Synthesis and Transdermal Properties of Acetylsalicylic Acid Derivatives

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Abstract

The skin is an amazing elastic and relatively impermeable barrier that provides protective, perceptive and communication functions to the body. The stratum corneum is widely accepted as the barrier of the skin – limiting the transport of molecules into and across the skin. It is evident that the transdermal permeation of drugs depend on a number of factors of which the physicochemical properties play the most prevalent role. The potential of using intact skin as the site of administration for dermatological preparations to elicit pharmacological action in the skin tissue has been well recognised. Transdermal drug delivery offers several advantages over oral and parenteral dosing. They include avoiding hepatic first pass metabolism, maintaining constant blood levels for longer periods of time, improving bioavailability, decreasing the administered dose, adverse effects and gastrointestinal side effects, easy discontinuation in case of toxic effects and improved patient compliance. Optimal transport through the skin requires a drug to possess lipophilic as well as hydrophilic properties. Research has indicated that the ideal log P value for optimal transdermal permeation is between 1 and 2.

Acetylsalicylic acid (aspirin) possesses anti-inflammatory, analgesic and antipyretic activity, and as an anti-inflammatory analgesic agent it is used in the treatment of musculoskeletal disorders, such as rheumatoid arthritis. Its use is limited to the relief of pain and inflammation, as it does not halt the progression of the pathological injury caused to the tissue. Acetylsalicylic acid is also used in the treatment of fever, prevention of thromboembolic disorders, reducing the incidence of colon cancer and it delays the onset of Alzheimer's disease. The most common adverse effect of acetylsalicylic acid occurring with therapeutic doses is gastro-intestinal disturbances.

The primary aim of this study was to determine the transdermal penetration of acetylsalicylic acid and some of its derivatives and to establish a correlation, if any, with selected physicochemical properties.

The ten derivatives of acetylsalicylic acid were prepared by esterification of acetylsalicyloyl chloride with ten different alcohols. The structures of the products were confirmed by mass spectroscopy (MS), nuclear magnetic resonance spectroscopy (NMR), infrared spectroscopy (IR) and differential scanning calorimetry (DSC) for methyl acetylsalicylate. Experimental aqueous solubility and partition coefficients were determined for acetylsalicylic acid and its different derivatives at a pH of 4,5. *In vitro* penetration was measured through excised female human abdominal skin in diffusion cells. The prediction software Interactive Analysis (IA) was used to predict aqueous solubility, while prediction software IA, K_{ow}Win and ACD Labs were

used to predict the log P values for each derivative. None of the predicted values correlated with the experimental values.

The experimental aqueous solubility, partition coefficient and transdermal flux values were determined for acetylsalicylic acid and its derivatives. The experimental aqueous solubility of acetylsalicylic acid (6,56 mg/ml) was higher than that of the synthesised acetylsalicylate derivatives (ranging from 1,76 x 10^{-3} to 3,32 mg/ml), and the partition coefficient of acetylsalicylic acid (-0,85) was lower than that of its derivatives (ranging from -0,25 to 1,95). There was thus a direct correlation between the aqueous solubility data and the partition coefficients. The experimental transdermal flux of acetylsalicylic acid (47,53 $\mu g/cm^2/h$) was much higher than that of its derivatives (ranging from 0,03 to 28,32 $\mu g/cm^2/h$). With the ethyl derivative (28,32 $\mu g/cm^2/h$) and the methyl derivative (10,06 $\mu g/cm^2/h$) being the only derivatives with appreciable flux. Pentyl acetylsalicylate (0,03 $\mu g/cm^2/h$) had the lowest flux.

The higher flux values of acetylsalicylic acid and its methyl and ethyl derivatives might be due to the fact that it is more hydrophilic and had better aqueous solubility, thus permeating through the proteins of the skin. Pentyl acetylsalicylate had a log P value of 1,95, but had the lowest flux (0,03 $\mu g/cm^2/h$), just proving once again that to cross the stratum corneum a drug should posses both hydrophilic and lipophilic properties. Tert-butyl acetylsalicylate had a flux (7,30 $\mu g/cm^2/h$) lower than that of methyl and ethyl acetylsalicylate, but a higher flux than the other synthesised derivatives which could be due to its log P value being slightly greater than 1 and having an average aqueous solubility. The low transdermal permeation may also be attributed to the fact that at the pH (4,5) chosen for transdermal studies, acetylsalicylate was only 9,09 % unionised. A higher degree of unionised species results in higher flux values.

This study has confirmed that transdermal flux is dependent on several factors including optimum solubility, partitioning, diffusion and the degree of ionisation in the stratum corneum in addition to a suitable partition coefficient and high aqueous solubility. The solution to the increased transdermal delivery of lipophilic drugs does not simply lie in producing a derivative with a higher aqueous solubility and more ideal partition coefficient. Other means of increasing the transdermal permeation of lipophilic acetylsalicylic acid derivatives will have to be investigated in further studies.

Opsomming

Die vel is 'n ongelooflike elastiese en relatief deurlaatbare skans wat beskermende, waarnemende en kommunikeerbare funksies in die liggaam verrig. Die stratum corneum word geredellik aanvaar as die skans van die vel wat die beweging van molekules in en deur die vel beperk. Dit is dus duidelik dat die transdermale aflewering van geneesmiddels afhanklik is van 'n aantal faktore, waarvan die geneesmiddel se fisies-chemiese eienskappe die belangrikste rol speel. Die potensiaal om intakte vel as die plek van toediening van dermatologiese preparate te gebruik om farmakologiese werking in die vel teweeg te bring, is goed bekend. Die transdermale toediening van asetielsalisielsuur het verskeie voordele bo die tradisionele toedieningsroetes, nl. oraal of parenteraal. Hierdie voordele is onder meer die uitskakeling van die eerstedeurgangseffek, onderhoud van konstante bloedvlakke vir langer tydsperiodes, beter biobeskikbaarheid, laer toegediende dosis, minder nadelige effekte en gastro-intestinale newe-effekte, maklike staking indien toksiese effekte vermoed word en beter pasïentmee-werkendheid. Vir optimale transdermale deurgang moet die geneesmiddel oor lipofiliese sowel as hidrofiliese eienskappe beskik. Navorsing toon dat die aangewese log P-waarde vir optimale transdermale penetrasie tussen 1 en 2 moet lê.

Asetielsalisielsuur (aspirien) besit anti-inflammatoriese, analgetiese en anti-piretiese aktiwiteit en word as anti-inflammatoriese analgetikum gebruik vir die behandeling van muskuloskeletale afwykings, soos rumatoïede artritis. Die gebruik van asetielsalisielsuur is beperk tot die behandeling van pyn en inflammasie, aangesien dit nie die vordering van patologiese besering, wat aan die vel veroorsaak is, rem nie. Asetielsalisielsuur word ook gebruik vir die behandeling van koors, voorkoming van tromboëmbolisme, verlaging van die insidensie van kolonkanker en vertraging van die aanvang van Alzheimer se siekte. Die mees algemene newe-effek wat by terapeutiese dosisse van asetielsalisielsuur voorkom, is gastro-intestinale afwykings.

Die hoofdoel van hierdie studie was om die transdermale penetrasie van asetielsalisielsuur en enkele derivate daarvan te bestudeer en om 'n korrelasie met sekere fisies-chemiese eienskappe, indien enige, te vind.

Die tien verskillende derivate van asetielsalisielsuur is berei deur die verestering van asetielsalisieloïelchloried met tien verskillende alkohole. Die strukture van die produkte van elke sintese is met behulp van massaspektroskopie (MS), kernmagnetieseresonans-spektroskopie (KMR), infrarooispektroskopie (IR) en differensiëleskanderingskalorimetrie (DSK) vir metielasetielsalisielaat bevestig. Die eksperimentele wateroplosbaarheid en verdelingskoeffisiënt van asetielsalisielsuur en sy verskillende derivate, by 'n pH van 4,5, is bepaal. *In vitro* penetrasie deur vroulike abdominale mensvel is in diffusieselle gemeet. Die

rekenaarprogram Interactive Analysis (IA) is gebruik om die wateroplosbaarheid te voorspel, terwyl die rekenaarprogramme IA, K_{ow}Win en ACD Labs gebruik is om die verdelingskoeffisiënte te voorspel. Geen voorspelde waardes het met die eksperimentele waardes gekorreleer nie.

Die eksperimentele wateroplosbaarheid, verdelingskoeffisiënt en transdermale fluks van asetielsalisielsuur en sy derivate is bepaal. Die eksperimentele wateroplosbaarheid van asetielsalisielsuur (6,56 mg/ml) was hoër as die van die gesintetiseerde derivate (1,76 x 10^{-3} tot 3,32 mg/ml), terwyl die verdelingskoeffisiënt van asetielsalisielsuur (-0,85) laer was as die van die gesintetiseerde derivate (-0,25 tot 1,95). Die eksperimentele wateroplosbaarheid korreleer met die van die verdelingskoeffisiënte. Die eksperimentele transdermale fluks van asetielsalisielsuur (47,53 $\mu g/cm^2/h$) is baie hoër as die van al sy derivate (0,03 tot 28,32 $\mu g/cm^2/h$) met die etielderivaat (28,32 $\mu g/cm^2/h$) en die metielderivaat (10,06 $\mu g/cm^2/h$) as die enigste derivate met noemenswaardige fluks. Pentielasetielsalisielaat (0,03 $\mu g/cm^2/h$) het die laagste fluks.

Die hoër fluks van asetielsalisielsuur en sy metiel- en etielderivate is moontlik as gevolg daarvan dat hierdie verbindings meer hidrofilies is en 'n beter wateroplosbaarheid het en dus deur die proteïene van die vel penetreer. Pentielasetielsalisielaat het 'n log P-waarde van 1,95, maar het die laagste fluks (0,03 $\mu g/cm^2/h$), wat net weereens toon dat 'n geneesmiddel oor beide lipofiliese en hidrofiliese eienskappe moet beskik om deur die stratum corneum te beweeg. Ters-butielasetielsalisilaat se fluks (7,30 $\mu g/cm^2/h$) was laer as dié van die metiel- en etielderivate, maar het 'n hoër fluks gehad as die ander gesintetiseerde verbindings. Die rede hiervoor is moontlik dat ters-butielasetielsalisilaat 'n log P-waarde het van amper 1 en 'n gemiddelde wateroplosbaarheid. Lae transdermale penetrasie kan moontlik ook toegeskryf word aan die pH (4,5) wat vir die transdermale studies gekies is, want asetielsalisielsuur was slegs 9,09 % ongeïoniseerd. 'n Hoër graad van geïoniseerde spesie lei tot 'n hoër fluks.

Hierdie studie bevestig dat transdermale fluks afhanklik is van 'n aantal faktore, waaronder optimale wateroplosbaarheid, verdelings- en diffusiekoeffisïent en graad van ionisasie in die stratum corneum, saam met verdelingskoeffisiënt en hoë wateroplosbaarheid, 'n rol in deurgang speel. Goeie transdermale penetrasie van lipofiele geneesmiddels kan dus nie bloot deur net die vervaardiging van derivate met hoër wateroplosbaarheid en meer ideale verdelingskoeffisiënte verkry word nie. Ander maniere om transdermale deurgang van lipofiele asetielsalisielsuur derivate te verhoog, sal in verdere studies ondersoek moet word.

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Chapter 1

Introduction and problem statement

1.1 Introduction

The skin is the most extensive and readily accessible organ in the body. Its chief functions are concerned with protection, temperature regulation, control of water excretion and sensation. In an average adult it covers an area of about 1,73 m^2 and receives one third of circulating blood through the body at any given time. The potential of using intact skin as the site of administration for dermatological preparations to elicit pharmacological action in the skin tissue has been well recognised (Barr, 1962). The permeation of chemicals, toxicants and drugs are much slower across the skin when compared to other biological membranes in the body, due to the outermost layer of the skin, the stratum corneum or horny layer. The lipophilic stratum corneum is responsible for the primary barrier function of the skin and provides an extensive challenge to scientists in their pursuit to develop drugs for transdermal delivery (Pefile & Smith, 1997).

In addition to the structure of the stratum corneum through which transdermal absorption occurs, the physicochemical properties of both the drug and the vehicle play an important role in determining the percutaneous absorption (Blank *et al.*, 1967; Abraham *et al.*, 1995). These factors include the molecular properties of the drug and the vehicle (Ritschel & Hussain, 1988; Blank *et al.*, 1967). Transdermal absorption is also dependent on the molecular weight, melting point, partition coefficient, pH of the drug solution in the vehicle and the concentration of the drug on the surface of the skin required to deliver a desired therapeutic effect (Barry, 1983; Bunge & Cleeck, 1995). Small molecules penetrate more rapidly than large molecules (Liron & Cohen, 1984). Compounds with lower melting points exhibit higher permeability coefficients (Roy & Flynn, 1988). According to Guy (1996) compounds with a log P value between 1 and 3, with relative low molecular weights and modest melting points, are likely to have decent passive skin permeabilities. The lipophilic stratum corneum is more permeable to drugs in their non-ionic state, because of their greater lipid solubility (Abdou, 1989). Drugs utilised for transdermal delivery should have a high potency, as the concentration, which is usually delivered transdermally, is very low (Naik *et al.*, 2000).

Acetylsalicylic acid (aspirin) possesses anti-inflammatory, analgesic and antipyretic activity. Acetylsalicylic acid as an anti-inflammatory analgesic agent is used in the treatment of musculoskeletal disorders, such as rheumatoid arthritis. Its use is limited to the relief of pain

and inflammation, as it does not halt the progression of the pathological injury caused to the tissue. Acetylsalicylic acid is also used in the treatment of fever, prevention of thromboembolic disorders, reducing the incidence of colon cancer and it delays the onset of Alzheimer's disease (Insel, 2001; Giovannucci *et al.*, 1995; Rang & Dale, 1999). The most common adverse effect of acetylsalicylic acid occurring with therapeutic doses is gastro-intestinal disturbances (Reynolds, 1984).

Transdermal drug delivery offers a few advantages over oral and parental delivery. They include avoiding hepatic first pass metabolism, maintaining constant blood levels for longer periods of time, improving bioavailablity, decreasing the administered dose, adverse effects and gastrointestinal side effects, easy to discontinue in case of toxic effects and improved patient compliance (Mitragotri, 2000).

1.2 Aim and objectives of this study

The aim of this study was primarily to determine the transdermal penetration of acetylsalicylic acid and some of its derivatives and to establish a correlation, if any, with selected physicochemical properties.

In order to achieve this goal, the following objectives were set:

- Synthesise esters of acetylsalicylic acid and verify their structures.
- Experimentally determine the aqueous solubility and the partition coefficient for acetylsalicylic acid and its synthesised derivatives.
- Compare the experimental aqueous solubility and the partition coefficient of synthesised acetylsalicylic acid derivatives with values calculated from commonly used prediction software.
- Experimentally determine the transdermal flux of acetylsalicylic acid and its derivatives.
- Compare the experimental flux data of the synthesised acetylsalicylic acid derivatives with values calculated from commonly used theoretical equations.
- Determine whether a correlation exists between the aqueous solubility, partition coefficient and transdermal flux data of the acetylsalicylic acid derivatives.

Acetylsalicylic acid as non-steroidal anti-inflammatory drug (NSAID)

2.1 Introduction

Acetylsalicylic acid (aspirin) is the most widely prescribed anti-inflammatory, analgesic and antipyretic drug and is the prototype for the comparison and evaluation of other NSAIDs, which share certain therapeutic actions and side effects (Insel, 2001). A survey of medication use in the United States reported that acetylsalicylic acid was taken by 17 % of adults. Based on market data provided by Information Resources, Inc., it is estimated that in the year 2001, approximately 14,5 billion tablets of OTC single-ingredient acetylsalicylic acid were purchased in the U.S.A. (Kaufmann, *et al*., 2002).

2.2 History

The history of analgesic and anti-inflammatory substances started with the use of decocted salicylate-containing plants by ancient Greek and Roman physicians. Willow bark was already mentioned in the Corpus Hippocraticum (a collection of medical scripts compiled by Alexandrian scholars in approximately 300 BC) as a substance for treating fever and pain conditions. Ancient Asian records indicate its use 2400 years ago (Osborne, 1998). In 1763, Reverend Edward Stone had collected observations from around England on the effect of willow bark for the relief of fever (Osborne, 1998). Salicin was isolated from willow bark, *Spirea ulmaria*, by Leroux in 1829. No truly useful therapeutic application was found from this glycoside until 1874 (Kennewell, 1990).

In 1870, Professor Von Nencki of Basle demonstrated that salicin was converted to salicylic acid in the body. Salicylic acid was then given to patients with fevers and symptoms were relieved. However, the compound caused severe irritation of the lining of the mouth, oesophagus and stomach (Osborne, 1998). Four years later, Maclagan used salicin for the treatment of rheumatic fever. Subsequently, he found its metabolite, salicylic acid, to be more efficacious in the treatment of a variety of rheumatic conditions. By this time, its antipyretic properties had also been recognised (Kennewell, 1990).

In 1875, chemists synthesised sodium salicylate to use in clinical studies. It reduced pain and fever with less irritation, but tasted awful. The large doses of sodium salicylate used in treating

rheumatism caused the patients to vomit (Osborne, 1998).

A German chemist, Felix Hoffmann, synthesised acetylsalicylic acid in the laboratories of Farbenfabriken Bayer, Elberfeld, Germany in 1897. Two years later Dreser tested the compound pharmacologically, while Wohlgemuth and Witthauer tested it clinically and documented the antirheumatic, antipyretic and analgesic properties free of the undesired side effects of salicylic acid. This new compound was called aspirin ('a' for acetyl and 'spir' for "spirsäure", which is German for salicylic acid) (Florey, 1979).

During World War I the British wanted acetylsalicylic acid, but as it was manufactured by the Germans (Bayer & Co), the British government offered a £20 000 reward to anyone who could develop a workable manufacturing process. George Nicolas, a Melbourne pharmacist, achieved this and subsequently named the tablet 'Aspro' (Osborne, 1998).

Nowadays more than 10 million kilograms of acetylsalicylic acid are manufactured per year in the U.S. Acetylsalicylic acid is not only used as a painkiller but has also been proposed as an effective drug in reducing the incidence of heart disease.

2.3 Mechanism of action of NSAIDs

Acetylsalicylic acid exerts its effect primarily by interfering with the biosynthesis of cyclic prostanoids, i.e. thromboxane A_2 (TXA_2), prostacyclin, and other prostaglandins. These prostanoids are generated by the enzymatically catalysed oxidation of arachidonic acid, which is itself derived from membrane phospholipids (Figure 2.1). Arachidonic acid is metabolised by the enzyme prostaglandin (PG) H-synthase, which, through its cyclooxygenase (COX) and peroxidase activities, results in the production of PGG_2 and PGH_2, respectively. PGH_2 is then modified by specific synthases, thus producing prostaglandins D_2, E_2, $F_{2\alpha}$, I_2 (prostacyclin), and TXA_2, all of which mediate specific cellular functions (Smith, 1992).

PGH-synthase, also referred to as COX, exists in 2 isoforms that have significant homology of their amino acid sequences (Williams & DuBois, 1996). A single amino acid substitution in the catalytic site of the enzyme confers selectivity to inhibitors of the COX isoforms (Gierse *et al.*, 1999; Hawkey, 1999). The first isoforms (COX-1) is constitutively expressed in the endoplasmic reticulum of most cells (including platelets) (Morita *et al.*, 1995) and results in the synthesis of homeostatic prostaglandins responsible for normal cellular functions, including gastric mucosal protection, maintenance of renal blood flow, and regulation of platelet activation and aggregation (Smith, 1992). The second isoform (COX-2) is not routinely present in most mammalian cells but, rather, is rapidly inducible by inflammatory stimuli and growth factors and results in the production of prostaglandins that contribute to the inflammatory response (Kujubu,

1991).

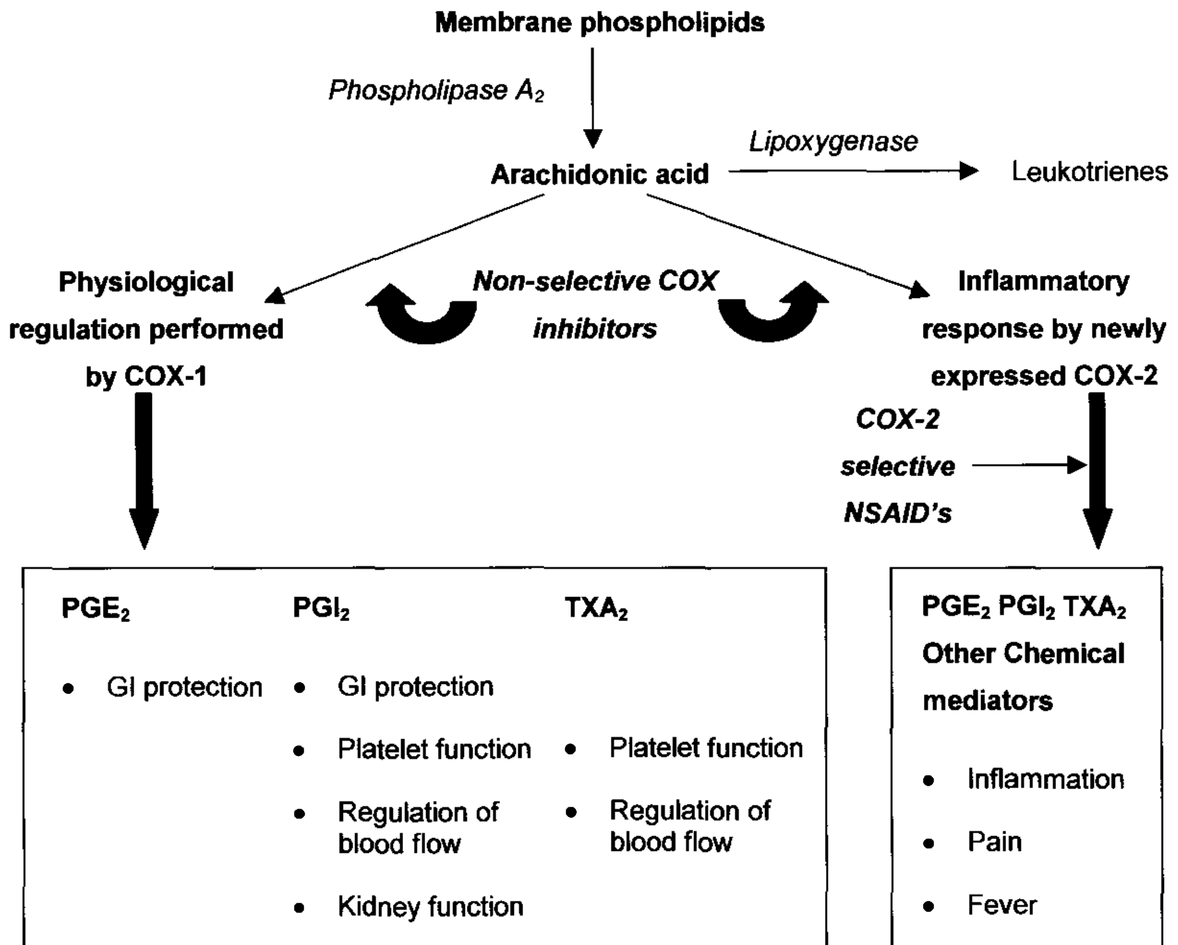


Figure 2.1: Mechanism of drug action of NSAIDs (Mesecar, 2001).

Acetylsalicylic acid imparts its primary antithrombotic effect through the inhibition of PGH-synthase/COX by the irreversible acetylation of a specific serine moiety (serine 530 of COX-1 and serine 516 of COX-2) (Roth & Majerus, 1975; Loll *et al.*, 1995). Acetylsalicylic acid is approximately 170 fold more potent in inhibiting COX-1 than COX-2 (Vane *et al.*, 1998).

Figure 2.2 shows the inactivating process through acetylation. In the presence of acetylsalicylic acid, COX-1 is completely inactivated, whereas COX-2 converts arachidonic acid not to PGH_2, but to 15(*R*)hydroxyeicosatetraenoic acid (15-*R*-HETE) (Smith & De Witt, 1995). The end result is that neither affected isoforms is capable of converting arachidonic acid to PGH_2, a necessary step in the production of prostanoids. The resultant decreased production of prostaglandins accounts for the therapeutic effects, as well as the toxicities of acetylsalicylic acid.

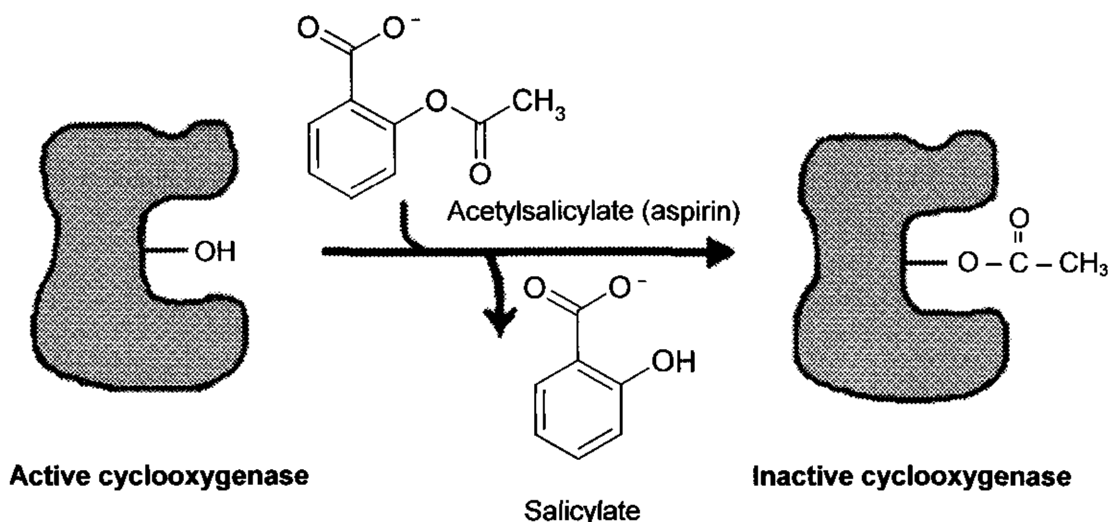


Figure 2.2: Inactivating cyclooxygenase (COX) through acetylation (Mesecar, 2001).

2.4 Clinical use and adverse effects of acetylsalicylic acid

In the design of any drug formulation it is highly desirable to have a detailed knowledge of the clinical use of the drug, for these considerations can well indicate if the formulation met certain specific requirements. Acetylsalicylic acid is the analgesic of choice for mild to moderate pain such as headaches, neuritis, toothache and dysmenorrhoea; it is relatively ineffective in visceral pain (Dollery, 1999 & Reynolds, 1984). The therapeutic effect is dose related. Doses of 300 – 600 mg every 4 – 6 h may be effective in mild pain, whereas to be more effective for severe pain (e.g. after dental extraction) doses of more than 1 g may be required (Dollery, 1999). Acetylsalicylic acid is used in the form of an anti-inflammatory agent in the treatment in musculoskeletal disorders, such as rheumatoid arthritis (Insel, 2001), a property that is not shown by certain other mild analgesics, for example, paracetamol, or by the potent analgesics, for example, pethidine (Bean, *et al.*, 1964). Antipyretic therapy is reserved for patients in whom fever in itself may be deleterious and for those who experience considerable relief when a fever is lowered (Dollery, 1999). Acetylsalicylic acid is also prescribed for the prevention of thromboembolic disorders, but the dose is only a quarter of that used for analgesia, namely 75 – 325 mg daily (Insel, 2001). Regular use of acetylsalicylic acid is associated with reduced incidence of colon cancer (Giovannucci *et al.*, 1995). There is also some preliminary evidence that acetylsalicylic acid delays the onset of Alzheimer's disease (Rang & Dale, 1999).

The most common adverse effects occurring with therapeutic doses of acetylsalicylic acid are gastro-intestinal disturbances such as nausea, dyspepsia and vomiting. Irritation of the gastric mucosa with erosion, ulceration, haematemesis and melaena may occur. Slight blood loss is not usually of clinical significance but may cause iron-deficiency anaemia during long-term salicylate therapy. Some patients, especially asthmatics, exhibit notable sensitivity to

acetylsalicylic acid which may provoke various reactions including urticaria and other skin eruptions, as well as angioneurotic oedema, rhinitis, and severe, even fatal, paroxysmal bronchospasm and dyspnoea (Reynolds, 1984).

2.5 Transdermal delivery of acetylsalicylic acid

A series of investigations have been done on the transdermal delivery of acetylsalicylic acid. The first study performed was to discover if acetylsalicylic acid would penetrate the skin by using human volunteers. Later rat and even porcine epidermis were used. Different types of studies were performed for example controlling pain associated with herpes zoster and post-herpetic neuralgia, modifying platelet function, determining the acetylsalicylic acid in transdermal perfusates after different compounds of aspirin were synthesised, and examining the effect that solvent systems have on *in vitro* transdermal absorption of acetylsalicylic acid.

Feldmann & Maibach (1970) studied the percutaneous penetration of 21 organic chemicals of which acetylsalicylic acid was one. The method involved applying the chemical (4 g/cm^2) to the ventral surface of the human forearm. Acetylsalicylic acid was dissolved in acetone and applied with a microliter syringe on unprotected skin sites. The subjects were not allowed to wash the area for 24 hours and all urine was collected for 5 days to measure the metabolites. All studies were performed with radiolabeled (^{14}C) tracer doses. The absorption was expressed as the percentage of applied dose over the 5 day period, values obtained was 21,81 with a standard deviation of 3,11 for acetylsalicylic acid.

Bronaugh *et al.* (1982) studied and compared the percutaneous absorption of radiolabeled acetylsalicylic acid by *in vivo* and *in vitro* techniques during a 5 day period. *In vivo* absorption was measured from urinary excretion data through female rat skin after radiolabeled acetylsalicylic acid was applied in a petroleum vehicle, while *in vitro* absorption was measured through excised rat skin in diffusion cells. The *in vivo* and *in vitro* absorption was expressed as the percentage of applied dose over a 5 day period, values obtained was $24{,}8 \pm 4{,}4$ and $29{,}0 \pm 3{,}1$, respectively. The permeability constant (cm/h) for acetylsalicylic acid *in vivo* was $5{,}2 \times 10^{-5}$ and *in vitro* $6{,}5 \times 10^{-5}$. Hence, good agreement was observed between the two methods.

King (1988) used acetylsalicylic acid in the control of pain associated with herpes zoster and post-herpetic neuralgia. Two (350 mg) acetylsalicylic acid tablets were crushed to a fine powder and 15 – 30 ml of chloroform or acetone were added and stirred. The suspension / solution was daubed onto the painful infected area. After the solution evaporated powdered acetylsalicylic acid covered the skin. Acetylsalicylic acid in water was ineffective, but in chloroform pain faded within 10 – 15 min and disappeared after 20 – 30 min. Chloroform had a cooling effect, while

acetylsalicylic acid had anti-inflammatory and analgesic effects. Chloroform not only functions as a solvent/suspension for acetylsalicylic acid, but also as a cleansing solvent of cutaneous fats, waxes and oils, thus allowing high concentration deposits of aspirin in close proximity to cutaneous nociceptors at the site of hepatic inflammation. Hence, the analgesic properties of acetylsalicylic acid became extraordinarily effective.

De Benedittis *et al.* (1992) used the same method originally pioneered by King (1988), except for using diethyl ether rather than chloroform. Diethyl ether also functioned as a solvent/suspensing agent for acetylsalicylic acid, as well as a cleansing solvent, but was preferred to chloroform because of lower hepatic, renal and cardiac toxicity. The acetylsalicylic acid/diethyl ether mixture has proved to be efficient in the treatment of acute herpetic neuralgia and post-herpetic neuralgia.

Keimowitz *et al.* (1993) used acetylsalicylic acid to modify platelet function percutaneously over a 10 day period. Acetylsalicylic acid was dissolved in ethanol or isopropyl alcohol and propylene glycol in the ratio of 1,7 to 1,0 (v/v), respectively, and the solution (250 mg or 750 mg acetylsalicylic acid) was applied on the forearm and upper arm of the volunteers. Urine was collected to measure 2,3-dinor-TXB_2 (TXM), the major enzymatic metabolite of TXA_2 and was determined by negative ion chemical ionisation, gas chromatography/mass spectrometry (NICI-GCMS) using authentic deuterated standards. They found that daily applied acetylsalicylic acid induced a dose-dependant inhibition of platelet cyclooxygenase, as measured by TXB_2. Maximum inhibition was achieved after 10 days and exceeded 95 % (of platelet cyclooxygenase inhibition) at the highest dose (750 mg acetylsalicylic acid). *In vivo* such a degree of suppression is necessary to inhibit TXA_2 biosynthesis and platelet function.

Steen *et al.* (1995) applied acetylsalicylic acid (60 mg/ml) or lactose (placebo) dissolved in diethyl ether (10 ml) on the palmar forearm of volunteers, where pain was induced. A continuous pressure infusion of an acidic phosphate buffered isotonic solution (pH 5,2) was used to produce a highly localised burning pain sensation in and around the injection site. Both treatments resulted in a sudden pain relief due to the cooling effect of the evaporating diethyl ether. With the placebo the pain returned after 6 – 8 min, while with acetylsalicylic acid it was significantly suppressed for the whole observation period (30 min).

Steen *et al.* (1996) used the same method to induce pain as in a previous study, but changed the vehicle from diethyl ether to a vaseline/paraffin ointment. The placebo (lactose in vaseline/paraffin ointment) was once again ineffective against pain, while acetylsalicylic acid decreased the pain which completely vanished after 28 min. They stated that low pH dose-dependent acetylsalicylic acid had the same analgesic effect as more highly concentrated ibuprofen cream in the treatment of cutaneous pain.

McAdam *et al.* (1996) examined the transdermal delivery of acetylsalicylic acid using two patch systems for suppressing platelet cyclooxygenase. The first patch (type A) was without and the second (type B) with limonene, a permeation enhancer. Type A patches had a total surface area of 100 cm^2 and contained 84 mg acetylsalicylic acid/patch. By day 14 serum TBX_2 resulted in 85 ± 6 % suppression and the residue drug in the patch showed that each patch delivered 18 ± 3 mg (day 1) and 17 ± 4 mg (day 14). Type B patches had a total surface area of 50 cm^2 and contained 120 mg acetylsalicylic acid/patch. By day 14 serum TBX_2 resulted in 60 ± 11 % reduction and 84 ± 9 % by day 21 and delivered 33 ± 3 mg of acetylsalicylic acid daily. Hence, platelet cyclooxygenase was suppressed and delivery was improved by limonene.

McMahon *et al.* (1998) synthesised four acetylsalicylic acid prodrugs, namely aspirin anhydride, an isosorbide ester, phenyl ester and nitrophenyl ester of acetylsalicylic acid, to determine the aspirin and salicylic acid in transdermal perfusates. *In vitro* transdermal studies were performed with mouse skin in Franz cells. The prodrug examined was diluted with ethanol, mixed with polyethylene glycol and topically applied to the skin. PBS buffer was used in the receptor phase at physiological pH and the entire receptor volumes were withdrawn and replaced with 37 °C fresh buffer solution after 2, 4 and 6 hours and injected directly onto the HPLC system and analysed. The aspirin anhydride was significantly more susceptible to hydrolysis than the ester prodrugs, yielding acetylsalicylic acid in the perfusate samples. Evidence of hydrolysis of the ester compounds to acetylsalicylic acid was seen, but it was not sufficient to warrant further investigation.

Levang *et al.* (1999) examined the effect that solvent systems, ethanol and propylene glycol, have on *in vitro* transdermal absorption of acetylsalicylic acid through porcine epidermis. They studied the biophysical changes in the stratum corneum lipids through the use of Fourier transform infrared (FTIR). Maximum flux of acetylsalicylic acid was achieved by 80 % ethanol in combination with 20 % propylene glycol that showed a maximum decrease in absorbance for asymmetric and symmetric C – H peaks. The aforementioned suggested a greater loss of lipids in the stratum corneum layers and each of the solvent systems significantly enhanced *in vitro* transepidermal water loss.

Winek *et al.* (2001) gave the following blood levels of acetylsalicylic acid for analgesic use:

- Therapeutic or normal blood level: 20 – 100 μg/ml
- Toxic blood level: 150 – 300 μg/ml
- Lethal blood level: 500 μg/ml

Chapter 3

Transdermal drug permeation

3.1 Introduction

There has been an increasing interest in percutaneous drug absorption over the past few years. The transdermal application of drugs is an alternative route with some biopharmaceutical benefits, such as bypassing hepatic first-pass elimination and improving compliance (Kai *et al.*, 1992; Ouriemchi & Vergnaud, 2000). As the largest and most external organ, the skin is constantly exposed to the hazards of the environment and is often viewed as a living protective envelope surrounding the body. It serves as a barrier, limiting the systemic exposure to the excessive loss of critical internal contents. However, it is becoming increasingly apparent that the skin is not a complete barrier; in fact, it's a readily accessible portal with a large surface area, through which a variety of substances can enter the body and subsequently pass into the systemic circulation (Kao, 1990).

