueous solubility of drug (S_t , mol/L) in the presence of a given total CD concentration (C_{CD} , mol/L) is described by the following equation:

$$S_t = S_o + \frac{K S_o}{1 + K S_o} [C_{CD}] \quad \text{Eq (9)}$$

where S_o is the aqueous solubility of the drug in the absence of CDs. For any formulation where complete drug solubilization by CD complexation is required, the practical utility of CDs as efficient solubilizers depends on (1) the binding constant, K , (2) the drug intrinsic solubility, S_o , (3) the dose of the drug, and (4) the maximum workable amount of CD. Thus a new dimensionless number, named the CD utility number (U_{CD}) has been introduced to assess the feasibility of the use of CDs in dosage forms depending on the above mentioned factors.

Let the dose of drug and total amount of workable CD be D_t (mol) and CD_t (mol), respectively. Also, let V (L) be the volume available for the formulation to dissolve. This may be the volume of injectable solution or the volume available in the GI tract for an immediate release oral dosage form to dissolve or volume inside a coated modified release dosage form (e.g., osmotic pump tablet). For the entire dose of drug to be in solution

$$\frac{D_t}{V} \leq S_t \quad \text{Eq (10)}$$

Substituting 9 into 10,

$$\frac{D_t}{V} \leq S_o + \frac{KS_o}{1+KS_o} \frac{CD_t}{V} \quad \text{Eq (11)}$$

Upon further rearrangement,

$$\frac{S_o V}{D_t} + \frac{KS_o}{1+KS_o} X \frac{CD_t}{D_t} \quad \text{Eq (12)}$$

The first term, $S_o V/D_t$, is the inverse of the dose number, a dimensionless number that has been used to classify drugs as poorly and highly water soluble. It is generally less than one for poorly soluble drugs and therefore it is reasonable to assume that the first term is negligible. The second term of eq. 12 can be defined as the CD utility number, U_{CD} and is expressed as:

$$U_{CD} = \frac{KS_o}{1+KS_o} \frac{CD_t}{D_t} = \frac{KS_o}{1+KS_o} \frac{m_{CD}}{m_D} \frac{MW_D}{MW_{CD}} \quad \text{Eq (13)}$$

where m_D and m_{CD} are the drug dose and workable amount of CD in mg, respectively, and MW_D and MW_{CD} are molecular weights of D and CD , respectively. When the dimensionless number, U_{CD} is greater than or equal to one, solubilization is adequately provided by complexation by CDs. When the dimensionless number is less than one, the complexation alone is not enough for complete solubilization. The

workable amount of CD, m_{CD} can also be fixed based on the dosage form type, weight or volume limit (tablet size), tonicity of the solution (parenteral or ophthalmic), toxicity, cost etc. For the application of Eq. 13 in determining the utility of CD for a specific drug formulation, only the value of the binding constant, K needs to be determined⁶⁰.

1.9.2 ASSOCIATION CONSTANT AND CYCLODEXTRINS

The association constant (K) is a constant that is determined by the association between the drug molecule (D) and the cyclodextrin molecule (CD). One molecule of (D) may be associated with one molecule of (CD), or two molecules of (D) may be associated with one molecule of (CD), or one molecule of (D) may be associated with two molecules of (CD). This can be explained by Figure 7 showing phase diagrams.

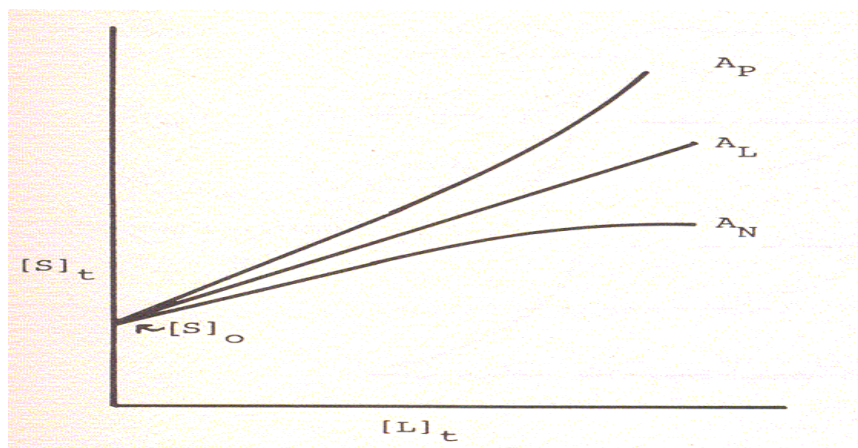


Figure 7: Schematic representation of the A-type phase diagrams

The type A phase diagram represents systems in which the complex formed is soluble and does not precipitate regardless of the amount of ligand. Depending upon the type of association between the (D) and the (CD) three types of variations are seen in the phase diagram. The A_L model represents the association constant of $K_{1:1}$ which means that one molecule of (D) forms a complex with one molecule of (CD) and a linear relationship exhibits. This can be explained by the following equation where m and $n=1$.



Type A_P system represents where one molecule of (D) forms a complex with two molecules of (CD) and a positive deviation from linearity is obtained. This is expressed as in the equation given below.



The type A_N exhibits a negative deviation which represents a decreasing dependence on CD added at higher CD concentration. This type is the least frequently encountered system²⁷.



Generally the most common stoichiometry of drug/CD complexes is 1:1, and is often studied by the phase-solubility method. However, in recent years it has becoming increasingly clear that solubilizing effects of CD's are frequently due to the formation of multiple inclusion and non-inclusion complexes. A study of the aqueous solubility of 38 different drugs in aqueous solution, aqueous buffer solutions and aqueous cyclodextrin solutions, found that the apparent stability constant ($K_{1:1}$) of the 1:1 drug/cyclodextrin complexes calculated by the phase-solubility method shows strong negative deviation from the intercept solubility (S_{int}) and the intrinsic solubility (S_0) for poorly soluble drugs with aqueous solubility <0.1mM (or approximately 0.03mg/mL). In the case of drugs with intrinsic solubility (S_0) values greater than 1mM the (S_{int}) nearly equals the (S_0). (S_0) is in general much larger for poorly soluble than the intercept of the phase-solubility diagram (S_{int}) resulting in non-linearity of otherwise linear (AL-type) phase-solubility diagram. This leads to erroneous $K_{1:1}$ values⁶¹.

It is not clear why the intercept of the phase-solubility diagram is below S_0 but it could be due to the non-ideality of water as a solvent. Usually we treat solvents as homogenous and ideal but somewhat random structure of solvent molecules that are more or less independent of each other. In recent years it has become increasingly clear that water is a highly structured solvent with many unique physicochemical

properties that have yet to be explained at a molecular level. For example, the molecular structure of water allows the water molecules to form a cage around non-polar solutes without sacrificing much of their hydrogen bonding capacity. Structured water can close on the solute like elastic net trapping one or more solute molecules. This physicochemical property of water can be responsible for some of the solubility irregularities observed in pure aqueous solutions⁶¹.

To avoid this discrepancy in solubilities a more accurate method for determination of the solubilizing efficiency of CD is to determine their complexation efficiency (CE), i.e. the concentration ratio between CD in a complex and free CD. CE is calculated from the slope of the phase-solubility diagrams, it is independent of both S_0 and S_{int} , and more reliable when the influences of different pharmaceutical excipients on the solubilization are being investigated⁶¹.

1.9.3 PHYSICOCHEMICAL ASPECTS OF DRUG AND COMPLEXES

Complexes form in aqueous solutions as the result of the additive effects of a variety of intermolecular interactions. The forces which act are London dispersion, dipolar (including hydrogen bonding), ionic, and hydrophobic forces. A single type of bonding is not dominant in a solution between the drug and complexing agent. However, most small molecules that form complexes exhibit molecular features by forming intermolecular hydrogen bonding which results in a non-planar configuration. It has been strongly suggested that planar configuration stack together and is generally associated with aromatic moieties. However, this is not the case with most of the drugs of pharmaceutical interest as they are non planar and/or non-aromatic. For a drug to form a complex with a suitable agent both the drug and the complexing agent should exhibit similar interaction chemically. Mostly this is exhibited by the formation of hydrogen bonding between a drug and a complexing agent²⁷.

1.9.4 FACTORS INFLUENCING INCLUSION COMPLEX FORMATION

The type of CD can influence the formation as well as the usefulness of drug/CD complexes. For complexation, the cavity size of the CD should be suitable to accommodate a drug molecule of a particular size⁶². Compared with neutral CDs, complexation can be improved when the CD and the drug carry opposite charges but

may decrease when they carry the same charge. For many acidic drugs forming anions, the cationic (2-hydroxy-3-[trimethylammonio] propyl)- β -CD has acted as an excellent solubilizer. In the case of ionisable drugs, the presence of charge may play a significant role in drug/CD complexation and hence a change in the solution pH can vary the complexation constant. In general, ionic forms of drugs are weaker complex forming agents than their nonionic forms⁶³ but in the case of mebendazole, the unionized form was less included in HP β CD than the cationic derivative⁶⁴.

Temperature changes can affect drug/CD complexation. In most cases, increasing the temperature decreased the magnitude of the apparent complexation constant of the drug/CD complex and the effect was reported to be a result of possible reduction of drug/CD interaction forces, such as van der Waals and hydrophobic forces with rise of temperature. However, temperature changes may have negligible effect when the drug/CD interaction is predominantly entropy driven (ie, resulting from the liberation of water molecules hydrated around the charges of guest and host molecules through inclusion complexation)⁶⁴.

The physicochemical properties of CDs, including their complexation ability, may be greatly affected by the type, number, and the position of the substituents on the parent CD molecule. The “degree of substitution” *per se* does not uniquely characterize a β -CD derivative such as HP β CD. When produced under different conditions, the physicochemical properties of HP β CD samples with the same degree of substitution may not be identical owing to the possible occupancy of hydroxypropyl groups at different positions on the parent CD molecule. Since the purity of CD can have a significant effect on the final quality of the drug product and its marketability, it is necessary to have a proper understanding of the following term that is used in identification of CD purity⁶⁵.

“Degree of substitution” (DS) is the average number of substituted hydroxyls per glucopyranose unit of the CD ring. Since the number of reactive hydroxyls per mole of glucopyranose unit is 3, the maximum numbers of substituents possible for α -, β -, and γ -CDs are 18, 21, and 24, respectively⁶⁵.

1.9.5 APPLICATIONS OF CYCLODEXTRINS IN THE PHARMACEUTICAL INDUSTRY

The supramolecular characteristics, water solubility and wide availability of CDs, plus their generally low toxicity, make them ideal candidates for various industrial applications, especially for pharmaceutical applications. Improved water solubility, bioavailability, or metabolic stability, have allowed the reformulation of many drugs or to reduce the therapeutic dose of the active drug substance. Some studies on CD also suggest the direct therapeutic use of CDs, for example, to facilitate the elimination of a drug, like barbiturates from the blood circulation, or to sequester bile acids in the gastrointestinal tract in order to reduce the endogenous cholesterol levels. Unlike the more commonly used CD in pharmaceuticals wherein CDs mainly act as excipients given together with an active drug substance, the direct therapeutic use of CDs is based on certain pharmacological effects resulting from *in vivo* CD-guest complexation⁶⁶. Cyclodextrins are growing in use in the pharmaceutical industry and this is not limited to a particular dosage form, but has spread to almost every dosage forms⁶⁶.

The enhancing effects of CDs on the solubility, the dissolution rate, and the bioavailability of the drug tacrolimus after oral administration to rats were examined and compared with those after administration of a PROGRAF capsule containing the solid dispersion formulation of tacrolimus. The study suggests that dimethyl β -CD (DM β CD) is particularly useful in designing oral preparations of tacrolimus with an enhanced bioavailability and a reduced variability in absorption⁶⁷.

Cyclodextrins have effectively being used in ocular formulations either to enhance the solubility or to increase the viscosity in conjunction with polyvinyl alcohol (PVA) of the drug solution, so that the drug-eye contact is maximised. For example the complexation of pilocarpine prodrug with sulfobutyl ether beta-CD (SBE7- β -CD), with and without PVA, on the miotic response and eye irritation were studied in pigmented rabbits. The pilocarpine prodrug formed 1:1 inclusion complexes with variably substituted sulfobutyl ether derivatives of β -CD (SBE4- β -CD and SBE7- β -CD), and 1:1 and 1:2 complexes with hydroxypropyl- β -CD (HP β CD) at pH 7.4.

Coadministered SBE7- β -CD eliminated the eye irritation due to the pilocarpine prodrug, but also decreased the miotic response. Ocular absorption of the prodrug was improved by increasing the viscosity of prodrug/SBE7-beta-CyD solution with PVA without inducing any eye irritation⁶⁸.

CD's, and in particular HP β CD, have been found to improve the solubility and stability of drugs for nasal delivery. A recent study evaluated the potential use of HP β CD in the solubilisation and stabilization of prostaglandin E₁ (PGE₁). The solubility and chemical stability of PGE₁ were found to improve significantly upon complexation with HP β CD. The nasal delivery of PGE₁ from the complex formulation when studied in Wistar rats and compared with intravenous administration was found to cause a rapid decrease of blood pressure and exhibit an obvious dose-efficacy relationship, showing results nearly similar to those obtained for the intravenous route. Besides, the *in vitro* effect of the PGE₁ complex on nasal mucociliary movement was also investigated with a toad palate model. The PGE₁ complex formulation exerted only minor effect on nasal mucociliary movement. The results of the study indicated that the PGE₁-HP β CD complex formulation for nasal delivery is a very promising preparation with advantages such as rapid and effective absorption, good chemical stability, ease of administration, and minor nasal ciliotoxicity⁶⁹.

Cyclodextrins were used for rectal administration of some drugs like cefmetazole. For example in a study an inclusion complex of decanoic acid (DA) with alpha-cyclodextrin (α -CD) was prepared as an additive of cefmetazole sodium (CMZ) suppository and rectally administered to rabbits. The complexation was examined by the phase solubility method, differential scanning calorimetry (DSC) and X-ray diffractometry. Plasma concentration and area under the curve (AUC) values of CMZ after rectal administration of a suppository containing DA/ α -CD complex to rabbits increased significantly over those without additive⁷⁰.

In recent years, CD's have being evaluated for their solubility enhancement property for many insoluble drugs for parenteral use and researchers have found a wide range

of interest in using cyclodextrins for the same. For example, phenytoin was studied to increase its solubility by complexing it with varying concentrations of HP β CD and creating an entirely aqueous formulation with a pH significantly closer to physiologic pH (7.4). The two most promising formulations consisting of 40% HP β CD at pH 10.4 and 20% HP β CD at pH 11.0 were selected for further study. Both formulations were aqueous with a significantly decreased pH compared to the original commercial formulation (Parke-Davis, pH 12.0). The formulations were also found to significantly decrease the tendency of phenytoin to precipitate *in vitro*. The tissue irritation potential of the 20% w/v HP β CD formulation at pH 11.0 was also reduced considerably compared to the commercial injection in a BALB/c mouse model⁷¹.

In another study, a parenteral formulation of the water-insoluble benzodiazepine, diazepam, was developed. Different CD's such as HP β CD, hydroxy-propyl-gamma-cyclodextrin (HP- γ -CD), SBE-7- β -CD and maltosyl-beta-cyclodextrin (malt- β -CD) were used as alternatives to cosolvents to increase the solubility for parenteral injection. The greatest improvements in solubility (3.5 mg/ml in 40% CD) were found by adding HP β CD, or SBE-7- β -CD. A parenteral aqueous diazepam solution was prepared containing 10 mg diazepam/5 ml 30% HP β CD or SBE-7- β -CD solution. The preparations met the requirements for parenteral administration. Sterilisation was by filtration since autoclaving degrades the active compound. The stability with and without pH adjustment to pH 5, was evaluated during an 18 months period and no noticeable degradation was found⁷².

The complexation technique in parenteral products has also been utilised to reduce toxicity and improve patient compliance. For example, mitomycin C (MMC), an anticancer drug, can cause severe dermatological problems upon injection. It can cause delayed erythema and/or ulceration occurring either at or distant from the injection site for weeks or even months after administration. In an attempt to reduce skin necrosis, complexation of MMC with HP β CD was studied in the presence and absence of mannitol in order to help increase patient compliance and acceptance. It was found that the mannitol present in the commercial formulation caused an increase in the binding of MMC to HP β CD. Also isotonicity adjustment of hypotonic MMC

formulations by the addition of normal saline did not change the degree of complexation with MMC. When evaluated for antitumor efficacy using the B-16 melanoma cell model no difference in antitumor activity between the complexed and uncomplexed MMC formulations was observed⁷³.

1.10 NEED FOR PARENTERAL FORMULATION OF PRILOCAINE AND LIGNOCAINE

Topical preparations of prilocaine hydrochloride and lignocaine hydrochloride have gained popularity over parenteral preparations commercially as well as in research, because of the ease in formulation and application by a patient. Parenteral local anaesthetics have had little research attention although they hold some advantages such as:

- 1) Being used in localized treatment on any part of the body to block the nerves locally to alleviate the underlying pain.
- 2) Providing a quick onset and longer duration of action compared to topical preparations.
- 3) Being able to use such drugs in combination with other analgesic drugs. For example, parenteral injection of lignocaine with adrenaline is used in many surgeries. Similarly, in epidural anaesthesia for labour pain local anaesthetics alone were used for many years, but are now generally administered in lower concentrations with opioids to provide effective, synergistic analgesia while reducing a side effect of motor nerve block associated with local anaesthetics⁷⁴.
- 4) Producing very effective analgesia for a longer period of time post operatively when given at the conclusion of a surgery.

Prilocaine and lignocaine show greater lipid solubility in their free base or molecular form. Lignocaine in its hydrochloride salt form has been used for producing a local anaesthetic effect parenterally. However, there has been very little research on

parenteral preparations of prilocaine and lignocaine in their molecular forms. These drugs in their free base form possess a solubility problem for parenteral formulation. These studies therefore seek to enhance the solubility of these drugs in their free base form. The expectation is to improve the solubilities of the drugs by formulating them as a solution using a complexing/solubilising agent. CD's in particular HP β CD can be used as effective complexing agents.