Transdermal therapy, however, has its limitations. Firstly, and most obviously, the skin acts as a two-way barrier, preventing the entry of harmful or unwanted molecules from the external environment, while controlling the loss of water, electrolytes and other body constituents. Secondly, there may be pharmacodynamic, physiological and/or physicochemical limitations. Compounds may act as irritants, cause allergic sensitisation, be keratolytic or cause hyperpigmentation. These pharmacodynamic effects are dependent on the extent of the percutaneous absorption of the substance in question, which, in turn, depends on the physiological characteristics of the skin and the physicochemical properties of the penetrant. Thus, the physicochemical properties of the drug have an influence on the rate and extent to which a number of drugs pass through the skin readily (Beckett, 1982).

The physicochemical features of a drug control the rates of diffusion and partitioning within the delivery system as well as the skin and include molecular mass, ionisation of the drug at physiological pH, the lipid/water partition coefficient, melting point, solubility and chemical structure. Predictive algorithms use the molecular volume and the hydrogen bond donor-acceptor activities to determine skin permeability (Potts & Guy, 1995). This is a clear indication of the importance of hydrogen bonding in skin permeation, a factor that was considered qualitatively by Roberts *et al.* (1977). Abraham *et al.* (1995) have also considered solute size, solute dipolarity/polarisability and hydrogen bond basicity, which produced some interesting relationships.

The percutaneous delivery of drugs is an effective way of achieving controlled drug delivery. Unfortunately it is only suitable for a limited number of drugs that possesses the appropriate physicochemical characteristics to allow them to cross the excellent barrier provided by the outermost layer of the skin, the stratum corneum (Harrison *et al.,* 1996). Histologically, the skin is a complex multilayered organ (Holbrook & Wolff, 1993) with a total thickness of 0,05 – 2,0 mm (Foldvari, 2000). The stratum corneum has physical barrier functions to most compounds, including drugs, while the viable skin is responsible for enzymatic bioconversion. Transdermal permeation involves drug molecules first partitioning onto the surface of the skin and subsequently diffuses across the stratum corneum toward the viable tissue. The diffusion into the stratum corneum is believed to be a rate-limiting step on transdermal absorption of most drugs that are stable in the skin. After penetration across the skin, drug molecules are efficiently taken away into the microcirculation located beneath the basal layer of the skin (Tojo, 1997).

Topical application of drugs for systemic therapy may have several advantages over the conventional oral route. It circumvents two of the main problems from oral drug administration:

1. It eliminates variables that may influence the gastro-intestinal absorption, such as food intake, the drastic change in pH along the gastro-intestinal tract, intestinal motility and illness such as nausea, which disables the patient to contain the drug for a long enough period thus inhibiting absorption.

2. It may eliminate systemic first-pass metabolism as it circumvents the liver. This may result in an increased bioavailability of the drugs susceptible to this bioconversion (Wiechers, 1989).

Except for these two main advantages, we can add that transdermal drug delivery:

- avoids peaks and valleys in serum levels often seen with discrete oral dosages and which can often cause undesirable side effects (Roy, 1997) and

- maintains zero-order delivery in many instances and can be sustained for longer periods of time, leading to less frequent dosing regimens. This would, in turn, improve patient compliance, since frequent drug intake is no longer necessary (Naik *et al*, 2000).

3.2 Percutaneous absorption

Percutaneous absorption can be defined as the uptake of a compound into the systemic circulation after dermal application, and it describes the movement through the various layers of the skin with respect to both rate and extent.

This whole process of absorption can be divided into three steps:

1. Penetration (the entry of a substance into a particular layer or organ).
2. Permeation (the penetration through one layer into another, which is both functionally and structurally different from the first layer).
3. Absorption (the uptake of a substance into the vascular system, lymph and/or blood vessels, which act as the central compartment) (Schaefer *et al.,* 1982).

Factors influencing the percutaneous absorption of chemicals through the skin are:

- the structure of the skin,
- the physicochemical characteristics of the penetrant,
- the physicochemical characteristics of the vehicle and
- the dosing conditions (Wiechers, 1989).

3.2.1 The skin as barrier to transdermal absorption

The largest organ of the body, the skin, covers an area of approximately 1,73 m^2 (Barr, 1962) and weighs an average of 3 – 4 kg (Schalla & Schaefer, 1982; Stuttgen, 1982). The average thickness of the skin is about 0,5 mm (ranging from 0,05 – 2,0 mm) (Foldvari, 2000). A square centimetre of skin contains 3 blood vessels, 10 hair follicles, 12 nerves, 15 sebaceous glands and 100 sweat glands (Asbill & Michniak, 2000).

Before reaching the systemic circulation, a penetrating chemical has to cross several potential barriers. These include the epidermis (consisting of the stratum corneum or horny layer and the viable layers of the epidermis) and the dermis.

A cross section of the skin (Figure 3.1) shows the anatomically distinguishable regions, from the outside of the skin inwards (Flynn, 1990).

- The ~ 10 μm thin nonviable epidermis or stratum corneum;
- The ~ 100 μm thin viable epidermis, which includes the germinal (basal) layer and everything living above up to the stratum corneum;
- The ~ 1000 μm thick dermis, and
- The hypodermis or subcutaneous fat layer.

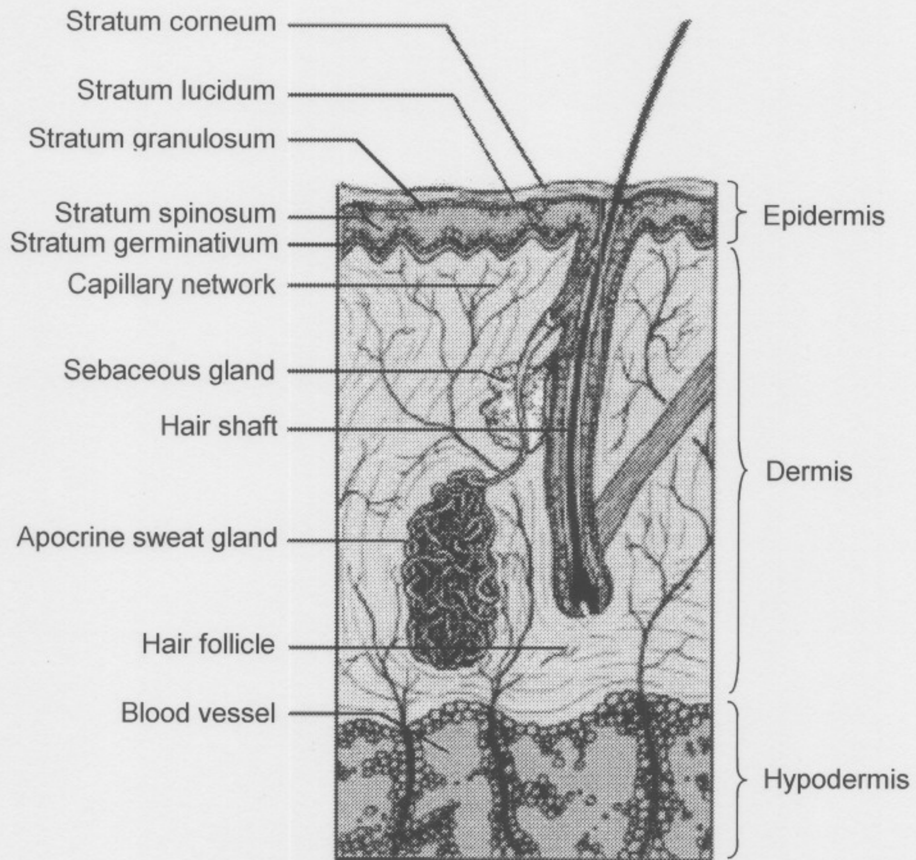


Figure 3.1: A cross section of the human skin (West & Nowakowski, 1996).

These structures are anatomically and functionally dissimilar. Each has a unique body distribution, and there are characteristic differences in histological appearance of the structures from place to place on the body. Also, penetrating the tissue, up to, but not into, the epidermis, is a complex network of blood vessels. Finally, the skin is interlaced with sensory nerves (Flynn, 1990).

3.2.1.1 Stratum corneum

The outmost layer of the skin is the stratum corneum or "cornified layer" of the skin and consists of keratinised epithelial cells, called corneocytes, physically isolated from one another by extracellular lipids arranged in multiple lamellae. It is a very dense tissue, about 1,4 g/cm^3 in the dry state. The stratum corneum is under continuous formation. A total turnover of cells in the stratum corneum occurs about every 2 weeks in normal adults.

The thickness of the stratum corneum under normal non-hydrated conditions ranges from 10 – 15 μm and contains 10 – 25 layers of corneocytes (Flynn, 1979; Foldvari, 2000). Although it is flexible, it's also impermeable. On the palms of the hands and on the foot soles, the stratum

corneum has an average thickness of 400 – 600 μm, with vertically stacked cells.

The stratum corneum tissue is often schematically represented as a brick wall (as shown in Figure 3.2). The terminally differentiated, keratin-filled corneocytes are the "bricks" while the lamellar, intercellular lipid domain represent the "mortar". The interstitial lipid is the residue of the membrane surrounding each epidermal cell; subsequently it becomes embodied into the stratum corneum (Elias, 1983; Menon, 2002; Roy, 1997).

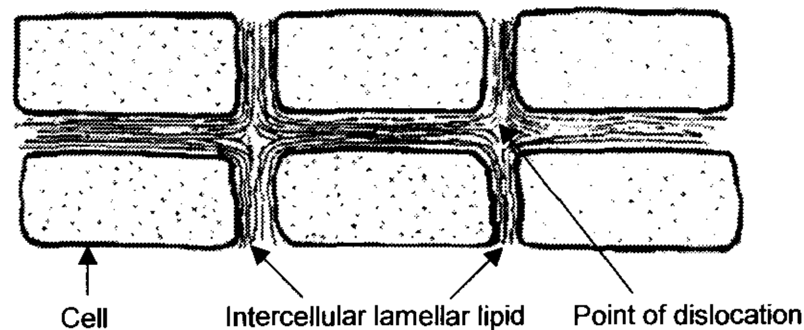


Figure 3.2: Proposed "Brick and Mortar" Two-Compartment Model (Elias, 1983).

In its normal state at ordinary relative humidities, the stratum corneum also contains moisture to the extent of 15 – 20% of its dry weight. The water content increases up to 300 – 400% of the dry weight on some areas of the body when the skin becomes waterlogged through soaking. The stratum corneum is thus a thin, ultra dense polyphasic epidermal covering made from dehydrated, highly filamented former cells (Flynn, 1990).

Lipids are also synthesised during keratinocyte epidermal transit. It is collected in vesicles and is visible in the granular layer. As the granular cells further transform and enter the stratum corneum, these vesicles migrate to the cell membrane, at which point their contents are passed through the cell wall and into the intercellular space. This lipid thus becomes a mortar that seals the total structure, making the stratum corneum an incredibly efficient moisture barrier. The lipid content of the horny layer is estimated to comprise as much as 20% of the stratum corneum's dry weight (Flynn, 1990).

The stratum corneum lipid bilayers (Figure 3.3) play an important role in the transdermal absorption of drugs. The intercellular lipid membranes constitute a barrier for the absorption of hydrophilic drugs (Matsuzaki *et al.*, 1993).

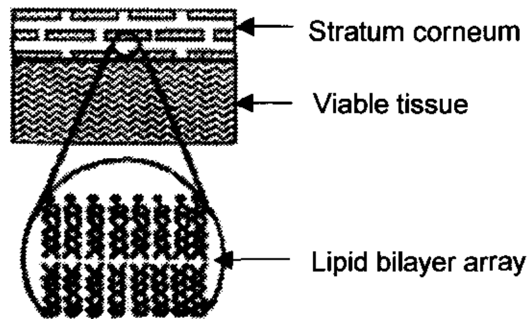


Figure 3.3: A schematic representation of the stratum corneum lipid bilayers (Hadgraft & Wolff, 1993).

The stratum corneum lipids are selectively enriched in ceramides, free acids, free sterols and lesser quantities of glycolipids, triglycerides, hydrocarbons, sterol esters and cholesterol sulphate, but contains no phospholipids (Elias, 1983). It is postulated that despite an absence of phospholipids, these lipids apparently can arrange themselves into membrane bilayers (Roy, 1997). All of the compounds of interstitial lipids, except for water-soluble proteins, essentially contribute to the variable function of the stratum corneum (Scheuplein & Blank, 1971).

The stratum corneum is generally regarded as the rate-limiting barrier for transport, of most solutes of pharmaceutical interest, across the skin. In spite of this well-documented heterogeneity, most studies of drug transport treat the stratum corneum as a homogeneous membrane. Thus, solute fluxes are assumed to be directly proportional to stratum corneum/water partition coefficients and diffusivities and inversely proportional to the macroscopic thickness of the stratum corneum (Raykar *et al.*, 1988).

Some experimental observations appear to conflict with predictions arising from the assumption of homogeneity. For example, the thickness of the stratum corneum and the rates of percutaneous transport across human skin, are not influenced by the number of cell layers but, instead, correlate inversely with the lipid content. These stratum corneum lipids may be pooled in the intercellular spaces, forming broad, multilamellar sheets, which constitute the barrier to diffusion. Similarly, in reaggregrated stratum corneum cell systems the effectiveness of the barrier function is directly proportional to the lipid content rather than the barrier thickness (Raykar *et al.*, 1988).

3.2.1.2 Viable epidermis

As shown in Figure 3.1, the viable epidermis lies between the stratum corneum and the dermis, and it has shown readily definable interfaces with each. In drug delivery considerations it is often regarded as a single stratum of living cellular tissue, although histologically it is

multilayered.

It is primarily aqueous in nature and its diffusional resistance resembles an aqueous protein gel (Scheuplein, 1986). It is about 75 – 150 µm thick and consists of various layers, characterised by various stages of differentiation (Roy, 1997). A penetrating chemical has to cross the stratum lucidum, the stratum granulosum (granular layer), the stratum spinosum (spinous layer) and the stratum basale (or basale layer). These are metabolically active cells undergoing systematic transitions which eventuate in cell death, for as they move toward the surface, they move away from the microcirculation in the dermal layer that supplies them with the necessary oxygen and nutrition.

The cellular structure of the viable epidermis is predominantly hydrophilic throughout its various layers, and substances can be transported in its intercellular fluids. Especially for polar substances, the resistance to penetrate is considerably lower than in the stratum corneum, because the tightly packed alternating hydrophilic and lipophilic layers are no longer present (Wiechers, 1989). It consists primarily of an aqueous cytoplasm encapsulated in cellular compartments by delicate cell membranes - the cells being fused together by tonofibrils. The water has the thermodynamic activity of a 0,9 % NaCl solution. As a slab, the density and the consistency are not much different from that of water (Flynn, 1990).

3.2.1.3 Dermis

The dermis is depicted in Figure 3.1 as a nondescript region lying between the epidermis and the subcutaneous fatty region. It consists mainly of a dense network of structural protein fibres, collagen, reticulum and elastin embedded in a semigel matrix of mucopolysaccharidic "ground substance" (the dermis is also penetrated by a network of sensory nerves and lymphatics) (Asbill & Michniak, 2000; Flynn, 1990). It ranges from 0,1 – 0,5 cm in thickness. The microcirculation that subserves the entire skin is located in the epidermis (Flynn, 1990).

The excellent blood supply in the dermis functions as a "sink" (constantly removing drugs from the absorption site) for diffusing molecules and keeps penetrating molecule concentrations very low, thereby amplifying concentration gradients across the skin layers and promoting percutaneous absorption (Danckwerts, 1991; Roy, 1997). Hence it is believed that the dermis offers no barrier for drug to permeate, except for molecules that might be substantive to specific dermal components (Rieger, 1993).

3.2.1.4 Hypodermis

The hypodermis or subcutaneous fatty layer is the innermost layer of the skin, provides a mechanical cushion for external blows and a thermal barrier from external variations in

temperature. It also synthesises and stores readily available high-energy chemicals (Danckwerts, 1991).

3.2.1.5 Skin appendages

In addition to the above three major layers of the skin, the skin has many other appendages that affect the percutaneous delivery of drug compounds (Danckwerts, 1991). The skin has interspersed hair follicles, nails and associated sebaceous glands, the so-called pilosebaceous glands, as well as in specific regions two types of sweat glands, the eccrine and apocrine glands. Collectively these are all called the skin appendages (Flynn, 1990) of which all, except the nails, lie in the dermis (Hunter *et al.,* 1996). The sebum, which is produced by the sebaceous glands, consists of a mixture of fatty acids, triglycerides, waxes, cholesterol and cellular debris (Montaga, 1965). The expanded lower part of the hair follicle contains the matrix from which new cells are formed. These cells move upward and cornify differently than the skin (Katz & Poulson, 1971).

As barrier to percutaneous drug delivery the skin can be generalised to conclude that it serves as a very effective barrier to chemical penetration, because the diffusional resistance is larger for virtually all molecular species. Transport across the skin, is thus obviously a complex phenomenon.

3.2.2 The process of percutaneous absorption

The quantitative prediction of the rate and the extent of transdermal penetration and absorption of topically applied drugs are complicated by the biological variability inherent to the skin. In order to gain perspective of this phenomenon, one should appreciate that mammalian skin is a dynamic organ with a myriad of biological functions. The most obvious is its barrier property, which is of primary relevance to transdermal absorption (Riviere, 1993).

Molecules moving from the environment across the intact skin of living humans must first penetrate the stratum corneum. They must then penetrate the viable epidermis, the papillary epidermis, and the capillary walls into the bloodstream or lymph channels, whereupon they are removed from the skin by flow of blood or lymph (Idson, 1975; Kalia & Guy, 2001). To move, molecules have to overcome a different resistance in each tissue (Idson, 1975).

When molecules move onto the intact skin, the diffusant then have three potential routes of entry to the subepidermal tissue as seen in Figure 3.4 (Barry, 2001).

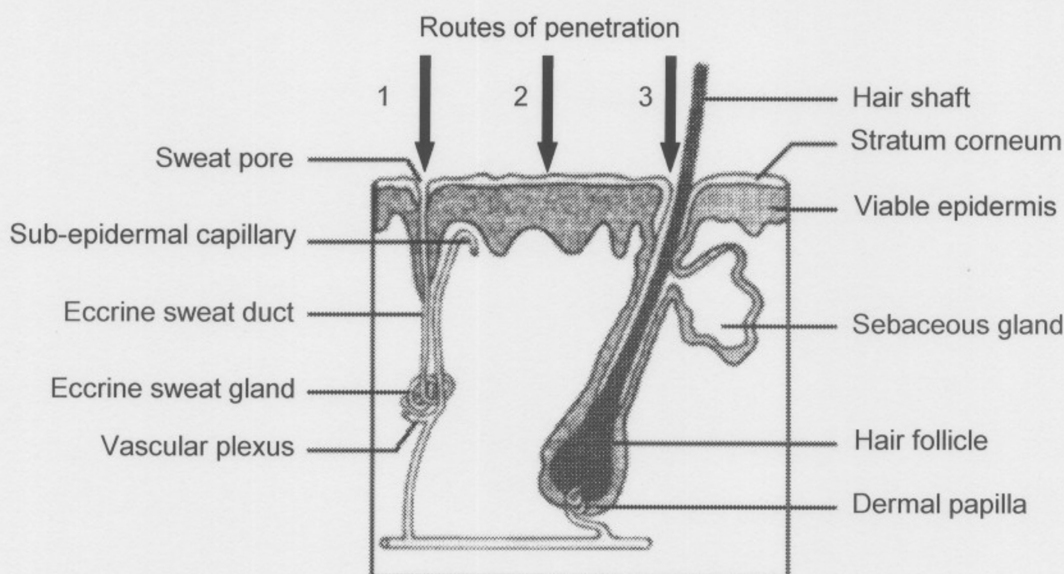


Figure 3.4: Pathways of penetration through the skin: (1) via the sweat gland ducts; (2) across the stratum corneum; or (3) through the hair follicles (Barry, 2001).

Guy & Hadgraft (1989) identified three possible permeation pathways across the stratum corneum. The first pathway involves crossing the stratum corneum by the most direct route and diffusing through the cornified cells and extracellular bilayers. It is known as the transcellular path. The second pathway involves passage through the lipids in the stratum corneum and is known as the intercellular path. The last pathway is the appendageal path. The aforementioned path permeates through the hair follicles and sweat gland ducts, bypassing the stratum corneum. This path is considered to be of substantially less importance as it accounts for less than 0,1 % of the total surface area of the skin (Schaefer & Hensby, 1990) and may be important for ions and large polar molecules that struggle to cross intact stratum corneum (Barry, 2001).

It is considered that for most compounds, the intercellular route predominates (Guy & Hadgraft, 1989). Irrespective of which route is favoured, the drug eventually works its way to the edge of the viable tissue. Ordinarily the viable tissue is not much of a diffusion impediment and net drug passes with facility through the living layer towards the closest capillary bed (Flynn & Weiner, 1993).

There are many factors that can alter the rate of extent of absorption into the skin. The mode of application, temperature and condition of the skin, influence of the vehicle, frequency and duration of application, concentration and physicochemical properties of the active ingredient are all examples that can affect the absorption. If all but the last of the aforementioned factors can be kept constant, then it will be possible to determine which physicochemical properties of the compound are most important in determining the absorption through the skin or into the skin

(Lien & Tong, 1973).

The whole process of dermal absorption has been modelled in a simplified approach (Guy & Hadgraft, 1989) and the diffusional and partitioning steps involved are depicted in Figure 3.5. The rate constant, K_1 (h^{-1}), is a first order approximation for diffusion and its magnitude is related to the molecular size through molecular weight, M, by Equation 3.1:

$K_1 = 0{,}9\ M^{-0.33}$ Equation 3.1

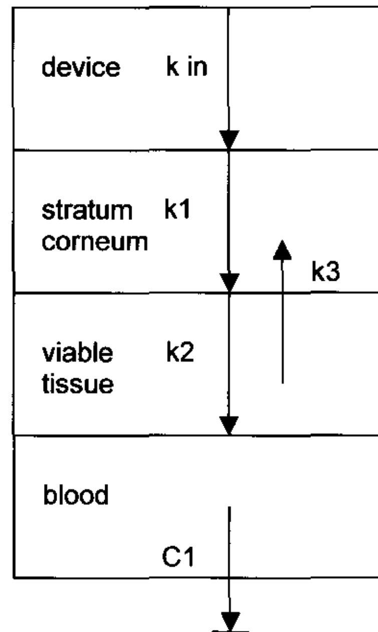


Figure 3.5: Kinetic model of skin (Guy & Hadgraft, 1989).

It follows that diffusion and partitioning are the key physical processes pertinent to dermal permeation (Guy & Hadgraft, 1989).

The diffusion is related to the number of hydrogen bonding groups on the solute, with the presence of zero to two groups having the most pronounced effect on the magnitude of the diffusion coefficient. More about hydrogen bonding and the diffusion coefficient will be discussed later (§ 3.3.6 and 3.3.2 respectively).

From the results obtained in the study of Lien & Tong (1973) on physicochemical properties and percutaneous absorption of drugs, it appears that the lipophilic character of the compound, as measured by the partition coefficient, plays the most important role in determining percutaneous absorption.

A concentration gradient is established through the skin via passive diffusion. The concentration gradient is very steep in the horny layer because of its barrier function and less

steep in the viable epidermis. As a consequence of the passive diffusion (there being no evidence for active transport mechanisms in the skin) a decreasing concentration gradient from the horny layer to the subcutaneous tissue is found. The driving force for absorption or transport of any drug is proportional to the concentration gradient of any drug within the skin (Flynn, 1989).

3.3 Physicochemical factors influencing transdermal absorption

The principal factors affecting penetration are the properties of the drug, the vehicle and the skin. The physical and chemical nature of each of these components and their collective interactions all influence the rate at which the drug penetrates the skin (Katz & Poulsen, 1971).

The physicochemical properties of a drug substance are very important determinants for its permeation through the skin. The most important processes to consider are the partitioning and diffusion steps that occur in the transport into, through and out of the stratum corneum (Hadgraft & Wolff, 1993).

3.3.1 Drug solubility in the stratum corneum

The released drug will partition into the outer layers of the stratum corneum. The degree to which this will happen is controlled by the amount applied and the solubility limit in the stratum corneum. The rate of partitioning from the vehicle to the skin will be more rapid than the diffusion into the skin and, in general, does not need to be taken into account (Hadgraft & Wolff, 1993).

The thermodynamic activity of a drug in a particular vehicle indicates the potential of the active substance to become available for therapeutic purposes (Kemken *et al.*, 1992). It has been shown that supersaturated solutions provide enhanced fluxes through model membranes and skin (Hadgraft, 1991). A saturated solution is therefore preferable for a topical drug delivery system as it represents maximum thermodynamic activity (Kemken *et al.*, 1992). The level of saturation is dependent on the solubility of the drug in the delivery formulation (Danckwerts, 1991). Less drug is released from sub-saturated solvents than from saturated ones.

In general, the flux of any given compound across a membrane from a saturated solution, irrespective of its concentration, is constant, provided that there are no interactions between the membrane and the components of the formulation. Therefore under normal circumstances, the flux of a drug is limited by its solubility, which, in turn, can also limit its bioavailability. Consequently, the preparation of stable supersaturated systems not only circumvents some of

the regulatory issues that are associated with other mechanisms of enhancement, but it can also lead to increased bioavailabilty (Pellet *et al.*, 1994).

The solubility of a drug can also be affected by the presence of a co-solvent in the formulation. Co-solvents that increase the solubility of the active drug can produce greater concentrations across the vehicle skin interface (Pellet *et al.*, 1994). However, enhanced solubility of the drug in the solvent may result in a reduced partitioning of the drug between the membrane and the vehicle (Danckwerts, 1991). As a result, there is a need to keep the solubility of the drug in the vehicle as close to the saturation point as possible. Therefore it is undesirable to use a drug that is highly soluble in the base, as the release of the drug will be retarded.

Solubility is dominant in skin penetration. Its importance was recognised early when it was found that compounds with both lipid and water solubilities penetrate better than substances with either high water or high lipid solubility (Liron & Cohen, 1984; Naik *et al.,* 2000; Pefile & Smith, 1997). The solubility characteristics of a substance greatly influence its ability to penetrate biological membranes. The lipid-water solubility pattern of the applied material was recognised at the beginning of this century in the Meyer-Overton theory of absorption. This theory stated that, because the epidermal cell membrane consists of a mosaic pattern of lipid and protein molecules, substances soluble in lipids pass through the cell membrane owing to its lipid content. While water soluble substances pass after the hydration of the protein particles in the cell wall, which leaves the cell permeable to water soluble substances (Naik *et al.,* 2000). In essence, the aqueous solubility of a drug, determines the concentration presented to the absorption site, and the partition coefficient strongly influences the rate of transport across the absorption site (Idson, 1975).

3.3.1.1 Solubility parameter

The solubility parameter is one of the indexes expressing energetics of molecular interaction, namely, higher miscibility can be realised when two solubility parameters of the components are closer in the binary system. By using the solubility parameter, the solubility of solute in solvent is almost predictable (Otha *et al.,* 1999).

The solubility parameter is defined as the square root of the cohesive energy density. The cohesive energy of a material is the energy which holds that substance together and is therefore also the net effect of all the intermolecular interactions. It is the amount of energy required to separate the constituent atoms or molecules of the material to an infinite distance and therefore it is a direct measurement of the attraction that atoms of molecules have for one another (Hildebrand & Scott, 1950).

The solubility parameter, δ, is an intrinsic physicochemical property of a substance, which has

been used to explain the drug action, structure-activity relationships, drug transport kinetics and *in situ* release of drugs. Hence, the precise value of the solubility parameter of the drug is of interest (Subrahmanyam & Sarasija, 1997).

The solubility parameter first defined by Hildebrand and Scott has been found to be a useful guide for solvent miscibility. The solubility parameter of an organic solute (δ_2) in the stratum corneum can be estimated using Equation 3.2, if the solubility of the solute in a non-polar organic solvent (like hexane) is known, as well as the solute's heat of fusion, the melting point, and the solubility parameter of the solvent (hexane) (Hildebrand *et al.*, 1970):

$$\ln X_2 = \frac{-\Delta H_f}{RT}\left(\frac{T_f - T}{T_f}\right) + \frac{\Delta C_p}{R}\left(\frac{T_f - T}{T} - \ln\frac{T_f}{T}\right) - \frac{V_2 \phi_1^{\,2}}{RT(\delta_1 - \delta_2)^2}$$ Equation 3.2

where X_2 is the solute's mole fraction solubility in hexane, ΔH_f is the heat of fusion of a solid, R is the gas constant, T_f is the melting point of the solid (Kelvin), T is experimental temperature < T_f, ΔC_p is the difference in heat capacity between the solid form and the hypothetical super cooled liquid form of the compound, both at the same temperature, V_2 is the molar volume of the liquid solute, ϕ_1 is the volume fraction of the solvent, δ_1 is the solubility parameter or square root of the cohesive energy density of the solvent (hexane) and δ_2 is the solubility parameter or square root of the cohesive energy density of the solute.

A low solubility parameter for a solute is synonymous with high lipophilicity (Roy & Flynn, 1989).

The solubility parameter of the skin has been estimated at approximately 10 (Liron & Cohen, 1984) and therefore drugs, which possess similar values would be expected to dissolve readily in the stratum corneum. Formulation components, which can diffuse into the skin, e.g. propylene glycol, will tend to and is expected to increase the value of the solubility parameter and would be expected to promote the solubility of polar drugs in the lipids (Hadgraft & Wolff, 1993).

3.3.1.2 Aqueous solubility

One of the most important factors influencing bioavailabilty is the drug's chemical structure, which in return influences the drug's aqueous solubility. Both the pH and the physical properties play a role in determining solubility. As a general rule, a drug substance with an aqueous solubility of less than 1 mg/ml may represent a potential bioavailability problem. In some instances, minor chemical modifications of the drug chemical, such as salt formation or esterification, are necessary (Abdou, 1989).

Aqueous solubility has long been recognised as key factor in controlling drug efficacy. There

seems to be a relationship between aqueous solubility and the chemical structure of the drug. The aqueous solubility of the drug is governed by three major factors (Yalkowsky & Valvani, 1980):

1. the entropy of mixing;
2. the difference between drug-water adhesive interactions and the sum of the drug-drug and water-water cohesive interactions; and
3. the additional drug-drug interactions associated with the lattice energy of crystalline drugs.

Aqueous solubilities of nonpolar organic compounds depend on their molecular surface areas, which are essentially hydrophobic in nature. Thus, the affinity for water decrease exponentially as molecular hydrophobic surface area increases (Barry, 2001). The aqueous solubility of drugs by convention is reported on a molar rather than a mole fraction scale. For poorly soluble compounds, the molar solubility is simply the mole fraction solubility multiplied by 55,5. The following equation enables the estimation for the aqueous solubility of either liquid or crystalline organic or crystalline nonelectrolytes (Yalkowsky & Valvani, 1980):

$$\log S_w \approx 1{,}00 \log PC - 1{,}11 \frac{\Delta S f\ (mp - 25)}{1364} + 0{,}54$$

Equation 3.3

where PC is the octanol-water partition coefficient, ΔSf is the entropy of fusion and are estimated from the chemical structure and mp is the melting point.

Compounds with low melting points usually have high solubillities and consequently higher dissolution rates.

This equation provides a means of assessing the role of crystal structure (as reflected by the melting point and the entropy of fusion) and the activity coefficient (as reflected by the octanol-water partition coefficient) in controlling the aqueous solubility of a drug (Yalkowsky & Valvani, 1980).

The intermolecular force factors, which lend polarity to molecules and tend to make permeability coefficients low, are the same factors that contribute positively to aqueous solubilities (Roy & Flynn, 1989).

3.3.2 Diffusion coefficient

Diffusion can be defined as the transport of matter resulting from movement of the substance within a substrate (Rieger, 1993). The diffusion coefficient can therefore be defined as the

number of moles of drug that diffuse across a membrane or within the various strata of a given area per time unit, and is influenced by the molecular size of the drug and the viscosity of the surrounding medium (Idson, 1983).

Particles move through membranes firstly by simple molecular permeation and secondly by movement through pores and channels (Martin *et al.,* 1983). Because of the dense nature of the stratum corneum, values of the diffusion coefficients in this tissue are 1000 times smaller than anywhere else in the skin. This factor contributes to a high resistance and low permeability (Flynn, 1990); hence a general rule is that molecules follow the path of least diffusional resistance (Flynn, 1989).

The three important factors influencing the penetration of drug into the skin are:

1. Concentration of dissolved drug, since penetration rate is proportional to concentration.

2. Partition coefficient K - between the skin and the vehicle.

3. Diffusion coefficients, which represent the resistance of the drug molecule movement through the vehicle and the skin barriers (Martin *et al.,* 1983).

Fick's laws are generally viewed as the mathematical description of the diffusion process through the membranes. Fick's laws are applicable whenever the chemical or physical nature of the membrane controls the rate of diffusion. In order to pass from the solvent to the skin, the diffusing molecule must have some affinity for the stratum corneum. Once the molecule is within that membrane it can diffuse in any direction. Progress is not random, because the permeant tends to move readily from the higher concentration to the lower concentration. Fick's first law can be applied to describe the diffusion processes in the stratum corneum (Wiechers, 1989):

$$J = K_p \,.\, \Delta C = \frac{D \,.\, K \,.\, \Delta C}{L}$$

Equation 3.4

where J is the steady state flux of the permeant through the stratum corneum ($\mu g.cm^{-2}.h^{-1}$), K_p is the permeability coefficient of the permeant in the stratum corneum ($cm.h^{-1}$), ΔC is the concentration gradient of the permeant across the stratum corneum ($\mu g.cm^{-3}$), D is the diffusion coefficient of the permeant in the stratum corneum ($cm^2.h^{-1}$), K is the apparent partition coefficient of the permeant between the stratum corneum and the vehicle and L is the length of the pathway through the stratum corneum (cm).

It is apparent from Equation 3.4 that the flux is constant if the permeability coefficient and the concentration difference are constant.

The concentration gradient over the stratum corneum will depend primarily upon chemical characteristics of the permeant including solubility, lipophilicity, ionisation and stability (Wiechers, 1989; Smith, 1990). It is assumed that ΔC will be the same as the donor concentration if "perfect sink" conditions exist within the dermal membrane, together with the dermal drug concentration never exceeding 10 % of the donor concentration (Roy, 1997).

Fick's laws are more correctly expressed in terms of the chemical potential of the diffusant rather than its concentration. In an ideal system, there should be a linear relationship between the rate of diffusion and the concentration of the diffusant. The maximum flux will occur when the concentration reaches the solubility limit.

The diffusion coefficient or diffusivity, D, is a rough measure of the ease with which a molecule can move about within a medium, in this case the stratum corneum (Smith, 1990). It is dependent on the molecular weight and volume, and the degree of interaction between the permeant and the stratum corneum. The larger the molecule, the more difficult it is to move about, and the lower the diffusivity. Up to a molecular weight of at least 500 daltons, and perhaps 5 000 daltons, the molecular size plays no crucial role (Wiechers, 1989). For molecules with similar polarity, those having the lower molecular weight permeate faster. This might be explained by the observed decrease in diffusivity in liquid media with increasing molecular volume according to Equation 3.5:

$$D = A \, . \, V_m \qquad \text{Equation 3.5}$$

where D is the diffusivity of a spherical penetrant, A is a constant and V_m is molar volume.

Non-specific and specific binding may occur in both the epidermis and dermis, reducing diffusivity and thereby decreasing skin permeability (Wiechers, 1989). Another important factor that influences the diffusion coefficient is the drug state, e.g. ionised or unionised, with unionised forms diffusing more freely than the ionised forms (Abdou, 1989).

Other parameters include the affinity of the drug for the vehicle, the temperature of the vehicle and the viscosity. The lower the affinity of the drug is for the vehicle, the higher the diffusion coefficient (Babar *et al.*, 1990). Diffusivity decreases with increasing viscosity and decreasing temperature of the vehicle. Equation 3.6 details the influence of the diffusion coefficient parameter on the permeability characteristics of the drug.

$$K = \frac{Ph}{D} \qquad \text{Equation 3.6}$$

where K is the partition coefficient, P is the permeability coefficient, h is the thickness of the barrier and D is the diffusion coefficient.

This whole process of absorption can be divided into three steps:

1. Penetration (the entry of a substance into a particular layer or organ).
2. Permeation (the penetration through one layer into another, which is both functionally and structurally different from the first layer).
3. Absorption (the uptake of a substance into the vascular system, lymph and/or blood vessels, which act as the central compartment) (Schaefer *et al.,* 1982).