1.10.1 OBJECTIVES OF THIS STUDY

1. To develop and validate an analytical high performance liquid chromatography method for three local anaesthetic drugs namely prilocaine hydrochloride, lignocaine hydrochloride and tetracaine hydrochloride.
2. To evaluate HP β CD as complexing agent to increase the solubility of prilocaine and lignocaine as their free bases leading to parenteral formulation as solutions for rapid onset of action.
3. To evaluate the solubility of prilocaine and lignocaine alone and in combination.
4. To study the effect of complexation with HP β CD on the solubility of prilocaine and lignocaine alone and in combination. In addition the temperature dependency will be evaluated.
5. To evaluate the complex formation using nuclear magnetic resonance spectroscopy (NMR).

2 MATERIALS AND METHODS

2.1 MATERIALS

Materials used were prilocaine hydrochloride (Lot no-39H0703, Sigma-Aldrich Chemie, Germany), lignocaine hydrochloride (Lot no-52774, ICN Biomedicals, Ohio, USA), tetracaine hydrochloride (Lot no 387H021, Hoechst Australia Ltd, Melbourne, Australia), lignocaine (lot No-074K1685, Sigma-Aldrich Chemie, Germany), hydroxy propyl β cyclodextrin (molecular weight - 1454.46 and degree of substitution - 5.5) (Lot no-Y1933, Cerestar[®], USA) sodium hydroxide (pellets) (Lot no-28353, Merck Pty Ltd, Victoria), sodium carbonate anhydrous AR Grade (Lot no-FOG151, Asia Pacific Speciality Chemicals Ltd, NSW, Australia). All other reagents used were of analytical grade.

The solvents used were deionised water, passed through a Milli Q Apparatus (Millipore Corporation, Bedford, USA), methanol (Labscan, Asia Co. Ltd, Bangkok, Thailand). All other solvents used were of HPLC grade.

2.2 METHODS

2.2.1 ULTRAVIOLET (UV) SPECTROPHOTOMETRY FOR PRILOCAINE HYDROCHLORIDE, LIGNOCAINE HYDROCHLORIDE, AND TETRACAINE HYDROCHLORIDE

A Hewlett-Packard diode array UV spectrophotometer (HP 8952A) California, USA was used to determine a common wavelength that could be used to detect the three local anaesthetic drugs simultaneously. The samples were diluted with Milli-Q water. Two wavelengths 220nm and 230nm were evaluated and 230nm was selected because the extinct coefficient of absorbance for all the three local anaesthetics namely prilocaine hydrochloride (HCl), lignocaine HCl and tetracaine HCl were found to be equal.

2.2.2 METHOD DEVELOPMENT FOR PRILOCAINE HYDROCHLORIDE, LIGNOCAINE HYDROCHLORIDE, AND TETRACAINE HYDROCHLORIDE

2.2.2.1 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

An HPLC method was developed for prilocaine hydrochloride, lignocaine hydrochloride and tetracaine hydrochloride using a Waters Associates Model 501 pump, IL, (Milford MA, USA) a 486 MS tunable absorbance detector and a Hewlett Packard integrator (HP 3396A), (CA, USA). An Apollo silica column, C18, 5 μ , 150 x 4.6mm ID, (Altech Associate, IL, USA) was used for separation of analytes.

The mobile phase was 60/40 v/v of methanol and water in 20mM acetate buffer, pumped at the flow rate of 1.5mL/min. A 20 μ L injector loop, Rheodyne, (CA, USA) was used. The detection was carried out at 230nm using an attenuation of 0.13 milli absorbance units full scale.

For each of the drugs 10mg was weighed accurately and dissolved in approximately 25mL of water and the volume was made up to 100mL with water. Using this as

stock solution further dilutions were obtained over the concentration range of 1-10 μ g/mL and injected onto the HPLC column. The calibration graph was prepared by plotting the peak areas against the concentration of each drug and correlation coefficients calculated.

The lower limit of detection was determined using the range of concentrations for lignocaine hydrochloride, prilocaine hydrochloride, and tetracaine hydrochloride with the signal to noise response ratio of 2H/h where H is the height of the peak corresponding to the component and h = the absolute value of the largest noise fluctuation from the baseline of the blank solution. The wavelength was 230 nm and the attenuation was at 5.0. The intra- and inter-day precision was calculated with samples of n = 6 and concentrations of 0.001, 0.004, 0.008, and 0.010mg/mL, whereas interday precision was calculated with samples of n = 5 and concentrations of 0.004, 0.008, 0.010mg/mL respectively.

2.2.2.2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

A second assay was developed with only prilocaine hydrochloride and lignocaine hydrochloride in combination.

The mobile phase consisted of 70:30 v/v methanol water in 10mM phosphate buffer strength (pH = 5.5) pumped at 1.5mL/min through a 20 μ L injector loop. The detection was carried out at 230nm.

The stock solution was prepared similar to the method described above and dilutions were made in the concentration range of 10-100 μ g/mL and injected onto the HPLC column. The calibration graph was prepared by plotting the areas under the curve against the concentration of the drugs and correlation coefficients calculated.

2.2.3 PREPARATION OF PRILOCAINE AND LIGNOCAINE BASES

2.2.3.1 METHOD I- PRILOCAINE BY FREEZE DRYING

Prilocaine hydrochloride and lignocaine hydrochloride were precipitated using drop wise addition of 5M sodium hydroxide prepared as a stock solution. The precipitate

obtained for prilocaine was an oily liquid. The oily liquid was collected and washed with milli Q water and then it was freeze dried. It was then collected in an amber bottle to protect it from light and sealed. For further protection aluminium foil was wrapped around the bottle.

The lignocaine base was obtained similarly and the precipitate was washed with water and filtered and dried at room temperature. It was then transferred to a glass bottle and sealed.

2.2.3.2 METHOD II- PRILOCAINE BY EXTRACTION WITH DICHLOROMETHANE (DCM)

The prilocaine obtained by the above method was not very pure and still contained traces of water after freeze drying, so an extraction method with dichloromethane (DCM) was employed to obtain prilocaine in pure form. This method involved addition of DCM along with 5M NaOH to prilocaine hydrochloride. The oily mixture was then shaken and washed with DCM thrice and the extract collected from the separator. Anhydrous sodium sulphate was added to DCM solution and left for one hour to take up the excess water content. Following filtration DCM was evaporated by distillation and the oil was transferred to the bottle.

2.2.3.3 METHOD III- PRILOCAINE BY PRECIPITATION METHOD

A third approach to obtain prilocaine in its base form involved using 0.5M sodium carbonate (Na_2CO_3) solution. To different concentrations of HP β CD solutions an excess amount of prilocaine hydrochloride was added, then 1mL of the Na_2CO_3 solution was added to each concentration to effectively change prilocaine hydrochloride to prilocaine base. The solutions were then shaken for 24 hours for solubility evaluation.

2.2.4 PREPARATION OF HP β CD SOLUTION

A 30 % solution of HP β CD was prepared by the following procedure.

HP β CD (molecular weight 1454.44 and degree of substitution 5.5) was triturated mildly in a glass mortar with a pestle to release the loose aggregates that might have

formed on standing. 30 g were then weighed accurately and the powder then transferred to a flask. A magnetic stirrer was used to stir the solution at all times. Small amounts of water were added each time and stirring adjusted to fast or medium as HP β CD went in solution. After all the HP β CD was in solution the stirring was continued for another 30 minutes to ensure a clear solution. It was transferred to a 100mL volumetric flask and washings were added to the volumetric flask to make up the volume to 100mL. This solution was used as a stock solution for further studies.

2.2.5 MAINTAINING THE pH OF PRILOCAINE BASE AND LIGNOCAINE BASE SOLUTION

A sodium carbonate solution was prepared and added to the stated concentrations of HP β CD and prilocaine or lignocaine base to ensure that the drugs remained in their molecular form. The pH was maintained at 10, which was 2 pH units above the pKa (\approx 8.0). The pH meter used was a Microprocessor Bench pH Meter (HI 8417), Hanna instruments, USA.

2.2.6 PREPARATION OF 0.5M SODIUM CARBONATE SOLUTION

A 50mL solution of 0.5M sodium carbonate was prepared as follows.

Sodium carbonate anhydrous (2.2g) was weighed accurately and dissolved with minimum quantity of Milli Q water. The solution was then transferred to a 50 mL volumetric flask and the washings transferred to the solution. This was then made to volume.

2.2.7 PREPARATION OF 5M SODIUM HYDROXIDE SOLUTION

The 5M stock solution of sodium hydroxide was prepared using following procedure. Sodium hydroxide was weighed accurately and transferred to a beaker. A minimum amount of Milli Q water was added to dissolve sodium hydroxide completely. This was then transferred to 100mL volumetric flask and the washings of the beaker added to it. The final volume was achieved by adding Milli Q water after bringing it to 25°C.

2.3 SOLUBILITY STUDIES OF PRILOCAINE AND LIGNOCAINE ALONE AND IN COMBINATION AT 25° C

2.3.1 SOLUBILITY OF PRILOCAINE WITH HPβCD

The solubility studies for prilocaine base and HPβCD were carried out using the phase solubility method. The pKa of prilocaine is 7.9⁷⁵ therefore the solubility studies were carried out at pH 10.0 using Na₂CO₃ buffer. The studies were carried out in the concentration range of 0-30% w/v of HPβCD.

Excess amounts of prilocaine were added to glass vials containing different concentrations of HPβCD with Na₂CO₃ as buffer. The vials were sealed with screw caps and were shaken at 25 ± 0.2°C for 24 h. A magnetic stirrer (Variomag model 20 P) at the speed of 300 was used for the samples. After 24 hours, the samples were withdrawn from the vials and were centrifuged using an Eppendorf[®] mini spin centrifuge at 10,000 rpm for 10 min. The samples were then filtered through a GHP Acrodisc 13 mm Minispike Outlet 0.2μm syringe filters, (Gelman Pall, New York, America). The first drops of the sample were rejected and the rest collected and further dilutions prepared. Two dilutions of 0.1 in 10mL (1 in 10,000) were used. These samples were then injected on to the HPLC column for analysis. The mobile phase was 55:45 methanol:water with 20mM phosphate buffer at pH 5.5. The mobile phase was changed from 70:30 methanol:water to 55:45 methanol:water and the buffer remained the same.

2.3.2 SOLUBILITY OF LIGNOCAINE WITH HPβCD

The solubility studies with lignocaine base and HPβCD were determined using the method described in Section 2.3.1. Lignocaine has a pKa of 7.8⁷⁵, and therefore the solubility studies were carried out at pH 10.0 using Na₂CO₃. The concentration range for solubility studies with HPβCD was from 0-30% w/v in buffer. The mobile phase was 55:45 methanol:water with 20mM phosphate buffer at pH 5.5.

2.3.3 SOLUBILITY OF PRILOCAINE AND LIGNOCAINE WITH HPβCD

These studies were performed with a combination of prilocaine and lignocaine to determine the complexation with HPβCD. The solubility studies were carried using the same method as described in Section 2.3.1.

The individual solubilities of prilocaine and lignocaine and the solubilities of each in combination were determined using the phase solubility diagram and the association constant, $K_{1:1}$, was calculated from Equation 1 given below:

$$K_{1:1} = \frac{Slope}{S_0(1-Slope)} \quad \text{Eq (16)}$$

where, S_0 is the solubility in buffer at pH 10.0.

2.3.4 SOLUBILITY STUDIES OF PRILOCAINE AND LIGNOCAINE ALONE AND IN COMBINATION AT ELEVATED TEMPERATURES

Solubility studies for prilocaine, lignocaine, alone and in combination were carried out using the method described in Section 2.3.1. A shaking incubator (Labline Incubator), with the accuracy of ($\pm 0.2^\circ\text{C}$) was used to maintain temperatures. All the micropipettes, glasswares including the volumetric flasks, and the GHP Acrodisc 13 mm Minispike Outlet 0.2 μm Syringe filters along with the syringes were maintained at the same temperatures of 35 and 42 $^\circ\text{C}$ respectively to avoid precipitation.

The solutions of prilocaine base and lignocaine base alone and prilocaine with lignocaine were centrifuged using an Eppendorf[®] centrifuge, at 4000 rpm set at the temperatures of 35 $^\circ\text{C}$ and 42 $^\circ\text{C}$ respectively. The dilutions were done immediately using glasswares and filters maintained at the same temperatures. The mobile phase used remained the same with 20mM phosphate buffer at pH 5.5.

2.4 NUCLEAR MAGNETIC RESONANCE (NMR)

One-dimensional ^1H NMR spectra of prilocaine-HP β CD complexes and lignocaine-HP β CD complexes were recorded with a 600.13 MHz Bruker AV-600 spectrometer at 25°C. The NMR's were performed by Dr Lindsay Byrne (School of Biomedical, Biomolecular and Chemical Sciences, University of Western Australia, WA). Samples were dissolved in D₂O. To each 1mL of 0.5M Na₂CO₃ buffer was added and the mixture freeze dried to remove moisture. The samples were prepared with and without HP β CD. The samples were finally redissolved in D₂O prior to NMR analysis and sealed in ampoules. The chemical shifts ($\Delta\delta$) were reported as ppm and were referenced to the residual water signal.

3 RESULTS

3.1 DETERMINATION OF UV SPECTRA FOR PRILOCAINE HYDROCHLORIDE, LIGNOCAINE HYDROCHLORIDE, AND TETRACAINE HYDROCHLORIDE

The UV spectra for prilocaine hydrochloride, lignocaine hydrochloride, and tetracaine hydrochloride showed absorbance between 200 nm and 250 nm. The solvent used was Milli Q water and eqimolar concentrations of all the three drugs were used. The method is given in Section 2.2.1. The extinction coefficient of UV was found to be adequate at 230nm for all the three drugs. Representative UV spectra of all the three compounds are presented in Figure 8.

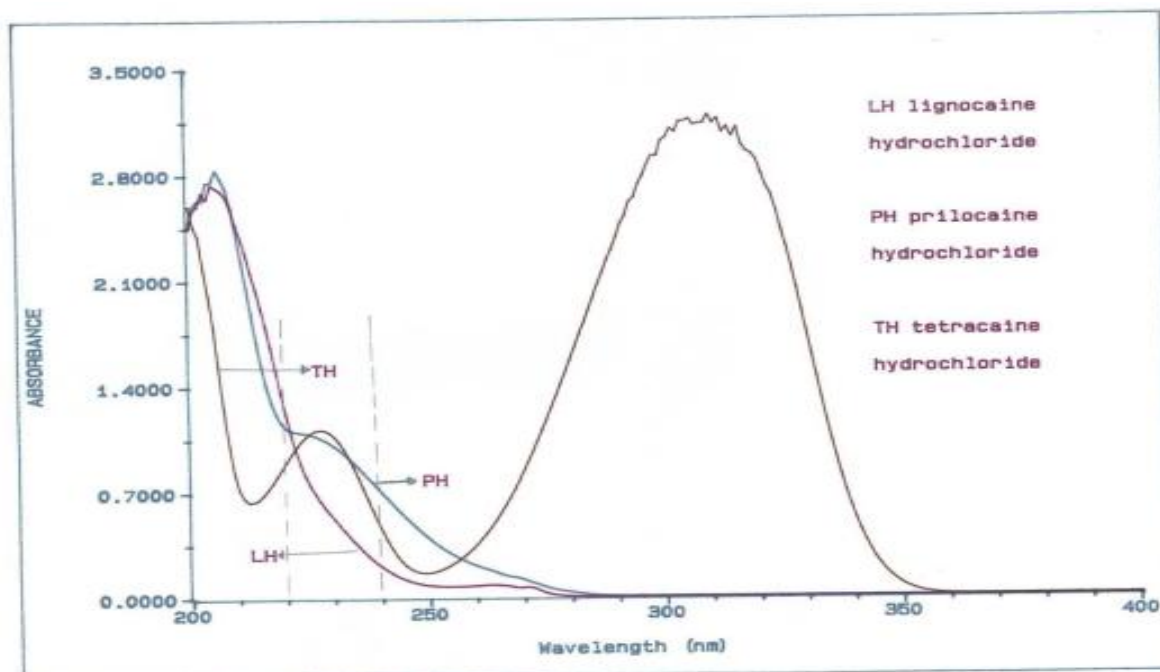


Figure 8: UV spectra of prilocaine hydrochloride, lignocaine hydrochloride, and tetracaine hydrochloride

3.1.1 STANDARD CURVES OF PRILOCAINE HYDROCHLORIDE, LIGNOCAINE HYDROCHLORIDE, AND TETRACAINE HYDROCHLORIDE

The calibration graphs for prilocaine hydrochloride, lignocaine hydrochloride and tetracaine hydrochloride were obtained using six different concentrations from 1-10 $\mu\text{g/mL}$ by the HPLC assay at 230 nm. The linear correlation coefficients R obtained for these three compounds were 0.999, 0.999, and 0.997 respectively for prilocaine hydrochloride, lignocaine hydrochloride, and tetracaine hydrochloride. Reproducible results were obtained with good separation of peaks. The retention times obtained for prilocaine hydrochloride, lignocaine hydrochloride, and tetracaine hydrochloride were 2.95 min, 6.38 min and 8.38 min respectively with a flow rate of 1.5 ml/min. The method is described in Section 2.2.2.1. Table 1 to Table 3 represents the six different concentrations of prilocaine hydrochloride, lignocaine hydrochloride, and tetracaine hydrochloride and Figure 9 to Figure 11 represent their calibration graphs.

The HPLC assay was validated. The lower limit of detection for all the three compounds was carried out at attenuation 5.0 and was estimated at 0.1 $\mu\text{g/mL}$ and varied below this concentration. The precision was calculated based on intraday and interday assays. The intraday assay was performed with samples of $n = 6$ of the same solutions at concentrations of 1.0, 4.0, 8.0, and 10.0 $\mu\text{g/mL}$. The intraday relative standard deviations (RSD) values for prilocaine hydrochloride, lignocaine hydrochloride and tetracaine hydrochloride were ranged from 0.43-2.90; 0.37-4.42, and 2.15-5.20 respectively. The interday RSD values for the above three drugs were based on $n = 5$ samples of the same solutions at the concentrations of 4.0, 8.0, and 10.0 $\mu\text{g/mL}$. The RSD values obtained varied between 0.59-1.46 for prilocaine hydrochloride, 1.34-3.67 for lignocaine hydrochloride and 2.82-5.60 for tetracaine hydrochloride. The retention times remained the same for all the three compounds. The method is described in Section 2.2.2.1.

Table 1: Concentrations and peak areas of prilocaine hydrochloride

Concentration ($\mu\text{g/mL}$)	Prilocaine hydrochloride area at 230 nm $\times 10^{-5}$
1	2.61
2	5.01
4	9.74
6	14.6
8	19.6
10	24.3

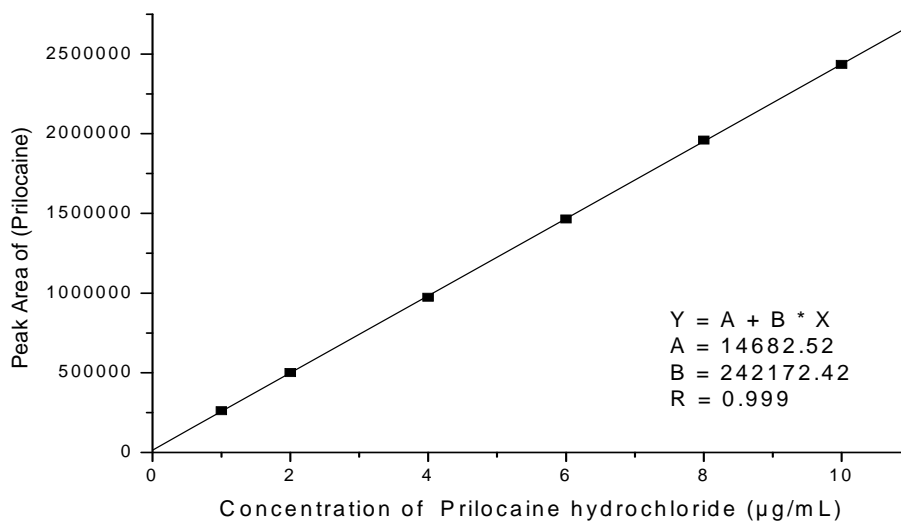


Figure 9: Calibration graph of prilocaine hydrochloride at 230 nm by HPLC

Table 2: Concentrations and areas of lignocaine hydrochloride

Concentration ($\mu\text{g/mL}$)	Lignocaine hydrochloride area at 230 nm x 10^{-5}
1	1.35
2	2.62
4	4.99
6	7.51
8	10.0
10	12.4

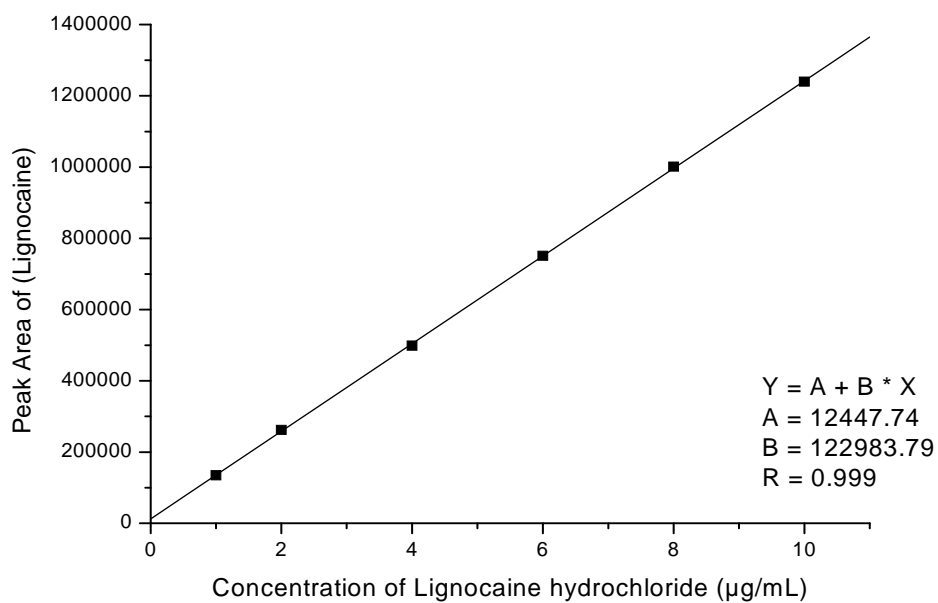


Figure 10: Calibration graph of lignocaine hydrochloride at 230 nm by HPLC

Table 3: Concentrations and peak areas of tetracaine hydrochloride

Concentration ($\mu\text{g/mL}$)	Tetracaine hydrochloride area at 230 nm $\times 10^{-5}$
1	2.85
2	5.10
4	8.61
6	15.0
8	20.1
10	25.9

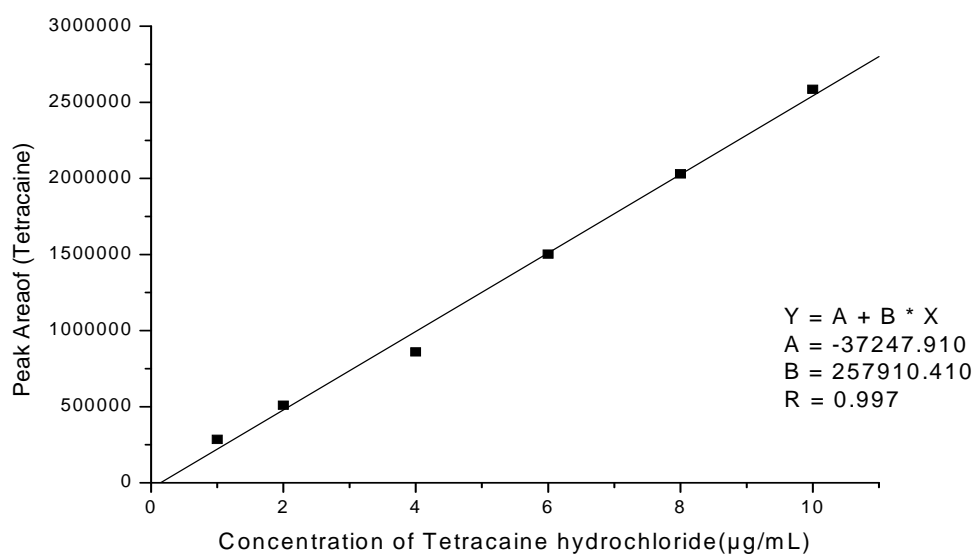


Figure 11: Calibration graph of tetracaine hydrochloride at 230 nm by HPLC

3.1.2 CALIBRATION ASSAYS FOR A COMBINATION OF PRILOCAINE HYDROCHLORIDE AND LIGNOCAINE HYDROCHLORIDE

The second assay was developed for a combination of prilocaine hydrochloride and lignocaine hydrochloride in the range of 10-100 $\mu\text{g/mL}$ (0.01-0.1 mg/mL) as described in Section 2.2.2.2. The wavelength used was 230 nm, and the coefficients of determination (R^2) obtained were 0.9999 for both prilocaine hydrochloride and lignocaine hydrochloride.

Table 4 shows the concentration range for prilocaine hydrochloride and lignocaine hydrochloride with the corresponding areas. Figure 12 represents the calibration graphs for prilocaine hydrochloride and lignocaine hydrochloride.

Table 4: Concentrations and areas of prilocaine hydrochloride and lignocaine hydrochloride

Concentration in mg/mL	Prilocaine hydrochloride area at 230 nm $\times 10^{-5}$	Lignocaine hydrochloride area at 230 nm $\times 10^{-5}$
0.01	2.41	1.19
0.02	4.02	1.96
0.04	7.22	4.07
0.06	10.84	6.09
0.08	14.21	7.91
0.10	17.37	9.81

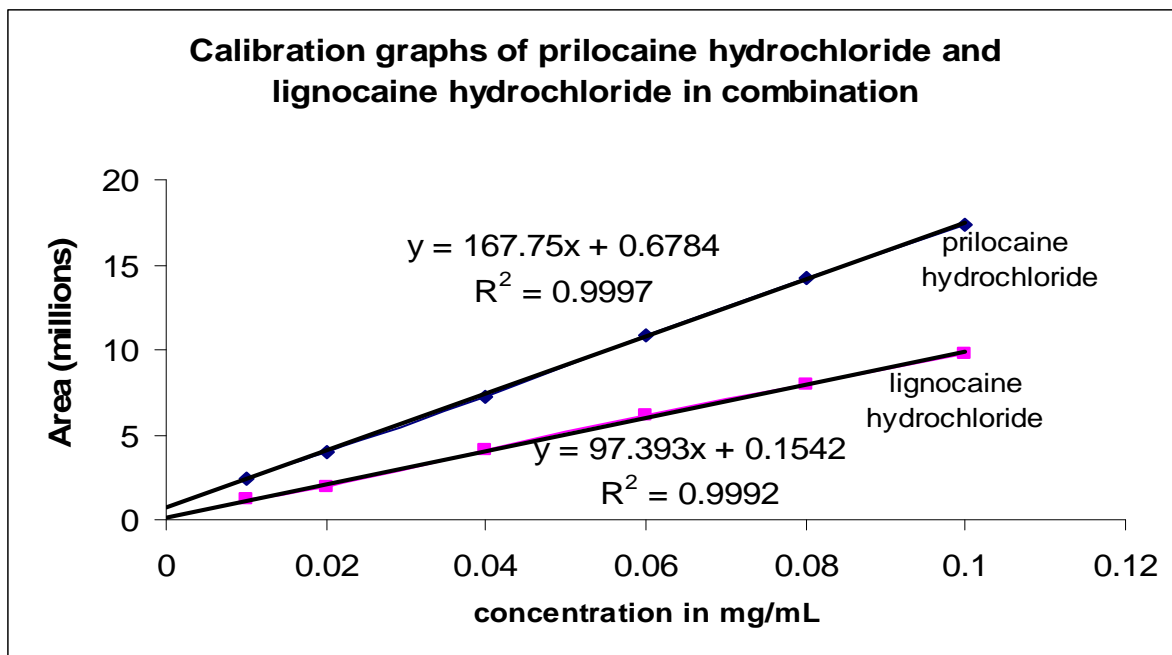


Figure 12: Calibration graphs of prilocaine hydrochloride and lignocaine hydrochloride in combination at 230 nm by HPLC

The mobile phase for the development of the first assay was 60:40 methanol:water with 20mM ammonium acetate buffer, whereas the mobile phase for the development of second assay was 70:30 methanol:water with 20mM phosphate buffer. The mobile phase concentration and buffer were changed to obtain sharper peaks and better results. The retention times for prilocaine hydrochloride and lignocaine hydrochloride changed from 2.95min and 6.38min to 2.92min and 6.30min respectively at the flow rate of 1.5 mL/min.

3.2 SOLUBILITY STUDIES

The solubility experiments for prilocaine and lignocaine were carried out in buffer solution at pH 10.0 at 25.0, 35.0, and 42.0°C. Section 3.2.4 represents solubilities and association constants calculated for prilocaine with HP β CD and lignocaine with HP β CD individually and prilocaine and lignocaine with HP β CD in combination. The solubility temperature relationship was obtained by plotting log S (S = Solubility) against the reciprocal of temperature in Kelvin. This is shown in Section 3.2.5.

3.2.1 SOLUBILITY STUDIES OF PRILOCAINE WITH HP β CD AT 25°C, 35°C, AND 42°C

High performance liquid chromatographic (HPLC) assays were used for the determination of prilocaine as described in Sections 2.3.1 and 2.3.4. The system provided a reproducible separation of prilocaine. The solubility curve of prilocaine in the presence of 0-30% HP β CD was obtained at 25°C. The solubility S determined over this concentration range of HP β CD and at 25°C was between 3.22-9.11 moles/L. At 35°C the solubility obtained was between 1.96-8.51 moles/L and at 42°C the solubility obtained was between 1.79-7.91 moles/L. The solubility S_0 in water (buffer) was calculated from the extrapolation of the line on the graph. The amount of prilocaine was calculated taking its hydrochloride salt determination from the assay into consideration. The mobile phase used at this stage was 55:45 methanol:water with 20mM phosphate buffer at pH 5.5. The change in mobile phase was made to move the prilocaine peak away from the void peak, and the retention time was thus changed from 2.95 min to 3.21 min.

3.2.2 SOLUBILITY DATA OF LIGNOCAINE WITH HP β CD AT 25°C, 35°C, AND 42°C

Solubility data for lignocaine were determined by HPLC at 25°C, 35°C, and 42°C over 0-30% HP β CD by the method described in Sections 2.3.2 and 2.3.4. The system provided a reproducible separation of lignocaine with the retention time of 7.9 min. The change in mobile phase from 70:30 methanol:water to 55:45 methanol:water moved the retention time of lignocaine from 6.30 min to 7.9 min. The solubilities S of lignocaine obtained over 0-30% concentration range of HP β CD at 25°C were between 1.69-4.41 moles/L, at 35°C the S value was between 1.04-4.22 moles/L, and

at 42°C the S value was between of 1.04-4.55 moles/L. Similar to prilocaine the solubilities of lignocaine base were done taking into consideration its assayed hydrochloride salt and the solubility graphs obtained at all temperatures were A_L type.

3.2.3 SOLUBILITY DETERMINATION OF A COMBINATION OF PRILOCAINE AND LIGNOCAINE WITH HPβCD AT 25°C, 35°C, AND 42°C

Solubilities of prilocaine and lignocaine at 25°C and at elevated temperatures were determined by HPLC using the method described in Sections 2.3.3 and 2.3.4. Reproducible results were obtained for prilocaine and lignocaine with retention times of 3.2 min and 7.9 min, respectively. The solubilities were obtained over the concentration range of 0-30% of HPβCD. At 25°C the solubilities S were between 0.91-2.67 moles/L and 1.03-2.82 moles/L for prilocaine and lignocaine, respectively. At 35°C the solubilities for prilocaine and lignocaine were 0.64-2.89 moles/L and 1.17-5.34 moles/L and at 42°C the solubilities for prilocaine and lignocaine were 0.66-3.68 moles/L and 1.80-8.35 moles/L, respectively. The curves obtained were of the A_L types (Figure 5).

Compared to the solubility data obtained alone for prilocaine and lignocaine the overall decrease in solubility was obtained for prilocaine but an increase in solubility was obtained for lignocaine in combination at higher temperatures.

3.2.4 SOLUBILITIES AND ASSOCIATION CONSTANTS OF PRILOCAINE AND LIGNOCAINE WITH HPβCD INDIVIDUALLY AND IN COMBINATION AT 25°C, 35°C AND 42°C

All the solubility isotherms obtained at different temperatures were classified as A_L type implying formation of soluble complexes, and showing linear increase in solubilities as a function of HPβCD concentration. The association constants

assuming a 1:1 stoichiometric complex ratio for each were calculated by Equation (17).

$$K_s = \frac{\text{slope}}{S_0(1 - \text{slope})} \quad \text{Eq (17)}$$

The association constants were calculated on the basis of moles/L and plotted as a function of concentration of HP β CD on X axis to concentration of prilocaine and/or lignocaine on Y axis. Table 14 represents the association constants for prilocaine and lignocaine alone and in combination.

Table 5 to

Table 7 show the values of solubilities in moles/L for prilocaine at 25°C, 35°C, and 42°C.

Table 8 to Table 10 show the values of solubilities in moles/L for lignocaine at elevated temperatures, and

Table 11 to Table 13 shows the values of solubilities in moles/L for prilocaine and lignocaine in combination at elevated temperatures. Figure 13 to Figure 15 represent graphs of linear fit for prilocaine, Figure 16 to Figure 18 represent graphs of linear fit for lignocaine. Figure 19 and Figure 20 represents polynomial fit for prilocaine and lignocaine in combination and Figure 21 to Figure 24 represent graphs of linear fit of prilocaine and lignocaine in combination.