Factors influencing the percutaneous absorption of chemicals through the skin are:

- the structure of the skin,
- the physicochemical characteristics of the penetrant,
- the physicochemical characteristics of the vehicle and
- the dosing conditions (Wiechers, 1989).

3.2.1 The skin as barrier to transdermal absorption

The largest organ of the body, the skin, covers an area of approximately 1,73 m^2 (Barr, 1962) and weighs an average of 3 – 4 kg (Schalla & Schaefer, 1982; Stuttgen, 1982). The average thickness of the skin is about 0,5 mm (ranging from 0,05 – 2,0 mm) (Foldvari, 2000). A square centimetre of skin contains 3 blood vessels, 10 hair follicles, 12 nerves, 15 sebaceous glands and 100 sweat glands (Asbill & Michniak, 2000).

Before reaching the systemic circulation, a penetrating chemical has to cross several potential barriers. These include the epidermis (consisting of the stratum corneum or horny layer and the viable layers of the epidermis) and the dermis.

A cross section of the skin (Figure 3.1) shows the anatomically distinguishable regions, from the outside of the skin inwards (Flynn, 1990).

- The ~ 10 μm thin nonviable epidermis or stratum corneum;
- The ~ 100 μm thin viable epidermis, which includes the germinal (basal) layer and everything living above up to the stratum corneum;
- The ~ 1000 μm thick dermis, and
- The hypodermis or subcutaneous fat layer.

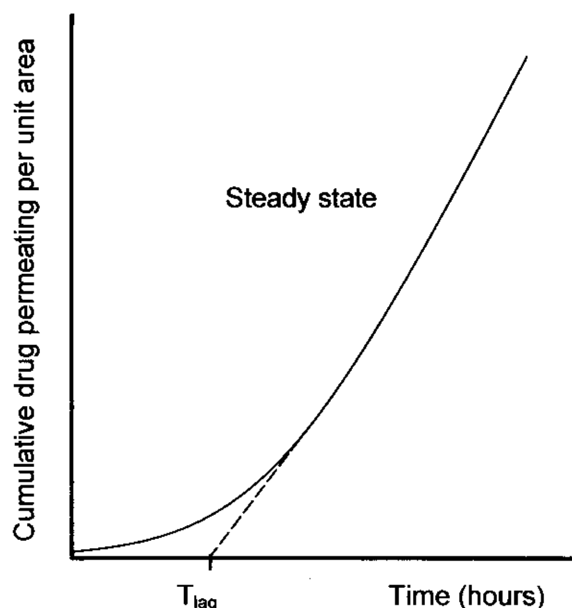


Figure 3.6: Typical permeation profile for a molecule diffusing across human skin (Williams & Barry, 1992; Smith, 1990).

The diffusivity, D, can be determined *in vitro* by simply measuring the initial transdermal flux until a steady state flux is reached using diffusion cells (§ 4.4.3). A representative plot of the cumulative amount of drug crossing the skin is shown in Figure 3.6. The time before steady state is reached is characteristic for the diffusivity of the permeant in the membrane, and can be used to calculate the diffusivity (Williams & Barry, 1992; Smith, 1990). The lag time, T_{lag}, is the time obtained from extrapolation of the steady state portion to the graph to the intercept on the time axis, and is defined by Equation 3.7 (Bach & Lippold, 1998; Williams & Barry, 1992; Smith, 1990):

$$T_{lag} = \frac{L^2}{6\,D} \qquad \text{Equation 3.7}$$

where L is the thickness of the membrane (cm) and D is the diffusivity ($cm.h^{-1}$).

It should be kept in mind, however, that L should represent the length of the pathway through the membrane, which most often does not correspond to the thickness of the membrane. Thus, in practice, this method for evaluating D has several disadvantages, as the exact length of pathway trough the membrane is difficult to measure. It may also vary with the constituents of the vehicle. Additionally, lag times obtained from permeation experiments with human skin tend to be very variable and may include a component arising from interactions between the stratum corneum and the permeant.

In order to increase the flux of drugs across the stratum corneum it is necessary to decrease the

diffusional resistance across in the structured lipids by making them more fluid. This can be achieved by the use of penetration enhancers to impart disorder into the structured bilayers (Hadgraft & Wolff, 1993).

3.3.3 Partition coefficient

Although only 10 – 15% of the total stratum corneum mass comprise lipids, these lipids largely dictate the overall skin permeability properties. The transfer of a drug across the stratum corneum is by passive diffusion and, because of barriers imposed by the skin, this process occurs very slowly. The tissue consists of aggregates of closely packed cells and contains both lipid and aqueous regions. Lipid soluble substances readily pass through the intercellular bilayers of the cell membranes, whereas water soluble drugs are able to pass through the skin because of hydrated intracellular proteins.

When a drug reaches the viable tissue it encounters a phase change. It has to transfer from the predominantly lipophilic intercellular channels of the stratum corneum into the living cells of the epidermis, which will be largely aqueous in nature and essentially buffered to pH 7,4 (Hadgraft & Wolff, 1993 and Smith, 1990). Therefore, skin permeants must have reasonable solubilities in oil and water, but should favour the oil (Guy & Hadgraft, 1989). For lipophilic drugs, transfer into the viable epidermis can be a slow process (Hadgraft & Wolff, 1993). That is why Hadgraft & Somers (1956) stated that maximal percutaneous absorption occurs when the medicament combines lipid solubility with a moderate solubility in water. A preferentially oil soluble drug may have difficulty leaving the stratum corneum and in contrast, an extremely polar drug will have trouble partitioning into the stratum corneum from its vehicle. Kai *et al.*, (1992) found that the partition coefficient to the membrane showed a linear correlation with drug lipophilicity, which suggested that drug permeation through membranes is governed mainly by the process of drug partitioning to the membrane.

Compounds with a high partition coefficient, i.e. high lipophilicity, are likely to be the best penetrants of the skin. Since there are difficulties in determining the actual skin/water partition coefficients of drugs, olive oil/water (Scheuplein & Blank, 1971) and octanol/water ($K_{octanol/water}$) (Scheuplein, 1986) partition coefficients are used for ranking the lipophilicities of compounds for skin permeation (Takahashi *et al.*, 1993). The octanol/water partition coefficient is generally believed to be a good representation of the partitioning of the drug between the lipophilic stratum corneum and the underlying hydrophilic viable epidermis (Tenjarla *et al.*, 1996). Dissolving drugs in aqueous solution with an organic solvent, and thereafter assaying each of the two phases for drug content determine partition coefficients. The partition coefficient is the organic solvent to water drug concentration ratio (Ansel, 1981).

The vehicle to stratum corneum partitioning often contributes to the rate-limiting step in transdermal drug delivery. Partitioning of the drug to the stratum corneum is dependent upon the affinity of the drug for the base formulation and the absorption of the drug through the skin (Watkinson *et al.*, 1995).

Other parameters, which are interrelated and important determinants in skin permeability, are the partition behaviour and the solubility characteristics of the drug between the vehicle and the skin. These can be affected quite significantly by the choice of formulation.

It is possible to make the following general comments regarding the optimal permeation of the drug. It should have reasonable solubility characteristics in both water and oils (this is generally associated with low melting point). It should have a log (octanol-water partition coefficient) in the range of 1 – 2 (Hadgraft, 1996).

The optimal log P value for NSAIDs is approximately 2,5 (Yano *et al.,* 1986). They found that below this optimum log P values (of ~ 2,5); the absorption rate increases. The higher partition coefficient provided a larger concentration gradient across the stratum corneum, while a decrease in the absorption rate occurred above this value of 2,5 (the latter possibly due to unfavourable solubility properties). Thus, for compounds with log P > 2, there are potential problems in achieving steady plasma concentrations in a reasonable time span (Guy & Hadgraft, 1989).

3.3.4 pH, pKa and ionisation

The lipoidal nature of cell membranes was first suggested in 1902. It was also found that membranes are selectively permeable by the free base or uncharged (unionised) form of the drug, as it tends to partition more rapidly than the charged (ionised) species, in the lipoidal membrane. The passage of drugs across the barrier, and hence there absorption, is dictated largely by physical processes and can be predicted from the dissociation constant and the lipid solubility of the undissociated drug moiety (Abdou, 1989).

Most drugs are weak acids or bases and according to the pH-partition theory, may exist in an ionised or unionised form, depending upon the pH of the vehicle. The activity coefficient of the molecular form of such drugs is rapidly changing as a function of pH, for pH values greater than pKa for acidic compounds, and less than pKw-pKb for alkaloid drugs (Barr *et al.,* 1962). Membranes are more permeable by the unionised forms, because of their greater lipid solubility (Abdou, 1989). Thus, the unionised moiety of a drug is more lipid soluble and may dissolve more rapidly in the lipid material of the skin, thereby facilitating transport by passive diffusion (Abdou, 1989 and Jack *et al.,* 1991). The ionised moiety, on the other hand, is usually less lipid soluble, limiting transdermal permeation (Ritschel, 1988). The pH of the vehicle in which the

penetrant is dosed, in combination with the penetrant ionisation constant, pKa, will determine the actual concentrations of the ionised and unionised species. The ionised molecule is believed to permeate by the intercellular route through the stratum corneum, whereas the unionised form is more likely to follow the intercellular spaces (Wiechers, 1989).

The drug concentration that exists in the unionised form is a function of both the dissociation constant of the drug and the pH at the absorption site (Abdou, 1989). The pH range of the stratum corneum is 4,2 – 5,6 and that of the viable epidermis is 7,3 –7,4 (Pardo *et al.,* 1992). Therefore weak acids and weak bases are dissociated to different degrees, depending on the pH and the pKa or pKb of the diffusant. The concept of pKa is derived from the Henderson-Hasselbach equation and is (Ansel, 1981):

For an acid:

$$pH = pKa + \log \frac{(\text{salt})\ (\text{ionised})}{(\text{acid})\ (\text{unionised})}$$ Equation 3.8

For a base:

$$pH = pKa + \log \frac{(\text{base})\ (\text{unionised})}{(\text{salt})\ (\text{ionised})}$$ Equation 3.9

Thus, the fraction of the unionised drug is a function of the pH (Barry, 1983).

This does not mean that ionic species cannot pass through the skin, for ion pairing is possible and, in the form of ion pairs, a salt can be soluble, to some extent, within a lipid continuum and, thereby, can diffuse through it (Flynn, 1989; Hadgraft & Valenta, 2000).

Because of the effect of pH on the relative concentrations of unionised and ionised species, it would appear to be possible to control the total flux of compounds by varying the pH of the drug containing vehicle applied to the skin.

3.3.5 Melting point

From the equation of Hadgraft *et al.* (1990), it is clear that the solubility parameter in the stratum corneum, δ_{sc}, can be estimated more accurately if the melting point of that drug is also taken into account.

$$\log \delta_{sc} = 1{,}911 \frac{10^3}{mp} - 2{,}956$$ Equation 3.10

where δ_{sc} is the solubility parameter in the stratum corneum and mp is the melting point (Kelvin).

A high level of crystallinity is expressed in the form of a high melting point and heat of fusion. This limits solubility itself, and thus also sets a limit on mass transfer across the skin. Generally, the greater a drug's innate tendency to dissolve, the more likely it is that the drug can be delivered at an appropriate rate across the skin (Ostrenga *et al.*, 1971). Hence, in order to obtain the best candidate for transdermal drug delivery, the melting point should be kept as low as possible.

Permeant melting point has been shown to be inversely proportional to lipophilicity (log P) and, therefore, transdermal flux. The melting point of a substance is often considered as an indicator of the maximum flux attainable through the skin and the correlation between flux and the reciprocal of the melting point have been attempted. Since the entropy of fusion of the permeant (ΔS_f) slowly varies with melting point, the ideal solubility increases exponentially with decreasing melting point for any given molecular weight. It follows that there should be an exponential increase in transdermal flux with decreasing melting point (Guy & Hadgraft, 1989; Stott *et al.*, 1998).

Roy & Flynn (1989) plotted the relationship between melting point and permeability coefficients of narcotic analgesics. They showed that the permeability coefficients systematically change with the melting point, with the compounds having the lowest melting points exhibiting the highest permeability coefficients.

Another study by Kommuru *et al.* (1998) showed that enantiomers with a lower melting point might exhibit higher solubility than that of the racemate, and consequently have higher skin permeation profiles. For example, the flux of the pure enantiomer of nivaldipine, a calcium channel blocker, across human cadaver skin was about 7 fold higher than that of the racemate. In this case, the melting point difference was about 34°C.

It is therefore clear that reduction in melting point of a permeant will have a direct effect on its solubility in skin lipids and so increase transdermal permeation (Stott *et al.*, 1998; Cleary, 1993).

3.3.6 Hydrogen bonding

The penetration of most compounds seems to be limited by the barrier function of the stratum corneum and in particular by the properties of the stratum corneum lipids (Potts & Guy, 1992; Surber *et al.*, 1993). The human skin consists of distinct layers: the dermis and epidermis. The outmost layer of the human epidermis, the stratum corneum, consists of terminally differentiated keratinocytes embedded in continuous array of extracellular lipid lamellae. These lamellae consist primarily of ceramides, fatty acids and cholesterol. Since the polar heads of ceramides are known to facilitate lateral hydrogen bonding with adjacent molecules, and since these lipids have acyl chains more than 24 carbons long, these extracellular lamellae form a rigid structure

at physiological temperatures. There is now considerable evidence that the stratum corneum lipids govern the permeability of water vapour through the stratum corneum. For example, lipid removal by solvent extraction leads to a 1000 fold permeability increase. More importantly, stratum corneum water permeability seems to be highly correlated with the mobility of the lipid acyl chains. This would suggest that the water barrier properties of the stratum corneum are associated with the carbon region of the extracellular lamellae. In this way the hydrocarbon regions of the extracellular lipid lamellae form a continuous barrier for water inside the stratum corneum, forcing water and ions to transverse a highly tortuous pathway and so extending the diffusion pathway (Pechtold *et al*., 1997).

The permeability coefficients of the solutes through the stratum corneum have previously been related to the presence of hydrogen bonding groups on the penetrant. It is suggested that, whereas lipophilicity of a solute is the major determinant for solute partitioning into the stratum corneum from aqueous solutions, the hydrogen bonding of the solute is the main determinant of solute diffusion across the stratum corneum (Roberts *et al.,* 1995).

Anderson & Raykar (1989) considered that the stratum corneum barrier could be modelled by hydrogen bonding organic solvent, and El Tayar *et al.* (1991) suggested the hydrogen bond donor potential of the penetrant to be the main determinant of permeability. Various proposals relating permeability coefficient of the stratum corneum to several penetrant properties (Roberts *et al.,* 1995) have been reviewed and concluded that both the donor (α) and acceptor (β) properties of the penetrant hydrogen bonding properties are important in determining the permeability coefficient. Pugh *et al.* (1996) showed that the stratum corneum is a predominantly hydrogen bond donor environment, with donor/acceptor properties in the ratio of 0,57:0,43.

In a study done by Potts & Guy (1995) on the effects of molecular size and hydrogen bond activity, the hydrogen bonding terms show that increased solute hydrogen bond acceptor and donor activity resulted in decreased partitioning into the organic phase; due to the free energy cost associated with the disruption of the hydrogen bonds in the aqueous phase. A smaller decrease was seen for octanol due to the limited hydrogen bonding ability and water solubility in this solvent. In short, hydrogen-bonding solutes are better accommodated in octanol than in alkene.

According to Roberts *et al.* (1995), the diffusion coefficient is dependent in terms of a maximal absorption of a solute to polar groups in the transport pathway of solutes through the stratum corneum. Accordingly, the diffusion coefficient of a solute decrease as the solute becomes bound to polar groups in the pathway until these groups are unable to associate with any additional hydrogen bonding groups on the solute. Above the saturated number of groups, the diffusion coefficient of the solute appears to be relatively constant.

The hydrogen bonding properties of a penetrant have a dominant effect on the diffusion across the stratum corneum, but a smaller influence on the partitioning, where lipophilicity might be an important factor (Roberts *et al.*, 1995).

3.3.7 Molecular size

Considering that the horny layer is a compact membrane and that diffusing molecules follow a tortuous path through it, it might seem obvious that the diffusion coefficient would be inversely related to molecular weight or some other measure of molecular size (Zatz, 1993; Naik *et al.*, 2000). Compounds of small molecular size may penetrate through the aqueous pathway more easily than larger molecules, which penetrate through the lipoidal pathway more readily (Zatz, 1993 and Takahashi *et al.*, 1993).

An inverse relationship appears to exist between the absorption rate and the molecular weight (Tregear, 1966). According to Potts & Guy (1995) increasing the molecular volume increases the hydrophobic surface area and that this will increase partitioning into, and hence, permeability through a lipid membrane. Conversely larger molecules diffuse more slowly, since they require more "space" to be created in the medium, and this in turn leads to diminished permeability. Small molecules penetrate more rapidly than larger molecules, but within a narrow range of molecular size, there is little correlation between size and penetration rate (Liron & Cohen, 1984).

There seems to be an inverse relationship between the permeant diffusivity and the permeant size. For the stratum corneum and the other lipid membranes, it has been suggested that the functional dependence of permeant diffusivity on molecular volume is exponential. Potts & Guy (1992) introduced a model for compounds ranging in molecular weight from 18 to > 750 and log K_{oct} from -3 to +6. They found that Equation 3.11 could predict the permeability through the human skin:

$$\log k_p = -2{,}7 + 0{,}71 \log K_{oct} - 0{,}0061\ MW \qquad \text{Equation 3.11}$$

where k_p is the permeability coefficient ($cm.sec^{-1}$), K_{oct} is the octanol/water partition coefficient and MW is the molecular weight.

They found that the substitution of molecular weight for molecular volume provides an equivalent fit in the model. In conclusion they found that the apparently sigmoidal dependence of log K_p upon log K_{oct} suggests a non-linear relationship between these parameters. However, when molecular volume is taken into account, the data lies on a three-dimensional surface defined by log K_p, log K_{oct} and molecular volume (Potts & Guy, 1992).

Pugh *et al.* (2000) confirmed the direct relationship between log K_p and log K_{oct}, but found that the relationships between log K_p and MW is also direct, and not inverse as was found by Potts & Guy.

The upper limit of molecular weight for permeation is still a matter of discussion. There seems to be a limit of about 5000, although some authors even mention weights of not more than 3000. Nevertheless, influx of compounds into the skin does decrease with increasing molecular weight due to the parallel decrease in the diffusion coefficient in water (Idson, 1975 and Schalla & Schaefer, 1982).

3.3.8 Lipophilicity

The stratum corneum is thought to be a lipophilic layer since it is composed mainly of keratin protein and lipids. Improvement of drug lipophilicity is expected to enhance the process of drug dissolution into lipophilic material of the stratum corneum, resulting in increased drug distribution into the skin (Hashiguchi *et al.,* 1997). Drugs with high distribution and low diffusion into the skin, will show high accumulation in the skin. Therefore, drug concentrations in the skin appear to increase with increasing drug lipophilicity.

The increased lipophilicity of derivatives of prednisolone contributed to their distribution into the stratum corneum of the skin. Although the permeation constants of the prednisolone derivatives were lower than that of prednisolone itself, drug retention in the skin increased and diffusion rate decreased with increase in lipophilicity of the derivatives (Hashiguchi *et al.,* 1997).

3.3.9 Hydrophilicity

Incorporating one or more hydroxy substituents at different positions on the steroidal skeleton progressively increased the hydrophilicity of progesterone, which is a lipophilic steroid itself. Effects of these hydrophilic substituents on the permeation of progesterone across the intact skin and stripped skin of the hairless mouse were studied. The steady state rate of permeation across the intact skin and stripped skin was found to be approximately proportional to the solubility of drugs in the stratum corneum or the viable skin, respectively. Furthermore, the solubility of progesterone and its hydroxyl derivatives in the stratum corneum was noted to decrease gradually as the hydrophilicity of the penetrant increased. However, the solubility of these progestrins in the viable skin was observed to be dependent not only on the penetrant hydrophilicity, but also on the position of the OH-group on the penetrant molecule. The diffusivity of progesterone and its hydroxyl derivatives across the stratum corneum was almost independent of the hydrophilicity of the drugs (Tojo *et al.*, 1987).

3.4 The influence of alkyl chain length on percutaneous absorption

It has long been recognised that a drug's physicochemical properties are very important in determining its biological and pharmaceutical characteristics. The major determinants of drug dissolution, distribution and availability are the aqueous solubility and the partition coefficient (Yalkowsky *et al.*, 1972). An understanding of the manner in which these and other properties change within a homologous series, i.e. with additions of methylene units, can be of use in choosing a derivative with optimum properties. It is recognised that in a homologous series, by increasing the nonpolar portion of a molecule by extending the length of the chain produces certain characteristic features, such as elevating boiling point, decreasing aqueous solubility and increasing the partition coefficient (Abdou, 1989).

As seen by Flynn & Yalkowsky (1972), relationships can be drawn for the influence of chain length on the partition coefficient and solubility. Partition coefficients of membranes of a homologous series between the immiscible polar and nonpolar phases, increase by a constant factor as the series ascend. This relationship is expressed in Equation 3.15:

$$\log K_n = \log K_o + \pi_n \qquad \text{Equation 3.15}$$

This is an especially useful relationship because it expresses a universal dependency, thereby allowing chain length n to be used in lieu of partition coefficients ($\log K_n$) in theoretical analysis. The value, π, is the log of the increase per methylene unit. They studied the permeation of several odd chain length *p*-aminobenzoates from saturated solutions.

They founded that (Flynn & Yalkowsky, 1972):

1 There is initially an increase in flux in the steady state as the homologous series is ascended.

2 The flux drops off markedly at longer chain lengths. Additionaly, lag times, which appear to be approximately constant initially, increase sharply for the longest esters.

As the alkyl chain length as well as the molecular weight increased, the aqueous solubility and the flux decrease. At the lower end of the range it takes an addition of three methylene units to produce a 10 fold increase in the partition coefficient. While partition coefficients are growing exponentially as the homologous series is ascended, there appears to be little current effect on diffusion coefficients (Flynn, 1989).

Yalkowsky *et al.* (1972) investigated the physicochemical properties of a homologous series of

alkyl-*p*-aminobenzoates and found that as chain length is increased, the melting point decreases almost linearly to the butyl ester and then increases gradually and irregularly. Thus, a change in melting behaviour relative to increasing chain length at butyl ester is clearly evident.

Hence, this all comes down to that an increase in chain length up to about 4 carbon atoms result in a lower melting point, lower solubility and lower crystallinity.

Chapter 4

Experimental

4.1 General experimental methods

4.1.1 Instrumentation

4.1.1.1 Nuclear magnetic resonance spectroscopy (NMR)

The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 spectrometer at a frequency of 300,075 MHz and 75,462 MHz, respectively. All the chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane ($\delta = 0$). The following abbreviations were used to describe the multiplicity of the ^{1}H signals: s = singlet, d = doublet, dd = doublet of doublets, ddd = doublet of doublets of doublets, t = triplet, q = quartet and m = multiplet.

4.1.1.2 Infrared spectroscopy (IR)

The IR spectra were recorded on a Nicolet Magna – IR 550 spectrometer using KBr pellets.

4.1.1.3 Mass spectroscopy (MS)

The MS spectra were recorded on an analytical VG 7070E mass spectrometer using fast atom bombardment (FAB) or electron impact (EI) at 70 eV as ionisation techniques.

4.1.1.4 Melting points

Melting points were determined by differential scanning calorimetry (DSC). DSC thermograms were recorded with a Shimatzu DSC – 50 instrument. Measurement conditions were as follows: sample weight of approximately 2 mg, an aluminium crimp cell sample holder, nitrogen gas flow at 40 ml/min and heating rate at 10 °C/min.

4.1.2 Chromatographic techniques

4.1.2.1 Thin-layer chromatography (TLC)

Analytical TLC was performed on 0,25 mm thick silica gel aluminium backed sheets (Merck® 5554 DC - Alufolien 60 F_{254}). Dichloromethane (DCM) was used as mobile phase. Chromatograms were examined under UV-light (254 nm) for the detection of the individual

compounds.

4.1.2.2 Column chromatography

Column chromatography was performed with a standard glass column with a length of 900 mm and an inner diameter of 30 mm. The stationary phase used was Merck® 7734 silica gel (0,063 - 0,200 mm). DCM was used as mobile phase.

4.1.2.3 High pressure liquid chromatography (HPLC)

The HPLC system consisted of a HP (Hewlet Packard) Agilent 1100 series auto sampler, HP Agilent 1100 series variable wave detector (VWD) and HP Agilent 1100 series isocratic pump. A Phenomenex (Luna C-18, 5 μ, 250 x 4,60 mm) column was used together with a Securityguard pre-column (C-18, 4 x 3 mm) insert (Phenomenex) in order to prolong column life and the Agilent Chemstation for LC Systems software package was used for data analysis. The flow rate, UV wavelengths, mobile phase compositions and retention times for each of the acetylsalicylic acid derivatives are presented in Table 4.1. Orthophosphoric acid (OPA) was used to adjust the pH of the mobile phase to 2,25. A volume of 100 μl was injected for each of the samples. It was determined that the recycling of the mobile phase did not adversely affect the HPLC analysis. Calibration curves were constructed ranging from concentrations of 0,125 μg/ml to 5,0 μg/ml.

Table 4.1: Data of the HPLC method

Compound	Flow rate (ml/min)	Wavelength (nm)	Mobile phase H_2O: acetonitrile	Retention time (min)
Acetylsalicylic acid	1,2	225	50:50	3,47
Methyl acetylsalicylate	1,2	225	50:50	6,25
Ethyl acetylsalicylate	1,2	225	50:50	8,65
Propyl acetylsalicylate	1,2	225	33:67	5,66
Isopropyl acetylsalicylate	1,2	225	33:67	5,38
Butyl acetylsalicylate	1,2	225	33:67	7,39
1-Methylpropyl acetylsalicylate	1,2	225	33:67	6,84
Tert-butyl acetylsalicylate	1,2	225	33:67	6,77
Pentyl acetylsalicylate	1,2	225	33:67	10,29
1-Methylbutyl acetylsalicylate	1,2	225	33:67	9,46
1-Ethylbutyl acetylsalicylate	1,2	225	33:67	9,15

4.1.3 Theoretical aqueous solubility

Interactive Analysis (http://www.logp.com/) prediction software was used to predict the water solubility for acetylsalicylic acid and its derivatives. These values are compared to experimental values (§5.2.1).

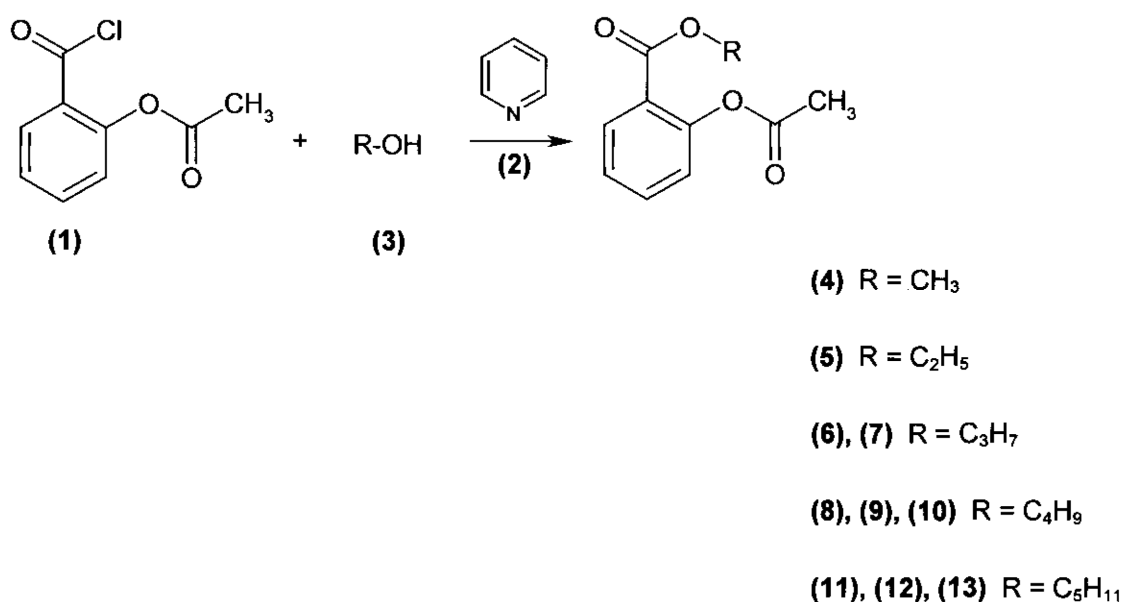
4.1.4 Theoretical partition coefficients

ACD Labs, K_{ow}Win (http://esc.syrres.com/interkow/kowdemo.htm) and Interactive Analysis (http://www.logp.com/) prediction software were used to predict the partition coefficients for acetylsalicylic acid and the acetylsalicylate derivatives. These values are compared to the experimental values (§5.2.3).

4.2 Synthesis and physical data of compounds

4.2.1 Esterification

A stirred solution of acetylsalicyloyl chloride (5,96 g; 30,0 mmol) **(1)** was dissolved in pyridine (1,2 ml; 14,9 mmol) **(2)** and esterified with ten different alcohols (30 mmol) **(4)** – **(13)**. Stirring was continued for 24 hours. 2 M Aqueous HCl (20 ml) was poured into the mixture, followed by an excess dichloromethane. The organic phase was collected and washed with 5 % $NaHCO_3$ (20 ml) and water (3 x 20 ml). The organic phase was then dried over anhydrous magnesium sulphate ($MgSO_4$). The dichloromethane was removed under vacuum and the resulting oil collected. The prepared compounds were purified using column chromatography.



Scheme 4.1: Synthesis of acetylsalicylic acid derivatives.

4.2.1.1 Methyl acetylsalicylate (4)

A yield of 1,2629 g (21,68 %) white crystalline compound was obtained; mp 46,80 °C (thermogram 1); R_f 0,71 (dichloromethane); $C_{10}H_{10}O_4$; M^+ 194; m/z (%; EI) (spectrum 1): 194 (14,2), 163 (83,0), 153 (92,5), 120 (52,8), 93 (67,0), 63 (100,0), 43 (49,8); ν_{max} (spectrum 15, KBr, cm^{-1}): 705,17; 753,04; 914,72; 1084,21; 1198,52; 1266,69; 1300,12; 1369,29; 1453,70; 1607,43; 1726,76; 1771,02; 2953,98; 3435,35; δ_H (spectrum 25, 300,075 MHz, $CDCl_3$): 2,32 (s; 3H; H-3''), 3,85 (s; 3H; H-3'), 7,08 (dd; 1H; J = 8,1; 1,1 Hz; H-6), 7,28 (ddd; 1H; J = 7,6; 7,6; 1,2 Hz; H-4), 7,53 (ddd; 1H; J = 7,4; 7,4; 1,7 Hz; H-5), 8,00 (dd; 1H; J = 7,9; 1,7 Hz; H-3); δ_c (spectrum 35, 75,462 MHz, $CDCl_3$): 20,90 (C-3''), 52,08 (C-3'), 123,16 (C-2), 123,76 (C-6), 125,92 (C-4), 131,70 (C-3), 133,78 (C-5), 150,68 (C-1), 164,84 (C-1'), 169,57 (C-2'').

4.2.1.2 Ethyl acetylsalicylate (5)

A yield of 0,2978 g (20,87 %) clear, light, orange-yellow oil was obtained; R_f 0,59 (dichloromethane); $C_{11}H_{12}O_4$; M^+ 208; m/z (%; EI) (spectrum 2): 208 (10,3), 163 (67,7), 121 (95,7), 92 (84,4), 65 (63,8), 43 (90,4), 28 (100,0); ν_{max} (spectrum 16, KBr, cm^{-1}): 704,79; 752,68; 772,13; 916,22; 1081,33; 1194,99; 1261,77; 1295,48; 1367,60; 1607,67; 1720,93; 1770,67; 2983,64; 3434,68; δ_H (spectrum 26, 300,075 MHz, $CDCl_3$): 1,34 (t; 3H; J = 7,1 Hz; H-4'), 2,32 (s; 3H; H-3''), 4,31 (q; 2H; J = 7,1 Hz; H-3'), 7,07 (dd; 1H; J = 8,1; 1,1 Hz; H-6), 7,28 (ddd; 1H; J = 7,6; 7,6; 1,2 Hz; H-4), 7,52 (ddd; 1H; J = 7,4; 7,4; 1,7 Hz; H-5), 8,00 (dd; 1H; J = 7,8; 1,8 Hz; H-3); δ_c (spectrum 36, 75,462 MHz, $CDCl_3$): 14,17 (C-4'), 20,93 (C-3''), 61,02 (C-3'), 123,57 (C-2), 123,69 (C-6), 125,88 (C-4), 131,68 (C-3), 133,60 (C-5), 150,55 (C-1), 164,47 (C-1'), 169,46 (C-2'').

4.2.1.3 Propyl acetylsalicylate (6)

A yield of 0,5610 g (8,41 %) clear, yellow oil was obtained; R_f 0,65 (dichloromethane); $C_{12}H_{14}O_4$; M^+ 222; m/z (%; EI) (spectrum 3): 222 (12,8), 181 (80,1), 163 (100,0), 65 (98,2), 41 (80,7), 39 (87,3), 27 (87,7); ν_{max} (spectrum 17, KBr, cm^{-1}): 704,35; 752,18; 914,70; 1009,58; 1081,15; 1193,25; 1294,24; 1368,39; 1485,31; 1607,55; 1721,37; 1771,86; 2969,12; 3429,34; δ_H (spectrum 27, 300,075 MHz, $CDCl_3$): 0,99 (t; 3H; J = 7,4 Hz; H-5'), 1,74 (m; 2H; H-4'), 2,32 (s; 3H; H-3''), 4,21 (t; 2H; J = 6,7 Hz; H-3'), 7,07 (dd; 1H; J = 8,1; 1,1 Hz; H-6), 7,28 (ddd; 1H; J = 7,6; 7,6; 1,2 Hz; H-4), 7,53 (ddd; 1H; J = 7,4; 7,4; 1,7 Hz; H-5), 8,00 (dd; 1H; J = 7,8; 1,7 Hz; H-3); δ_c (spectrum 37, 75,462 MHz, $CDCl_3$): 10,38 (C-5'), 20,95 (C-3''), 22,00 (C-4'), 66,66 (C-3'), 123,56 (C-2), 123,74 (C-6), 125,90 (C-4), 131,66 (C-3), 133,62 (C-5), 150,63 (C-1), 164,50 (C-1'), 169,52 (C-2'').

4.2.1.4 Isopropyl acetylsalicylate (7)

A yield of 0,4425 g (6,64 %) clear, dark, yellow oil was obtained; R_f 0,68 (dichloromethane); $C_{12}H_{14}O_4$; M^+ 222; m/z (%; EI) (spectrum 4): 222 (12,5), 180 (99,0), 163 (79,9), 121 (100,0), 92 (88,0), 65 (68,5), 39 (60,2); ν_{max} (spectrum 18, KBr, cm^{-1}): 705,29; 752,48; 913,26; 1009,65; 1079,30; 1108,29; 1295,77; 1371,83; 1485,23; 1607,78; 1716,45; 1770,85; 2982,69; 3512,48; δ_H (spectrum 28, 300,075 MHz, $CDCl_3$): 1,32 (d; 6H; J = 3,7 Hz; H-4', H-5'), 2,32 (s; 3H; H-3''), 5,20 (m; 1H; J = 6,3 Hz; H-3'), 7,06 (dd; 1H; J = 8,0; 1,1 Hz; H-6), 7,27 (ddd; 1H; J = 7,6; 7,6; 1,2 Hz; H-4), 7,51 (ddd; 1H; J = 7,4; 7,4; 1,7 Hz; H-5), 7,98 (dd; 1H; J = 7,8; 1,7 Hz; H-3); δ_c (spectrum 38, 75,462 MHz, $CDCl_3$): 20,99 (C-3''), 21,82 (C-4', C-5'), 68,50 (C-3'), 123,63 (C-2), 124,05 (C-6), 125,86 (C-4), 131,64 (C-3), 133,44 (C-5), 150,44 (C-1), 164,06 (C-1'), 169,41 (C-2'').

4.2.1.5 Butyl acetylsalicylate (8)

A yield of 0,5138 g (7,25 %) clear, light, orange-yellow oil was obtained; R_f 0,64 (dichloromethane); $C_{13}H_{16}O_4$; m/z (%; EI) (spectrum 5): 194 (100,0), 138 (96,3), 121 (92,3), 92 (74,2), 43 (86,1), 29 (61,3); (FAB, spectrum 11): 237 (($M+H^+$) 30,0%), 195 (78,0%), 163 (54,0%), 149 (31,5%), 138 (53,0%), 121 (100,0%); ν_{max} (spectrum 19, KBr, cm^{-1}): 704,60; 752,79; 915,29; 1010,19; 1081,58; 1295,82; 1368,30; 1452,70; 1485,24; 1607,60; 1723,48; 1770,54; 2960,94; 3428,93; δ_H (spectrum 29, 300,075 MHz, $CDCl_3$): 0,95 (t; 3H; J = 7,4 Hz; H-6'), 1,43 (m; 2H; H-5'), 1,70 (m; 2H; H-4'), 2,32 (s; 3H; H-3''), 4,26 (t; 2H; J = 6,7 Hz; H-3'), 7,07 (dd; 1H; J = 8,1; 1,1 Hz; H-6), 7,28 (ddd; 1H; J = 7,6; 7,6; 1,2 Hz; H-4), 7,53 (ddd; 1H; J = 7,4; 7,4; 1,7 Hz; H-5), 7,99 (dd; 1H; J = 7,8; 1,7 Hz; H-3); δ_c (spectrum 39, 75,462 MHz, $CDCl_3$): 13,66 (C-6'), 19,15 (C-5'), 20,95 (C-3''), 30,67 (C-4'), 64,94 (C-3'), 123,55 (C-2), 123,74 (C-6), 125,89 (C-4), 131,66 (C-3), 133,61 (C-5), 150,64 (C-1), 164,49 (C-1'), 169,51 (C-2'').

4.2.1.6 1-Methylpropyl acetylsalicylate (9)

A yield of 0,5876 g (8,29 %) clear, dark, yellow oil was obtained; R_f 0,73 (dichloromethane); $C_{13}H_{16}O_4$; M^+ 236; m/z (%; EI) (spectrum 6): 236 (9,3), 194 (89,5), 163 (84,7), 121 (100,0), 92 (84,0), 43 (94,9), 29 (71,3); ν_{max} (spectrum 20, KBr, cm^{-1}): 704,63; 751,54; 917,00; 1079,20; 1192,46; 1293,48; 1368,37; 1452,44; 1485,20; 1607,62; 1716,34; 1770,54; 2975,77; 3419,58; δ_H (spectrum 30, 300,075 MHz, $CDCl_3$): 0,94 (t; 3H; J = 7,4 Hz; H-5'), 1,29 (d; 3H; J = 6,3 Hz; H-6'), 1,66 (m; 2H; H-4'), 2,32 (s; 3H; H-3''), 5,04 (m; 1H; H-3'), 7,07 (dd; 1H; J = 8,0; 1,0 Hz; H-6), 7,28 (ddd; 1H; J = 7,6; 7,6; 1,2 Hz; H-4), 7,51 (ddd; 1H; J = 7,4; 7,4; 1,7 Hz; H-5), 7,99 (dd; 1H; J = 7,9; 1,7 Hz; H-3); δ_c (spectrum 40, 75,462 MHz, $CDCl_3$): 9,66 (C-5'), 19,42 (C-6'), 21,01 (C-3''), 28,87 (C-4'), 73,06 (C-3'), 123,70 (C-2), 124,05 (C-6), 125,88 (C-4), 131,62 (C-3),

133,45 (C-5), 150,53 (C-1), 164,16 (C-1'), 169,48 (C-2'').

4.2.1.7 Tert-butyl acetylsalicylate (10)

A yield of 0,4998 g (7,05 %) clear, light, orange-yellow oil was obtained; R_f 0,63 (dichloromethane); $C_{13}H_{16}O_4$; m/z (%; EI) (spectrum 7): 181 (60,6), 163 (100,0), 138 (56,5), 121 (71,2), 43 (57,3); (FAB, spectrum 12): 237 (($M+H^+$) 35,0%), 195 (87,5%), 163 (61,0%), 149 (32,0%), 138 (55,0%), 121 (100,0%); ν_{max} (spectrum 21, KBr, cm^{-1}): 704,00; 759,88; 848,03; 916,20; 1082,16; 1195,46; 1308,09; 1369,27; 1483,71; 1607,40; 1716,20; 1770,01; 2980,52; 3413,74; δ_H (spectrum 31, 300,075 MHz, $CDCl_3$): 1,55 (s; 9H; H-4', H-5', H-6'), 2,32 (s; 3H; H-3''), 7,04 (dd; 1H; J = 8,1; 1,2 Hz; H-6), 7,26 (ddd; 1H; J = 7,6; 7,6; 1,2 Hz; H-4), 7,46 (ddd; 1H; J = 7,4; 7,4; 1,8 Hz; H-5), 7,89 (dd; 1H; J = 7,8; 1,8 Hz; H-3); δ_c (spectrum 41, 75,462 MHz, $CDCl_3$): 21,08 (C-3''), 28,13 (C-4', C-5', C-6'), 81,54 (C-3'), 123,46 (C-2), 125,41 (C-6), 125,77 (C-4), 131,47 (C-3), 132,96 (C-5), 150,12 (C-1), 163,89 (C-1'), 169,39 (C-2'').

4.2.1.8 Pentyl acetylsalicylate (11)

A yield of 0,6023 g (8,02 %) clear, light, yellow oil was obtained; R_f 0,69 (dichloromethane); $C_{14}H_{18}O_4$; m/z (%; EI) (spectrum 8): 209 (75,1), 208 (94,9), 163 (77,7), 138 (90,2), 121 (86,4), 120 (100,0); (FAB, spectrum 13): 251 (($M+H^+$) 30,5%), 209 (82,0%), 163 (55,0%), 138 (63,0%), 121 (100,0%); ν_{max} (spectrum 22, KBr, cm^{-1}): 704,40; 752,41; 915,25; 1082,33; 1194,38; 1294,39; 1368,04; 1452,66; 1485,27; 1607,50; 1723,12; 1771,10; 2958,60; 3432,31; δ_H (spectrum 32, 300,075 MHz, $CDCl_3$): 0,90 (t; 3H; J = 7,1 Hz; H-7'), 1,37 (m; 4H; H-5', H-6'), 1,72 (m; 2H; H-4'), 2,32 (s; 3H; H-3''), 4,25 (t; 2H; J = 6,8 Hz; H-3'), 7,07 (dd; 1H; J = 8,1; 1,1 Hz; H-6), 7,28 (ddd; 1H; J = 7,6; 7,6; 1,2 Hz; H-4), 7,52 (ddd; 1H; J = 7,4; 7,4; 1,8 Hz; H-5), 7,99 (dd; 1H; J = 7,8; 1,7 Hz; H-3); δ_c (spectrum 42, 75,462 MHz, $CDCl_3$): 13,89 (C-7'), 20,97 (C-3''), 22,30 (C-6'), 28,07 (C-5'), 28,34 (C-4'), 65,25 (C-3'), 123,58 (C-2), 123,75 (C-6), 125,91 (C-4), 131,67 (C-3), 133,61 (C-5), 150,65 (C-1), 164,51 (C-1'), 169,53 (C-2'').

4.2.1.9 1-Methylbutyl acetylsalicylate (12)

A yield of 0,2368 g (15,46 %) clear, colorless oil was obtained; R_f 0,69 (dichloromethane); $C_{14}H_{18}O_4$; M^+ 250; m/z (%; EI) (spectrum 9): 250 (7,9), 208 (88,4), 163 (83,1), 139 (58,1), 121 (100,0), 92 (80,9), 65 (58,2); ν_{max} (spectrum 23, KBr, cm^{-1}): 704,53; 753,03; 915,97; 1079,52; 1194,41; 1294,91; 1368,14; 1452,07; 1484,90; 1607,68; 1719,58; 1772,76; 2961,49; 3423,08; δ_H (spectrum 33, 300,075 MHz, $CDCl_3$): 0,92 (t; 3H; J = 7,3 Hz; H-6'), 1,30 (d; 3H; J = 6,3 Hz; H-7'), 1,38 (m; 2H; H-5'), 1,60 (m; 2H; H-4'), 2,32 (s; 3H; H-3''), 5,12 (m; 1H; H-3'), 7,07 (dd; 1H; J = 8,1; 1,1 Hz; H-6), 7,28 (ddd; 1H; J = 7,6; 7,6; 1,2 Hz; H-4), 7,51 (ddd; 1H; J = 7,4; 7,4; 1,8 Hz; H-5), 7,98 (dd; 1H; J = 7,8; 1,7 Hz; H-3); δ_c (spectrum 43, 75,462 MHz, $CDCl_3$): 13,88 (C-

6'), 18.62 (C-7'), 19,95 (C-5'), 21,01 (C-3''), 38,15 (C-4'), 71,65 (C-3'), 123,70 (C-2), 124,06 (C-6), 125,88 (C-4), 131,62 (C-3), 133,44 (C-5), 150,53 (C-1), 164,14 (C-1'), 169,46 (C-2'').

4.2.1.10 1-Ethylbutyl acetylsalicylate (13)

A yield of 0,1611 g (10,88 %) clear, colorless oil was obtained; R_f 0,69 (dichloromethane); $C_{14}H_{18}O_4$; m/z (%; EI) (spectrum 10): 208 (80,7), 163 (94,2), 138 (98,3), 121 (100,0), 92 (74,3), 43 (63,4); (FAB, spectrum 14): 251 ((M+H$^+$) 23,0%), 209 (10,0%), 181 (16,5%), 163 (35,0%), 139 (21,0%), 121 (100,0%); ν_{max} (spectrum 24, KBr, cm^{-1}): 703,86; 751,49; 914,17; 1079,17; 1193,89; 1292,42; 1367,83; 1452,30; 1484,97; 1607,59; 1716,54; 1771,13; 2970,85; 3422,79; δ_H (spectrum 34, 300,075 MHz, $CDCl_3$): 0,92 (t; 6H; J = 7,5 Hz; H-5', H-7'), 1,65 (m; 4H; H-4', H-6'), 2,32 (s; 3H; H-3''), 4,96 (m; 1H; H-3'), 7,07 (dd; 1H; J = 8,1; 1,1 Hz; H-6), 7,28 (ddd; 1H; J = 7,6; 7,6; 1,2 Hz; H-4), 7,52 (ddd; 1H; J = 7,4; 7,4; 1,8 Hz; H-5), 8,00 (dd; 1H; J = 7,8; 1,7 Hz; H-3); δ_c (spectrum 44, 75,462 MHz, $CDCl_3$): 9,56 (C-5', C-7'), 20,99 (C-3''), 26,47 (C-4', C-6'), 77,54 (C-3'), 123,75 (C-2), 124,02 (C-6), 125,88 (C-4), 131,59 (C-3), 133,44 (C-5), 150,60 (C-1), 164,33 (C-1'), 169,47 (C-2'').

4.3 Physicochemical properties and solubility

4.3.1 Solubility determination

The aqueous solubility of acetylsalicylic acid and its derivatives was obtained by preparing saturated solutions in a tris-(hydroxymethyl)aminomethane buffer (TRIS) at pH 4,5. The slurries were stirred with magnetic bars in a water bath at 37 °C for 24 hours. An excess of solute was present at all times to provide saturated solutions. After 24 hours, the solutions were filtered and analysed directly by HPLC to determine the concentration of solute dissolved in the solvent. The experiment was done in triplicate. Results are presented in section 5.2.1.

4.3.2 Experimental partition coefficient

Equal volumes of *n*-octanol and TRIS buffer (pH 4,5) were saturated with each other under vigorous stirring for at least 24 hours. Each of the acetylsalicylic acid derivatives (10 mg/ml) was dissolved in 3 ml pre-saturated *n*-octanol, stoppered and agitated for 10 min. 3 ml pre-saturated TRIS buffer was transferred to assay tubes containing before mentioned solutions. The tubes were stoppered and agitated for 45 min. thereafter they were centrifuged at 4000 rpm for 30 min. The *n*-octanol and aqueous phases were separately analysed by HPLC. The aqueous phase was analysed directly by HPLC and the *n*-octanol solutions were diluted 1:1000 with acetonitrile (solvent) prior to being analysed by HPLC. The partition coefficients (K_{oct}) were calculated as logarithmic ratios of the acetylsalicylic acid derivative concentrations in the *n*-

octanol phase to the concentrations in the TRIS buffer. The experiment was done in triplicate. Results are presented in section 5.2.3.

4.4 Transdermal permeation

4.4.1 Skin preparation

Female human abdominal skin was used for the permeation studies and was obtained from Sunwardpark Clinic (Boksburg, South Africa) after cosmetic procedures. A scalpel was used to separate the skin from the fat layer; subsequently the stratum corneum was removed by means of immersion in 60 °C HPLC water for 60 seconds. The stratum corneum was gently teased away from the skin with forceps. Special care was taken that the integrity of the stratum corneum was not ruptured, as this would compromise the validity of the results. The stratum corneum was placed in a bath filled with HPLC water and carefully set on Whatman® filter paper, left to air dry and was wrapped in foil. The foil containing the stratum corneum was stored in a freezer at –20 °C and was used within a year after being prepared. Prior to use, the stratum corneum was thawed and examined for any defects, before it was mounted on the Franz diffusion cells.

4.4.2 Preparation of donor solutions

Donor solutions of the acetylsalicylate derivatives were obtained by the equilibration of excess amounts of solvent in TRIS buffer (pH 4,5). The slurries were prepared in stoppered flasks; stirring in a water bath at 37 °C over a period of 24 hours, in order for solvent saturation to occur. An excess amount of solute was present at all times.

4.4.3 Skin permeation method

Vertical Franz diffusion cells (Figure 4.1) with 2,0 ml receptor compartments and 1,0751 cm^2 effective diffusion area was used for the permeation studies. The epidermal skin layer was carefully mounted on the lower half of the Franz cell with the stratum corneum facing upwards. A clamp was used to fasten the upper and lower parts of the Franz cell together, with the stratum corneum separating the donor and receptor compartments. The receptor compartments were filled with isotonic TRIS buffer (pH 7,4). Special care was taken that no air bubbles came between the buffer solution and the stratum corneum. The Franz cells, containing buffer solution, were equilibrated for one hour in the water bath at 37 °C, prior to the addition of the saturated solutions to the donor compartments. Only the receptor compartments were submerged in the water and were equipped with stirring magnets. After a period of one hour, 1 ml of freshly prepared saturated solution was added to each donor compartment, which

was immediately covered with Parafilm® to prevent the evaporation of any constituents within the saturated solution for the duration of the experiment. An excess amount of solute was present in the donor compartments at all times during the experimental procedure.

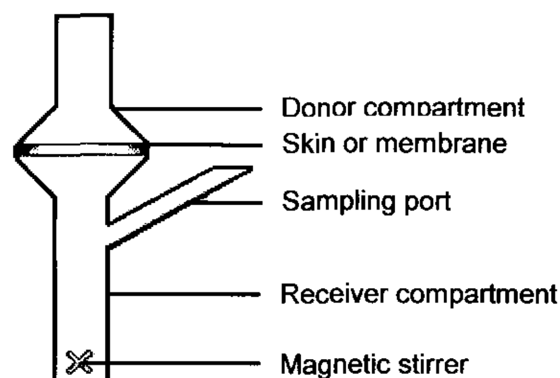


Figure 4.1: Schematic representation of the standard, original diffusion cell developed by Franz (Bronaugh & Collier, 1993).

The entire receptor volumes were withdrawn and replaced with 37 °C fresh buffer solution (pH 7,4) after 2, 4, 6, 8, 10, 12 and 24 hours, except for acetylsalicylic acid **(3)** the entire receptor volumes was withdrawn and replaced two hourly for 2 – 24 and 39 hours. The entire receptor volumes were withdrawn to mimic sink conditions as they occur in the human body. The experiments were conducted over 24-hour periods.

The withdrawn samples were assayed directly by HPLC to determine the drug concentration, which had permeated through the stratum corneum.

The experiment was done until six values within 30 – 40 % of one another were obtained.

Chapter 5

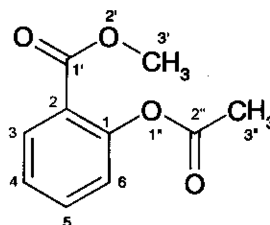
Results and discussion

5.1 Acetylsalicylate derivative esterification

The acetylsalicylic acid derivatives were synthesised, isolated and analysed by MS, IR and NMR, as well as DSC for **(4)**. It was clear from TLC that esterification had occurred between acetylsalicyloyl chloride **(1)** and the ten different alcohols to give products **(4)** – **(13)**. Purification by column chromatography was successful and the NMR analyses of theses samples indicated that they were all pure compounds. The ^{1}H and ^{13}C NMR data of all the acetylsalicylate derivatives were similar to that of acetylsalicylic acid and thus only the differences will be discussed.

5.1.1 Structures of the products

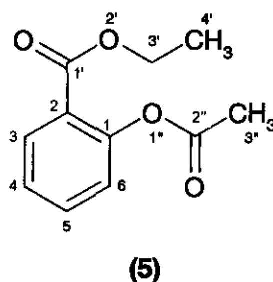
5.1.1.1 Methyl acetylsalicylate (4)



(4)

The MS data confirmed the presence of the molecular ion of **(4)** at m/z 194, corresponding to a molecular formula of $C_{10}H_{10}O_4$. The ^{13}C NMR data of **(4)** were similar to that of acetylsalicylic acid, except that the signal of the carbonyl carbon atom (C-1') has shifted to a slightly lower magnetic field at δ 164,84 in the ^{13}C NMR spectrum, while the presence of the methyl group was indicated by the signal at δ 52,08 representing the carbon atom C-3'. In the ^{1}H NMR spectrum the singlet at δ 3,85 represents H-3'. In the IR spectrum the alkane (CH) stretching vibration was at 2953,98 cm^{-1} and the stretching vibrations of the two ester groups were at 1726,76 and 1771,02 cm^{-1}.

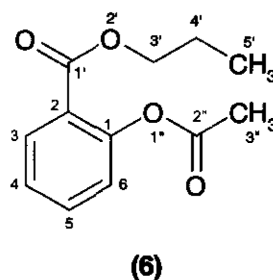
5.1.1.2 Ethyl acetylsalicylate (5)



(5)

The MS data confirmed the presence of the molecular ion of **(5)** at m/z 208, corresponding to a molecular formula of $C_{11}H_{12}O_4$. The ^{13}C NMR data of **(5)** were similar to that of acetylsalicylic acid, except that the signal of the carbonyl carbon atom (C-1') has shifted to a slightly lower magnetic field at δ 164,47 in the ^{13}C NMR spectrum, while the presence of the methyl and methylene groups was indicated by signals at δ 14,17 and 61,02 representing the carbon atoms C-4' and C-3', respectively. In the ^{1}H NMR spectrum the triplet at δ 1,34 and quartet at δ 3,41 represent H-4' and H-3', respectively. In the IR spectrum the alkane (CH) stretching vibration was at 2983,64 cm^{-1} and the stretching vibrations of the two ester groups were at 1720,93 and 1770,67 cm^{-1}.

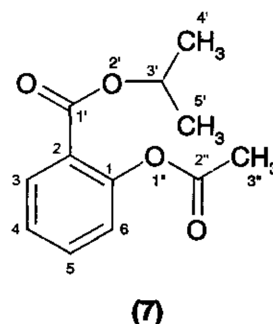
5.1.1.3 Propyl acetylsalicylate (6)



(6)

The MS data confirmed the presence of the molecular ion of **(6)** at m/z 222, corresponding to a molecular formula of $C_{12}H_{14}O_4$. The ^{13}C NMR data of **(6)** were similar to that of acetylsalicylic acid, except that the signal of the carbonyl carbon atom (C-1') has shifted to a slightly lower magnetic field at δ 164,50 in the ^{13}C NMR spectrum, while the presence of the methylene groups were indicated by signals at δ 22,00 and 66,66 representing the carbon atoms C-4' and C-3', respectively, with the methyl group, C-5', represented by the peak at δ 10,38. In the ^{1}H NMR spectrum the triplets at δ 0,99 and 4,21 together with the multiplet at δ 1,74 represent H-5', H-3' and H-4', respectively. In the IR spectrum the alkane (CH) stretching vibration was at 2969,12 cm^{-1} and the stretching vibrations of the two ester groups were at 1721,37 and 1771,86 cm^{-1}.

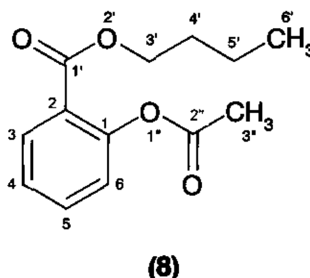
5.1.1.4 Isopropyl acetylsalicylate (7)



(7)

The MS data confirmed the presence of the molecular ion of (7) at m/z 222, corresponding to a molecular formula of $C_{12}H_{14}O_4$. The ^{13}C NMR data of (7) were similar to that of acetylsalicylic acid, except that the signal of the carbonyl carbon atom (C-1') has shifted to a slightly lower magnetic field at δ 164,06 in the ^{13}C NMR spectrum, while the presence of the methyl groups were indicated by the signal at δ 21,82 representing the carbon atoms C-4' and C-5', with the methine group, C-3', represented by the peak at δ 68,50. In the ^{1}H NMR spectrum the doublet at δ 1,32 represents H-4' and H-5', the multiplet at δ 5,20 represents H-3'. In the IR spectrum the alkane (CH) stretching vibration was at 2982,69 cm^{-1} and the stretching vibrations of the two ester groups were at 1716,45 and 1770,85 cm^{-1}.

5.1.1.5 Butyl acetylsalicylate (8)

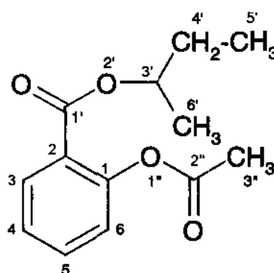


(8)

The molecular ion was not shown in the EI MS data, but FAB MS confirmed the presence of the molecular ion of (8) at m/z 237, corresponding to a molecular formula of $C_{13}H_{16}O_4$ and one proton. The ^{13}C NMR data of (8) were similar to that of acetylsalicylic acid, except that the signal of the carbonyl carbon atom (C-1') has shifted to a slightly lower magnetic field at δ 164,49 in the ^{13}C NMR spectrum, while the presence of the methylene groups were indicated by signals at δ 19,15; 30,67 and 64,94 representing the carbon atoms C-5', C-4' and C-3', respectively, with the methyl group, C-6', represented by the peak at δ 13,66. In the ^{1}H NMR spectrum the triplets at δ 0,95 and 4,26 represent H-6' and H-3', respectively. The multiplets at δ 1,43 and 1,70 represent H-5' and H-4', respectively. In the IR spectrum the alkane (CH)

stretching vibration was at 2960,94 cm^{-1} and the stretching vibrations of the two ester groups were at 1723,48 and 1770,54 cm^{-1}.

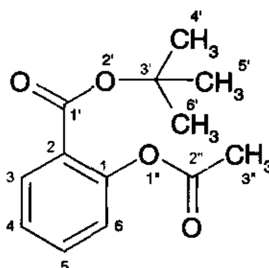
5.1.1.6 1-Methylpropyl acetylsalicylate (9)



(9)

The MS data confirmed the presence of the molecular ion of **(9)** at m/z 236, corresponding to a molecular formula of $C_{13}H_{16}O_4$. The ^{13}C NMR data of **(9)** were similar to that of acetylsalicylic acid, except that the signal of the carbonyl carbon atom (C-1') has shifted to a slightly lower magnetic field at δ 164,16 in the ^{13}C NMR spectrum, while the presence of the methyl groups were indicated by signals at δ 9,66 and 19,42 representing the carbon atoms C-5' and C-6', respectively, with the methine group, C-3', and the methylene group, C-4', represented by the peaks at δ 73,06 and 28,87, respectively. In the ^1H NMR spectrum the triplet at δ 0,94 and the doublet at δ 1,29 represent H-5' and H-6', respectively. The multiplets at δ 1,66 and 5,04 represent H-4' and H-3', respectively. In the IR spectrum the alkane (CH) stretching vibration was at 2975,77 cm^{-1} and the stretching vibrations of the two ester groups were at 1716,34 and 1770,54 cm^{-1}.

5.1.1.7 Tert-butyl acetylsalicylate (10)

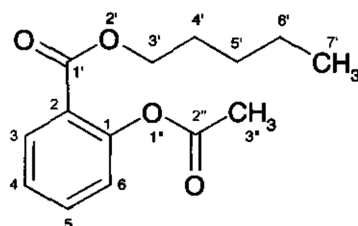


(10)

The molecular ion was not shown in the EI MS data, but FAB MS confirmed the presence of the molecular ion of **(10)** at m/z 237, corresponding to a molecular formula of $C_{13}H_{16}O_4$ and one proton. The ^{13}C NMR data of **(10)** were similar to that of acetylsalicylic acid, except that the signal of the carbonyl carbon atom (C-1') has shifted to a slightly lower magnetic field at

δ 163,89 in the ^{13}C NMR spectrum, while the presence of the methyl groups were indicated by the signal at δ 28,13 representing the carbon atoms C-4', C-5' and C-6', with C-3' represented by the peak at δ 81,54. In the ^{1}H NMR spectrum the singlet at δ 1,55 represent H-4', H-5' and H-6'. In the IR spectrum the alkane (CH) stretching vibration was at 2980,52 cm^{-1} and the stretching vibrations of the two ester groups were at 1716,20 and 1770,01 cm^{-1}.

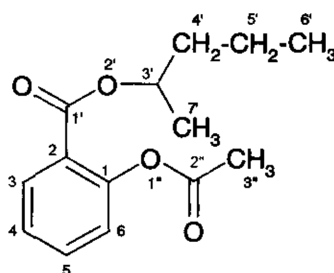
5.1.1.8 Pentyl acetylsalicylate (11)



(11)

The molecular ion was not shown in the EI MS data, but FAB MS confirmed the presence of the molecular ion of **(11)** at m/z 251, corresponding to a molecular formula of $C_{14}H_{18}O_4$ and one proton. The ^{13}C NMR data of **(11)** were similar to that of acetylsalicylic acid, except that the signal of the carbonyl carbon atom (C-1') has shifted to a slightly lower magnetic field at δ 164,51 in the ^{13}C NMR spectrum, while the presence of the methylene groups were indicated by signals at δ 22,30; 28,07; 28,34 and 65,25 representing the carbon atoms C-6', C-5', C-4' and C-3', respectively, with the methyl group, C-7', represented by the peak at δ 13,89. In the ^{1}H NMR spectrum the triplets at δ 0,90 and 4,25 represent H-7' and H-3', respectively. The multiplet at δ 1,37 represent H-5'and H-6', while the multiplet at δ 1,72 represent H-4'. In the IR spectrum the alkane (CH) stretching vibration was at 2958,60 cm^{-1} and the stretching vibrations of the two ester groups were at 1723,12 and 1771,10 cm^{-1}.

5.1.1.9 1-Methylbutyl acetylsalicylate (12)

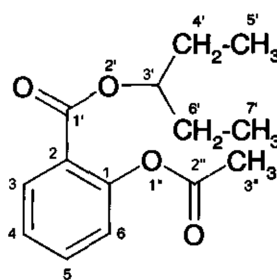


(12)

The MS data confirmed the presence of the molecular ion of **(12)** at m/z 250, corresponding to a molecular formula of $C_{14}H_{18}O_4$. The ^{13}C NMR data of **(12)** were similar to that of acetylsalicylic

acid, except that the signal of the carbonyl carbon atom (C-1') has shifted to a slightly lower magnetic field at δ 164,14 in the ^{13}C NMR spectrum, while the presence of the methyl groups were indicated by signals at δ 13,88 and 18,62 representing the carbon atoms C-6' and C-7', respectively, with the methine group, C-3', represented by the peak at δ 71,65. The methylene groups were indicated by signals at δ 19,95 and 38,15 representing the carbon atoms C-5' and C-4'. In the ^{1}H NMR spectrum the triplet at δ 0,92 and the doublet at δ 1,30 represent H-6' and H-7', respectively. The multiplets at δ 1,38; 1,60 and 5,12 represent H-5', H-4' and H-3', respectively. In the IR spectrum the alkane (CH) stretching vibration was at 2961,49 cm^{-1} and the stretching vibrations of the two ester groups were at 1719,58 and 1772,76 cm^{-1}.

5.1.1.10 1-Ethylpropyl acetylsalicylate (13)



(13)

The molecular ion was not shown in the EI MS data, but FAB MS confirmed the presence of the molecular ion of **(13)** at m/z 251, corresponding to a molecular formula of $C_{14}H_{18}O_4$ and one proton. The ^{13}C NMR data of **(13)** were similar to that of acetylsalicylic acid, except that the signal of the carbonyl carbon atom (C-1') has shifted to a slightly lower magnetic field at δ 164,33 in the ^{13}C NMR spectrum, while the presence of the methyl groups were indicated by the signal at δ 9,56 representing the carbon atoms C-5' and C-7', with the methine group, C-3', represented by the peak at δ 77,54. The methylene groups were indicated by the signal at δ 26,47 representing the carbon atoms C-4' and C-6'. In the ^{1}H NMR spectrum the triplet at δ 0,92 represent H-5' and H-7', respectively. The multiplet at δ 1,65 represent H-4' and H-6', while the multiplet at δ 4,96 represents H-3'. In the IR spectrum the alkane (CH) stretching vibration was at 2970,85 cm^{-1} and the stretching vibrations of the two ester groups were at 1716,54 and 1771,13 cm^{-1}.

5.1.2 Conclusion

The ^{1}H and ^{13}C NMR, MS and IR data confirmed that the acetylsalicylic acid derivatives were successfully synthesised.

5.2 Physicochemical properties

5.2.1 Aqueous solubility

The experimentally determined aqueous solubility together with predicted aqueous solubility values, obtained from IA (interactive analysis) prediction software (Parham, 2000), are presented in Table 5.1.

Table 5.1: The aqueous solubility of acetylsalicylic acid and acetylsalicylate derivatives.

	Aqueous solubility (mg/ml)	
Compound	**Experimental** [a]	**Predicted** [b] **(IA)**
Acetylsalicylic acid	6,56	3,70
Methyl acetylsalicylate	3,26	2,22
Ethyl acetylsalicylate	3,32	1,04
Propyl acetylsalicylate	1,60	0,47
Isopropyl acetylsalicylate	0,52	0,57
Butyl acetylsalicylate	0,07	0,22
1-Methylpropyl acetylsalicylate	0,20	0,31
Tert-butyl acetylsalicylate	0,76	0,28
Pentyl acetylsalicylate	$1,76 \times 10^{-3}$	0,12
1-Methylbutyl acetylsalicylate	0,05	0,14
1-Ethylpropyl acetylsalicylate	0,11	0,16

[a] experiments conducted at pH 4,5 in TRIS buffer
[b] calculated using IA (interactive analysis) (Parham, 2000)

5.2.2 Discussion

As expected, the water solubility of acetylsalicylic acid is higher than that of the synthesised acetylsalicylate compounds. The above results show that the solubility in general decreases with an increase in chain length in accordance with data in the literature (§ 3.4) (Abdou, 1989). For the butyl derivatives the solubility increased from butyl to tert-butyl. One can speculate that this is most probably because of branching of the chain. The same phenomenon is observed for the pentyl acetylsalicylate derivatives. For the propyl derivatives the previously explained phenomenon had not occurred, this might be that (1) the chain length is too short for branching to make a difference and (2) it only happens from a chain length with four or more carbons.

Acetylsalicylic acid is a relatively strong acidic drug with a pKa of 3,50 (Florey, 1979). Hence, for it to be 99,99 % unionised it should be dissolved in an acidic pH of - 0,5. Acetylsalicylic acid

is 50 % unionised at a pH of 3,5. This provides a problem for transdermal application, for if the pH is lower than 4,5 it will irritate the skin. The aforementioned states the reason why a pH of 4,5 was chosen, where acetylsalicylic acid was 9,09 % unionised. To compare the compounds with acetylsalicylic acid, it was necessary to determine aqueous solubility at the same pH. Compounds can only permeate the stratum corneum successfully if the proposed transdermal candidates are in an unionised state. Ionised molecules do not penetrate the lipophilic stratum corneum easily and have delayed transdermal permeation.

Experimentally determined aqueous solubility is higher than predicted values, and the same trend with branches versus linear side chains is observed. The difference between the data may be attributed to the method of calculation, the fact that the software does not specify the pH at which they were calculated and neither the temperature nor the buffer. Different temperature, pH values and buffers are likely to influence the solubility values.

5.2.3 Partition coefficient

The experimentally determined partition coefficients (log P) together with predicted partition coefficient values, obtained from the ACD Labs, IA and K_{ow}Win prediction software, are presented in Table 5.2.

Table 5.2: Partition coefficients of acetylsalicylic acid and acetylsalicylate derivatives (log P).

Compound	Experimental [a]	Predicted [b] (IA)	Predicted [c] (K_{ow}Win)	Predicted [d] (ACD)
Acetylsalicylic acid	-0,85	1,55	1,13	1,19
Methyl acetylsalicylate	-0,25	1,55	1,42	1,35
Ethyl acetylsalicylate	0,30	2,04	1,92	1,88
Propyl acetylsalicylate	0,86	2,55	2,41	2,41
Isopropyl acetylsalicylate	0,75	2,32	2,33	2,23
Butyl acetylsalicylate	1,32	3,05	2,90	2,94
1-Methylpropyl acetylsalicylate	1,23	2,80	2,82	2,76
Tert-butyl acetylsalicylate	1,09	2,77	2,79	2,58
Pentyl acetylsalicylate	1,95	3,57	3,39	3,47
1-Methylbutyl acetylsalicylate	1,64	3,37	3,32	3,29
1-Ethylpropyl acetylsalicylate	1,65	3,18	3,32	3,29

[a] experiments conducted at pH 4,5 in TRIS buffer
[b] calculated using IA (interactive analysis) (Parham, 2000)
[c] calculated using K_{ow}Win (http://esc.syrres.com/interkow/kowdemo.htm)
[d] calculated using ACD software

5.2.4 Discussion

The experimentally determined partition coefficients are lower than those predicted. There is a difference of approximately 1,5 log. The difference between the data may be attributed to the method of calculation, the fact that the software does not specify the pH at which they were calculated, or the buffers used. Different pH values and buffers are likely to influence the partition coefficient values. In work done in our laboratories to investigate the percutaneous delivery of different NSAIDs, it was found that K_{ow}Win programs gave values close to the experimental partition coefficient (Swart, 2003). The experimental partition coefficients done by Swart (2003) were conducted at pH 7,0. Hence, for K_{ow}Win programmes to correlate with the experimental values it might predict values at pH 7,0. In this study experimental partition coefficients were conducted at pH 4,5 which might be the reason for predicted values not correlating with experimental values.

As expected, the partition coefficient of acetylsalicylic acid is lower than that of the synthesised acetylsalicylate compounds, due to the increase in alkyl chain length that leads to an increase in partition coefficients in accordance with data from the literature (§ 3.4) (Abdou, 1989). For the butyl derivatives the partition coefficient decreased from butyl to tert-butyl, this is most probably because of branching of the chain. The same trend with branches versus linear side chains was observed. for the propyl and pentyl acetylsalicylate derivatives, although 1-methylbutyl and 1-ethylpropyl acetylsalicylate didn't differ much.

The results of the experimental partition coefficients indicate that esterification of acetylsalicylic acid results in a higher partition coefficient. These results are as expected and validate the aqueous solubility data, whereby compounds with higher partition coefficients present with an increased lipophilicity and lower aqueous solubility.

5.3 Transdermal permeation of acetylsalicylic acid and its synthesised derivatives

5.3.1 Transdermal permeation

Experimental values obtained for aqueous solubility, log P and the molecular weight (MW) can be used to estimate the flux values (J_{max}) for acetylsalicylic acid and its esters. The Potts and Guy equation (Equation 5.1) can be used to calculate the log k_p, from where the permeability coefficient (k_p) may be obtained (Hadgraft *et al.*, 2000). The estimated flux (μg/cm^2/h) can be obtained from the product of the permeability coefficient and the aqueous solubility at the same pH (Equation 5.2).