Table 5: Solubility data for prilocaine determined at 25°C

Concentration of HP β CD moles/L x 10 ²	Concentration of prilocaine moles/L x 10 ²
0.00	3.22
3.44	4.11
6.87	5.22
10.30	6.17
13.70	6.94
17.20	8.16
20.60	9.11

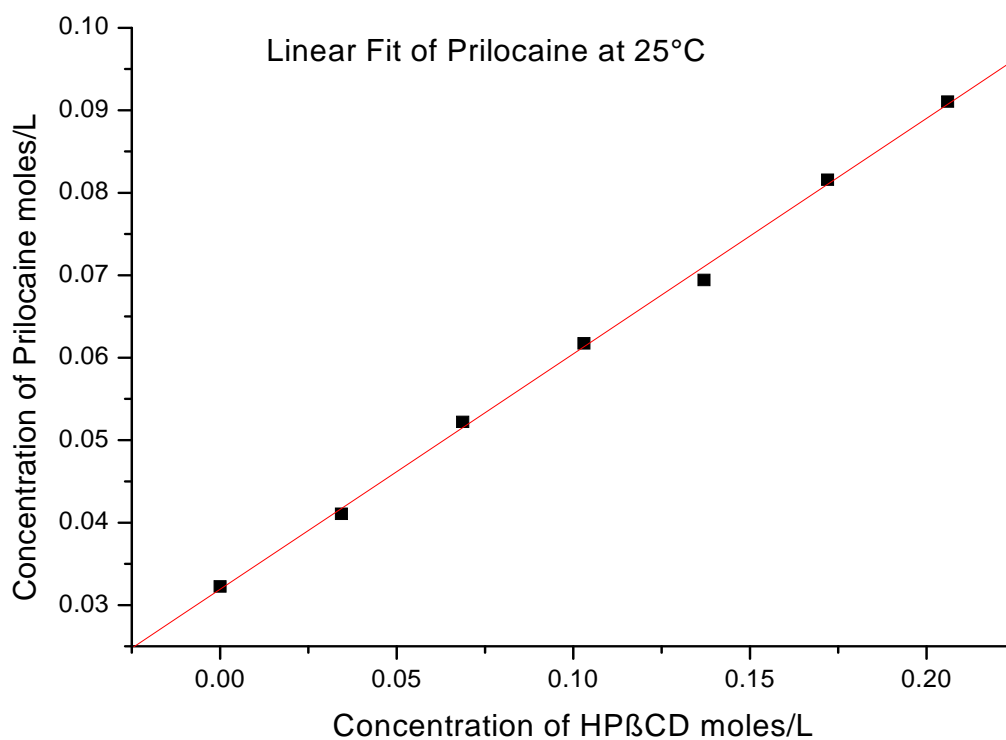


Figure 13: Solubility data for prilocaine at 25°C

Table 6: Solubility data for prilocaine determined at 35°C

Concentration of HP β CD moles/L x 10 ²	Concentration of prilocaine moles/L x 10 ²
0.00	1.96
3.44	3.14
6.87	4.44
10.30	4.98
13.70	7.04
17.20	7.44
20.60	8.51

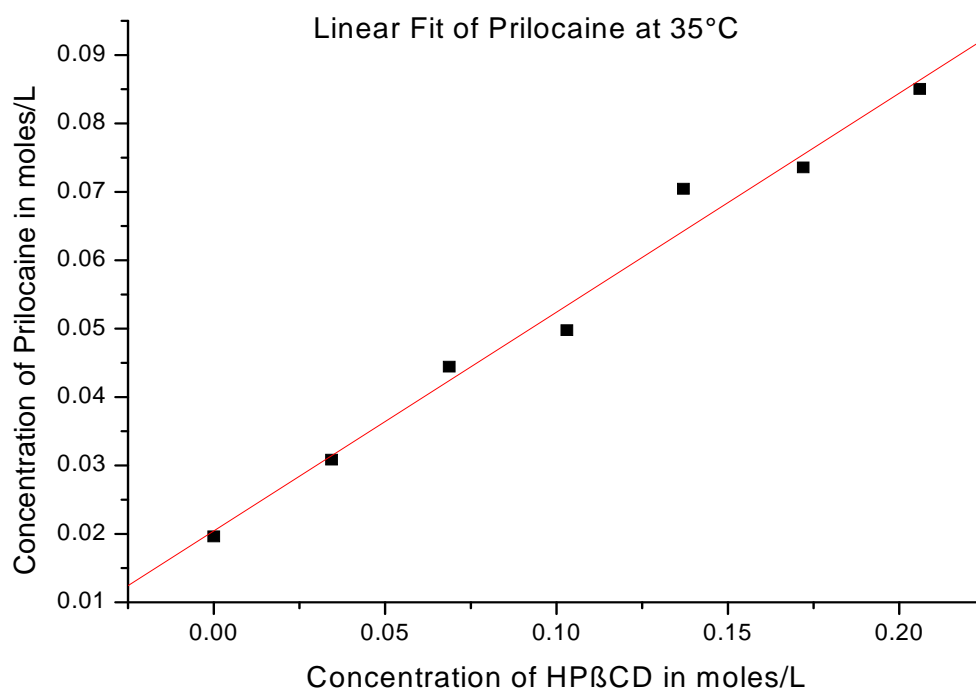


Figure 14: Solubility data for prilocaine at 35°C

Table 7: Solubility data for prilocaine determined at 42°C

Concentration of HP β CD moles/L x 10 ²	Concentration of prilocaine moles/L x 10 ²
0.00	1.79
3.44	2.96
6.87	3.51
10.30	4.58
13.70	6.23
17.20	7.31
20.60	7.91

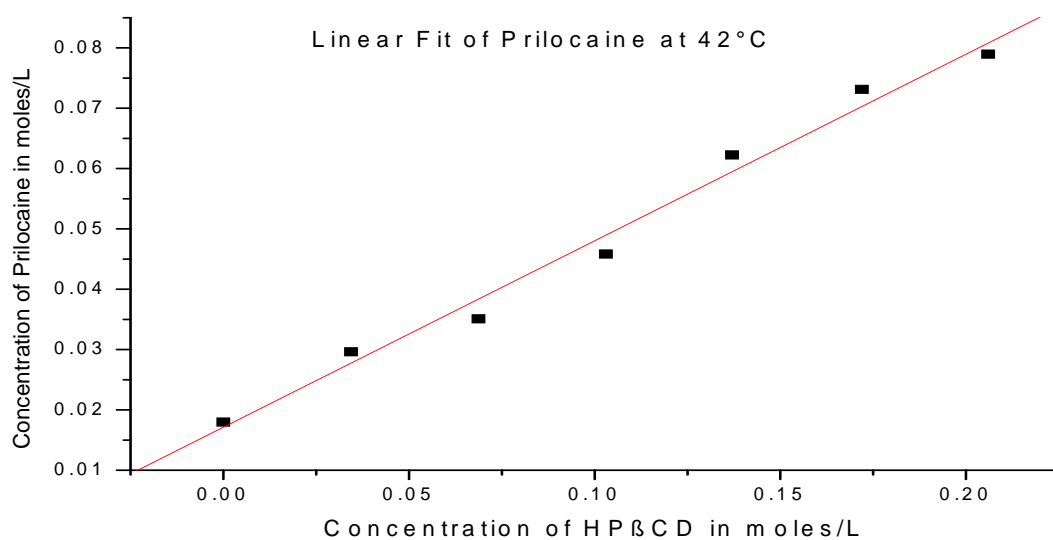


Figure 15: Solubility data for prilocaine at 42°C

The molar solubilities of prilocaine-HP β CD complex were found to decrease with respect to the elevated temperatures. This suggests an exothermic reaction of the complex at higher temperatures which is enthalpically dependent on temperature. The complex is proposed to be formed between the hydrophobic moieties of the drug (the

aromatic ring of prilocaine) and HP β CD giving out heat in the process of complexation.

Table 8: Solubility data for lignocaine determined at 25°C

Concentration of HP β CD moles/L x 10 ²	Concentration of lignocaine moles/L x 10 ²
0.00	1.69
3.44	1.89
6.87	2.72
10.30	3.38
13.70	3.75
17.20	4.17
20.60	4.41

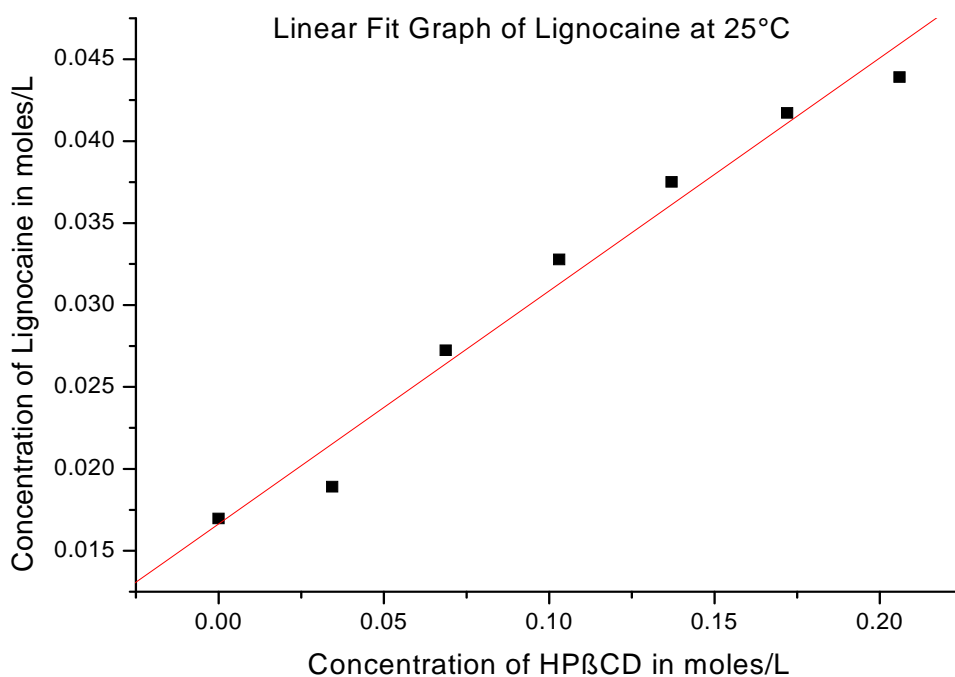


Figure 16: Solubility data for lignocaine at 25°C

Table 9: Solubility data for lignocaine determined at 35°C

Concentration of HPβCD moles/L x 10 ²	Concentration of lignocaine moles/L x 10 ²
0.00	1.04
3.44	1.33
6.87	2.14
10.30	2.76
13.70	3.70
17.20	4.29
20.60	4.22

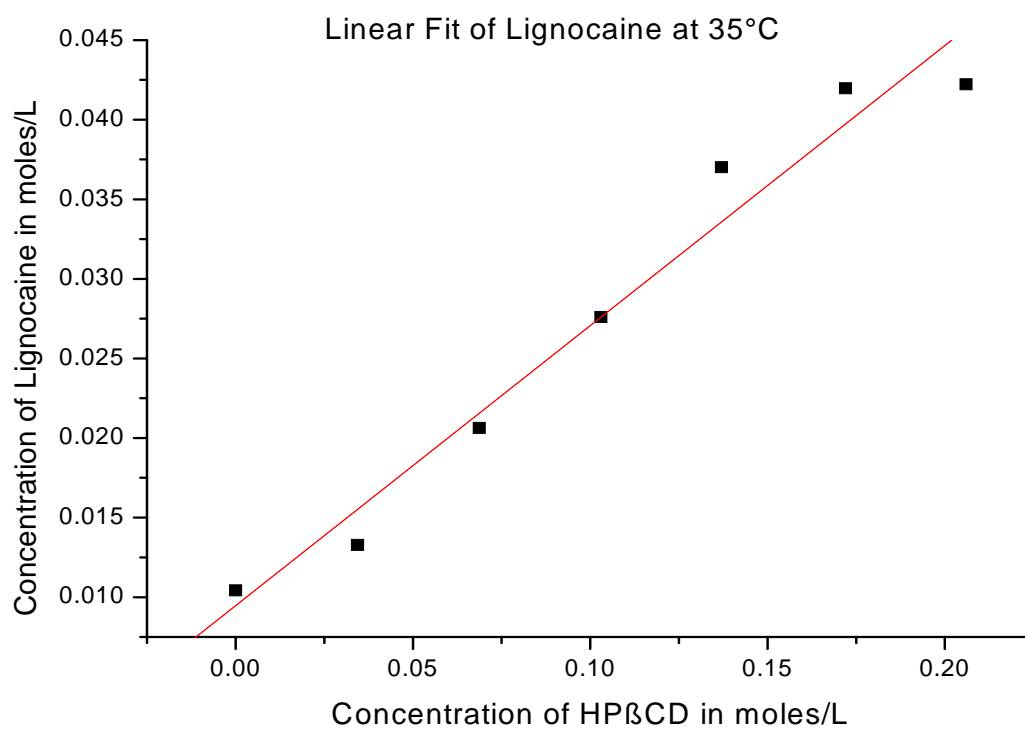


Figure 17: Solubility data for lignocaine at 35°C

Table 10: Solubility data for lignocaine determined at 42°C

Concentration of HP β CD moles/L x 10 ²	Concentration of lignocaine moles/L x 10 ²
0.00	1.04
3.44	1.43
6.87	2.11
10.30	3.12
13.70	4.17
17.20	4.41
20.60	4.55

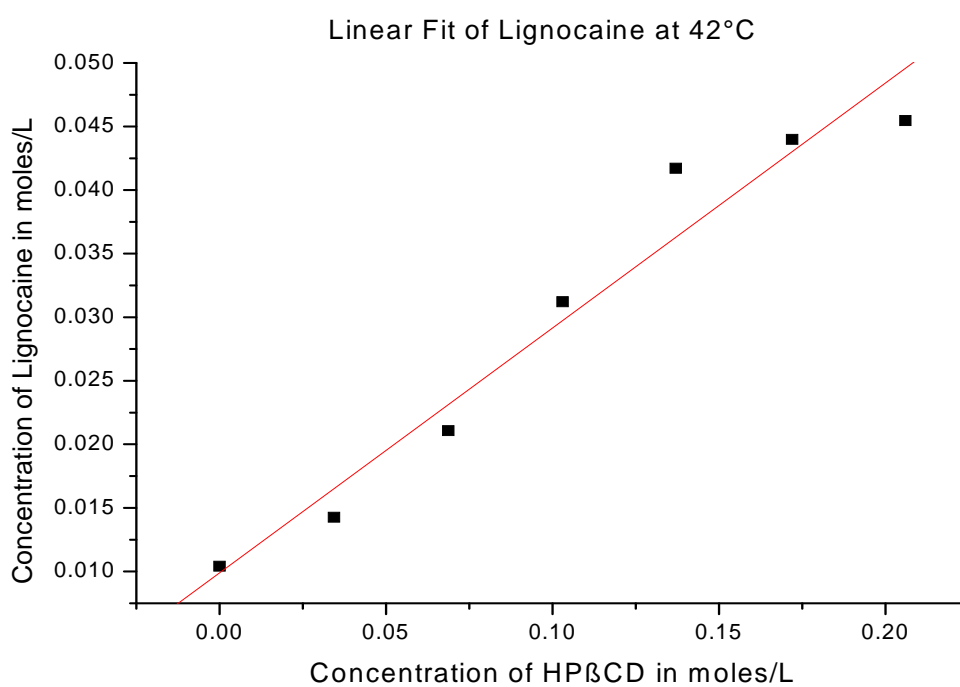


Figure 18: Solubility data for lignocaine at 42°C

Lignocaine do not show a significant change in molar solubility with lignocaine-HP β CD complex at higher temperature to any extent. This suggests that the reaction is almost independent of temperature change.

Table 11: Solubility data's for prilocaine and lignocaine when in combination at 25°C

Concentration of HP β CD moles/L x 10 ²	Concentration of prilocaine moles/L x 10 ²	Concentration of lignocaine moles/L x 10 ²
0.00	0.91	1.03
3.44	1.69	1.58
6.87	2.29	2.20
10.30	2.49	2.45
13.70	2.66	2.59
17.20	2.75	2.77
20.60	2.67	2.82

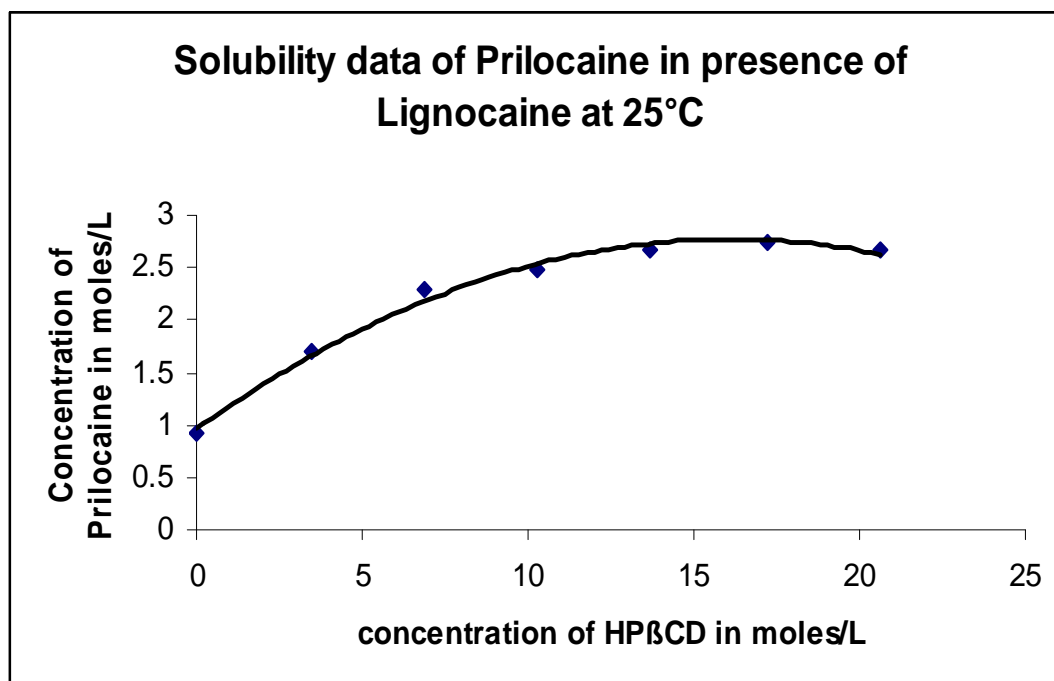


Figure 19: Solubility data for prilocaine at 25°C

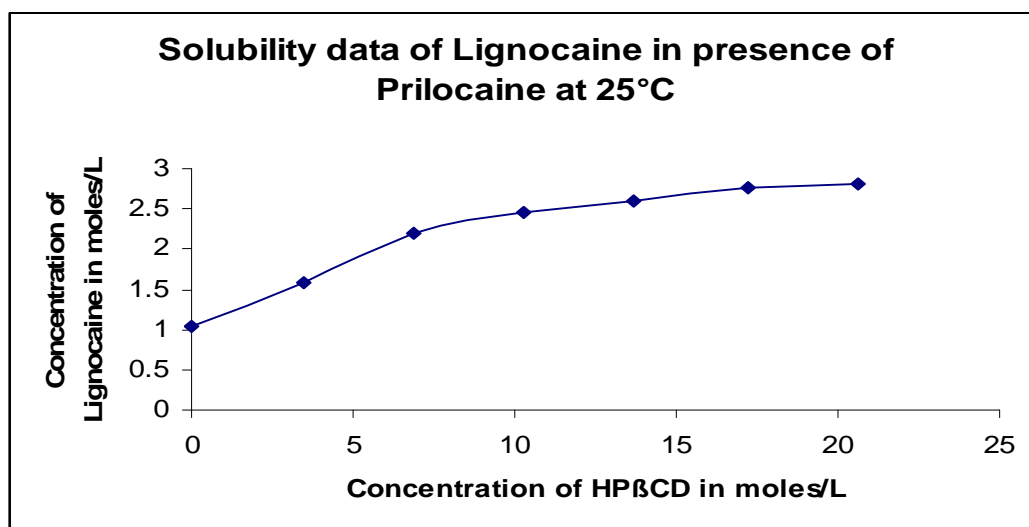


Figure 20: Solubility data for lignocaine at 25°C

In Figure 17 and Figure 18, the first three points are linear, and the complex formation is increasing with increasing concentrations of HPβCD. The figures do not represent linear fit graphs. All the other S shaped concentrations of lignocaine and prilocaine were linearly fit without much error.