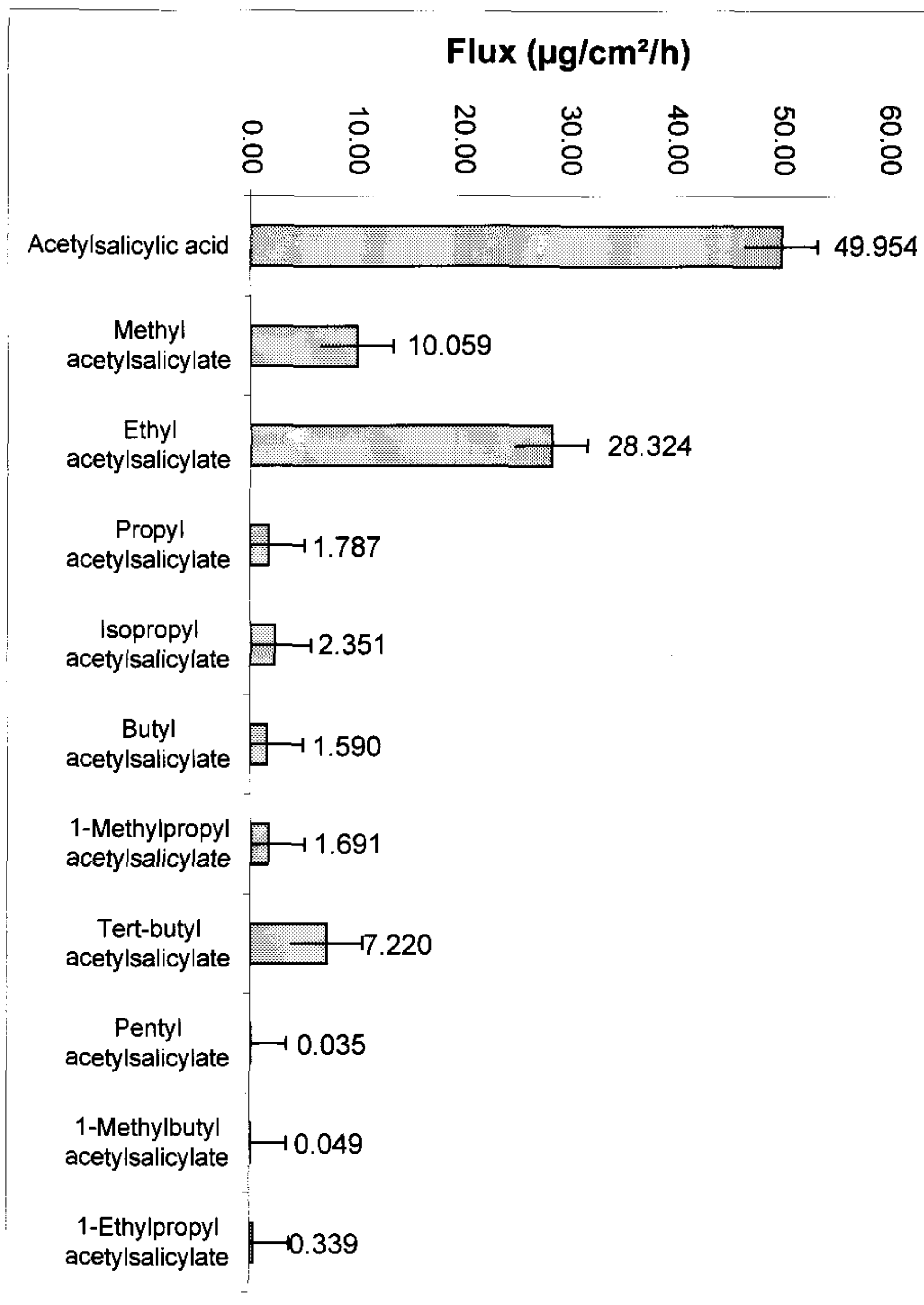


Figure 5.1: Experimental acetylsalicylic acid and acetylsalicylate derivative flux data.

5.3.2 Discussion

The experimental transdermal flux of acetylsalicylic acid (47,53 μg/cm²/h) was much higher than that of its derivatives, with the ethyl derivative (28,32 μg/cm²/h) and the methyl derivative (10,06 μg/cm²/h) being the only derivatives with appreciable flux. Acetylsalicylic acid, methyl and ethyl acetylsalicylate had higher aqueous solubilities and lower partition coefficients, thus being more hydrophilic than the other compounds. According to mosaic theory, proteins in the skin may take up water and the resulting swollen membranes may become permeable to water-soluble substances (Rothman, 1954). These three compounds most probably penetrated through the protein rich spaces between the corneosytes of the stratum corneum (Foldvari, 2000; Schalla & Schaefer, 1982), resulting in higher fluxes. This could be possible, because the stratum corneum is hydrated in a polar solution for 24 hours making it easier for hydrophilic compounds to penetrate (Morganti *et al.*, 2001; Hull, 2002).

Acetylsalicylic acid and methyl acetylsalicylate are both crystalline compounds, the rest of the acetylsalicylate derivatives are oils. Therefore, in the supersaturated buffer solutions acetylsalicylic acid and methyl acetylsalicylate had crystals at the bottom of the donor phase, on top of the skin, keeping the resultant solution homogenous which may explain the higher flux observed for these compounds. The other derivatives were found floating on top of the saturated solution, and as there was no stirring in the donor phase to keep the resultant solution homogenous, the solution closest to the skin became unsaturated and this may describe the decreased flux observed for these compounds.

From the plotted transdermal permeation data (see Appendix 1), it is noticeable that a plato in transdermal permeation formed, and as the alkyl chain lengthened the plato resulted sooner. Ethyl acetylsalicylate has a slight plato forming after 12 hours where 1-ethylpropyl acetylsalicylate's plato started after 8 hours. The reason for the plato forming may be because all the acetylsalicylate derivatives are oils, except methyl acetylsalicylate, and floated on top of the saturated solution, not being able to keep the solution closest to the skin saturated, which may cause the lower flux values for these compounds.

According to the literature (§ 3.3.3) a drug should have optimal permeation if it has reasonable solubility in both water and oils and has a log P in the range of 1 – 2 (Hadgraft, 1996). Acetylsalicylic acid, the methyl, ethyl and propyl derivatives have high aqueous solubility (ranging from1,60 to 6,56 mg/ml) and log P values lower than 1, while the butyl and pentyl derivatives have very low aqueous solubility (ranging from1,76 x 10^{-3} to 0,76 mg/ml) and log P values between 1 and 2. Pentyl acetylsalicylate had a log P value of approximately 2, but had the lowest flux (0,03 μg/cm²/h); this can be ascribed to the low aqueous solubility (1,76 x 10^{-3} mg/ml). Tert-butyl acetylsalicylate had a flux (7,30 ± 1,0 μg/cm²/h) lower than

methyl and ethyl acetylsalicylate, but a higher flux than the other synthesised derivatives which could be due to its log P value being slightly higher than 1 and having an average aqueous solubility, just proving once again that to cross the stratum corneum a drug should posses both hydrophilic and lipophilic properties.

As the pKa values of the synthesised derivatives are unknown and the compounds have to be at the same pH as acetylsalicylic acid for comparison, acetylsalicylic acid was used to determine the pH where it would be unionised. Acetylsalicylic acid was 50 % unionised at a pH of 3,5. Since using a pH lower than 4,5 burns and irritates the skin the pH chosen for transdermal studies were 4,5 where acetylsalicylic acid was only 9,09 % unionised. According to the literature (§ 3.3.4) a higher degree of ionisation leads to lower concentrations of unionised drug available for transdermal penetration. Hence, unionised species permeate the stratum corneum better than the ionised form (Abdou, 1989 and Jack *et al.,* 1991). Thus by choosing a lower pH the derivatives might have been more unionised which would have resulted in higher flux.

The predicted flux values were obtained by using a) experimentally predicted partition coefficient data and b) predicted partition coefficient values obtained from ACD software in the Potts and Guy equation (Equations 5.1 and 5.2). The predicted flux values didn't correlate well with the experimental flux values. The model used to predict transdermal flux values does not regard the state of ionisation and makes it hard to predict flux to compare with experimental flux values. The results clearly indicate that the degree of ionisation of intended transdermal candidates has to be considered when selecting the donor solution pH.

A series of investigations have been done on the transdermal delivery of acetylsalicylic acid. The following flux values for acetylsalicylic acid were obtained: 21,81 ± 3,11 μg/cm^2/h (Feldmann & Maibach, 1970), and *in vivo* 24,8 ± 4,4 μg/cm^2/h and *in vitro* 29,0 ± 3,1 μg/cm^2/h (Bronaugh *et al.*, 1982). The previous studies were all done at neutral pH. In this study the flux for acetylsalicylic acid was 47,53 ± 5,8 μg/cm^2/h at pH 4,5, which is a greater flux value than the values obtained in previous studies. Since acetylsalicylic acid is 99,99 % ionised at pH 7,4 and 91,91 % ionised at pH 4,5, it makes sense that the flux values obtained in this study was higher, due to the fact that acetylsalicylic acid is more unionised.

(Winek *et al.,* 2001) gave the normal blood level of acetylsalicylic acid for analgesic use as 20 – 100 μg/ml. If hydrolysis of the synthesised derivatives occurred to form acetylsalicylic acid, the only derivative that would be able to be used would be ethyl acetylsalicylate, and it will give a blood level concentration of 24,5 μg/ml.

Chapter 6

Summary and final conclusions

The skin, although an ideal site for drug administration, is also a major barrier to this process. Effective drug therapies must therefore overcome the challenge of finding a technology to administer, measure and deliver the required quantity of drug into or through the skin.

Acetylsalicylic acid holds a 25 % share of the worldwide analgesic market and is commonly used in the treatment of moderate pain such as headaches, neuritis, toothache, dysmenorrhoea, musculoskeletal disorders, such as rheumatoid arthritis, thromboembolic disorders and fever. The most common adverse effects occurring with acetylsalicylic acid are gastro-intestinal disturbances, irritation of the gastric mucosa and hypersensitivity reactions.

The adverse effects encourage a study into the development of a transdermal delivery system for acetylsalicylic acid with the aim of avoiding hepatic first pass metabolism, improving patient compliance and bioavailability and decreasing the administered dose. Much attention has been given to the use of penetration enhancers and delivery vehicles to improve transdermal permeation. The development of pro-drugs with enhanced physicochemical properties for greater transdermal delivery has been of interest recently.

In work done in our laboratories it was indicated that the analogues of thalidomide with the highest aqueous solubility presented with the greatest transdermal fluxes (Goosen et al., 2002). Research has also found that a drug should have optimal permeation if it has reasonable solubility in both water and oils and has a log P in the range of 1 – 2 (Hadgraft, 1996).

During this study, the aim was primarily to determine the transdermal permeation of acetylsalicylic acid and some its derivatives as well as the correlation, if any, with selected physicochemical properties.

The following objectives were set in this study:

- Synthesise esters of acetylsalicylic acid and verify their structures.
- Experimentally determine the aqueous solubility and the partition coefficient for acetylsalicylic acid and its synthesised derivatives.
- Compare the experimental aqueous solubility and the partition coefficient of synthesised acetylsalicylic acid derivatives with values calculated from commonly used prediction software.

- Experimentally determine the transdermal flux of acetylsalicylic acid and its derivatives.
- Compare the experimental flux data of the synthesised acetylsalicylic acid derivatives with values calculated from commonly used theoretical equations.
- Determine whether a correlation exists between the aqueous solubility, partition coefficient and transdermal flux data of the acetylsalicylic acid derivatives.

The acetylsalicylic acid derivatives were successfully synthesised and the structures were verified by ^{1}H and ^{13}C NMR, MS and IR spectroscopy.

As expected, the aqueous solubility of acetylsalicylic acid (6,56 mg/ml) was higher than that of the synthesised acetylsalicylate derivatives (ranging from 1,76 x 10^{-3} to 3,32 mg/ml). This was in accordance with data in the literature (§ 3.4), which proved that the solubility in general decreases with an increase in chain length.

The experimental partition coefficient of acetylsalicylic acid (-0,85) was lower than that of its derivatives (ranging from -0,25 to 1,95) and correlated with the aqueous solubility. The experimental partition coefficients were also compared to values obtained from the ACD Labs, IA and K_{ow}Win prediction software, but did not correlate with the experimental values. There was a difference of approximately 1,5 log which might be attributed to the method of calculation, the fact that the software does not specify the pH at which they were calculated, or the buffers used.

The transdermal flux of acetylsalicylic acid (47,53 $\mu g/cm^2/h$) was much higher than that of its derivatives (ranging from 0,03 to 28,32 $\mu g/cm^2/h$). With the ethyl derivative (28,32 $\mu g/cm^2/h$) and the methyl derivative (10,06 $\mu g/cm^2/h$) being the only derivatives with appreciable flux. These three compounds were more hydrophilic and most probably penetrated through the protein rich spaces between the corneosytes of the stratum corneum, resulting in higher fluxes. Acetylsalicylic acid and methyl acetylsalicylate are both crystalline compounds, the rest of the acetylsalicylate derivatives are oils. Therefore in the supersaturated buffer solutions the crystals were at the bottom of the donor phase, keeping the resultant solution homogenous which may explain the higher flux observed for these compounds, while the oils floated on top of the saturated solution, and as there was no stirring in the donor phase, to keep the resultant solution homogenous, the solution closest to the skin became unsaturated and this may describe the decreased flux observed for these compounds. The aforementioned resulted in a plato forming as seen in the plotted transdermal permeation data.

The reason for low flux values for the acetylsalicylate derivatives can be ascribed to the fact that at the pH (4,5) chosen for transdermal studies acetylsalicylate was only 9,09 % unionised. A

higher degree of unionised species results in higher flux values. Thus by choosing a lower pH the compounds might have been more unionised which may have resulted in higher flux values, but would have caused skin irritation.

The predicted flux values didn't correlate well with the experimental flux values. The model used to predict transdermal flux values did not regard the state of ionisation and made it hard to predict flux to compare with experimental flux values. The results clearly indicated that the degree of ionisation of intended transdermal candidates had to be considered when selecting the donor solution pH.

This study has confirmed that transdermal flux is dependant on several factors including optimum solubility, partitioning, diffusion and the degree of ionisation in the stratum corneum in addition to a suitable partition coefficient and high aqueous solubility. The solution to the increased transdermal delivery of lipophilic drugs does not simply lie in producing a derivative with a higher aqueous solubility and more ideal partition coefficient. Other means of increasing the transdermal permeation of lipophilic acetylsalicylic acid derivatives will have to be investigated in further studies.

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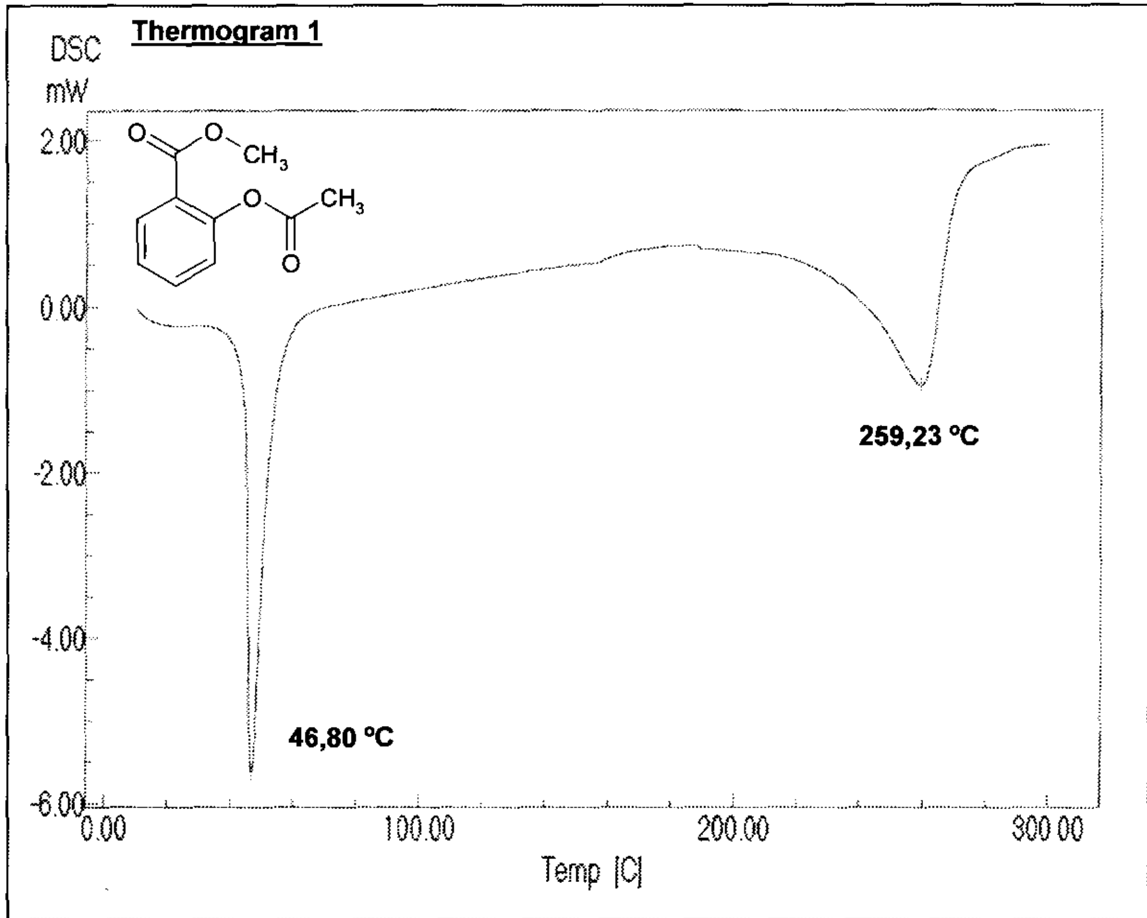
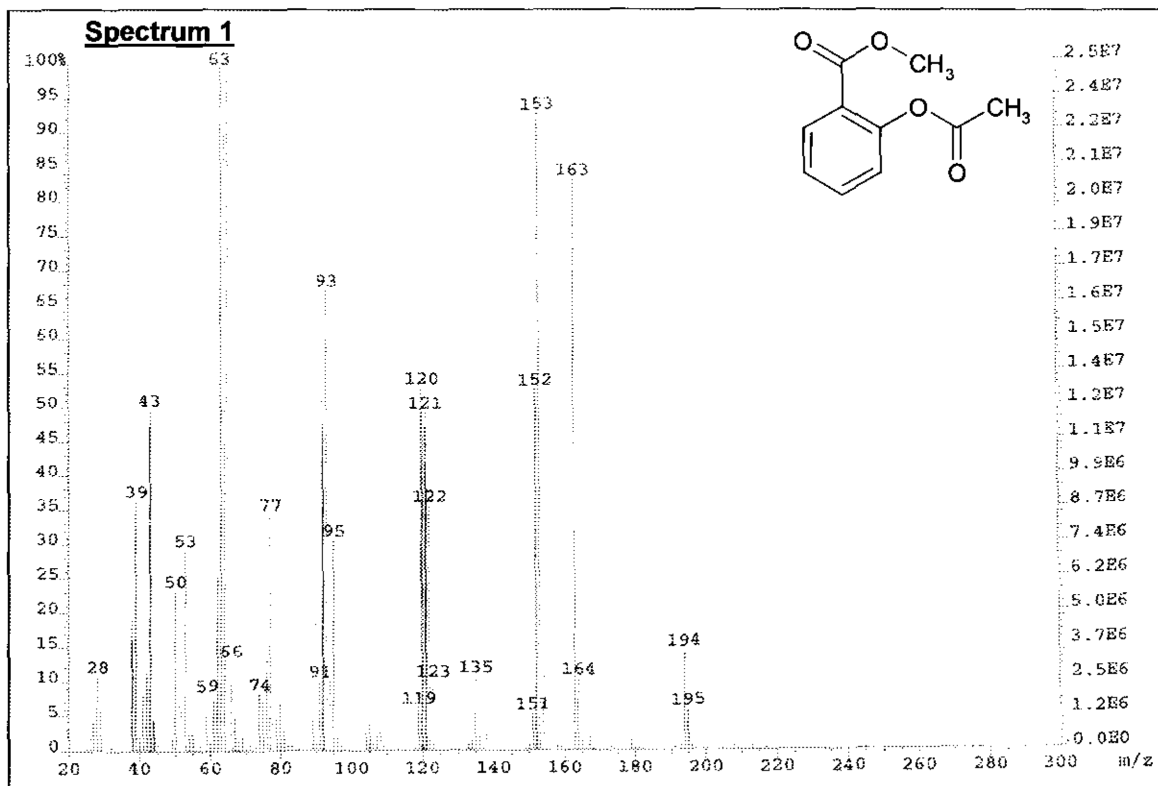

Thermogram 1
DSC
mW
2.00
0.00
-2.00
-4.00
-6.00
0.00
100.00
200.00
300.00
Temp [C]
46,80 °C
259,23 °C
O
O
CH3
O
CH3
O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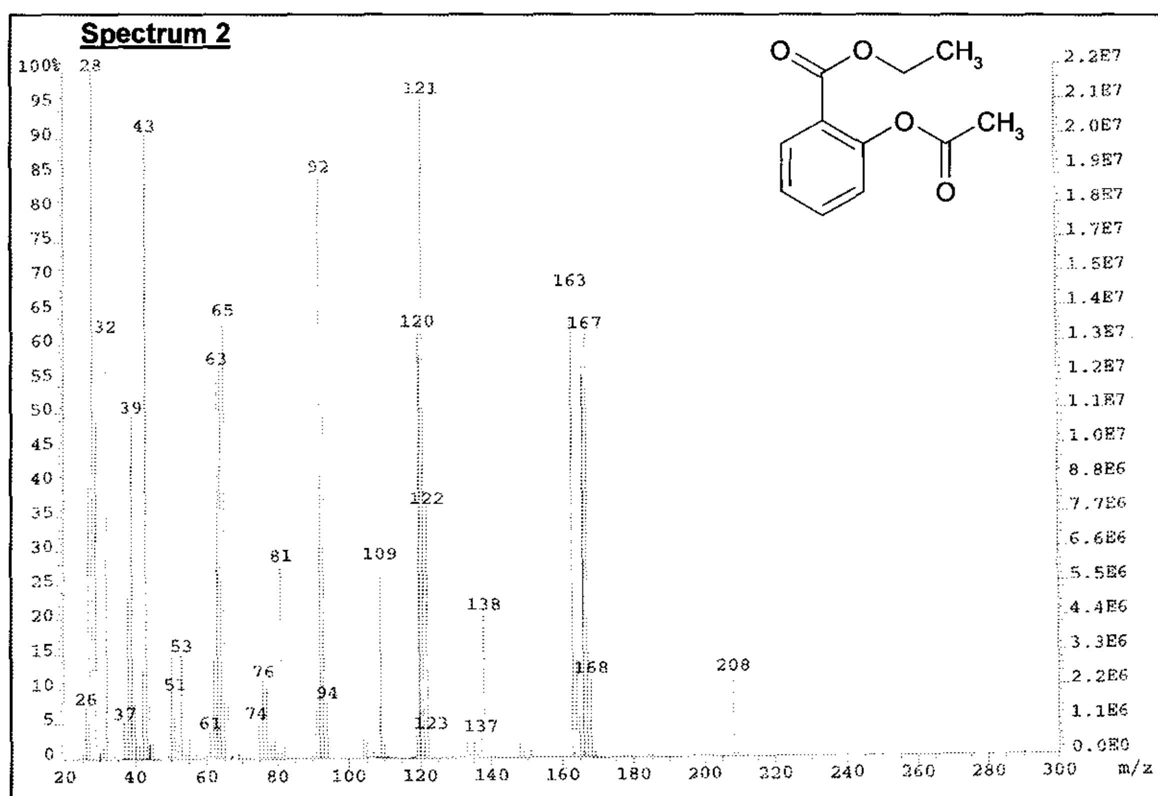
O
O
CH3
O
CH3
O
100%
63
153
163
93
120
152
121
43
39
77
122
95
53
50
194
66
28
91
123
135
164
59
74
119
151
195
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
m/z
2.5E7
2.4E7
2.2E7
2.1E7
2.0E7
1.9E7
1.7E7
1.6E7
1.5E7
1.4E7
1.2E7
1.1E7
9.9E6
8.7E6
7.4E6
6.2E6
5.0E6
3.7E6
2.5E6
1.2E6
0.0E0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O
CH3
O
CH3
O
100%
28
121
43
92
163
65
32
120
167
63
39
122
81
109
138
53
168
208
76
51
26
94
37
74
61
123
137
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
m/z
2.2E7
2.1E7
2.0E7
1.9E7
1.8E7
1.7E7
1.5E7
1.4E7
1.3E7
1.2E7
1.1E7
1.0E7
8.8E6
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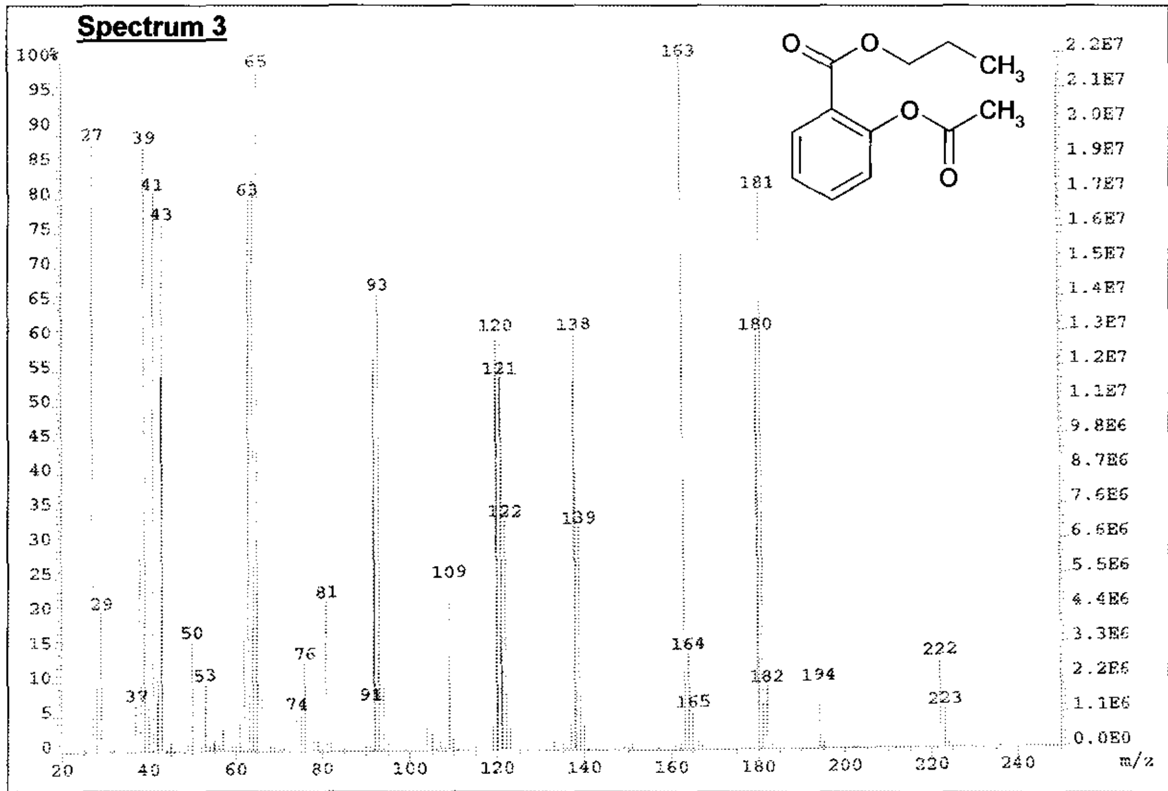
6.6E6
5.5E6
4.4E6
3.3E6
2.2E6
1.1E6
0.0E0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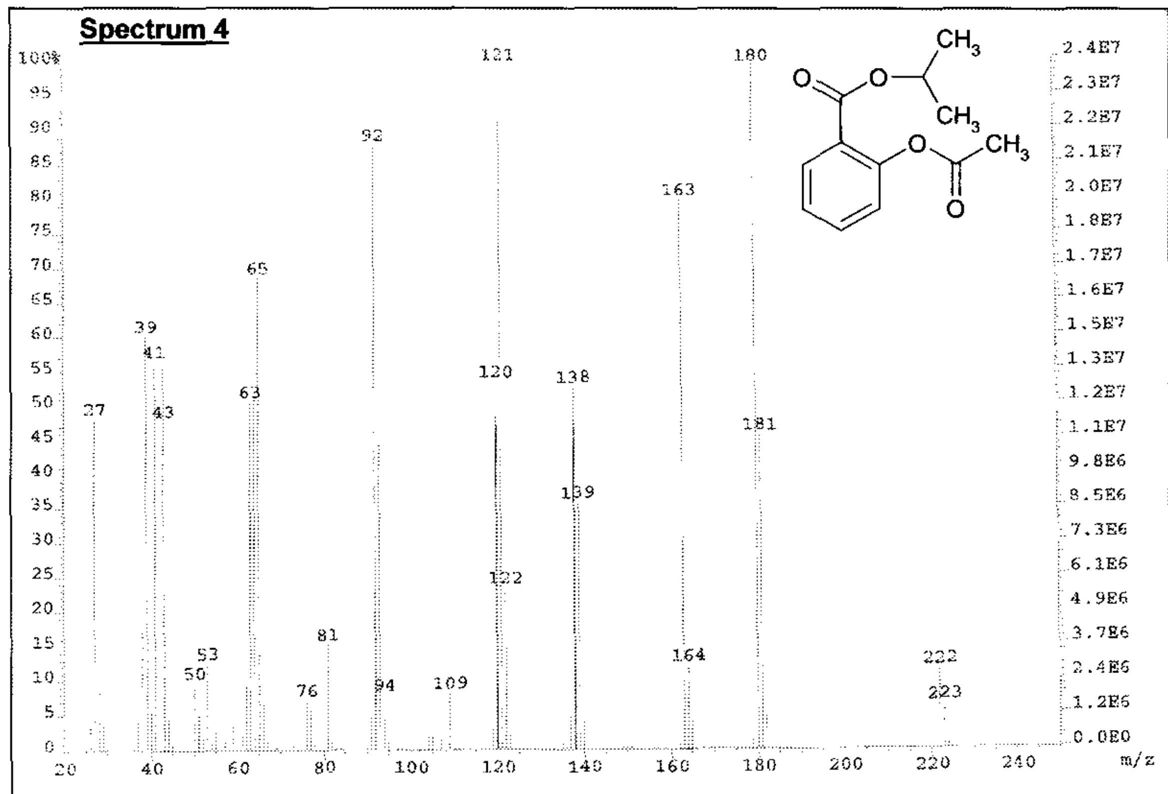
100%
m/z
163
181
93
120
138
180
121
122
139
109
81
50
76
53
74
91
164
182
194
222
223
165
27
39
41
43
63
65
29
37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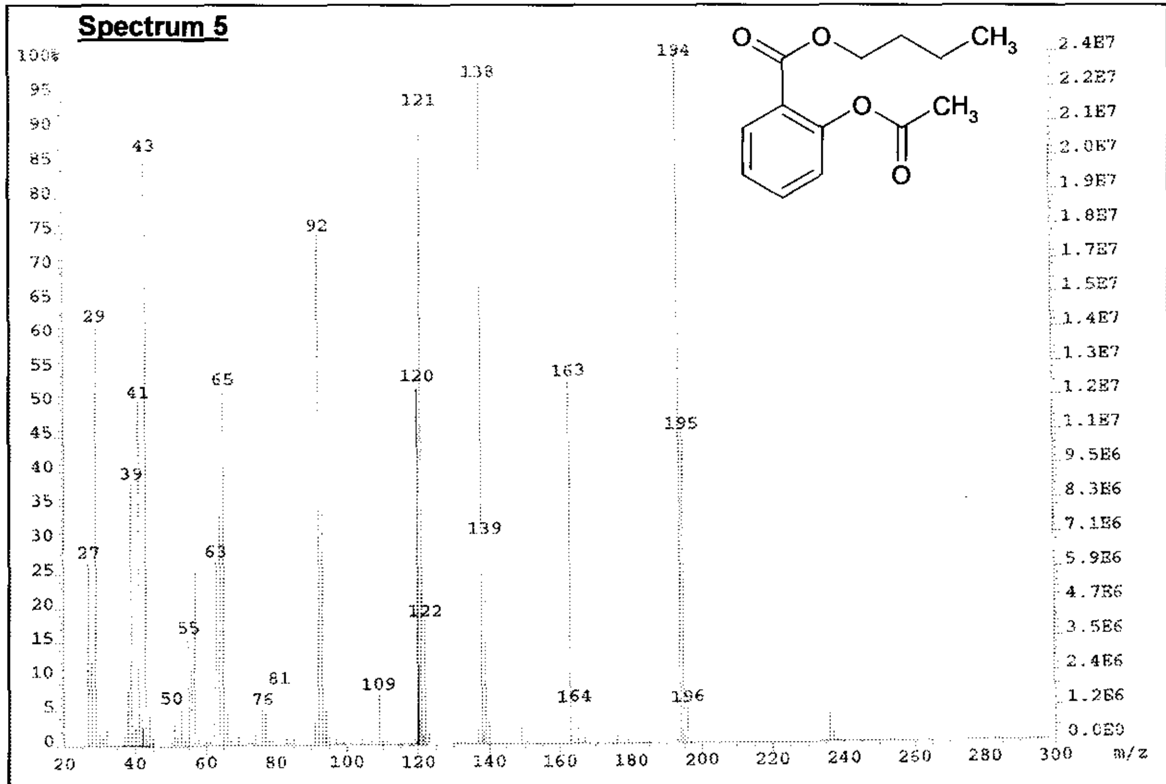
100%
m/z
121
180
92
163
65
39
41
43
27
63
120
138
181
139
122
81
53
50
76
94
109
164
222
223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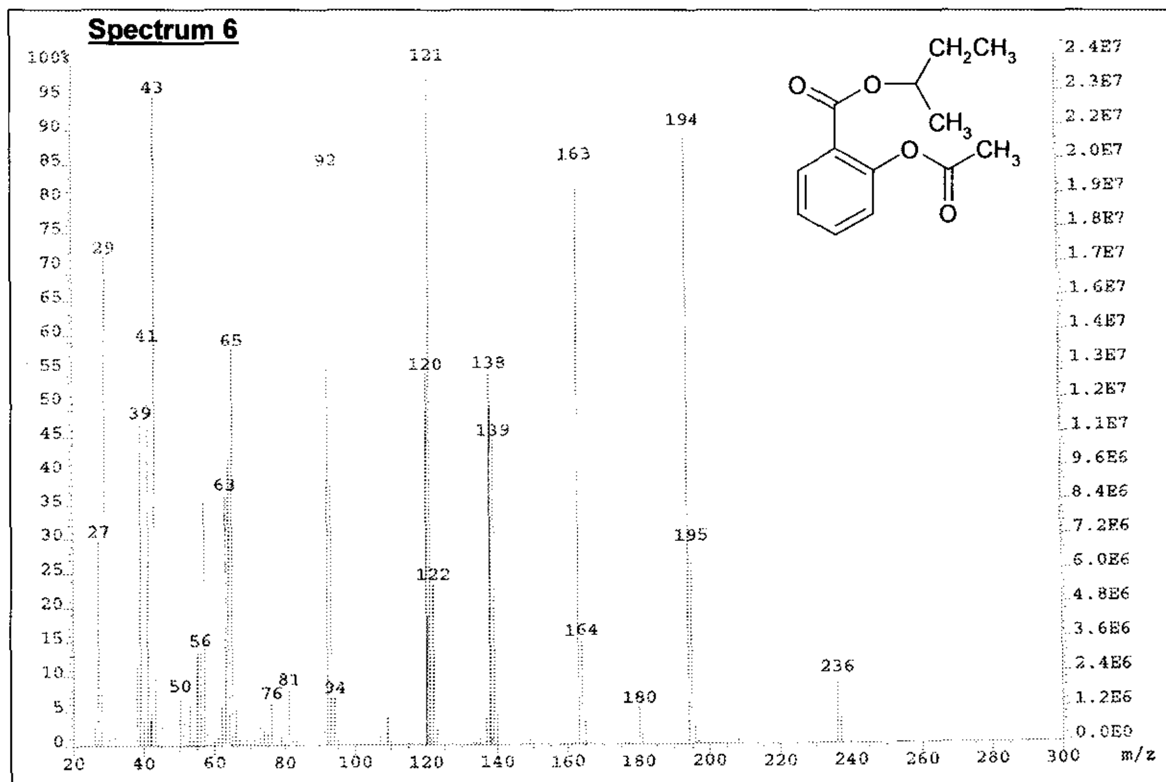
m/z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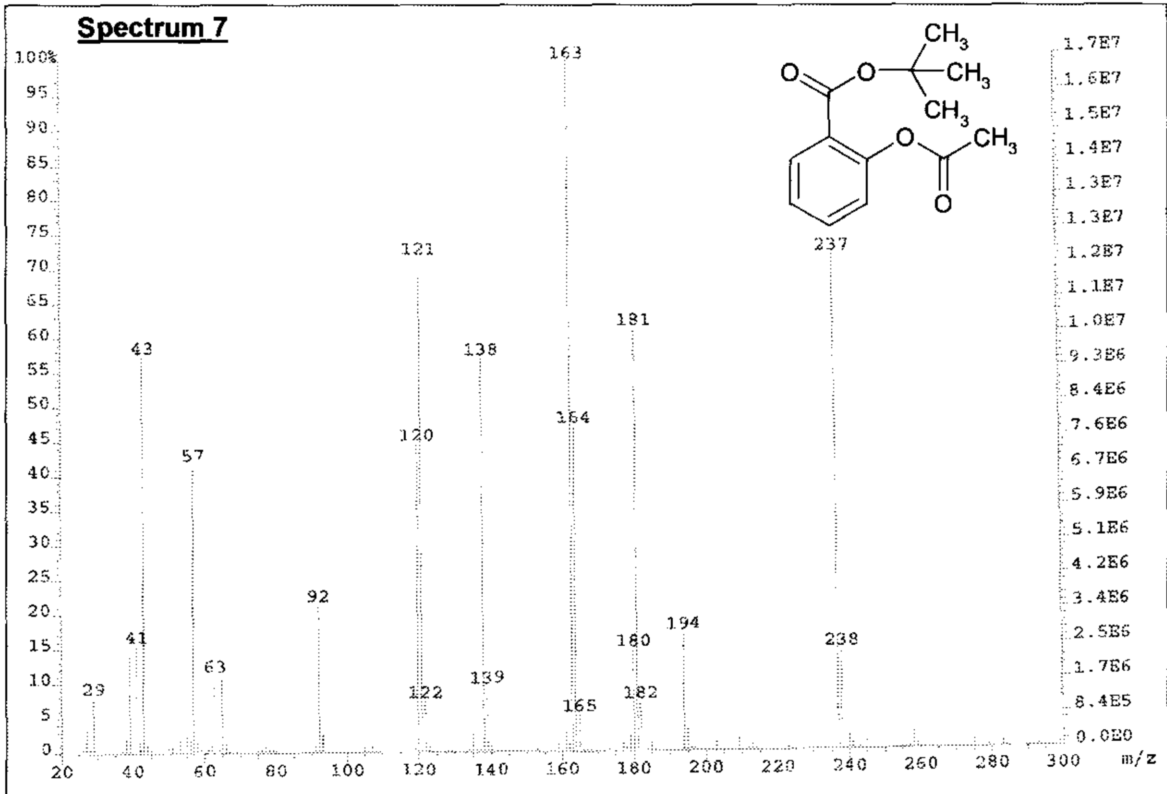
m/z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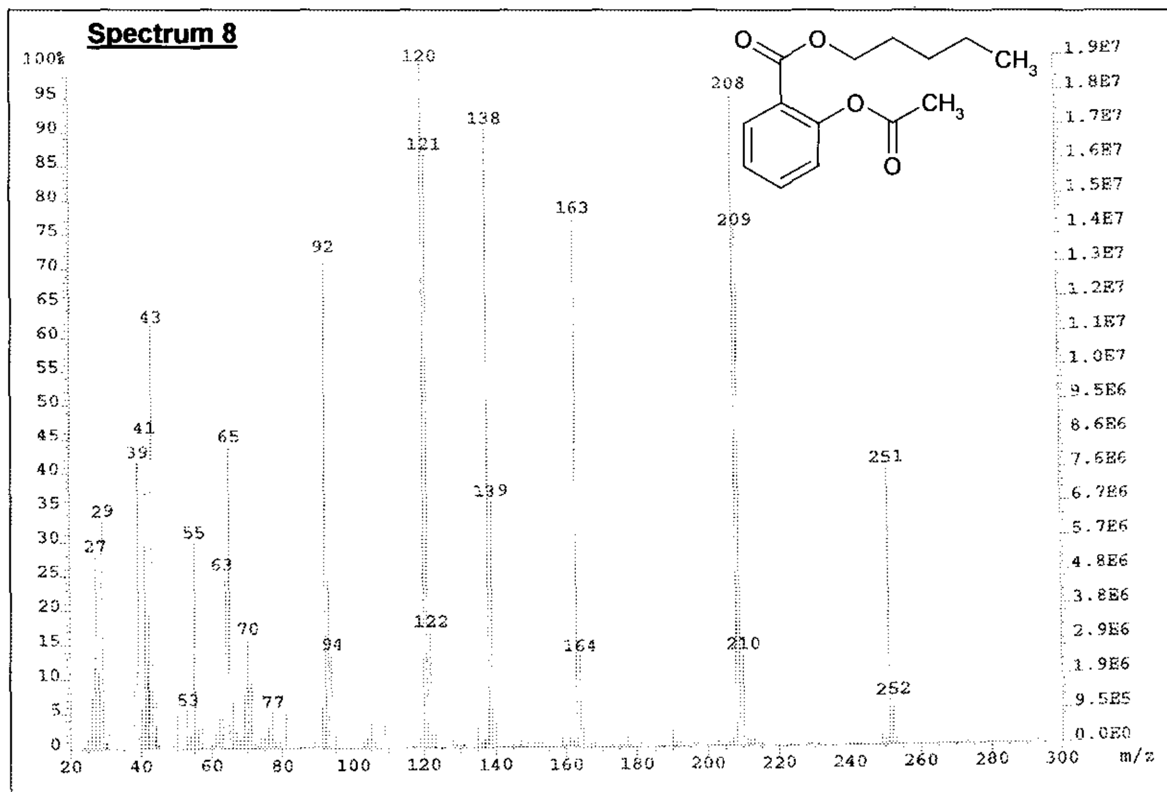
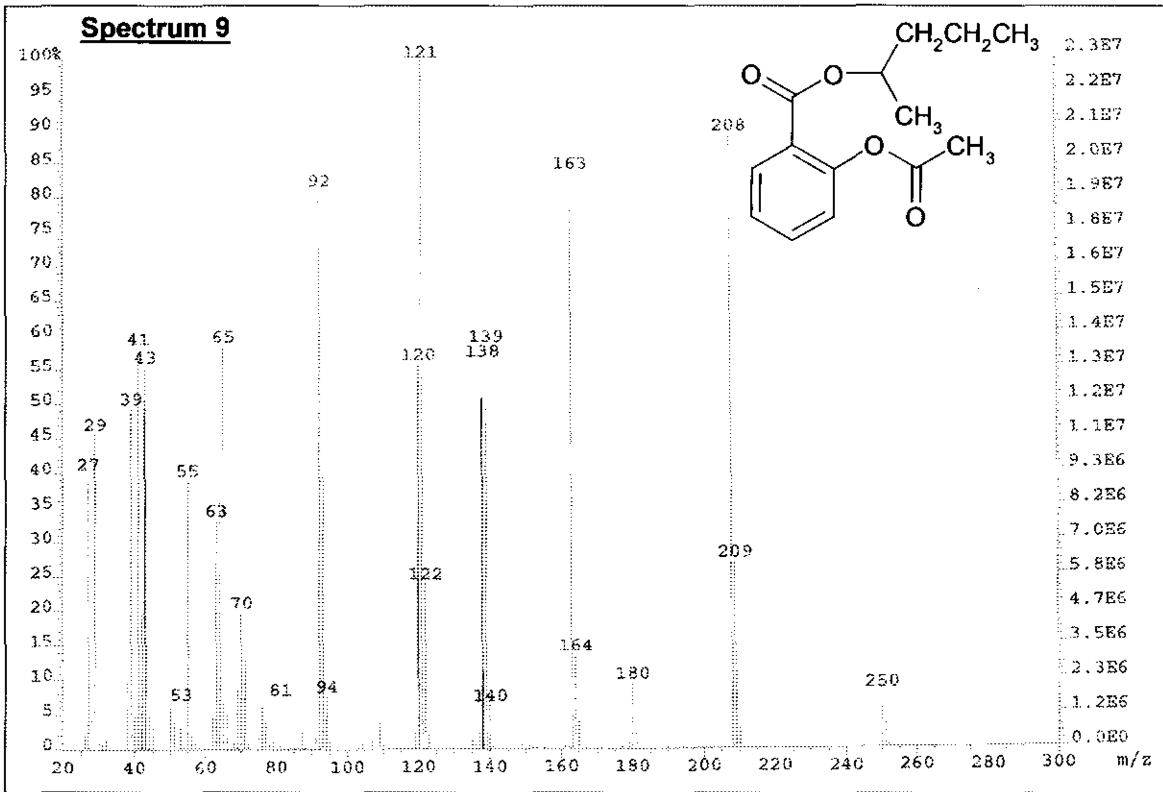
100%
163
121
237
181
43
138
164
120
57
92
194
41
180
238
63
139
29
122
182
165
1.7E7
0.0E0
m/z


Spectrum 8
100%
120
208
138
121
163
209
92
43
41
65
39
251
139
29
55
27
63
70
122
94
164
210
77
53
252
1.9E7
0.0E0
m/z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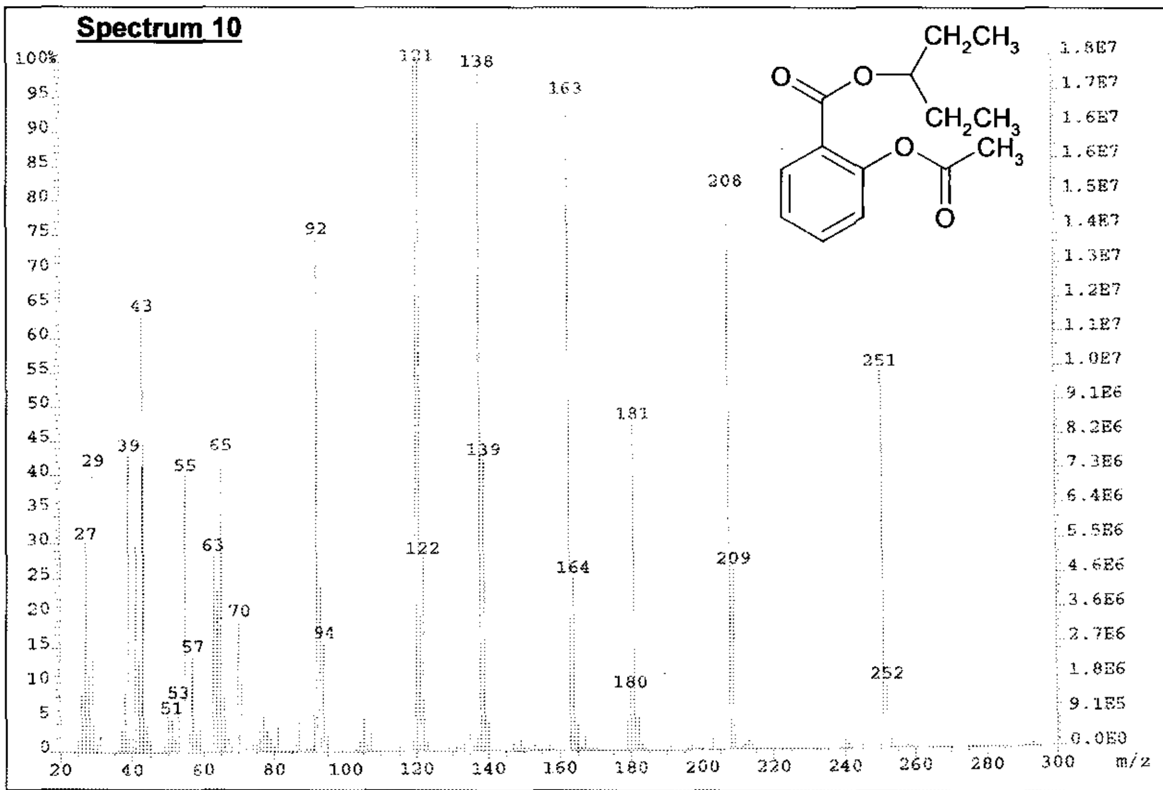
O
O
CH3
O
CH3
O
100%
m/z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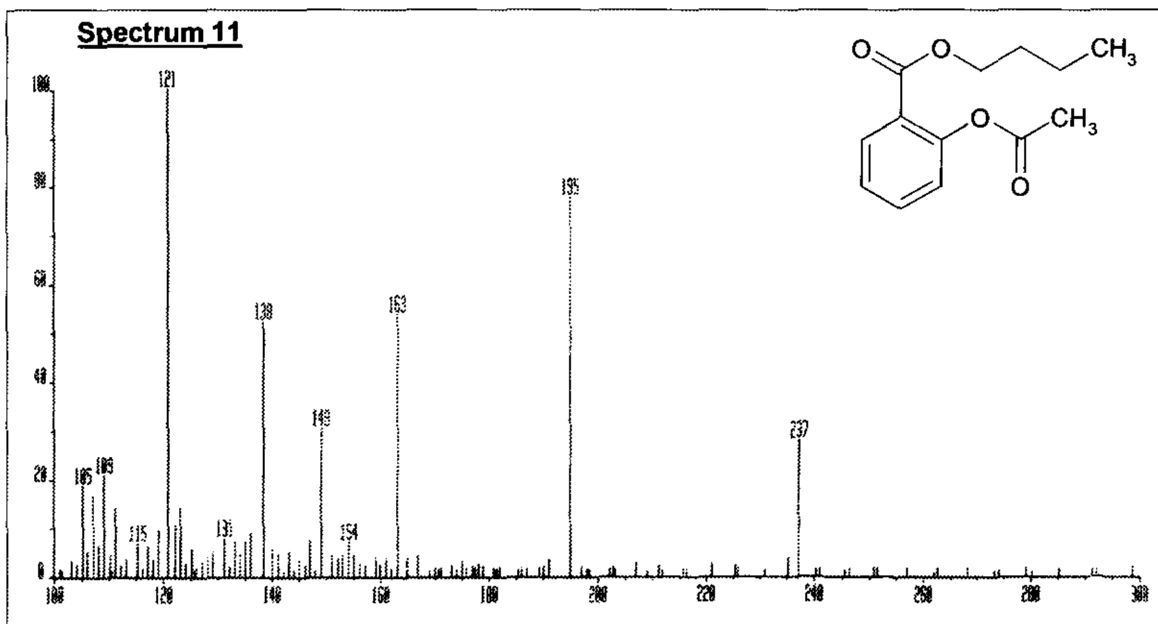
CH2CH3
O
O
CH2CH3
O
CH3
O
100%
m/z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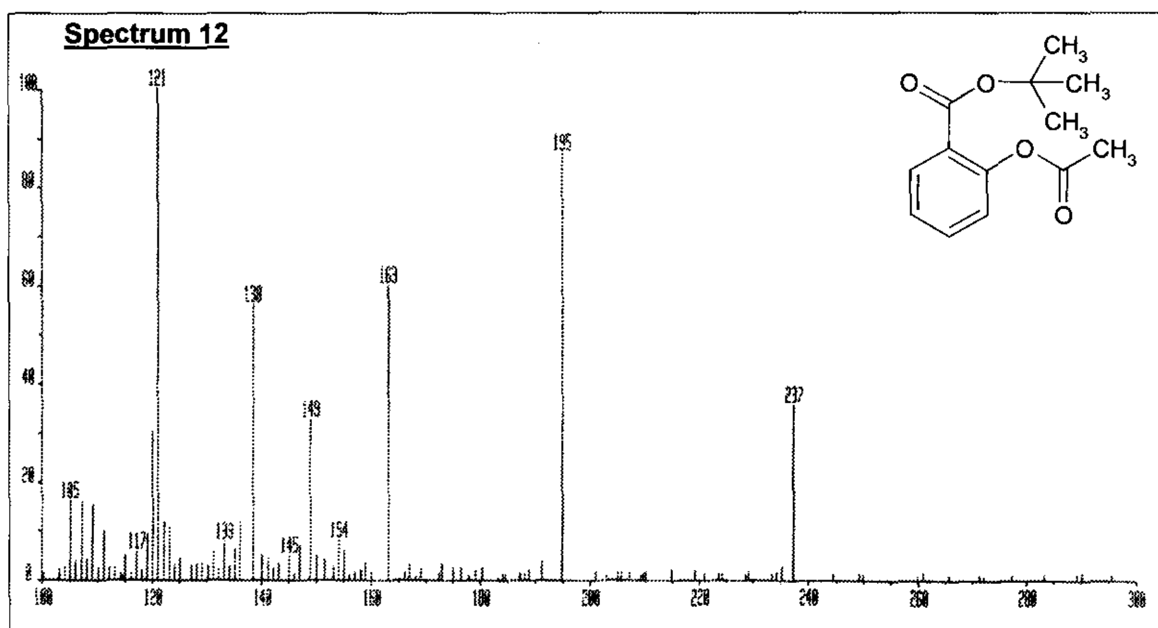
O
CH3
O
121
195
138
163
149
237
105
109
115
131
154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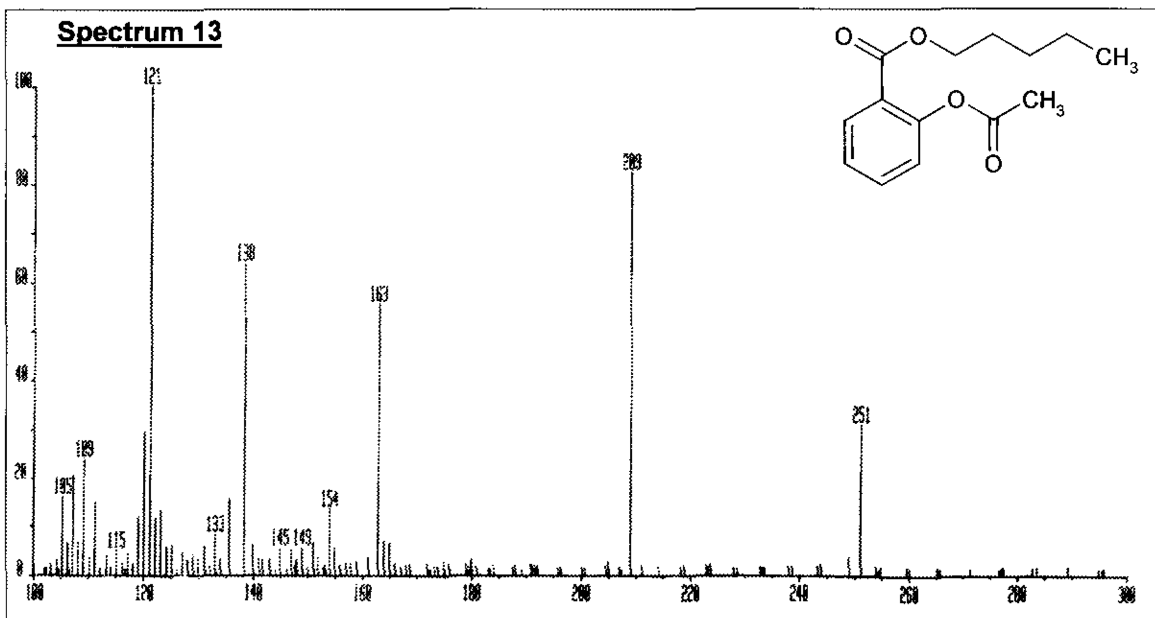
CH3
CH3
O
CH3
O
121
195
138
163
149
237
105
117
133
145
154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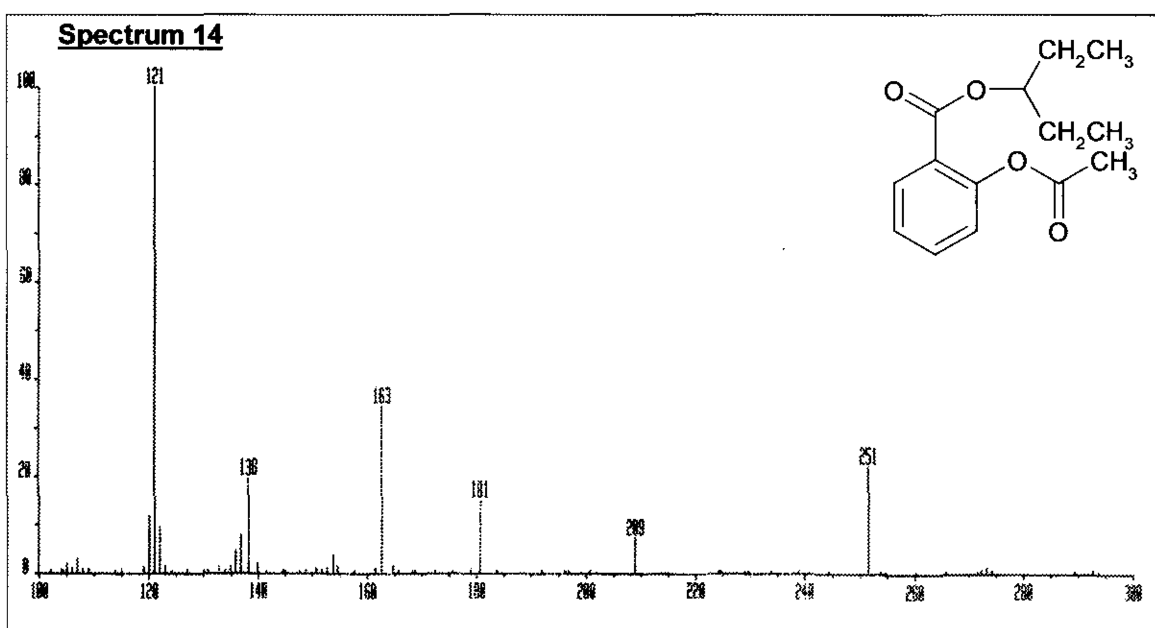
209
138
163
251
109
105
115
133
154
145 149
CH3
CH3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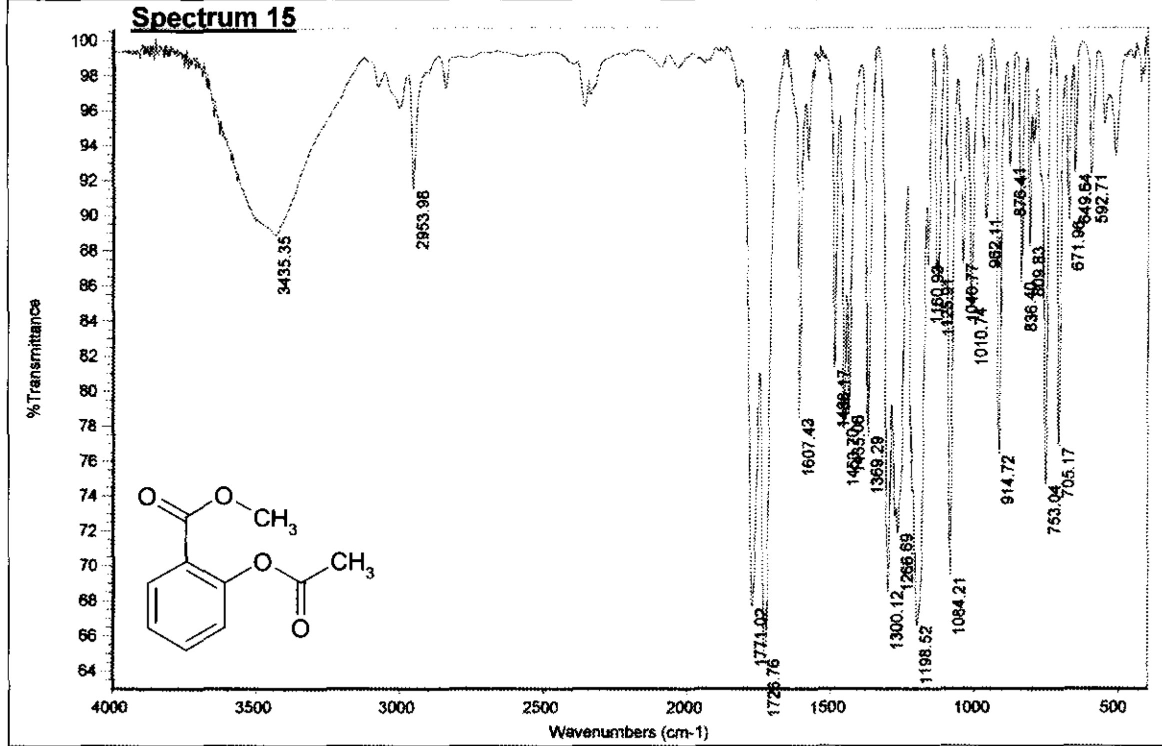
163
138
181
209
251
CH2CH3
CH2CH3
CH3


Spectrum 15
%Transmittance
Wavenumbers (cm-1)
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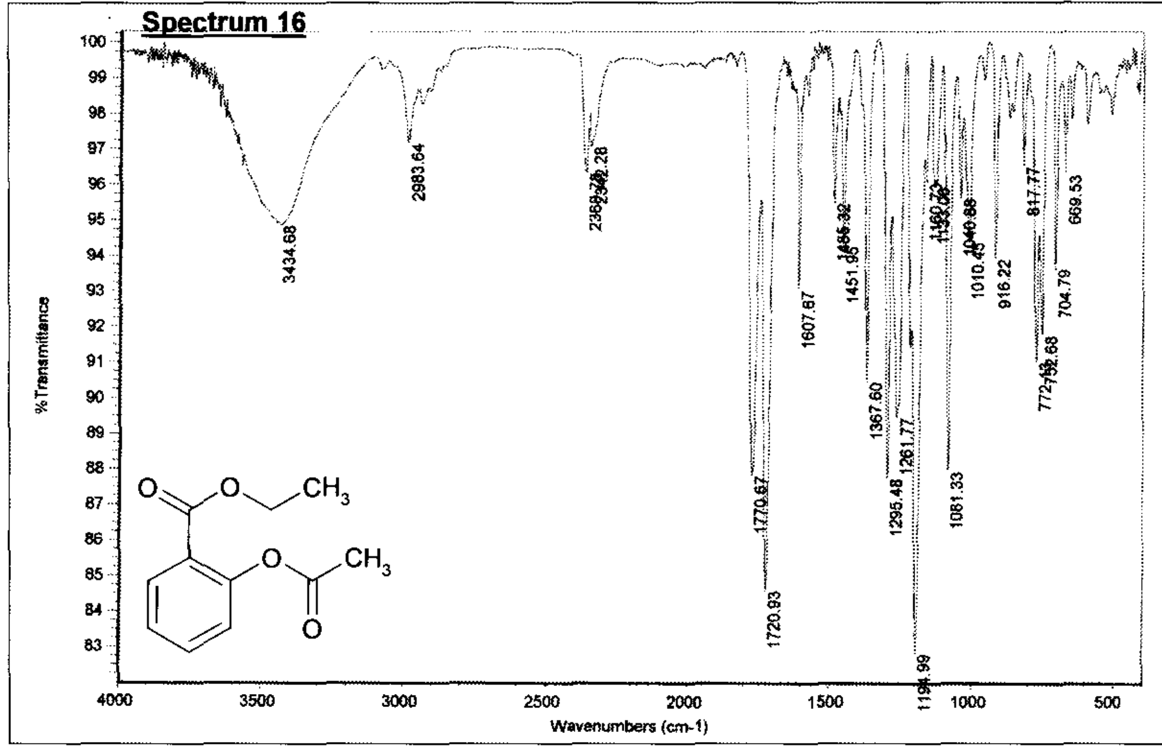
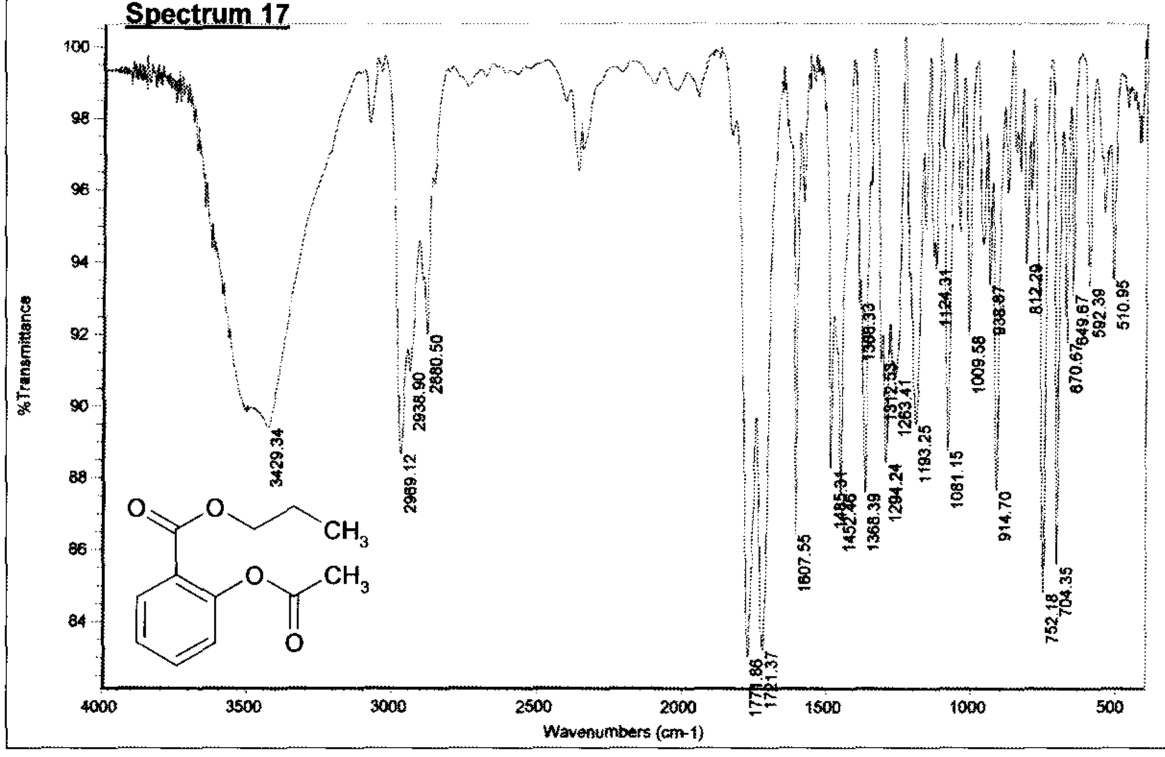
2953.98
1771.02
1607.43
1369.29
1300.12
1266.69
1198.52
1084.21
1010.74
914.72
753.04
705.17
592.71


Spectrum 16
%Transmittance
Wavenumbers (cm-1)
3434.68
2983.64
1770.67
1720.93
1607.67
1367.60
1295.48
1261.77
1081.33
916.22
817.77
704.79
669.53


Spectrum 17
%Transmittance
Wavenumbers (cm-1)
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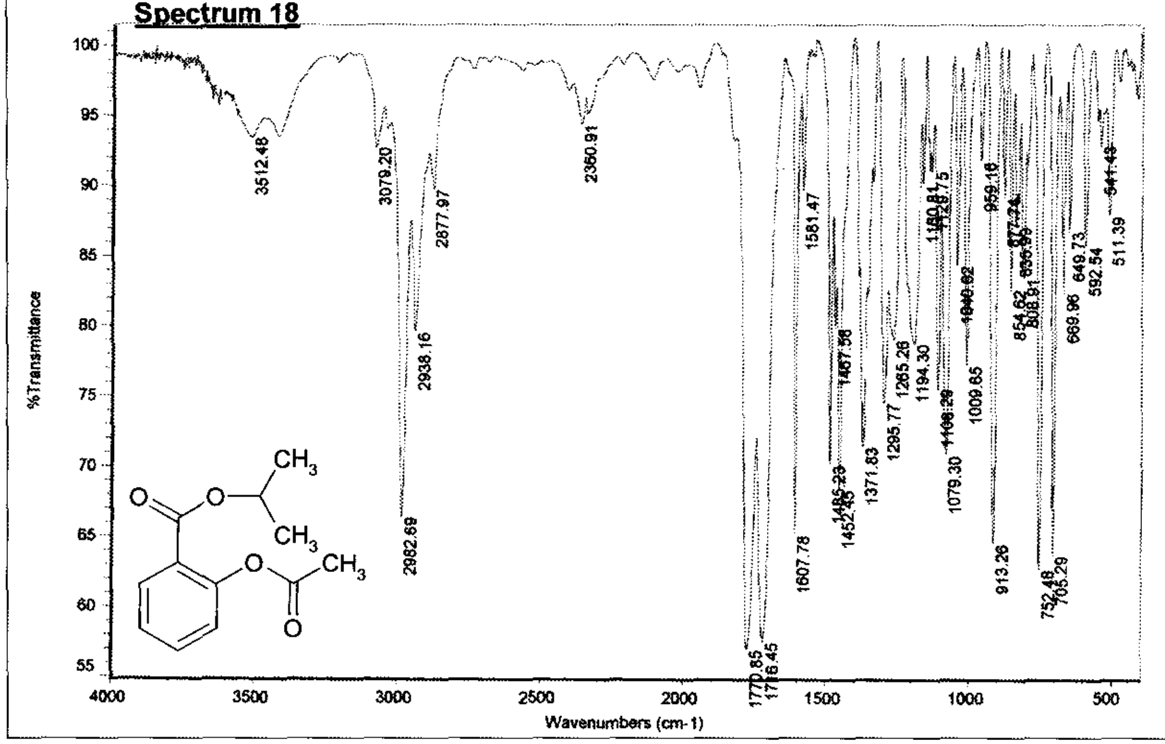
2969.12
2938.90
2880.50
1771.86
1721.37
1607.55
1485.34
1452.46
1368.39
1366.33
1312.53
1294.24
1263.41
1193.25
1124.31
1081.15
1009.58
938.87
914.70
812.29
752.18
704.35
670.67
649.67
592.39
510.95
O
CH3


Spectrum 18
%Transmittance
Wavenumbers (cm-1)
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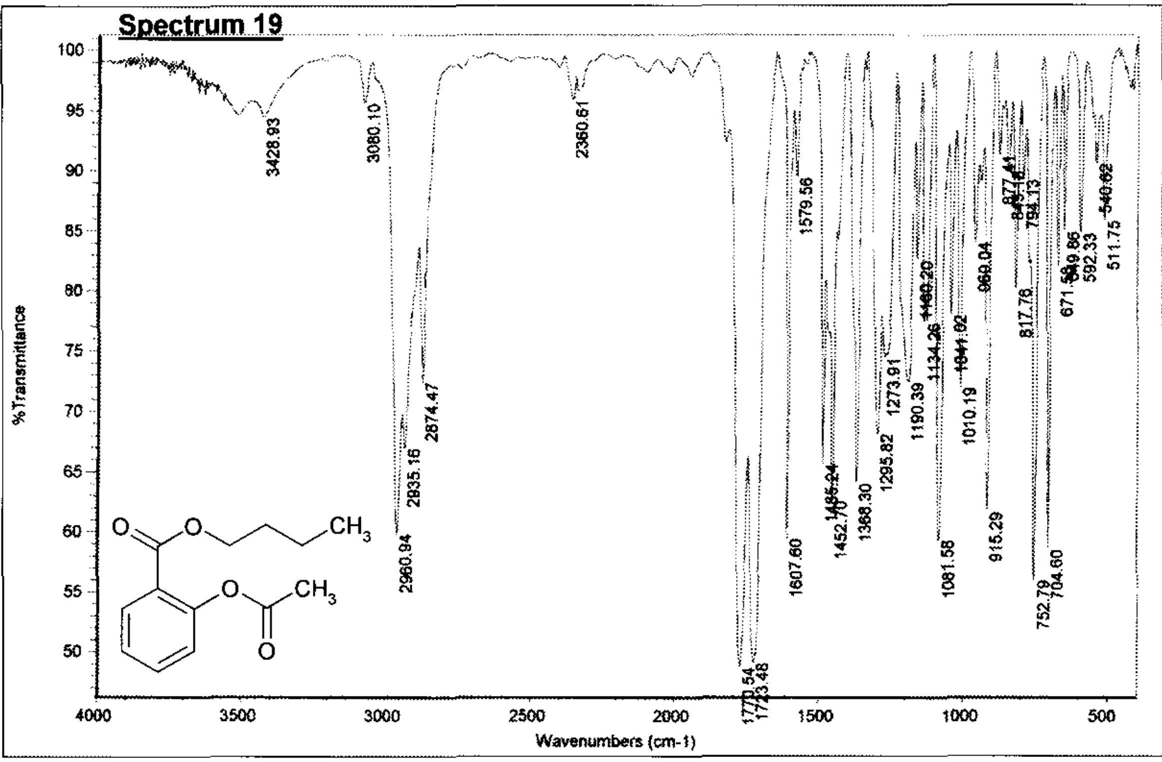
3079.20
2982.69
2938.16
2877.97
2360.91
1770.85
1716.45
1607.78
1581.47
1485.23
1452.45
1467.58
1371.83
1295.77
1265.26
1194.30
1160.81
1128.75
1108.26
1079.30
1040.62
1009.65
959.16
913.26
877.74
854.62
836.99
808.91
752.48
705.29
669.96
649.73
592.54
541.43
511.39
O
CH3