Table 12: Solubility data's for prilocaine and lignocaine when in combination at 35°C

Concentration of HPβCD moles/L x 10 ²	Concentration of Prilocaine moles/L x 10 ²	Concentration of Lignocaine moles/L x 10 ²
0.00	0.64	1.17
3.44	0.84	1.33
6.87	1.47	2.12
10.30	1.45	2.67
13.70	1.69	3.27
17.20	2.13	4.42
20.60	2.89	5.34

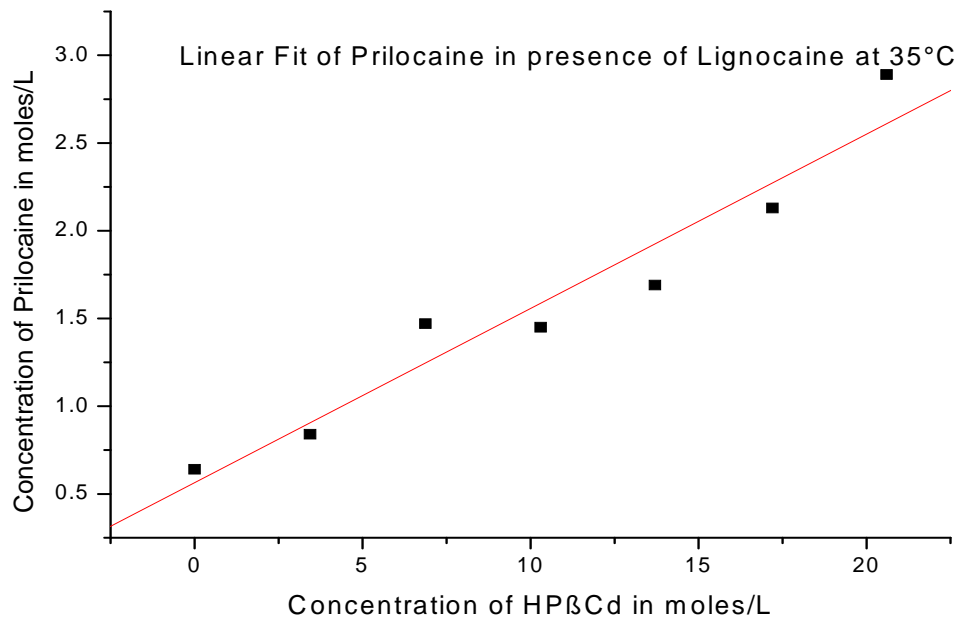


Figure 21: Solubility data for prilocaine at 35°C

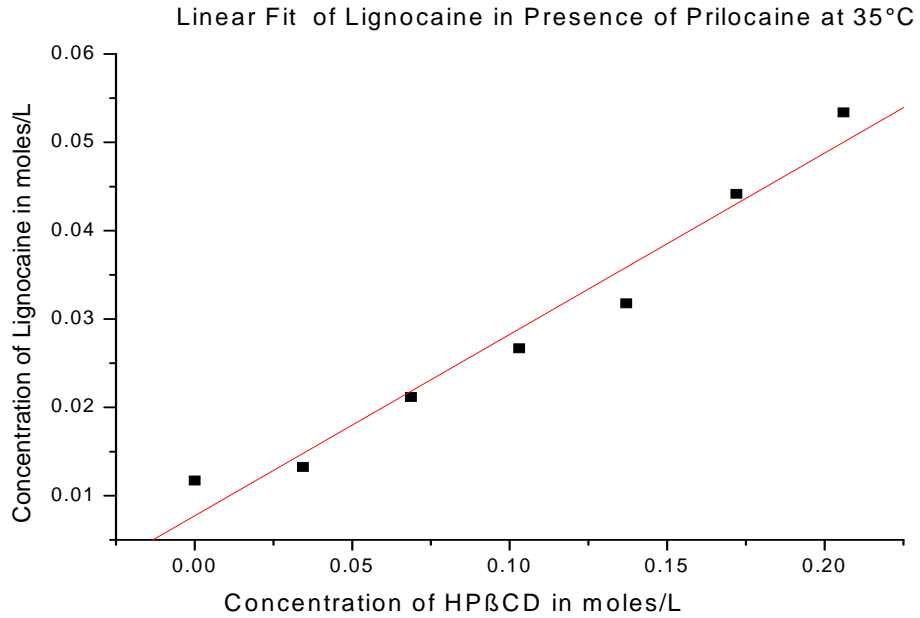


Figure 22: Solubility data for lignocaine at 35°C

Table 13: Solubility data's for prilocaine and lignocaine when in combination at 42°C

Concentration of HPβCD moles/L x 10 ²	Concentration of prilocaine moles/L x 10 ²	Concentration of lignocaine moles/L x 10 ²
0.00	0.66	1.80
3.44	1.06	2.98
6.87	1.67	3.95
10.30	1.96	5.57
13.70	2.45	6.38
17.20	3.40	7.72
20.60	3.68	8.35

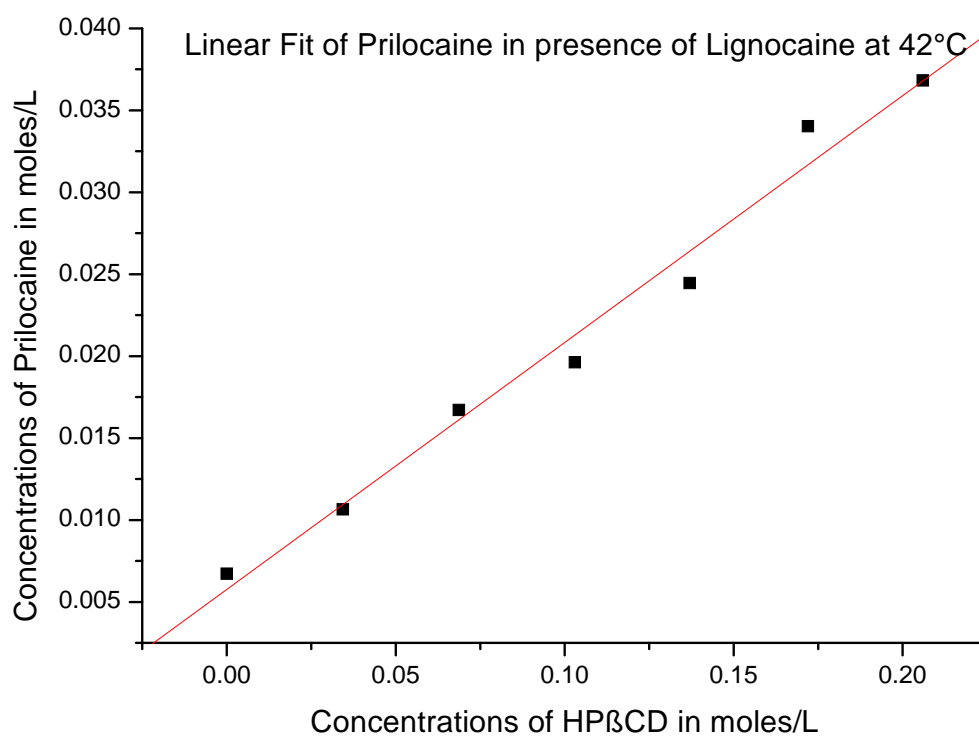


Figure 23: Solubility data for prilocaine at 42°C

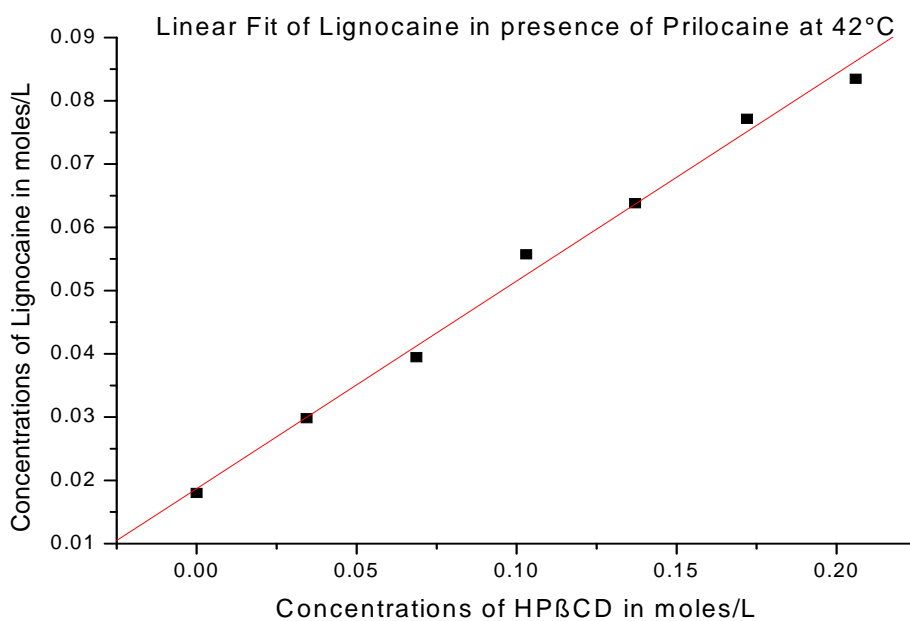


Figure 24: Solubility data for lignocaine at 42°C

Table 14: Association constants (K) of prilocaine, lignocaine and prilocaine and lignocaine at different temperatures

Drug	Temperature °C	Association Constants
Prilocaine	25°C	12.7
	35°C	23.2
	42°C	22.8
Lignocaine	25°C	9.9
	35°C	30.8
	42°C	21.1
Prilocaine and Lignocaine	25°C	27.5 and 19.0
	35°C	12.5 and 18.1
	42°C	27.3 and 17.5

In combination, the overall association constant values for prilocaine seem to decrease from 25°C to 35°C and then increase between 35°C and 42°C, whereas for lignocaine it seems to decrease with rise in temperatures. Individually for prilocaine, the association constants show an increase from 25°C to 35°C and then a slight decrease, the same is true for lignocaine too. This suggests that there is probably more than one mechanism involved in the formation of prilocaine-HPβCD and lignocaine-HPβCD complexes. Also, it is noted that the association constants are relatively small in value.

3.2.5 EFFECT OF ENTHALPY, ENTROPY AND FREE ENERGY ON SOLUBILITIES STUDIES OF PRILOCAINE AND LIGNOCAINE ALONE AND IN COMBINATION WITH HPβCD.

The enthalpy (ΔH°), entropy (ΔS°), and the free energy (ΔG°) for the drugs were calculated using the following equations, where R = gas constant in joules/calories

$$\text{Log } S = \frac{-\Delta H^\circ}{RT} + \text{constant} \quad \text{Eq (18)}$$

$$\Delta G^\circ = -2.303RT \log S \quad \text{Eq (19)}$$

$$\Delta S^\circ = \frac{\Delta H^\circ - \Delta G^\circ}{T} \quad \text{Eq (20)}$$

The standard states were defined as unit molarity and the ΔH° was determined by plotting reciprocal of temperature (T) in Kelvin versus log solubilities for prilocaine and lignocaine. These data are listed in Table 15 to Table 18.

Table 15: Log of solubilities of prilocaine at different temperatures

Temperature K ⁻¹	Log Solubility	of Prilocaine	in moles/L	
	0 % HPβCD	10 % HPβCD	20 % HPβCD	30 % HPβCD
3.17 x 10 ⁻³	-1.769	-1.456	-1.208	-1.108
3.25 x 10 ⁻³	-1.721	-1.357	-1.155	-1.071
3.35 x 10 ⁻³	-1.495	-1.284	-1.161	-1.041

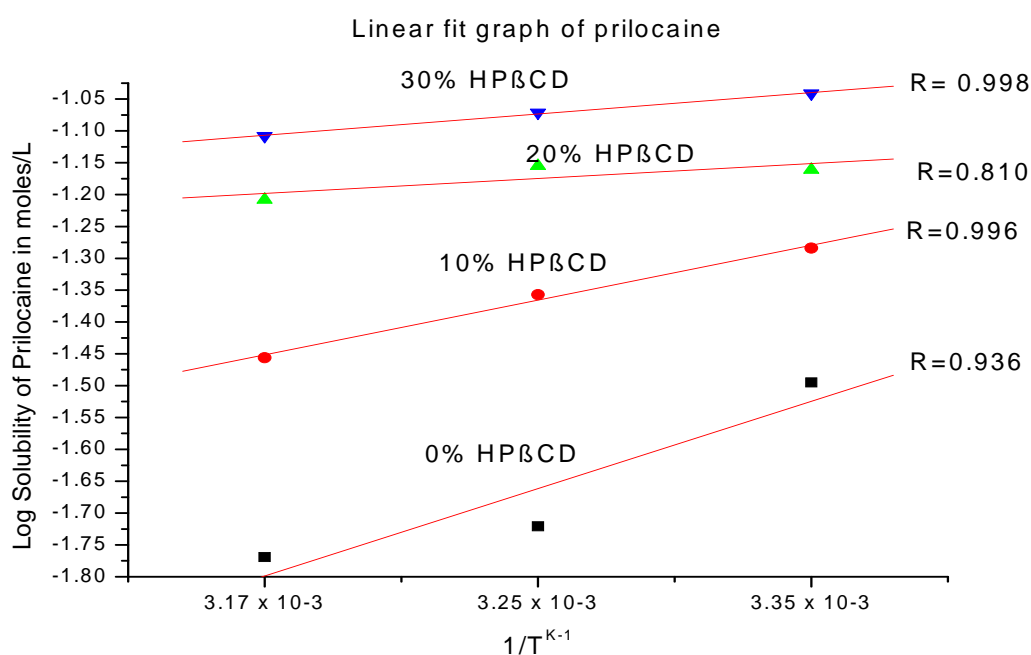


Figure 25: Linear fit graph showing R values for Prilocaine.

In figure 23 the linear fit graph showing R values for prilocaine obtained a linear relationship for 10% HPβCD and 30% HPβCD. R values greater than 0.95 were considered to give an adequate linear relationship following the van't Hoff equation. The ΔH° , ΔG° , and ΔS° values are given in Table 19. The log solubility of prilocaine seems to decrease with increase in temperatures as can be seen from Table 15. However the log solubility values are increased with increase in concentration of HPβCD. This suggests that although solubility of prilocaine is temperature dependent

an increase in solubility is obtained at higher concentrations of HP β CD. The enthalpy and entropy values as in Table 19 give an idea of how the complexation of prilocaine-HP β CD occurs. As is seen in Table 19 the ΔH° values are relatively similar and small difference exists between enthalpies dependent upon the concentration of HP β CD. A greater difference in entropy values was obtained and this suggests that complexation is governed more by entropy rather than enthalpy. Hence the orientation of the drug in the complex may be an important factor.

Table 16: Log of solubilities of lignocaine different temperatures

Temperature K ⁻¹	Log Solubility	of Lignocaine	in moles/L	
	0 % HP β CD	10 % HP β CD	20 % HP β CD	30 % HP β CD
3.17×10^{-3}	-2.000	-1.678	-1.387	-1.347
3.25×10^{-3}	-2.000	-1.699	-1.432	-1.377
3.35×10^{-3}	-1.796	-1.596	-1.432	-1.367

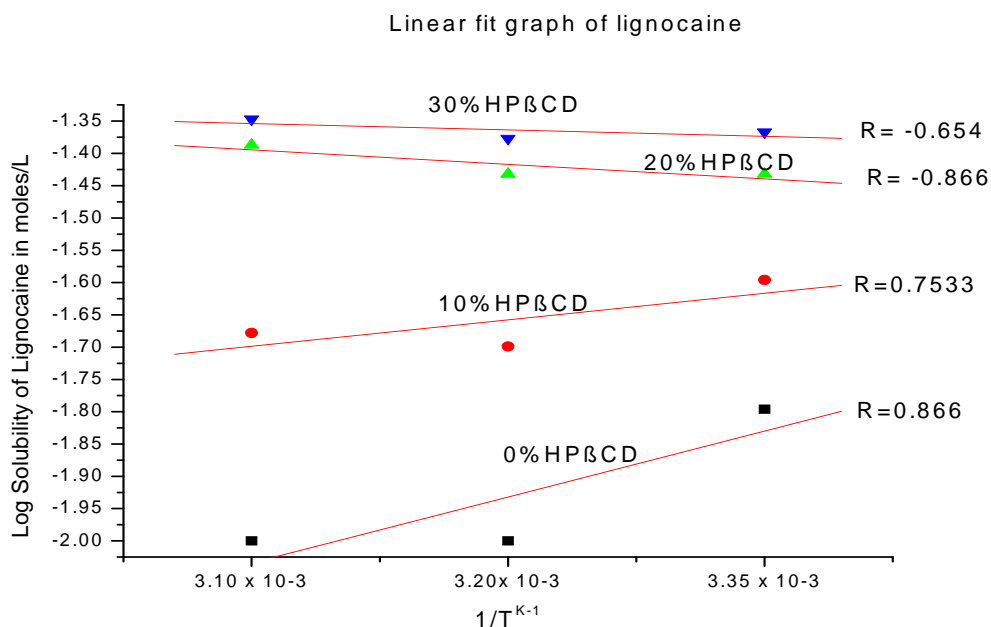


Figure 26: Linear fit graph showing R values for lignocaine.