Spectrum 19
%Transmittance
Wavenumbers (cm-1)
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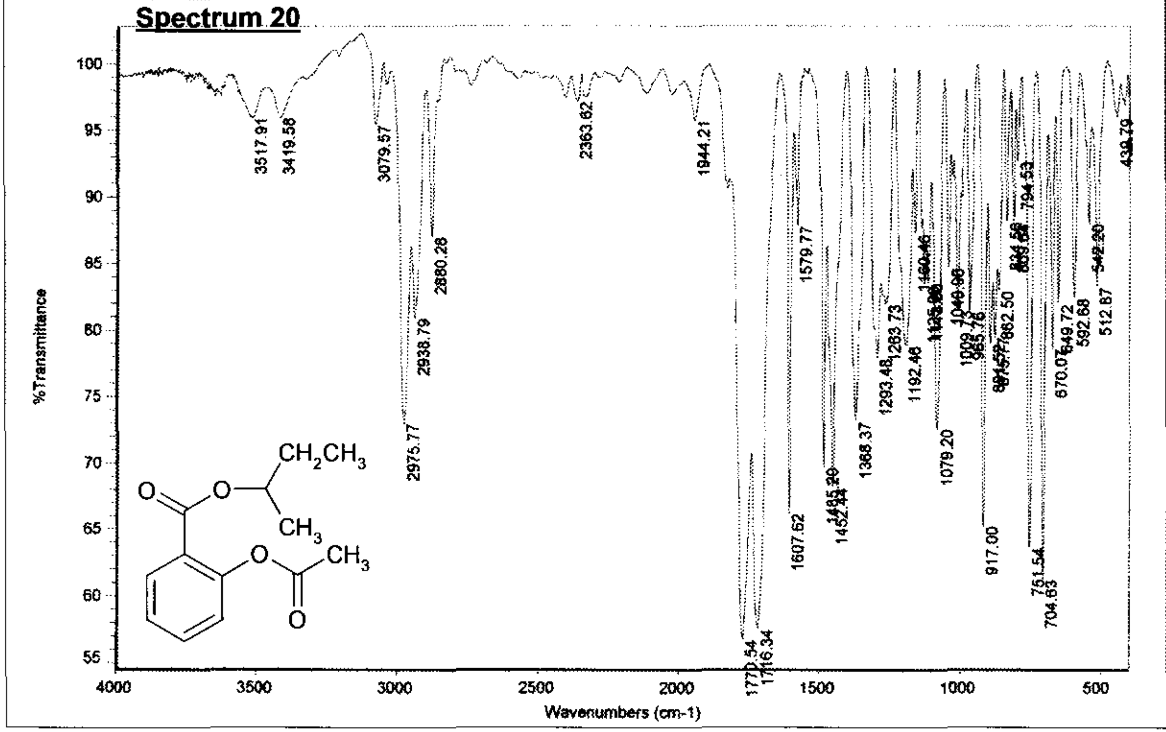
3080.10
2960.94
2935.16
2874.47
2360.61
1770.54
1723.48
1607.60
1579.56
1485.24
1452.70
1368.30
1295.82
1273.91
1190.39
1134.26
1010.19
1081.58
915.29
817.76
794.13
752.79
704.60
592.33
511.75
CH3
O
O
O
O
CH3


Spectrum 20
%Transmittance
Wavenumbers (cm-1)
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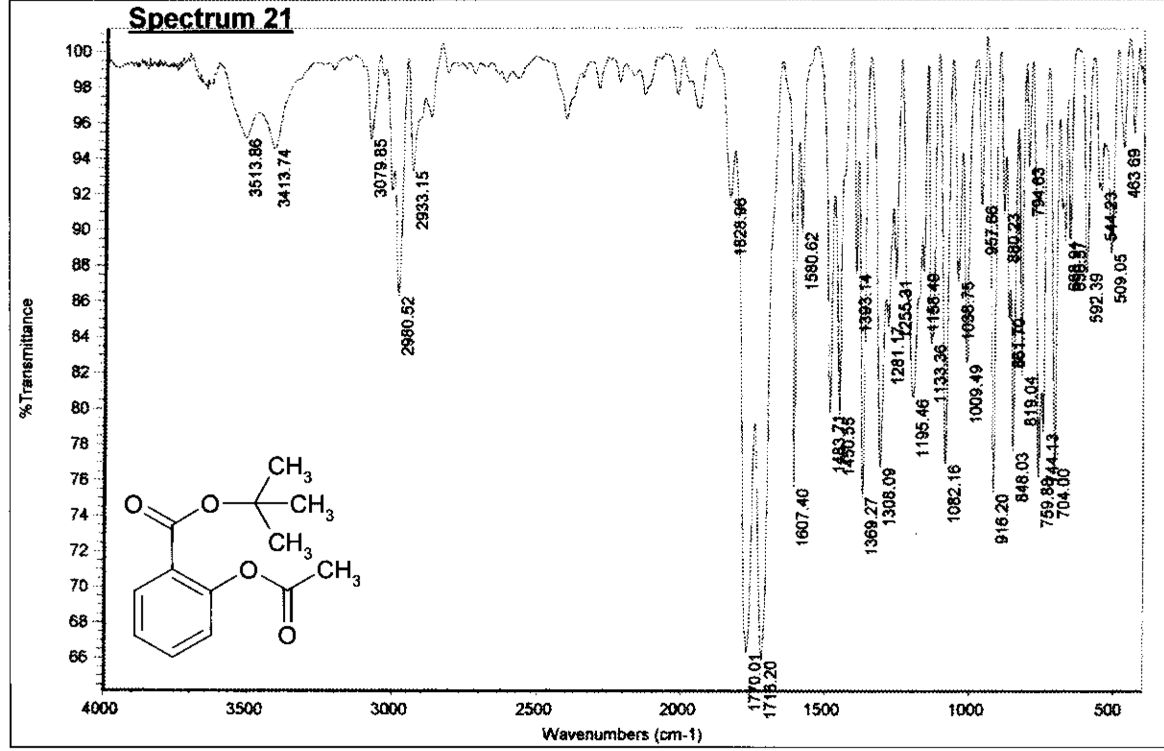
3419.58
3079.57
2975.77
2938.79
2880.28
2363.62
1944.21
1770.54
1716.34
1607.62
1579.77
1452.44
1368.37
1293.48
1263.73
1192.46
1079.20
917.00
862.50
794.53
751.54
704.63
670.07
649.72
592.68
512.87
439.79
CH2CH3
CH3
O
O
O
O
CH3


Spectrum 21
%Transmittance
Wavenumbers (cm-1)
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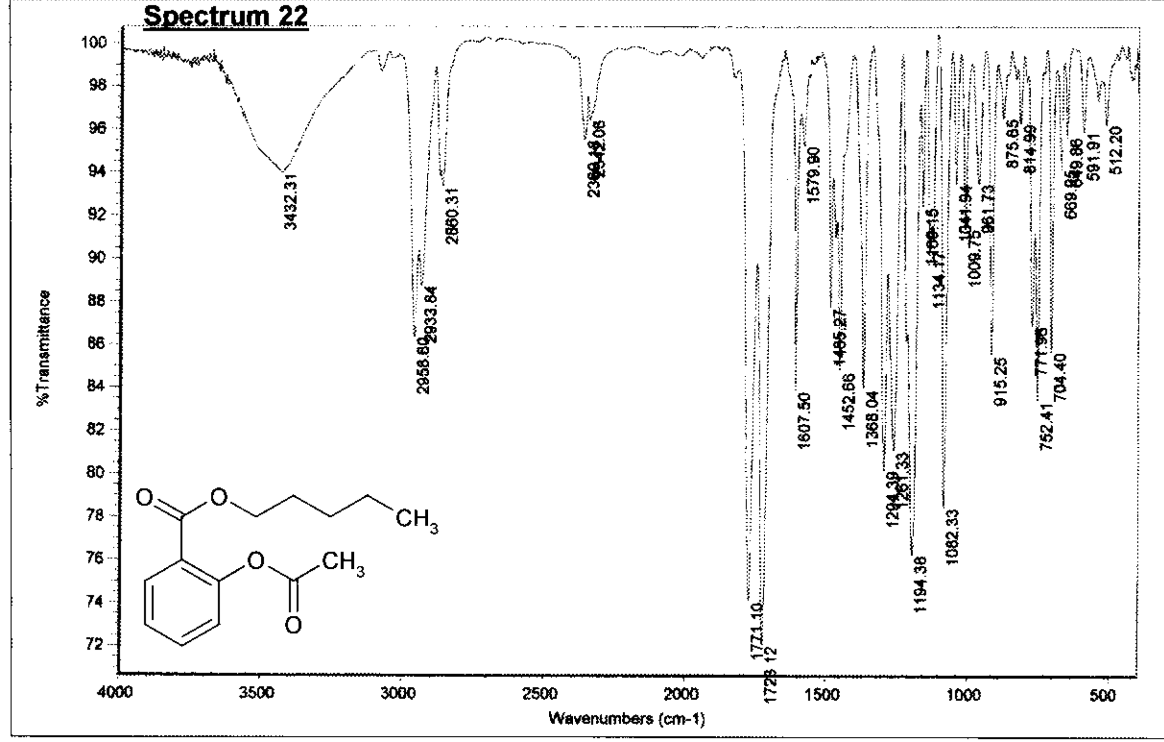
3079.85
2980.52
2933.15
1828.96
1770.01
1607.40
1580.62
1369.27
1308.09
1281.17
1255.31
1195.46
1133.36
1082.16
1009.49
916.20
848.03
819.04
759.89
704.00
592.39
509.05
463.69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%Transmittance
Wavenumbers (cm-1)
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2958.60
2933.84
2860.31
1771.10
1607.50
1579.90
1452.66
1368.04
1194.38
1082.33
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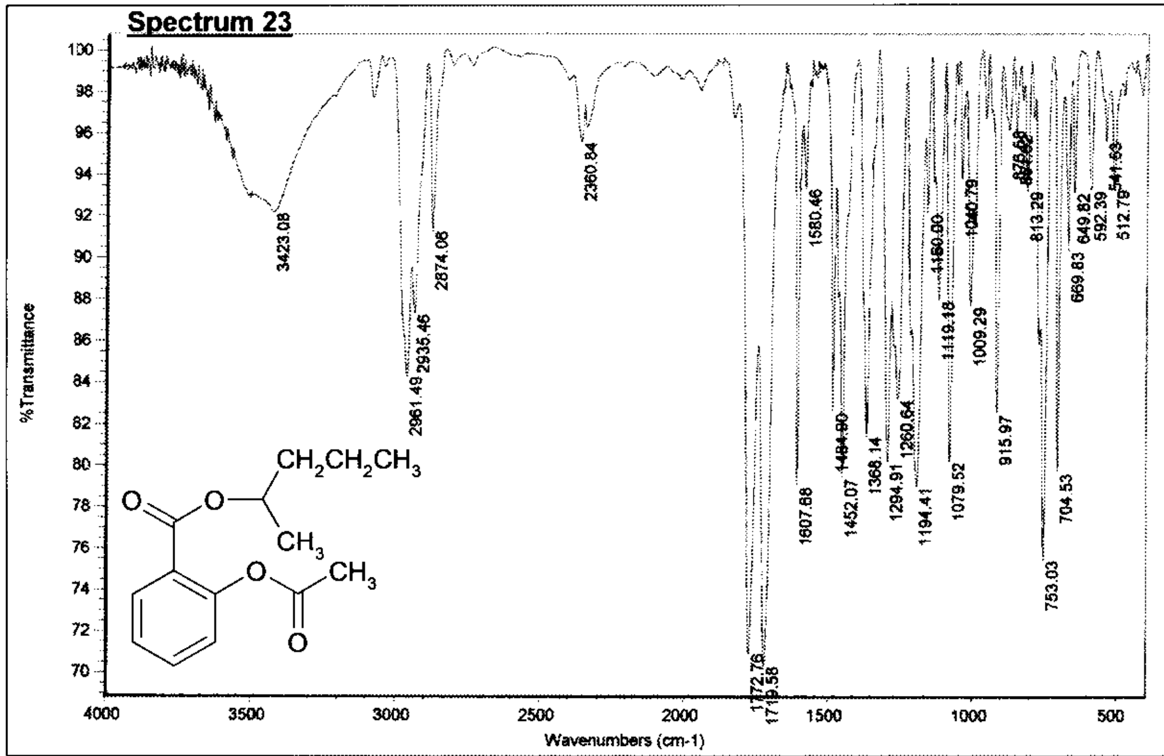
1009.75
961.73
915.25
875.65
814.99
752.41
704.40
591.91
512.20


Spectrum 23
%Transmittance
Wavenumbers (cm-1)
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CH_3
O
3423.08
2961.49
2935.46
2874.06
2360.84
1772.76
1719.58
1607.68
1580.46
1452.07
1368.14
1294.91
1260.64
1194.41
1119.18
1079.52
1009.29
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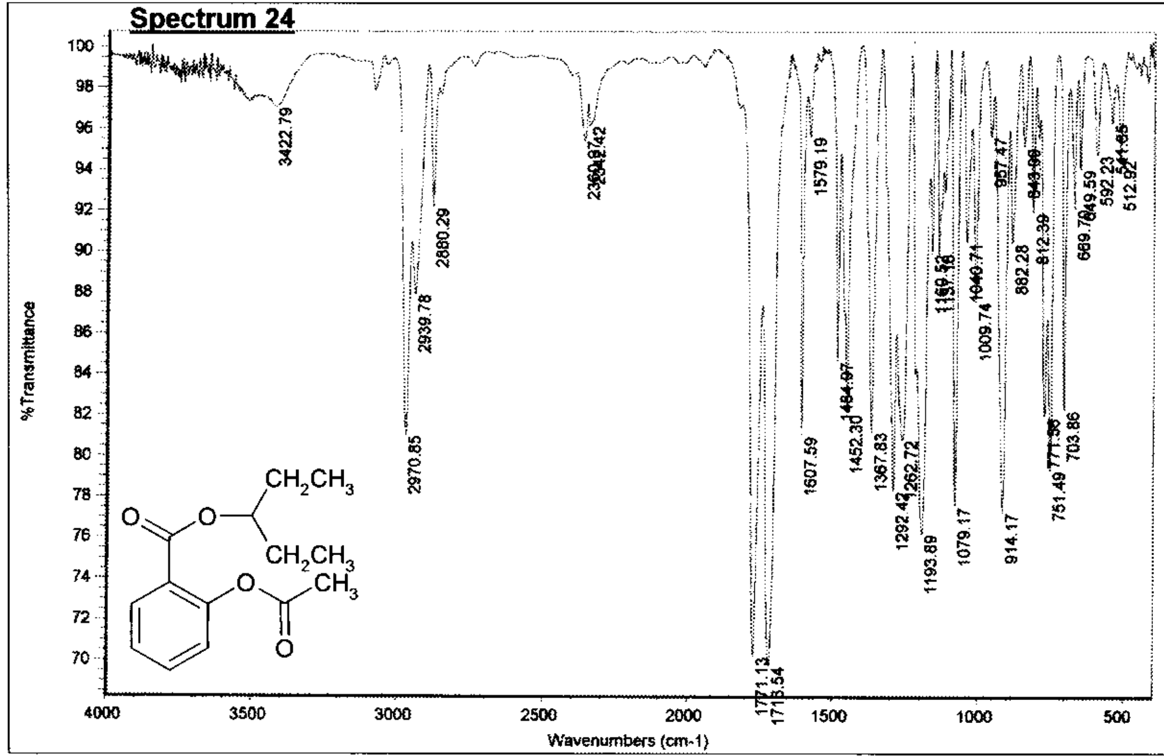
915.97
813.29
753.03
704.53
669.83
649.82
592.39
541.63
512.79


Spectrum 24
%Transmittance
Wavenumbers (cm-1)
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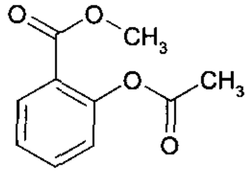
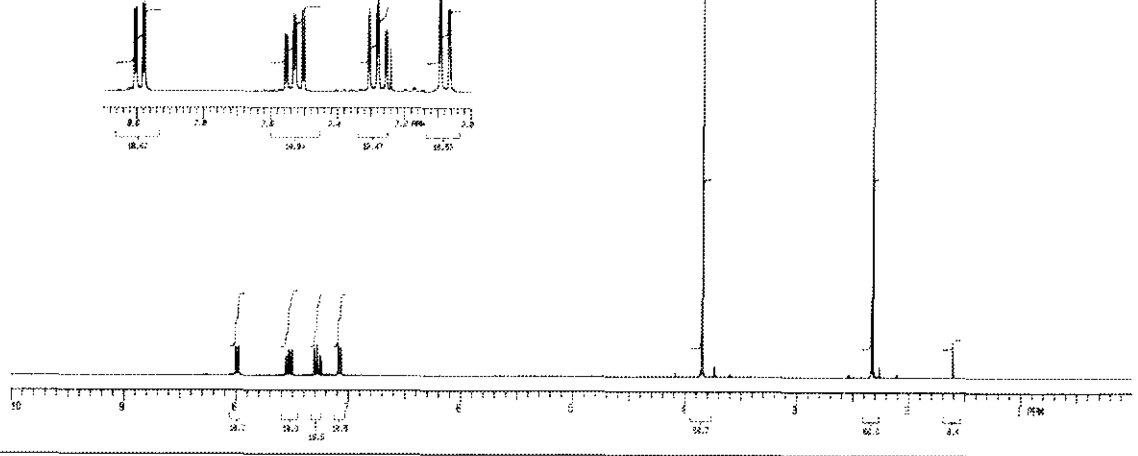
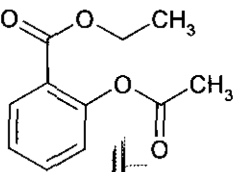
CH_2CH_3
CH_3
O
3422.79
2970.85
2939.78
2880.29
1771.13
1607.59
1579.19
1452.30
1367.83
1292.42
1262.72
1193.89
1079.17
1009.74
1040.71
914.17
882.28
812.39
751.49
703.86
592.23
512.92