In Figure 24 the linear fit graph shows that solubility of lignocaine increases with increase in concentration of HP β CD, however it is not very significant change and therefore when the temperature was raised almost no change in the log solubility values were obtained when plotted against absolute temperature. The nonlinear relationship was obtained for all the concentrations of HP β CD and thus van't Hoff's equation was not followed between any two of the temperatures.

Table 17: Log solubilities of prilocaine and lignocaine in combination at different temperatures

Temperature K ₁	Log Solubility	of Prilocaine	in presence of	Lignocaine in moles/L
			0 % HP β CD	10 % HP β CD
			20 % HP β CD	30 % HP β CD
3.17×10^{-3}	-2.174	-1.795	-1.619	-1.444
3.25×10^{-3}	-2.193	-1.833	-1.770	-1.540
3.35×10^{-3}	-2.046	-1.641	-1.575	-1.574

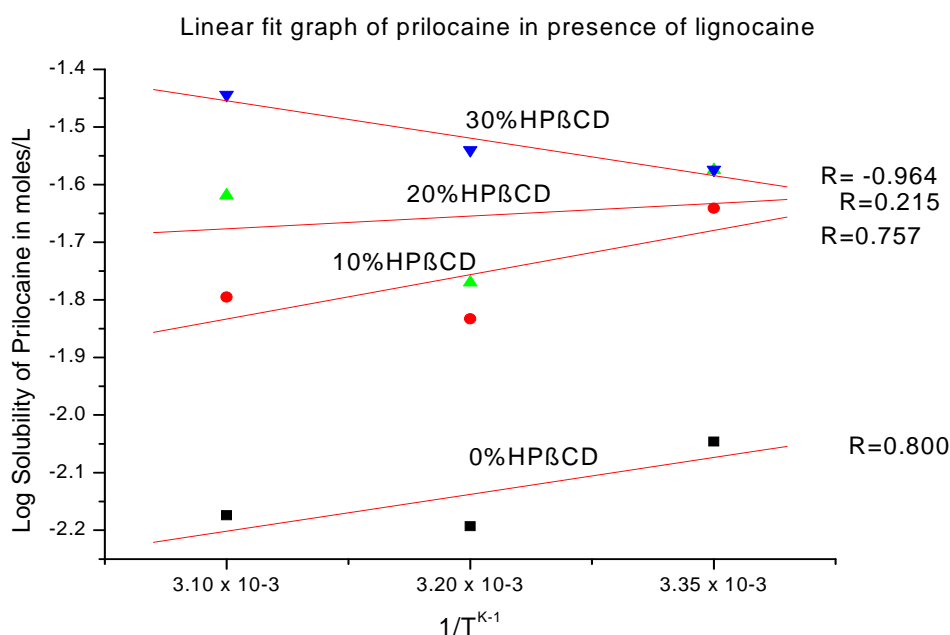


Figure 27: Linear fit graph showing R values for Prilocaine in presence of Lignocaine.

Linear fit graph showing R values for Prilocaine in presence of Lignocaine that shows the complex formation of prilocaine in presence of lignocaine with HP β CD. The log solubility seems to increase with increase in concentrations of HP β CD from 0 to 30%. However the log solubility was found to decrease with increase in temperature. The concentration of HP β CD (0%-20%) showed nonlinear relationships whereas a linear relationship was obtained for 30% HP β CD and was associated with the negative ΔH° . The ΔH° value obtained was very small and ΔS° value was very large suggesting hydrophobic interactions during complex formation.

Table 18: Log solubilities of lignocaine in combination with prilocaine at different temperatures

Temperature K ⁻¹	Log Solubility	of Lignocaine	in presence	of Prilocaine
	0 % HP β CD	10 % HP β CD	20 % HP β CD	30 % HP β CD
3.17×10^{-3}	-1.745	-1.408	-1.201	-1.081
3.25×10^{-3}	-1.958	-1.678	-1.495	-1.276
3.35×10^{-3}	-2.076	-1.748	-1.677	-1.641

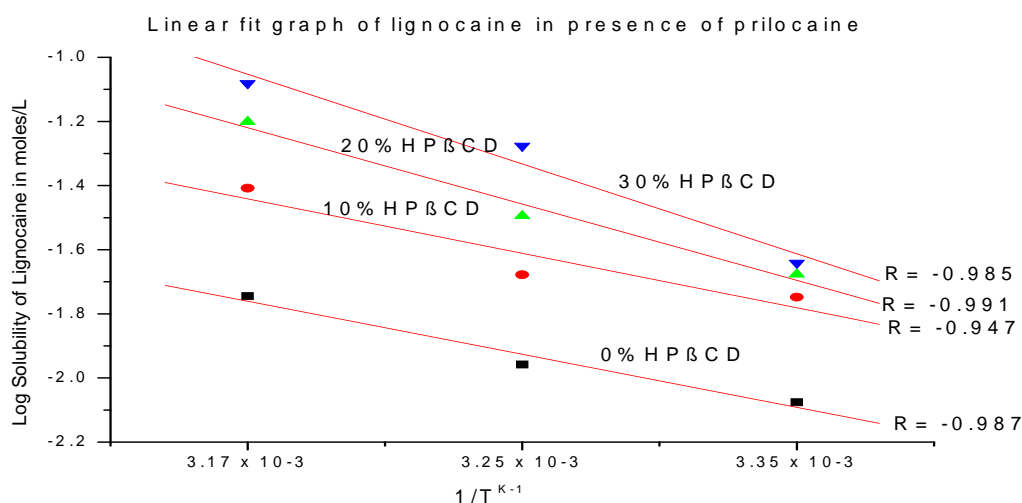


Figure 28: Linear fit graph showing R values of Lignocaine in presence of Prilocaine.

Linear fit graph showing R values of lignocaine in presence of prilocaine suggesting that a linear relationship is followed at all the concentrations of HP β CD and van't Hoff equation is followed. The log solubility increases with increase in concentration of HP β CD and with rise in temperature. However when ΔH° and ΔS° values were calculated (Table 19) a small positive ΔH° and a large ΔS° was obtained suggesting that the complex formation is driven by entropy. The negative R values gave a negative intercept.

Table 19: Thermodynamic parameters of prilocaine and lignocaine following a linear relationship at 25°C, 35°C and 42°C

Prilocaine	$\Delta H^\circ \text{ Jmol}^{-1}$	$\Delta G^\circ \text{ Jmol}^{-1}$	$\Delta S^\circ \text{ JK}^{-1}\text{mol}^{-1}$
0% HP β CD	-2.62	20761.11	-65.92
10% HP β CD	-1.65	14978.25	-47.55
30% HP β CD	-0.64	9099.50	-28.89
Prilocaine in presence of Lignocaine	$\Delta H^\circ \text{ Jmol}^{-1}$	$\Delta G^\circ \text{ Jmol}^{-1}$	$\Delta S^\circ \text{ JK}^{-1}\text{mol}^{-1}$
30% HP β CD	-25.20	247650.25	786.27
Lignocaine in presence of Prilocaine	$\Delta H^\circ \text{ Jmol}^{-1}$	$\Delta G^\circ \text{ Jmol}^{-1}$	$\Delta S^\circ \text{ JK}^{-1}\text{mol}^{-1}$
0% HP β CD	2.44	-10063.91	-39.94
10% HP β CD	2.38	-11487.31	-36.46
20% HP β CD	3.84	-25215.26	-80.04
30% HP β CD	4.50	-31825.02	-101.02

3.3 INVESTIGATION OF HPβCD COMPLEX FORMATION WITH PRILOCAINE AND LIGNOCAINE BY NMR

Complex formation of prilocaine and lignocaine with HPβCD was also investigated by NMR. NMR spectroscopy is one of the most used techniques to obtain information about the geometry of inclusion complexes. The chemical formula of prilocaine is $C_{13}H_{20}N_2O$ and the chemical formula of lignocaine is $C_{14}H_{22}N_2O$. Figure 29 and Figure 30 represents the chemical structures of prilocaine and lignocaine for evaluating the chemical shift and changes in characterisation of peaks obtained by complexation with HPβCD. Table 20 and Table 21 represents the protons of prilocaine and lignocaine involved in a chemical shifts with and without HPβCD and their $\Delta\delta$ (ppm).

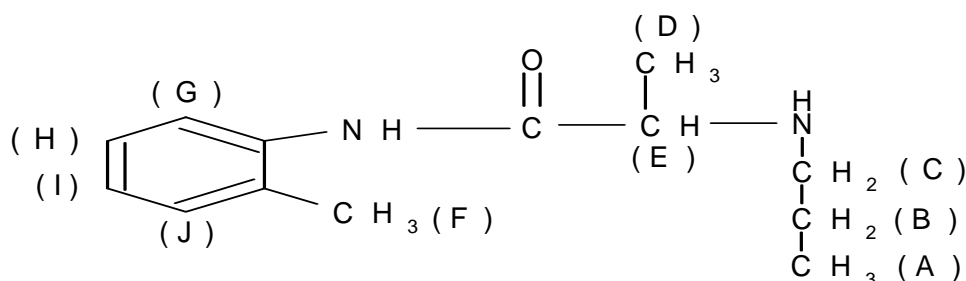


Figure 29: Chemical structure of prilocaine.

Table 20: 1H NMR showing chemical shift for prilocaine with and without HPβCD

Assignments	Prilocaine without HPβCD (ppm)	Prilocaine with HPβCD (ppm)	$\Delta\delta$ (ppm)
A- triplet	0.905	0.763	- 0.142
B- multiplex	1.515	1.373	- 0.142
C- triplet	2.569	2.424	- 0.145
D- doublet	1.370	1.235	- 0.135
E- quadruplet	3.505	*	*
F- singlet	2.214	2.132	- 0.082
G and I- broad doublet	7.296	7.113	- 0.183
H and J- broad multiplex	7.297	7.193	- 0.104

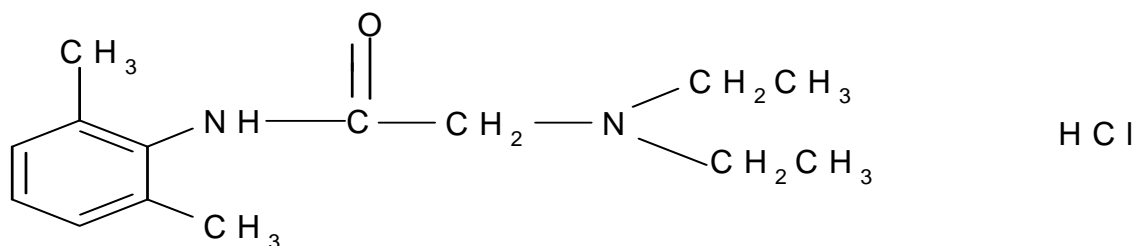


Figure 30: Chemical structure of lignocaine

Table 21: ¹H NMR showing chemical shift for lignocaine with and without HPβCD

Assignments	Lignocaine without HPβCD (ppm)	Lignocaine with HPβCD (ppm)	Δδ (ppm)
A – triplet	1.113	*	*
B – quadruplet	2.710	2.570	- 0.140
C – singlet	3.404	*	*
D – singlet	2.180	2.062	- 0.118
E – broad doublet	7.180	7.033	- 0.147
F – broad multiplex	7.218	7.065	- 0.153

An (*) means no chemical shift was obtained.

In the ¹H-NMR peak assignments for free and complex prilocaine and lignocaine the corresponding shifts Δδ is represented as Δδ = δ (complex) – δ (free). The prilocaine and prilocaine HPβCD and ligocaine and lignocaine HPβCD complexes were prepared as previously described in Section 2.4 and were dissolved in D₂O. Chemical shifts were given in parts per million relative to the signal obtained. ¹H NMR studies showed upfield shifts for all the protons from A-H for prilocaine and A-F for lignocaine.

Figure 31 (a), (b), (c), (d), and (e) represents the NMR peaks where,

(a) NMR of HP β CD

(b) NMR of prilocaine

(c) NMR of prilocaine with HP β CD

(d) NMR of lignocaine

(e) NMR of lignocaine with HP β CD

Fig 29 (a) NMR of HP β CD

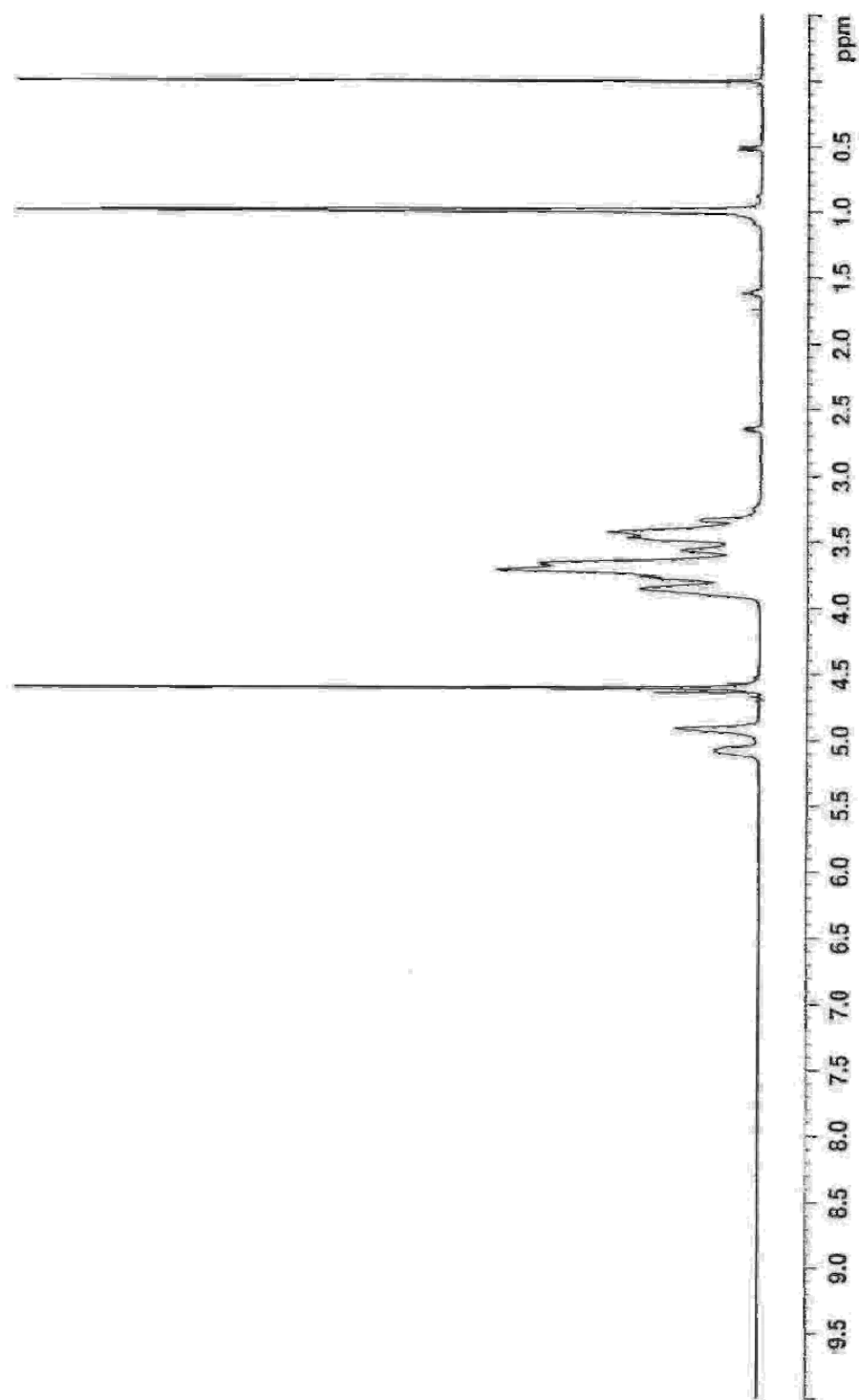


Fig 29 (b) NMR of Prilocaine

A- 0.905
B- 1.515
C- 2.569
D- 1.370
E- 3.505
F- 2.214
G- 7.296
H- 7.297

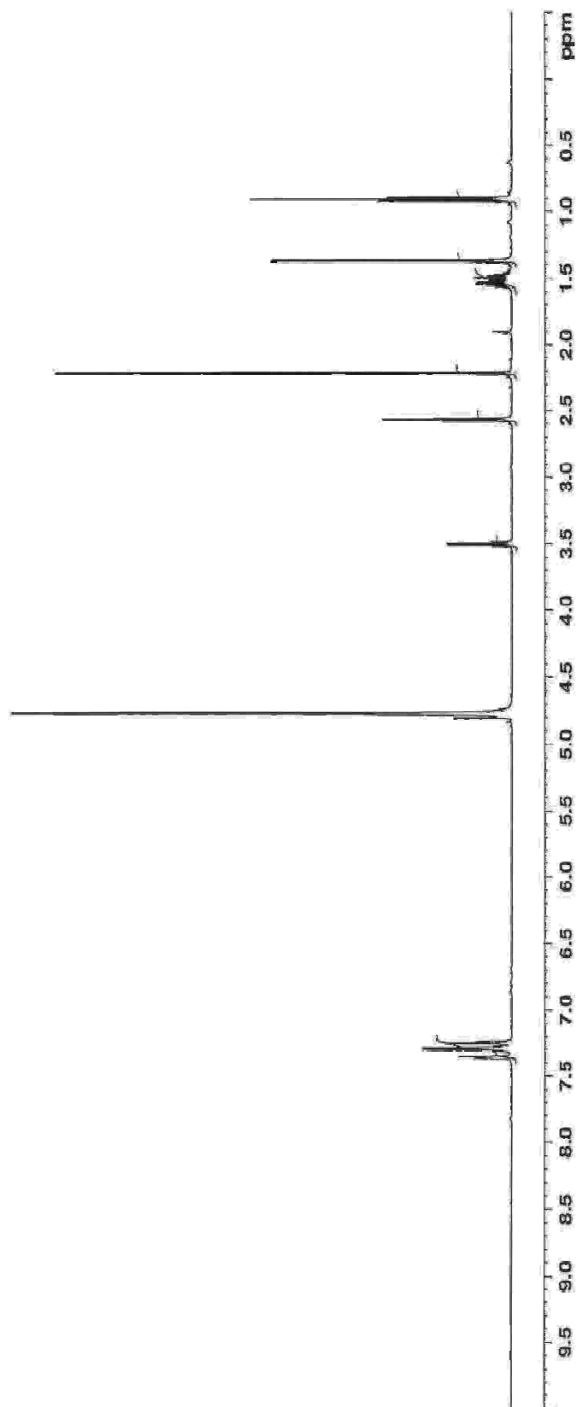


Fig 29 (c) NMR of Prilocaine with HPβCD

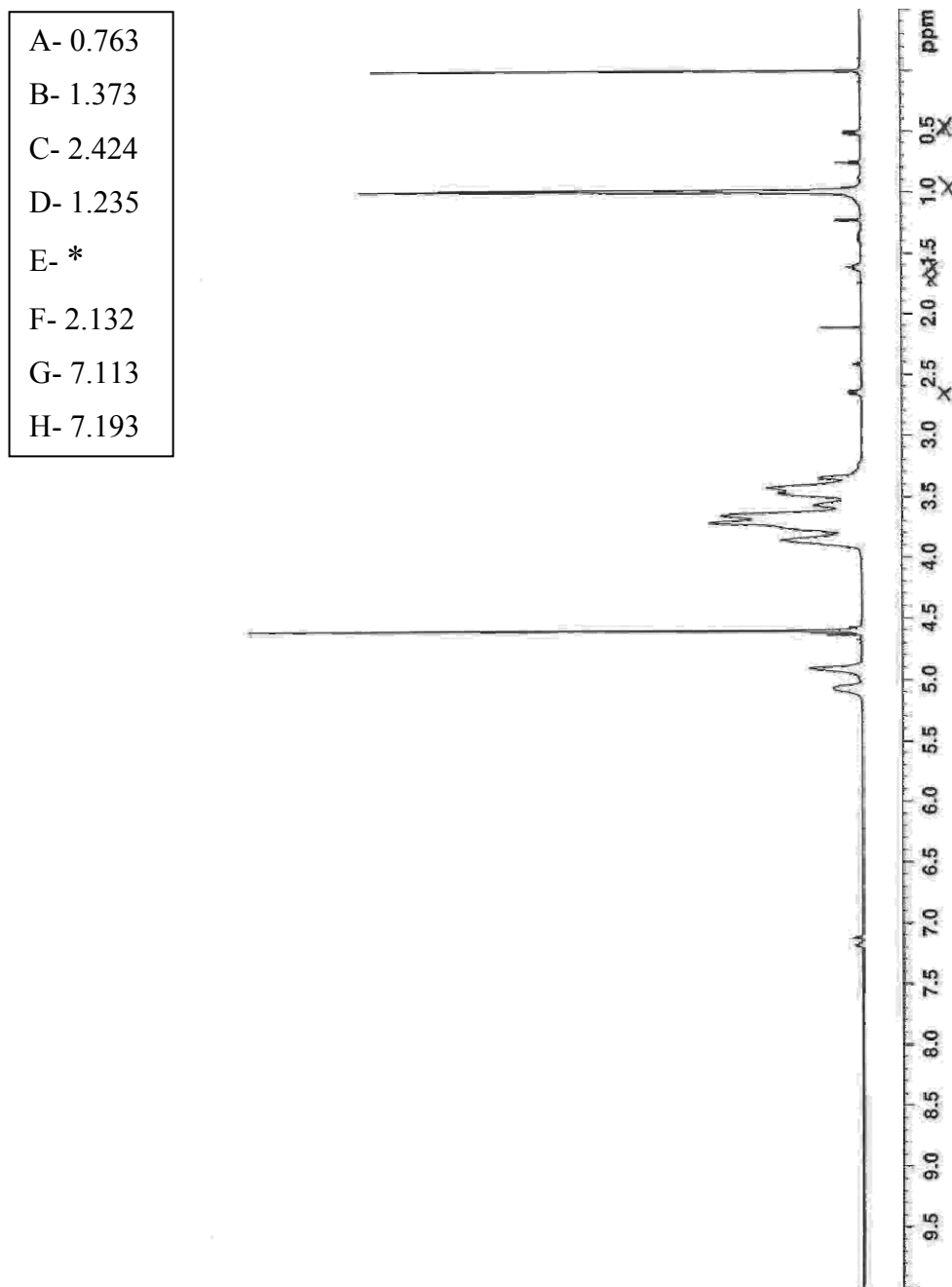


Fig 29 (d) NMR of Lignocaine

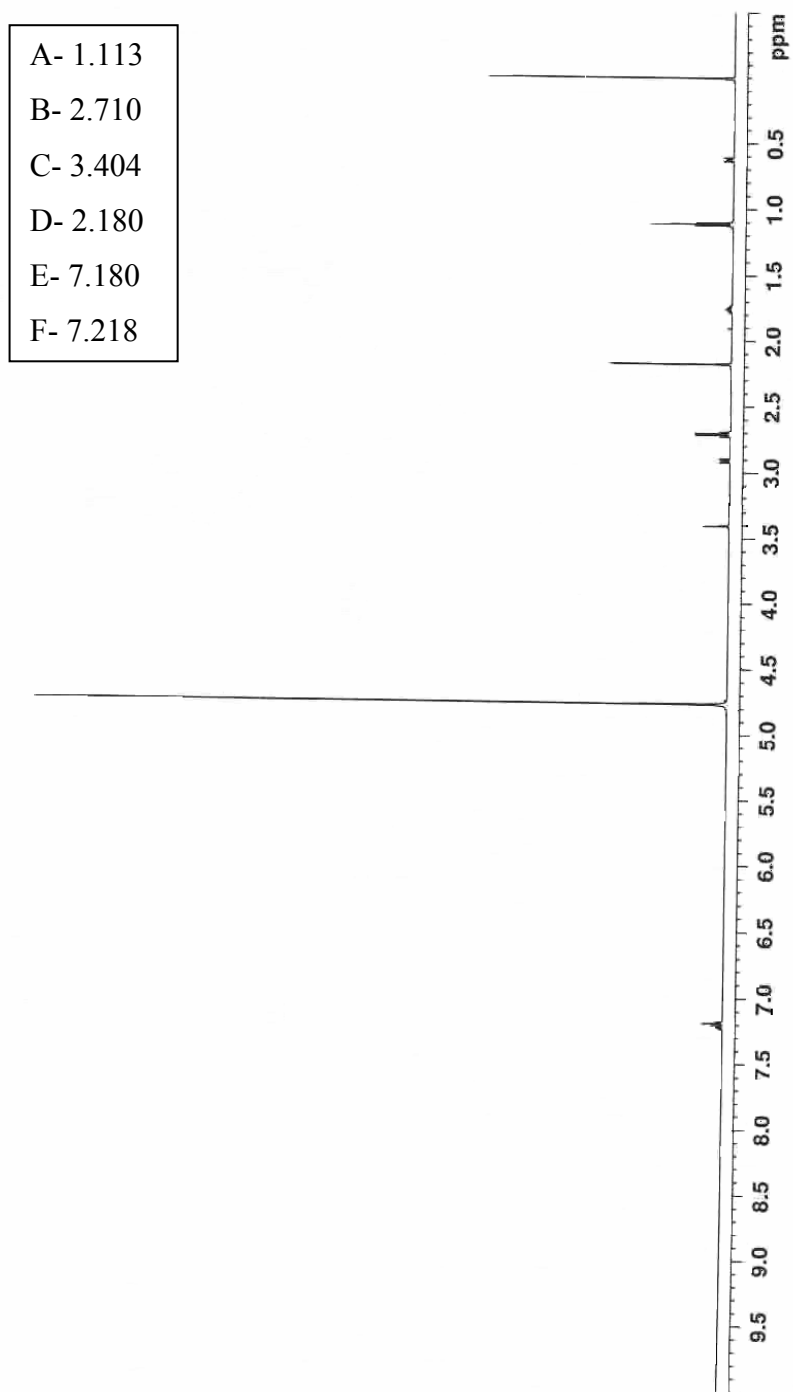


Fig 29 (e) NMR of Lignocaine with HPBCD

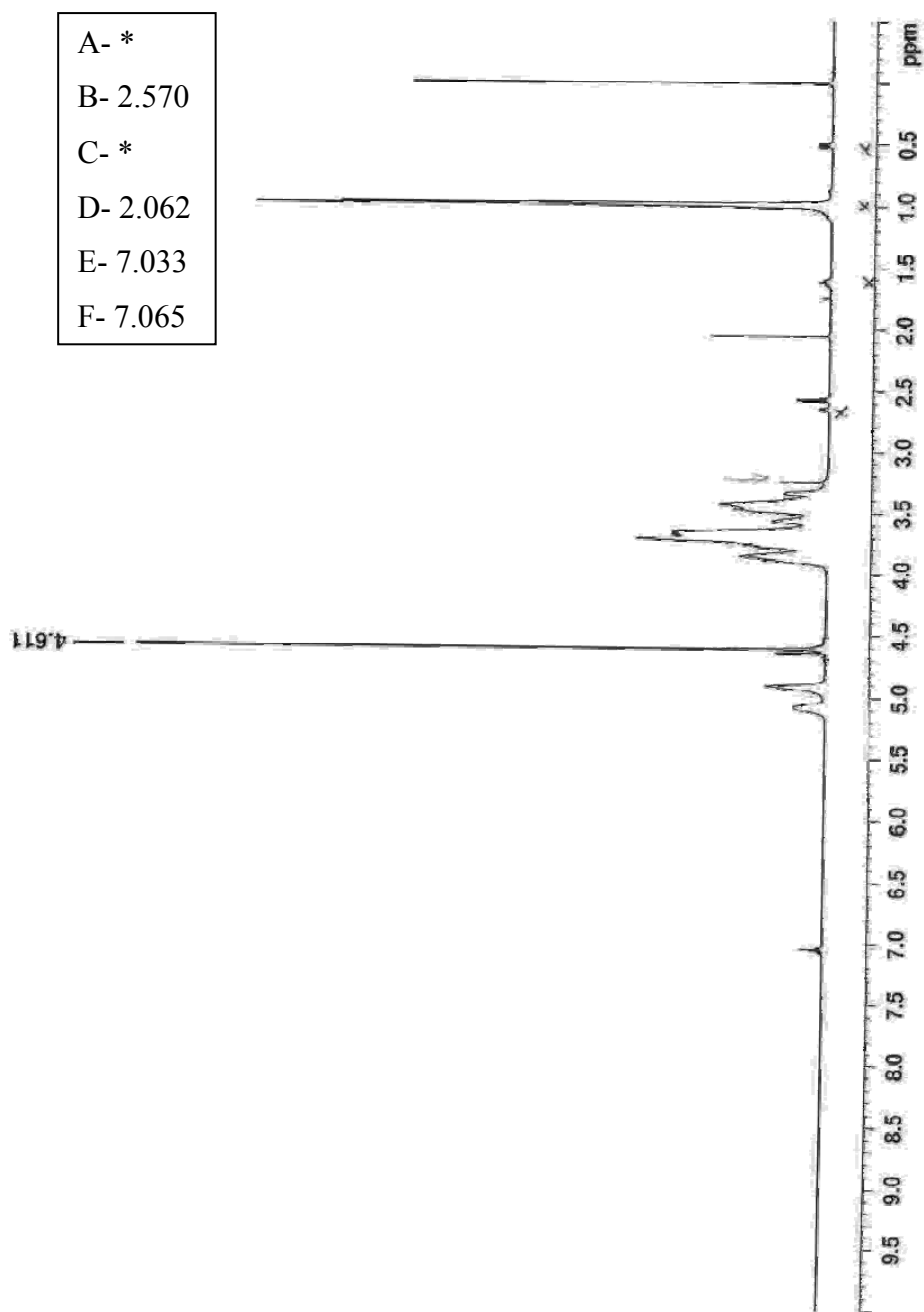


Figure 31: 1H NMR spectra of (a) HPBCD, (b) prilocaine, (c) lignocaine, (d) prilocaine-HPBCD complex and (e) lignocaine-HPBCD complex.

3.4 GRAPHICAL COMPARISON OF CHEMICAL SHIFTS OF PROTONS OF PRILOCAINE AND LIGNOCAINE IN THE PRESENCE AND ABSENCE OF HP β CD

Chemical shifts of protons for prilocaine and lignocaine in the presence and absence of HP β CD are represented graphically in Figure 32 and Figure 33. The chemical shift and change in peak character was observed where complexation for prilocaine and lignocaine occurred through HP β CD. The NMR's are given in Section 3.3. The peak suppression due to complexation for prilocaine with HP β CD is represented graphically in Figure 30. At 'E' no chemical shift was observed.

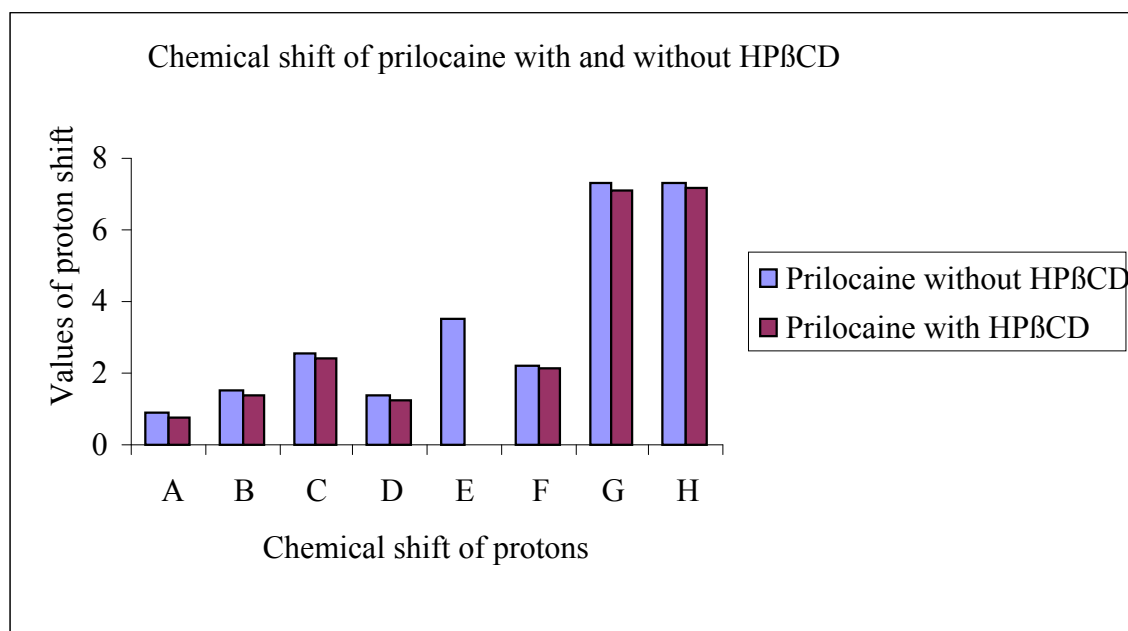


Figure 32: Chemical shift of prilocaine in the presence and absence of HP β CD

Lignocaine with HP β CD showed changes in peak characterisation at 'B', 'D', 'E' and 'F' suggesting the formation of complex through inclusion of lignocaine in the torus structure of HP β CD. Figure 31 represents the graphical interpretation of lignocaine-HP β CD and shows that at 'A' and 'C' no chemical shift is observed.

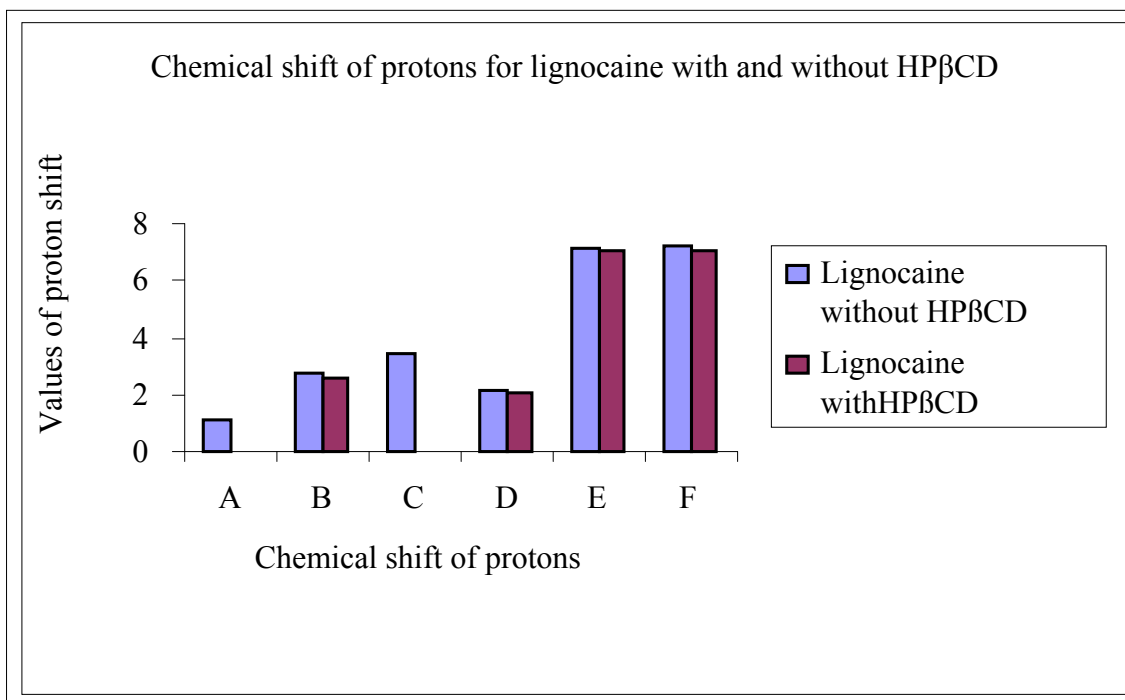


Figure 33: Chemical shift of lignocaine protons in the presence and absence of HP β CD

4 DISCUSSION

The validated method was initially developed to separate three local anaesthetic drugs namely prilocaine HCl, lignocaine HCl, and tetracaine HCl. The R values obtained for prilocaine HCl, lignocaine HCl, and tetracaine HCl were 0.999, 0.999, and 0.997 respectively. Tetracaine HCl was later excluded from the studies as complex formation with the combination of three drugs seemed unfeasible. A second method was then developed only for prilocaine HCl and lignocaine HCl. Reproducible results were obtained and the next step than was to determine the solubility of prilocaine and lignocaine and to enhance this by complexing it with HP β CD.

Solubility is the basic requirement for any formulation that needs to be prepared in a solution form. Also solubility enhancement has broad implications in parenteral formulation design. Although many drugs are fairly soluble in aqueous solutions, lipophilic drugs impose a solubility problem to be able to formulate as a solution. A variety of solubilisation techniques has been studied and widely used including pH adjustment, cosolvent addition, surfactant addition and cyclodextrin addition⁴⁰. Complexation with cyclodextrins is now a commonly used technique to improve drug solubility from number of other methods.

Although ionised drugs show greater aqueous solubility, the unionised form (molecular form) of drugs can be solubilised by complexation and pH adjustment. Prilocaine and lignocaine have the pKa values of 7.9 and 7.8 respectively therefore the pH of the solutions were maintained at 10.0. The lipophilic form of the drug may be advantageous for rapid onset of action if sufficient can be delivered in a solution formulation.

Complex formation with CD involves the release of enthalpy-rich water from the CD cavity as the main driving force for complexation. Other mechanisms that may be involved in complex formation include van der Waals interactions, hydrogen bonding and hydrophobic interactions. In the case of α -CD, release of ring strain is also thought to be involved with the driving force for compound-CD interaction⁷⁶.

Accordingly, different types of solubility isotherms are obtained as a result of complex formation with cyclodextrins which include A_L , A_P , and A_N types of isotherm. The A_L type of solubility isotherms was obtained for prilocaine and lignocaine with HP β CD. As mentioned above, the solubilities of prilocaine and lignocaine at higher temperatures were found to differ individually. One reason that can be proposed for an unexpected decrease in solubility of prilocaine at higher temperatures is its existence as R and S stereoisomer's. The spatial arrangements of these two stereoisomers of prilocaine at higher temperatures may limit its complex formation with HP β CD and affects its solubility and perhaps potency⁷⁷.

Many drugs studied in combination have either shown increase in solubility as expected or variations from solubility. Sometimes not the other drug but the cosolvent used along with the complexing agent was found to compete with drug for the entry into the HP β CD cavity decreasing the drug solubility. This has been found with the drug fluasterone where an overall decrease in solubility and complex formation occurred when complexed with HP β CD⁴⁰. Although cosolvent can act as a space regulating molecule for the drug molecule to fit into cyclodextrin, research done by Pitha et al. reported that the complexation constant of testosterone with HP β CD was 10,000 fold lowered in 80% ethanol than in water⁴⁰.

Almost similar solubility constants were obtained for prilocaine in combination with lignocaine when complexed with HP β CD. The solubility of prilocaine was again found to be decreased in the presence of lignocaine with a rise in temperature, but the differences obtained for solubilities between the temperatures were very small. The above proposed mechanism may hold true for prilocaine solubility in combination or it may be true again that in the presence of the other drug prilocaine showed hinderence in complex formation and thus its solubility decreased. However lignocaine solubility was found to rise effectively with rise in temperature as compared to its no change in solubility individually. It seems that in combination with prilocaine, lignocaine solubility was favoured.

Although prilocaine and lignocaine share structural similarities the difference's in solubility of these two drugs individually and in combination suggests varying patterns of complexation and the possibility of specific interactions or mixed modes of binding between different components or functional groups in the structure of the substrate and cyclodextrin. Earlier studies have reported the varying modes of complex formation with the relatively common structures of steroids, when complexed by SBE-7- β -CD, which only differed in the D ring substituents⁷⁸.

Thus the theory of mixed mode interactions for complex formation with HP β CD in for prilocaine and lignocaine individually and in combination at higher temperatures cannot be overruled.

The variability in complex formation with cyclodextrins can again be explained by the well documented experimental work of two hydrophobic drugs, indomethacin and griseofulvin, which have structural resemblance to each other. This was proved by various methods such as X-ray powder diffraction, modulated temperature differential scanning calorimetry and dissolution tests, which were used for the characterisation of solid state inclusion complexes of indomethacin and griseofulvin with β -CD, α -CD, and γ -CD in PEG 6000. The results showed that for griseofulvin no complex was formed with β -CD and this was partly represented by the steric hindrance of griseofulvin, whereas indomethacin in the presence of polyethylene glycol 6000 (PEG 6000) showed complex formation with β -CD and γ -CD, but not with α -CD. This was because α -CD showed good complexation with PEG 6000 in the melt and β -CD and γ -CD showed minimum interaction with the polymer and therefore could complex with indomethacin⁷⁶.

The variability of CD-drug complex can be further explained regarding to polymer complexation with different types of CD's. An aqueous solution of propylene glycol at low concentrations reduces the CD complexation of some drugs by acting as a competing guest molecule⁷⁹. The α -CD forms complexes with PEG of various molecular weights but, β -CD does not form complexes with PEG. This might promote the inclusion complex formation for the drug in this type of CD. It was

shown that β - and γ -CD form complexes with other polymers like polypropylene glycol (PPG) and polyisobutylene, while α -CD does not form complexes with either of these polymers. β - and γ -CD might therefore be more alike concerning their interactions with different polymers⁸⁰. Selection of the correct CD's therefore favours the complex formation with the drug molecule further enhancing its solubility effect.

In yet another study it was found that co-administration of hydroxypropyl methyl cellulose (HPMC) with SBE-7- β -CD sodium salt reduced the amount of β -CD needed in a solid dosage form of glibenclamide, without reducing its bioavailability and solubility. Compared to formulation without HPMC, more of the β -CD was needed for inclusion formation though the bioavailability was not affected in both of the formulations⁸¹.

The above findings in regard to complex formation with HP β CD suggest that different interactions at the molecular level exist during complexation and use of different CD's with different substituents can give different results. These findings lend support to the results obtained for solubilities of prilocaine and lignocaine.

The relative thermodynamic parameters for the complexation process are the standard free energy change (ΔG°), the standard enthalpy change (ΔH°), and the standard entropy change (ΔS°). As revealed in the earlier data complexation of prilocaine and lignocaine with HP β CD was accompanied by negative ΔH° , reflecting an exothermic process driven enthalpically. The enthalpy driven processes are associated with intermolecular interactions via hydrogen bonding and van der Waal's interactions. However the ΔH° and ΔS° values were not very significant for prilocaine and lignocaine and overall they suggested that an endothermic reaction is involved driven by entropy and involving hydrophobic interactions. Also nonlinearity observed at some of the HP β CD concentrations suggested that van't Hoff's equation was not followed.

Lignocaine alone and prilocaine in the presence of lignocaine showed non-linearity for the van't Hoff equation. It was noted that the solubilities were little affected by

temperature changes. But for lignocaine in presence of prilocaine the rise in solubilities was seen with rise in temperature and all the concentrations of HP β CD showed that van't Hoff equation was essentially followed as R value was above 0.95 for all.

The thermodynamic parameter ΔH° was relatively small as little change occurred in the solubility with a rise in temperature for both prilocaine and lignocaine. On the other hand ΔS° values were significantly large and so it can be said that complex formation was largely governed by entropy change.

The solubilities obtained for lignocaine individually showed little temperature effect, whereas in combination the solubilities seemed to increase to a greater extent. In contrast the solubilities of prilocaine decreased individually and also in combination. This indicated that more than one mechanism is involved in the complexation process and there may be a structural influence and stereoisomeric factors playing a role along with the hydrogen bonding giving varied solubility results. Also the theory proposed below can give an insight between the solution and the drug-HP β CD (guest-HP β CD) complex formation and the difference that exists in the mechanism of complexation.

The thermodynamic effects of β -CD and factors that result in different patterns of complexation apart from what is expected is well discussed in the complex formation of β -CD with different derivatives of azoalkanes leading to a host-guest relationship. Derivatives of the azoalkane 2,3-diazabicyclo[2,2,2]oct-2-ene, 1,4-dialkyl, 1,4-dichloro, 1-hydroxymethyl, 1-aminomethyl, and 1-ammoniummethyl substituents form host-guest inclusion complexes with β -cyclodextrin. In that experimental work they were employed as probes to assess substituent effects on the kinetics and thermodynamics of this complexation by using time-resolved and steady state fluorimetry, UV spectrophotometry, induced circular dichroism (ICD) measurements, and ^1H NMR spectroscopy. The ICD was employed for the assignment of the solution structures of the complexes, in particular the relative orientation of the guest in the host (co-conformation).

Accordingly the structure of the β -CD and 1-aminomethyl complex was assumed to give rise to the negative ICD signal, but the ICD spectra revealed that the complex of azoalkane 1-aminomethyl differed from the other derivatives and gave rise to an ICD band, which was significantly distorted, and also showed a bathochromic shift relative to the UV spectrum. Moreover, the ICD of 1-amino methyl complex showed a small positive component at shorter wavelengths in addition to the stronger negative ones. These irregularities suggested that a large co-conformational variability exist, which meant that complexes with different tilt angles may have been present in solution. It was found based on the results that for 1-aminomethyl, in contrast to 1-hydroxymethyl and 1-ammoniummethyl, the co-conformational spaces were not restricted by hydrogen bonding with the upper rim. The co-conformers with different tilt angles had quite different UV spectra (which reflected the depth of inclusion into the nonpolar environment) and ICD effects (which reflected the geometrical alignment with respect to the azo chromophore) and, thus, accounted for the observed shifts for 1-aminomethyl.

The hydrophobicity order of the examined guest molecules was established to be 1,4-dichloro > 1,4-dialkyl > 2,3-diazabicyclo[2,2,2]oct-2-ene > 1-aminomethyl > 1-hydroxymethyl and > 1-ammoniummethyl. A higher hydrophobicity was expected to result in a larger binding constant. Azoalkane 1,4-dialkyl did not show a higher binding affinity than 2,3-diazabicyclo[2,2,2]oct-2-ene despite its higher hydrophobicity. This was rationalized in terms of the solution structures, which indicated that the lateral co-conformation was energetically most favourable one and could not be attained for 1,4-dialkyl. Instead, a frontal mode of inclusion was adapted for 1,4-dialkyl which was energetically less favourable. This lessened the increased driving force due to its higher hydrophobicity and resulted in essentially the same binding constant as for 2,3-diazabicyclo[2,2,2]oct-2-ene.

Regarding the hydrophilicity generally, the more hydrophilic guests 1-aminomethyl and 1-ammoniummethyl should show lower binding constants than 2,3-diazabicyclo[2,2,2]oct-2-ene and 1,4-dichloro as the ionic ammonium guest displays

the weakest binding. The difference in binding constants between 1-aminomethyl (250 M^{-1}) and 1-ammoniummethyl (20 M^{-1}) was in line with the general observation that the ionization of the guest causes a destabilization of CD complexes in water. But for the hydroxyl and ammonium derivatives 1-hydroxymethyl and 1-ammoniummethyl, for which hydrogen bonding was presumed, it actually showed a weaker binding than the amino derivative, for which hydrogen bonding to the CD should have not played a role at all. This could be adequately accounted for in terms of guest hydrophobicity and a change in the co-conformation due to the introduction of bridgehead substituents⁸².

The above mentioned findings support the variability in solubilities obtained for prilocaine and lignocaine and confirms that expected solubilities may not be obtained as a result of complex formation with HP β CD and that variation in results can be obtained due to structural differences between two drugs, intermolecular changes because of the presence of other drug in combination and/or stereoisomeric interferences and again the type of structural interactions taking place between the guest molecule and CD. Competitive binding between prilocaine and lignocaine for CD sites may be another factor.

To study the complex formation of prilocaine and lignocaine with HP β CD further, NMR spectra's for prilocaine and lignocaine were obtained at 600 Hz. The NMR suggested that different chemical shifts were involved for both prilocaine and lignocaine with few similarities. The NMR details of free prilocaine and prilocaine-HP β CD complex and lignocaine and lignocaine-HP β CD were presented earlier in Section 3.3.

The NMR of prilocaine showed involvement of aromatic protons, along with other methene and methyl groups and the NMR of lignocaine showed involvement of aromatic protons along with aromatic methyl and ethyl amide groups.

When lignocaine HP β CD NMR was compared with the similar NMR studies of another local anaesthetic benzocaine and β -CD the results have shown similarities

between the complex formation that occurred at ethyl-CH₃, ethyl-CH₂, and at the aromatic protons H₁-H₆ for benzocaine⁸³.

In another study done for the selectivity in the binding and detection of charge diffuse ions, the neutral, lipophilic peralkylated cyclodextrin derivatives were found to selectively bind the onium ions like R-NH₃⁺ and NMe₄⁺. When local anaesthetics such as lignocaine were studied in the presence of 2, 6- didodecyl -β-cyclodextrin as the sensing ionophore it gave a Nernstian response at the NH⁺Et₂ binding site.

The response was obtained down to 10^{-5.6} mol dm⁻³ concentrations. The nernstian behaviour obtained showed that NH⁺Et₂ ion is potentially active site for binding with the cyclodextrin resulting in complex formation. The study showed that neutral, lipophilic cyclodextrin derivatives can be used for the chemoselective detection of a variety of aryl and alkyl ammonium ions⁸⁴. Similarly a chemical shift of a triplet and a multiplet NH⁺Et₂ for lignocaine HPβCD was observed at the NH⁺Et₂ binding site along with the chemical shifts of aromatic protons and aromatic methyl group. This would be less likely at high pH values used in this study.

The NMR details of prilocaine located from earlier studies in the literature found the involvement of chiral carbon. The chiral solvating agent, 2,2,2-trifluoro-1-(9-anthryl) ethanol, was used to determine the diastereomeric interactions and assignments of the configuration was based on the relative field position of resolved enantiomeric signals⁸⁵.

At present preparations of parenteral dosage forms of local anaesthetics in solution is achieved by means of their hydrochloride salts. Therefore the pH of these preparations can be as low as 3.0 to 4.5. A possible precipitation of the base at the injection site may occur due to an increase in pH to the tissue pH after injection exacerbated by a lowering of free base solubility at body temperature⁸⁶. A formulation therefore of higher pH range say about pH 10.0, will keep the drugs in unprotonated form and intact in a complex until it reaches its site of action. Tissue and blood pH will have little effect on the drug formulation as they are not used in

their salt forms. Research has shown that the pH of above 11.5 and below 4.0 can produce some pain during administration of anaesthetics, but no pain was observed in individuals at pH 10.0. Therefore the parenteral formulation of prilocaine and lignocaine base at pH 10.0 would be safe for parenteral purpose⁸⁷.

5 CONCLUSIONS

Prilocaine and lignocaine share structural similarities as both are amide type local anaesthetics. It was expected that similar solubility results would be obtained for both individually and in combination, but the difference's in solubility of these two drugs individually and in combination suggests varying patterns of complexation and the possibility of specific interactions or mixed modes of binding between different components or functional groups in the structure of the substrate and cyclodextrin.

The complex formation showed that entropy rather than enthalpy was governing the reaction for complexation form individually and in combination for both prilocaine and lignocaine as ΔH° values were very small. There was only a small temperature effect on solubility limiting the accuracy with which these effects could be described

The A_L type solubility isotherm was obtained for all the solubilities suggesting that binding involves 1:1 association between guest and CD molecule.

The NMR data showed that aromatic rings of both prilocaine and lignocaine are involved in complex formation apart from the methene and methyl groups for prilocaine and ethyl amide and aromatic methyl groups for lignocaine.

Solubilities of prilocaine determined over the concentration range of 0-30% HP β CD were found to increase with increasing concentration of HP β CD and solubilities of lignocaine showed little increase with increase in concentration of HP β CD. With the rise in temperature the solubilities of prilocaine decreased, whereas again temperature showed little effect on the solubilities of lignocaine. The solubilities obtained for prilocaine at the temperatures 25°C, 35°C, and 42°C were between 3.22-9.11 moles/L, 1.96-8.51 moles/L and 1.79-7.91 moles/L respectively. The solubilities obtained for lignocaine at the temperatures from 25°C-42°C were 1.69-4.41 moles/L, 1.04-4.22 moles/L and 1.04-4.55 moles/L respectively.

In combination, the solubilities of prilocaine and lignocaine showed variation in complexation with HP β CD from the solubilities obtained individually. Prilocaine in combination showed increase in solubility with increase in concentration of HP β CD but showed decrease in solubilities with rise in temperature. Lignocaine on the other hand showed increase in solubilities with increase in concentrations of HP β CD and with rise in temperatures. The solubilities obtained for prilocaine at 25°C, 35°C, and 42°C were 0.91-2.67 moles/L, 0.64-2.89 moles/L and 0.66-3.68 moles/L respectively and the solubilities obtained for lignocaine were 1.03-2.82 moles/L, 1.17-5.34 moles/L and 1.80-8.35 moles/L respectively.

From the data obtained in combination of prilocaine and lignocaine one can say that solubilities of prilocaine showed little change with the rise in temperature but the solubilities of lignocaine was found to increase in contrast to its solubilities obtained individually.

A pH of 10.0 used for the development of parenteral formulation should not cause irritation at the site of injection, but more studies need to be done regarding the cell permeability of HP β CD and drug complex formation and how fast the onset of action is achieved compared to the dissociation of local anaesthetics as their hydrochloride salts.

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