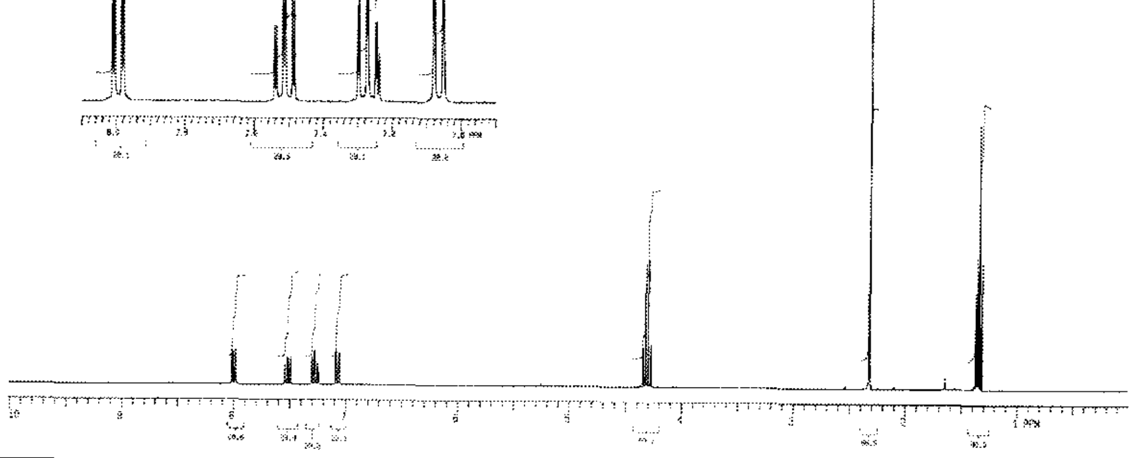
Spectrum 25

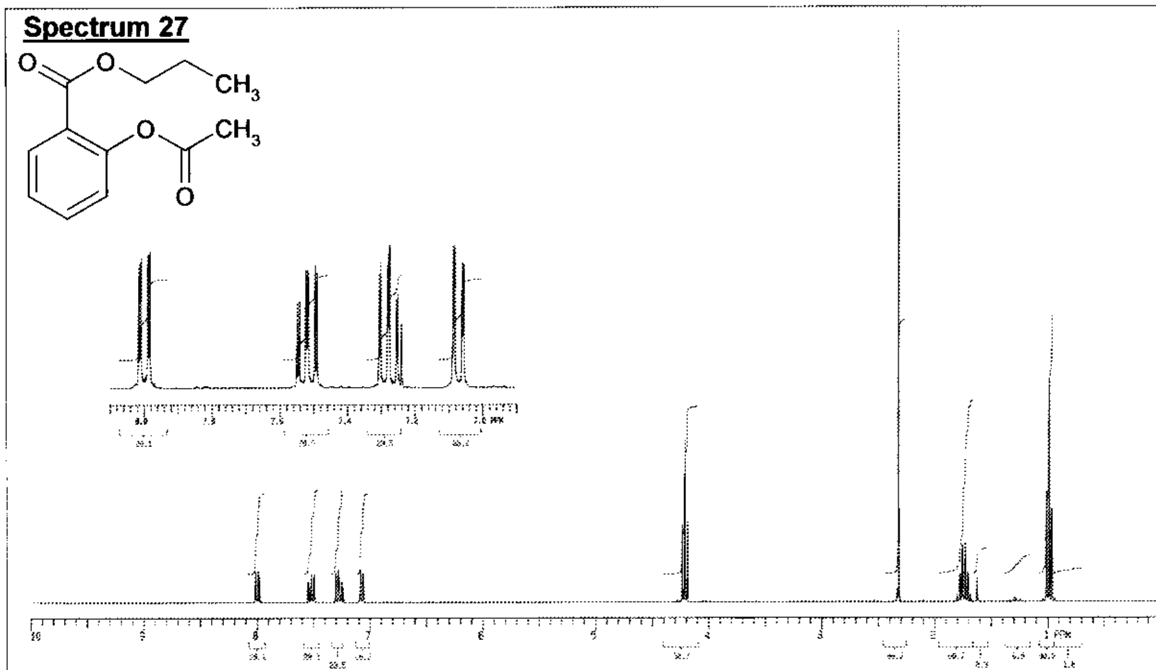


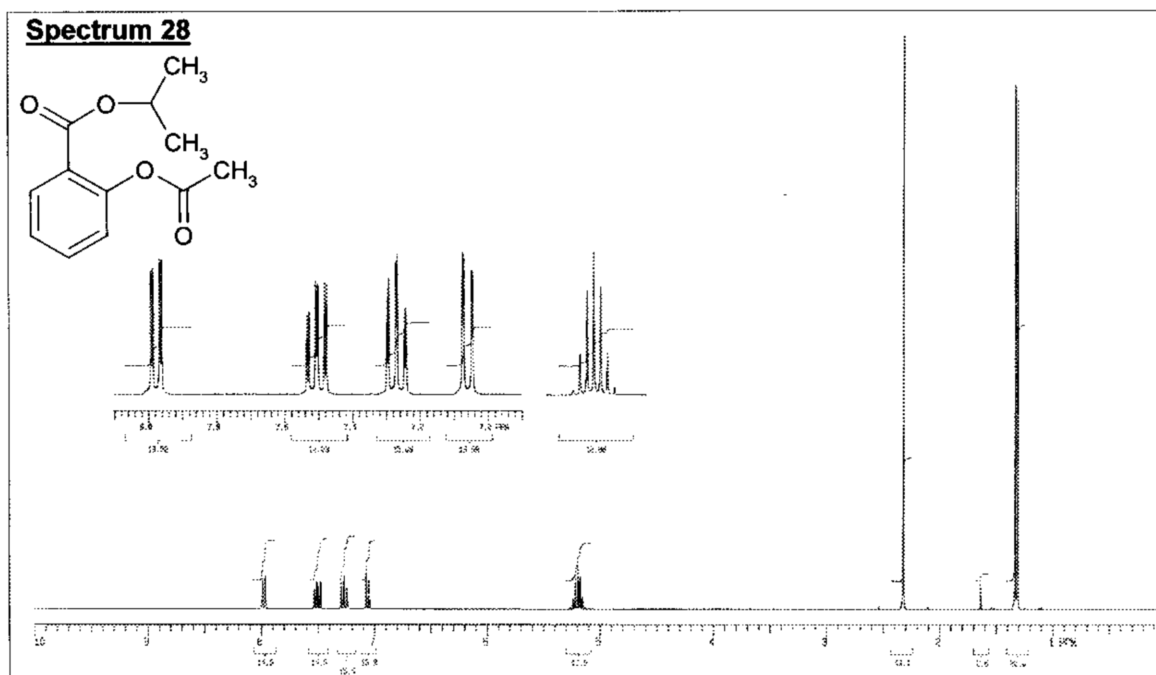


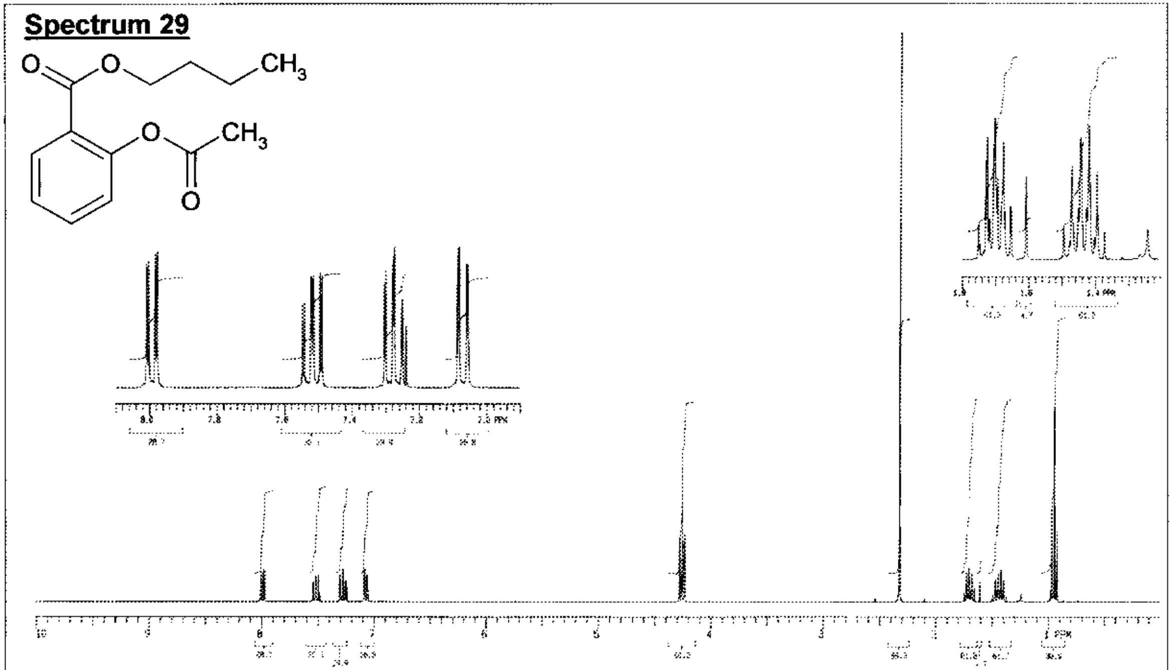
Spectrum 26

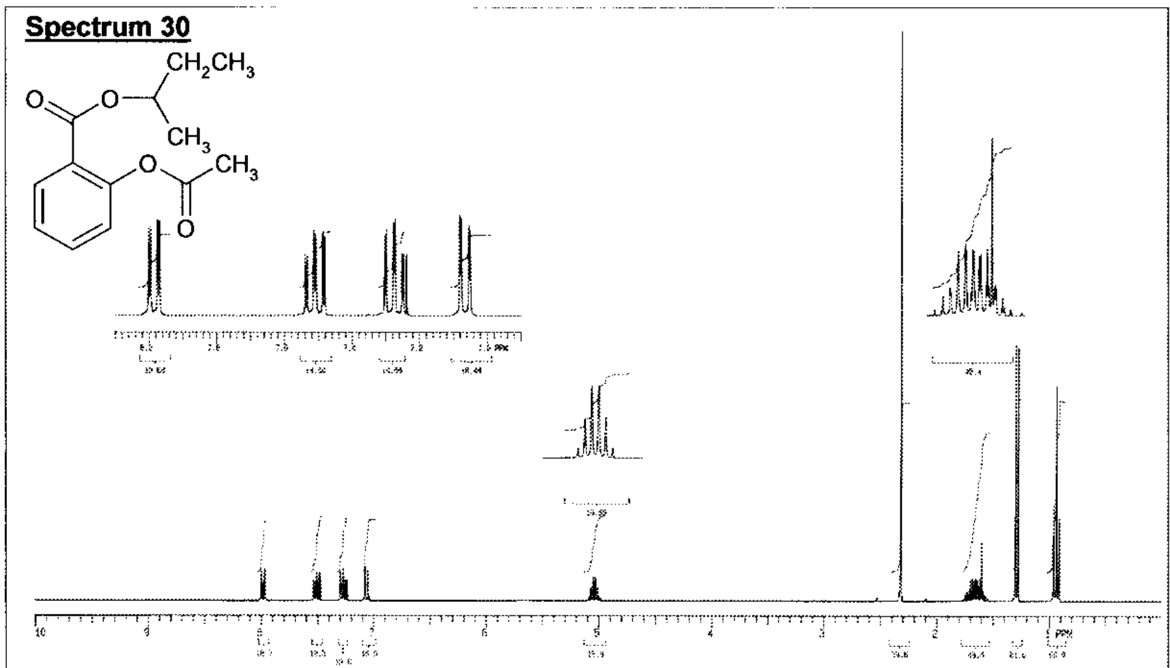


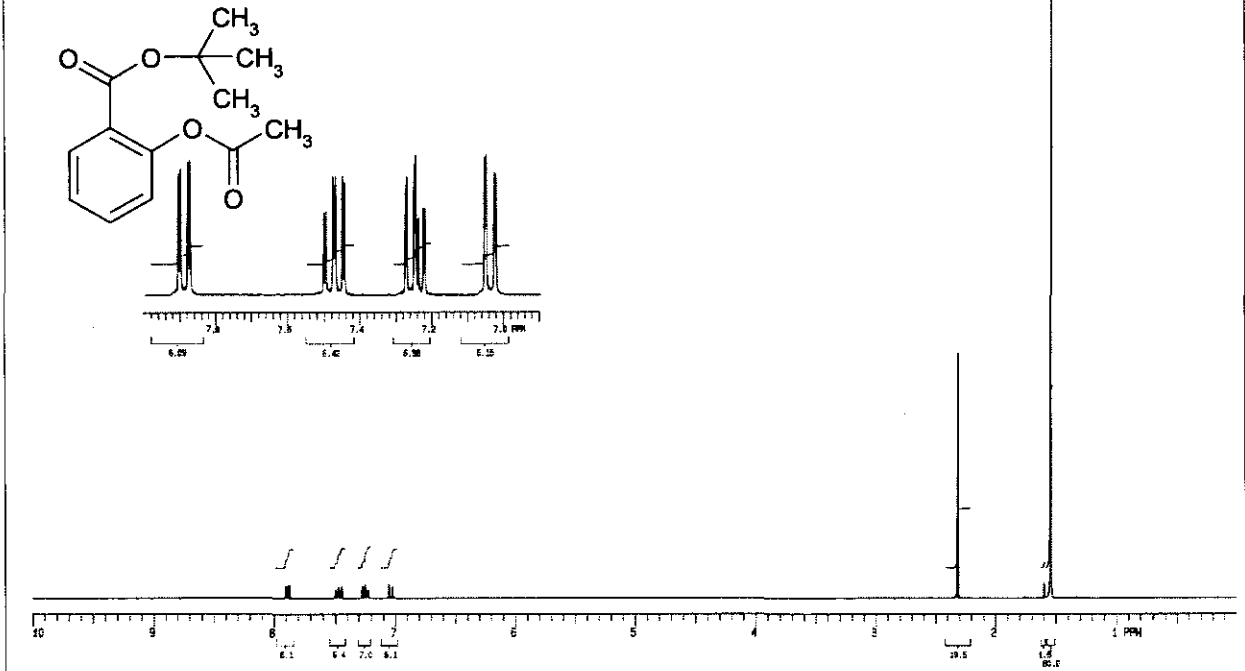


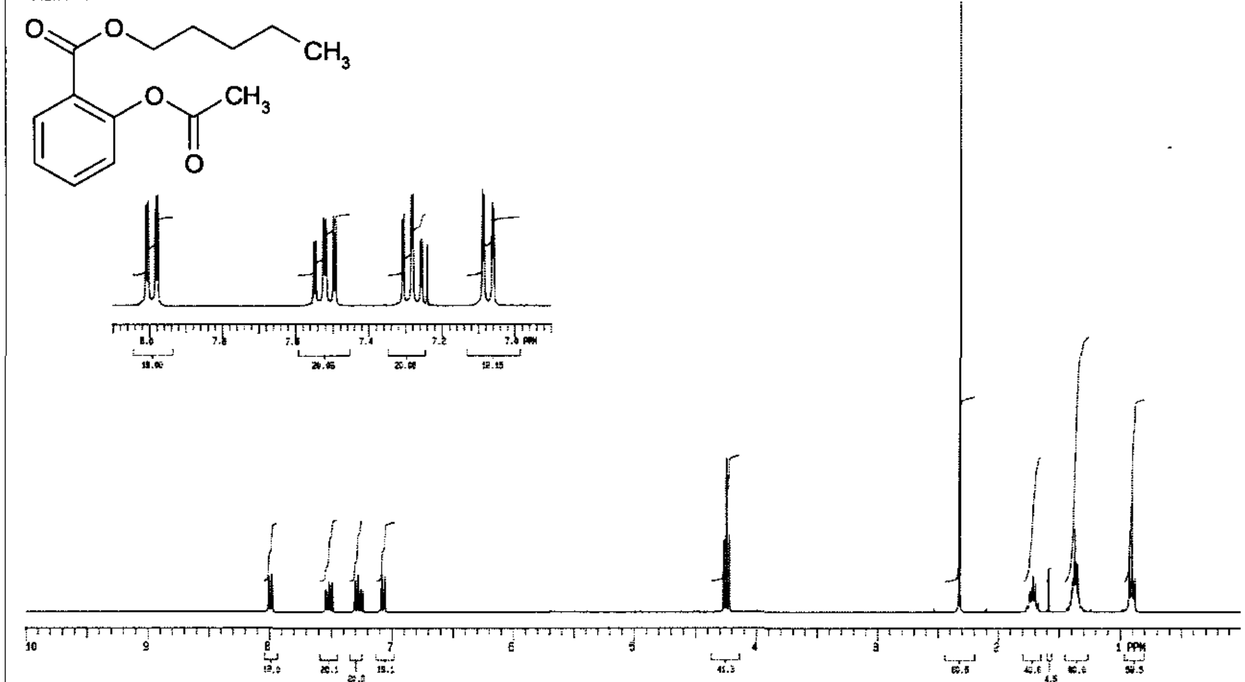

Spectrum 27
O
O
CH3
O
CH3
O

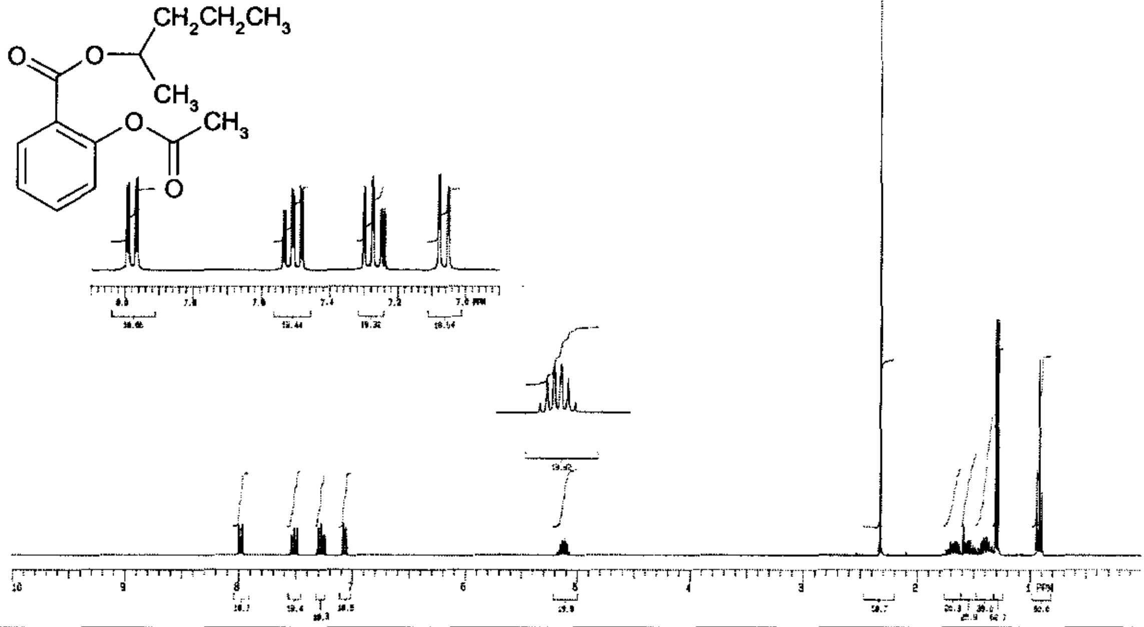

Spectrum 28
CH3
O
O
CH3
O
CH3
O

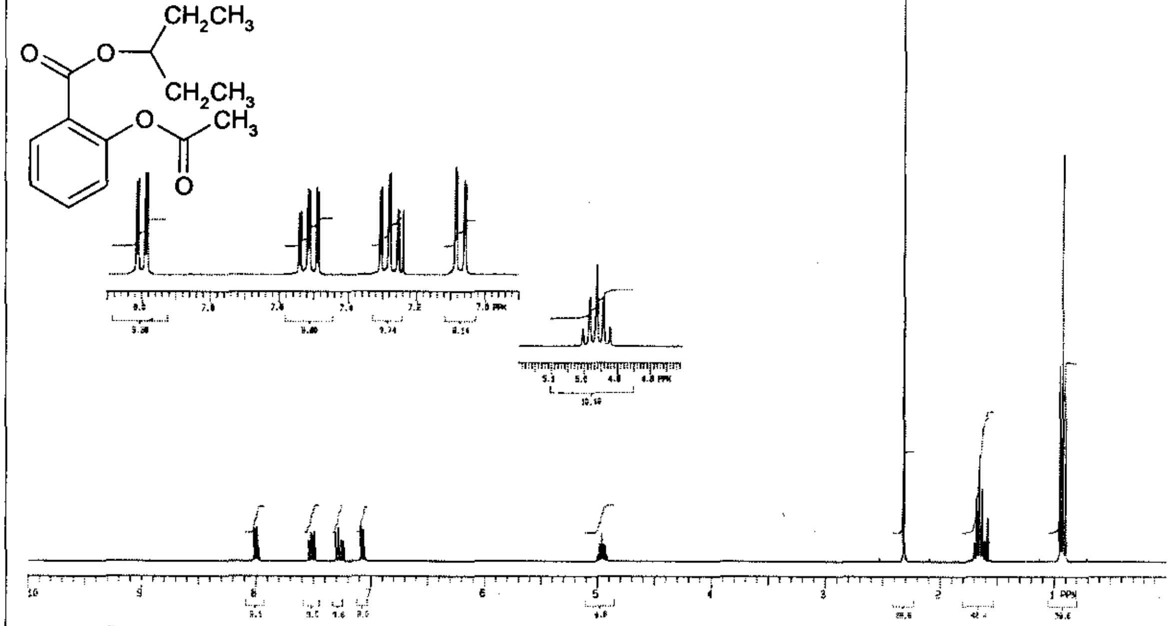

Spectrum 29
O
O
CH3
O
CH3
O
PPM


Spectrum 30
CH2CH3
O
O
CH3
O
CH3
O
PPM

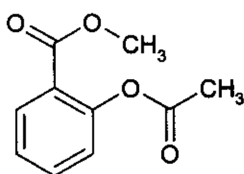

Spectrum 31
CH3
O
O
CH3
CH3
O
CH3
O
PPM

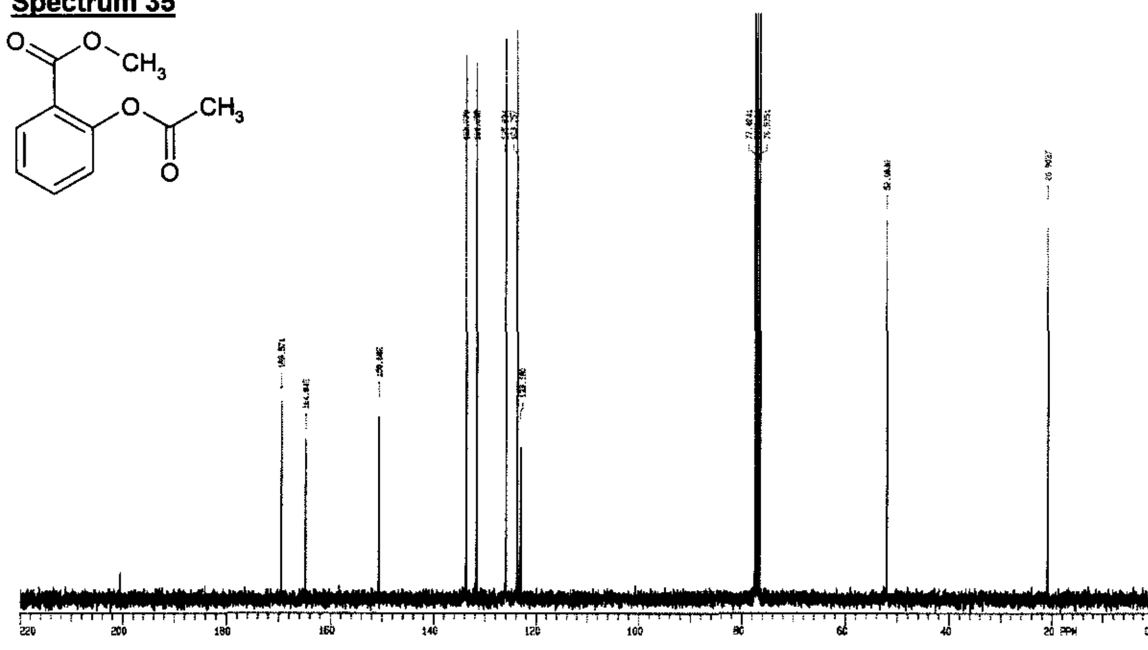

Spectrum 32
O
O
CH3
O
CH3
O
PPM


Spectrum 33
CH2CH2CH3
O
O
CH3
O
CH3
O
PPM

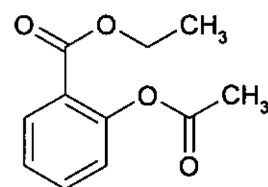

Spectrum 34
CH2CH3
O
O
CH2CH3
O
CH3
O
PPM

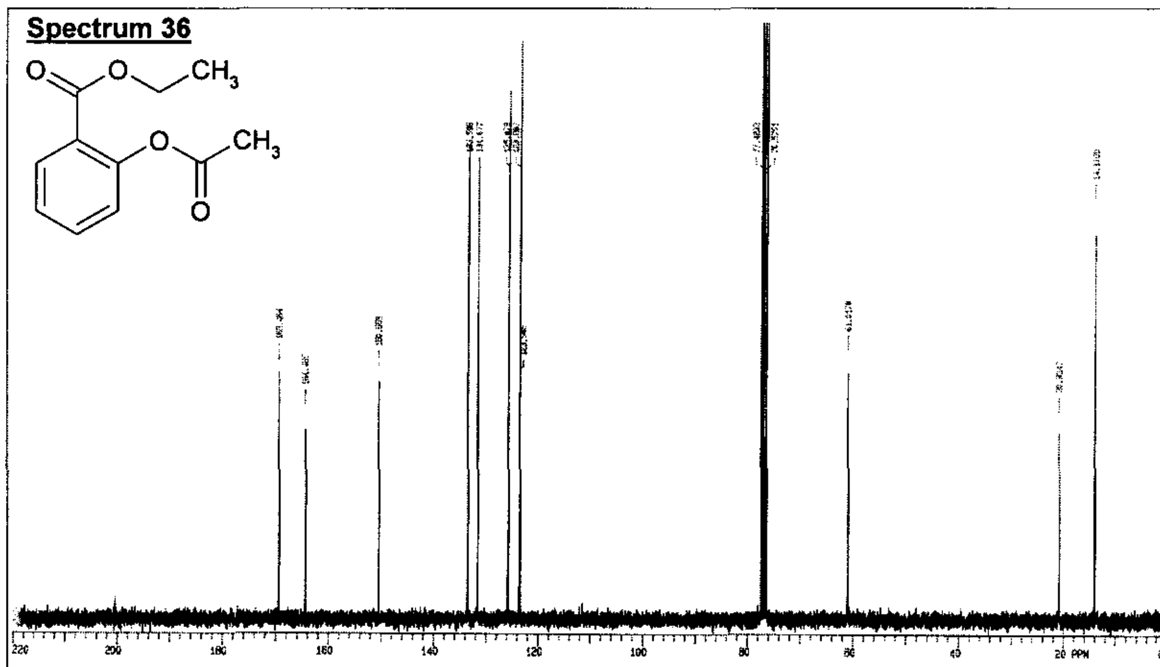
Spectrum 35



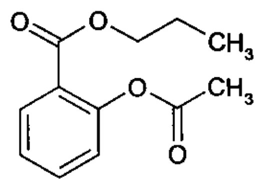


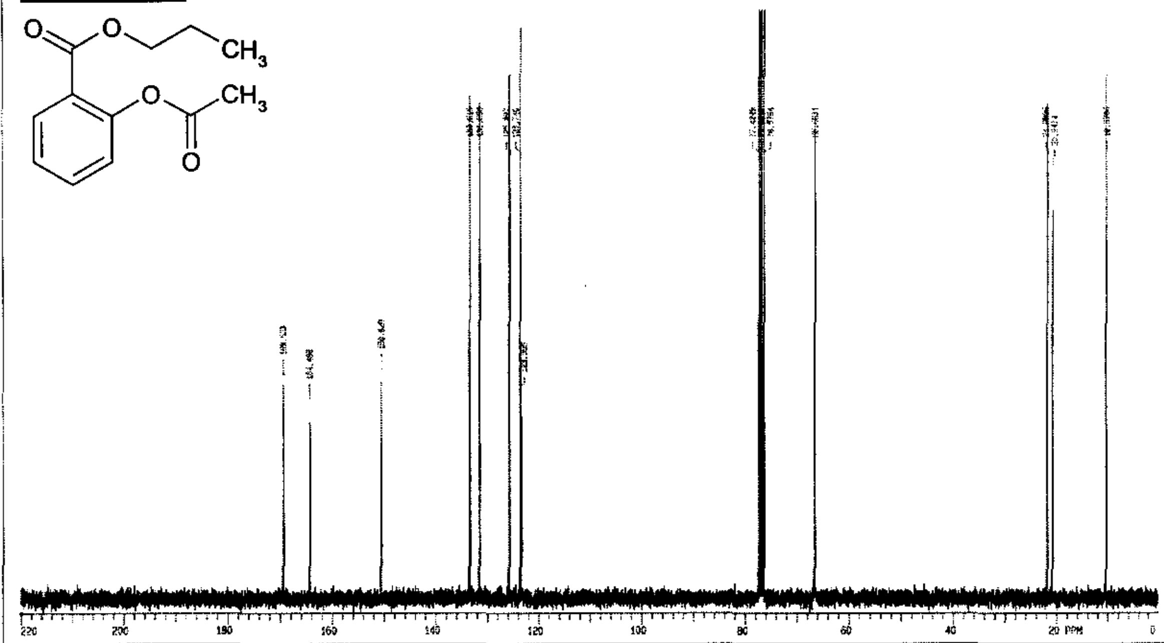
Spectrum 36



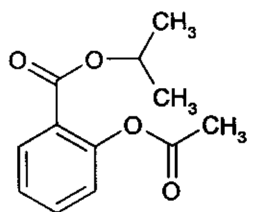


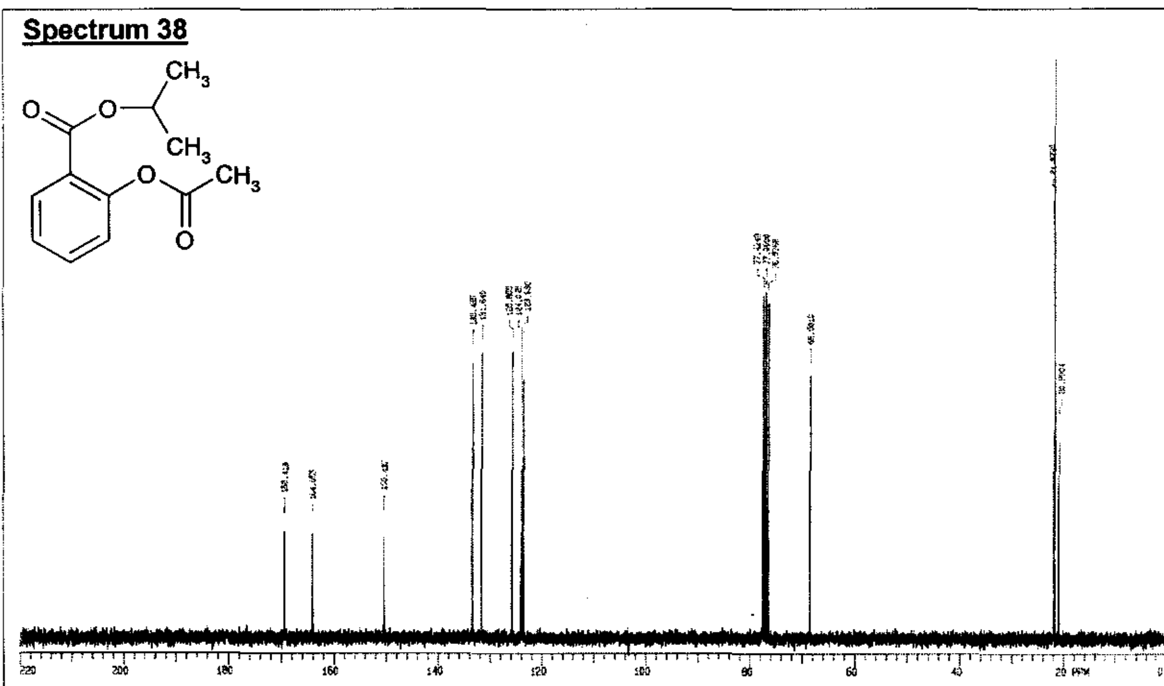
Spectrum 37



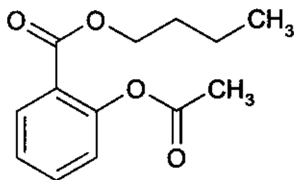


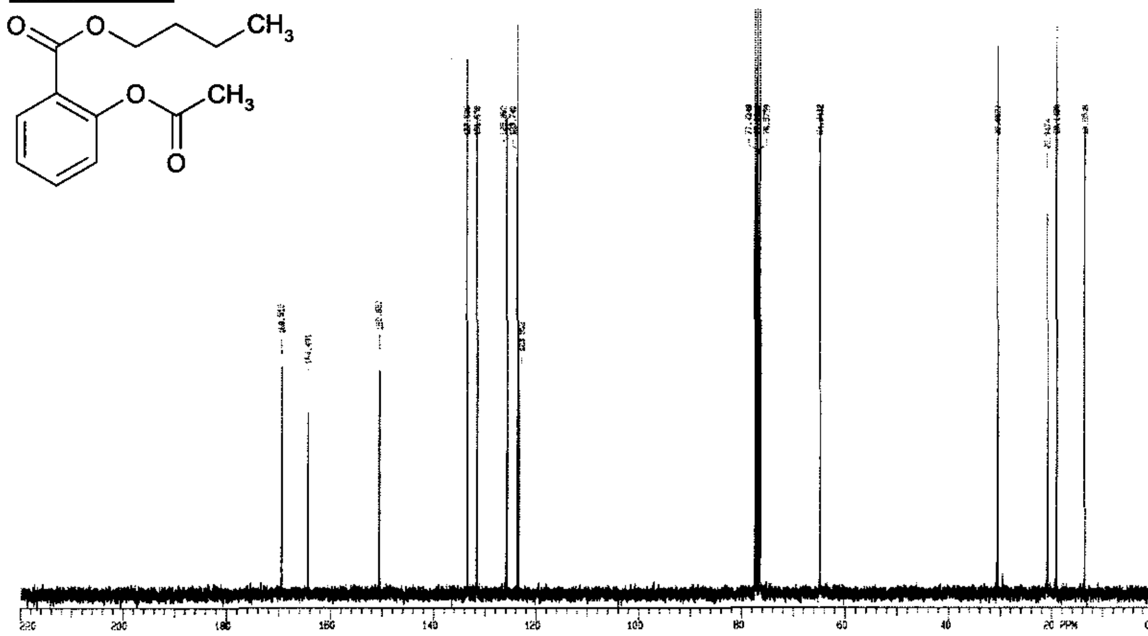
Spectrum 38



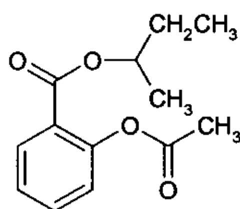


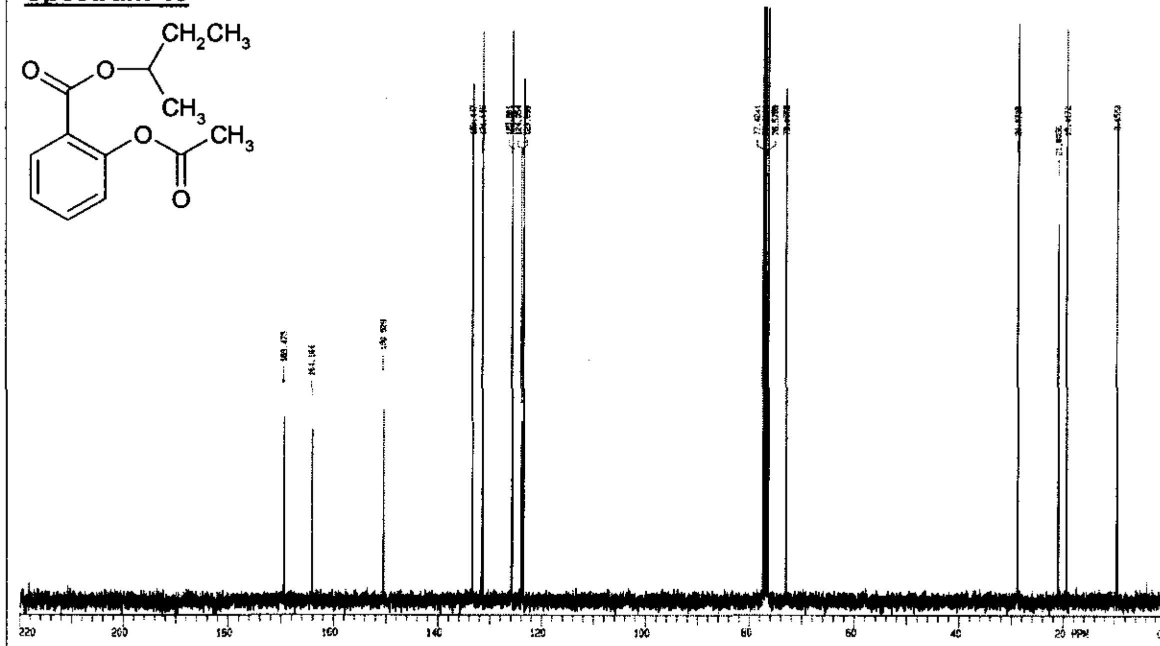
Spectrum 39

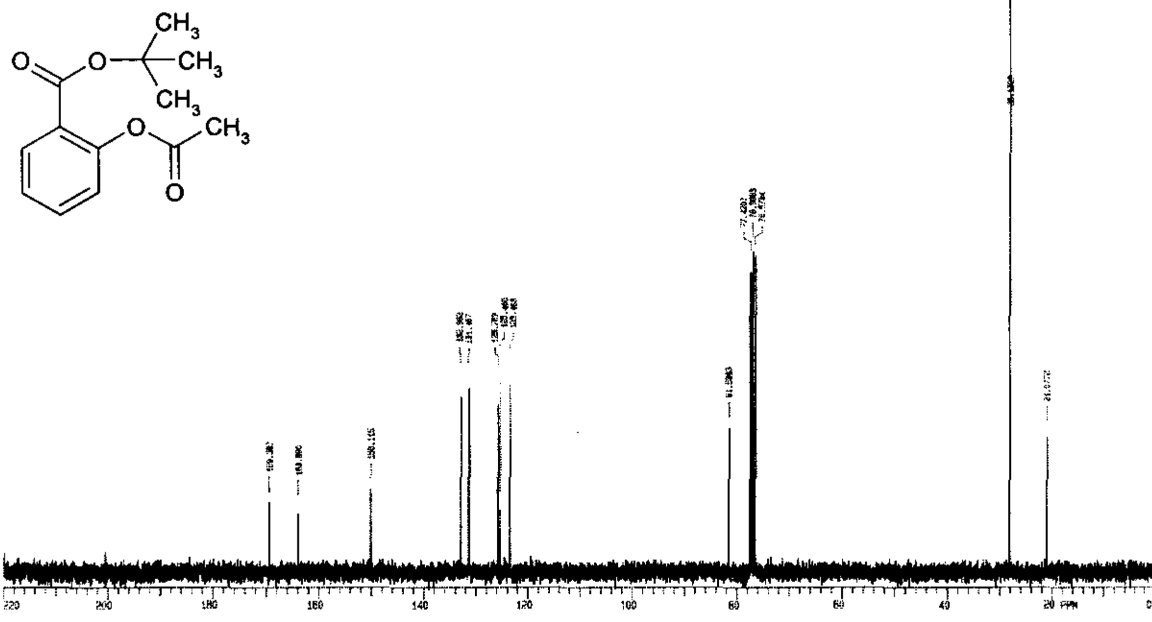


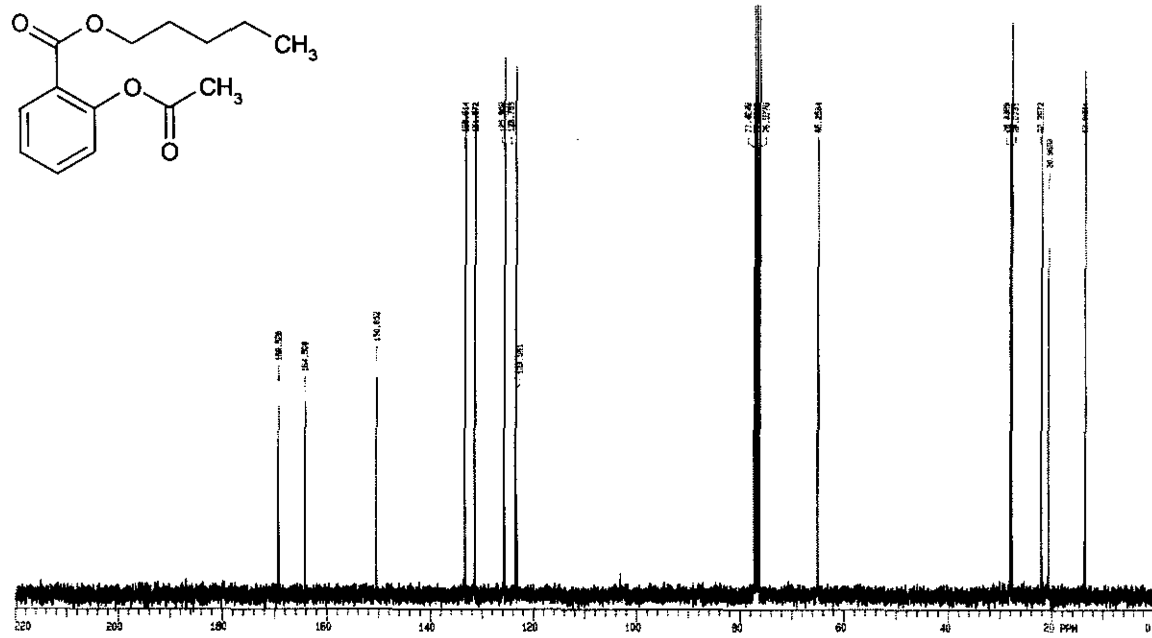


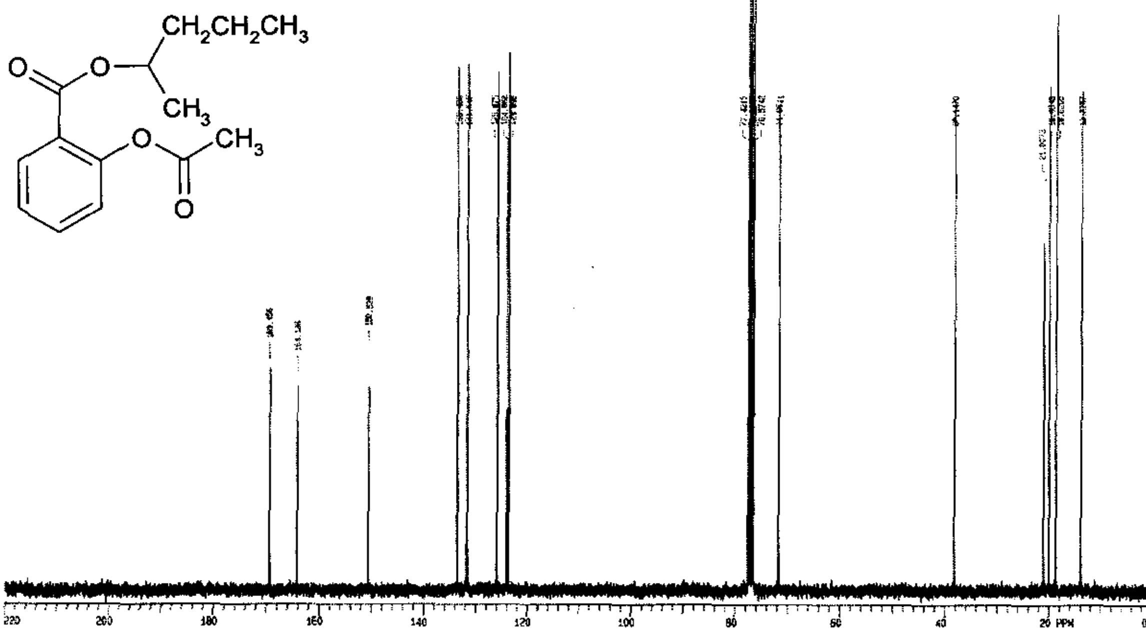
Spectrum 40

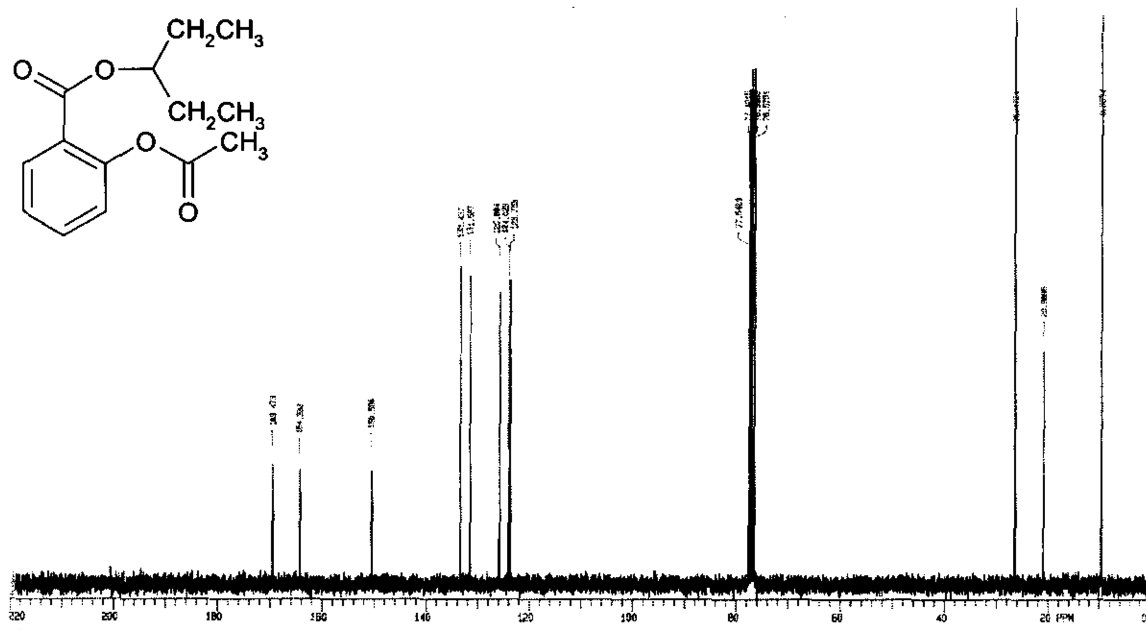





Spectrum 41
CH3
O
O
CH3
CH3
O
CH3
O
220
200
180
160
140
120
100
80
60
40
20 PPM
0


Spectrum 42
O
O
CH3
O
CH3
O
220
200
180
160
140
120
100
80
60
40
20 PPM
0


Spectrum 43
$CH_2CH_2CH_3$
O
O
CH_3
O
CH_3
O
PPM


Spectrum 44
CH_2CH_3
O
O
CH_2CH_3
O
CH_3
O
PPM

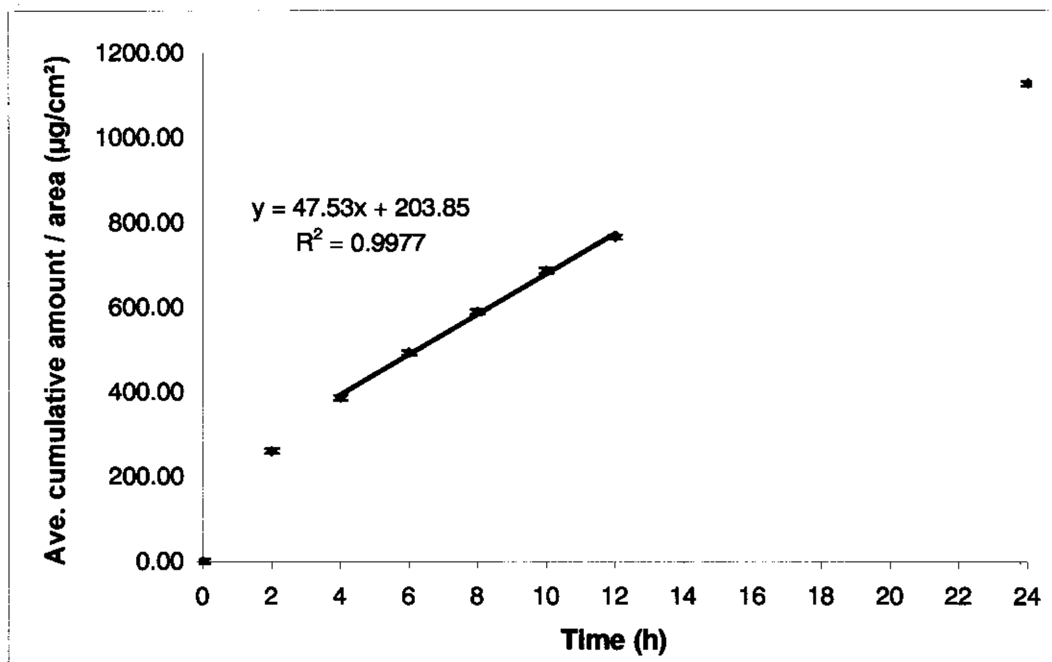


Figure A.1: Average cumulative amount of acetylsalicylic acid penetrated through the skin as a function of time.

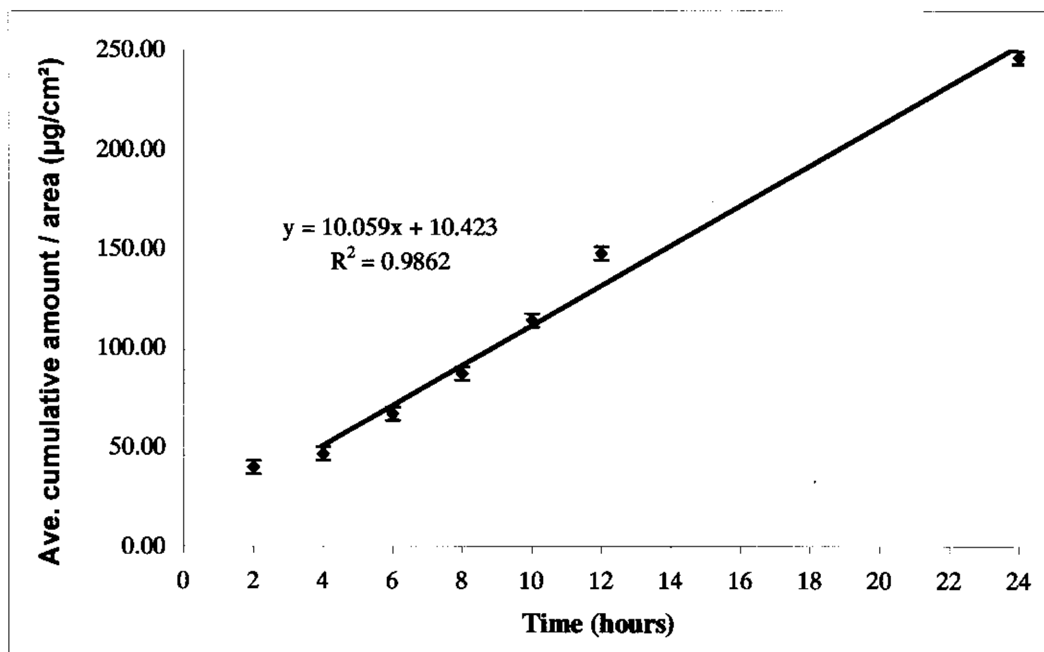


Figure A.2: Average cumulative amount of methyl acetylsalicylate penetrated through the skin as a function of time.

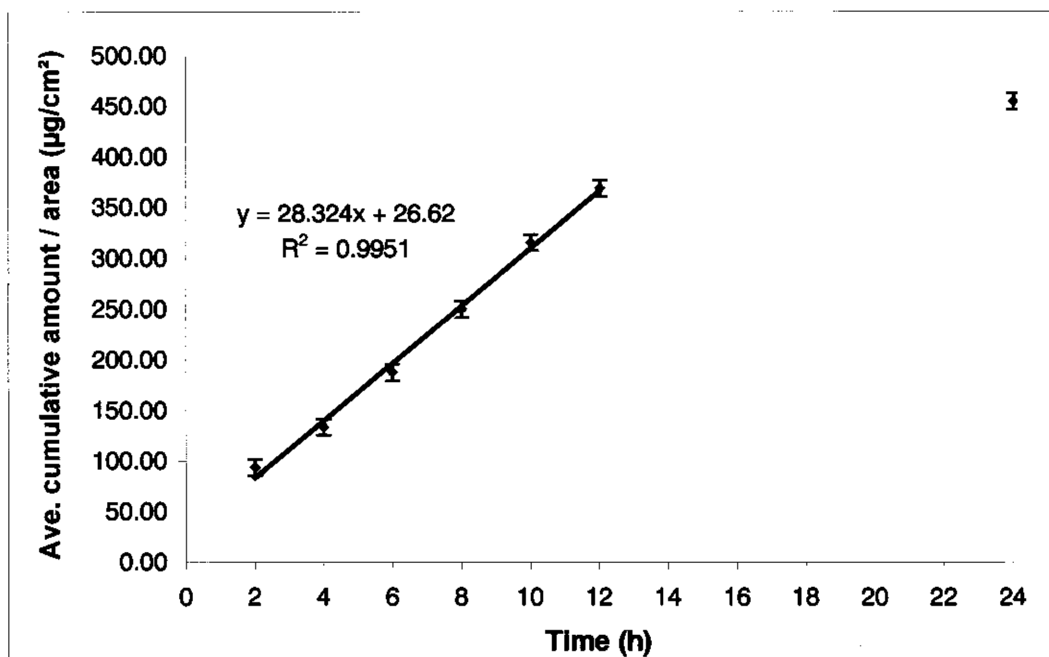


Figure A.3: Average cumulative amount of ethyl acetylsalicylate penetrated through the skin as a function of time.

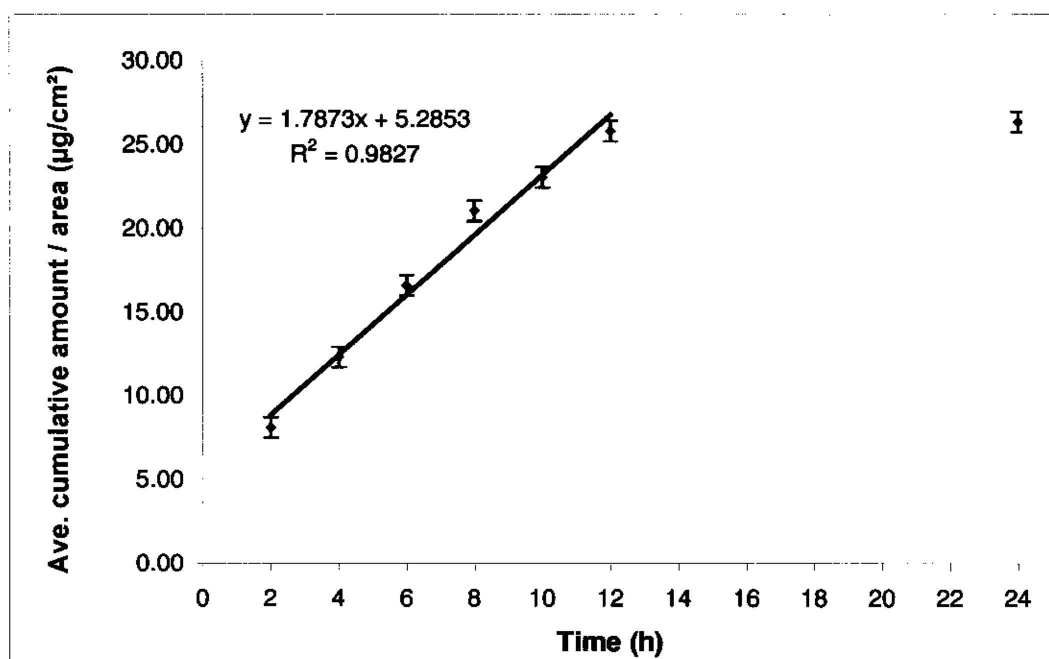


Figure A.4: Average cumulative amount of propyl acetylsalicylate penetrated through the skin as a function of time.

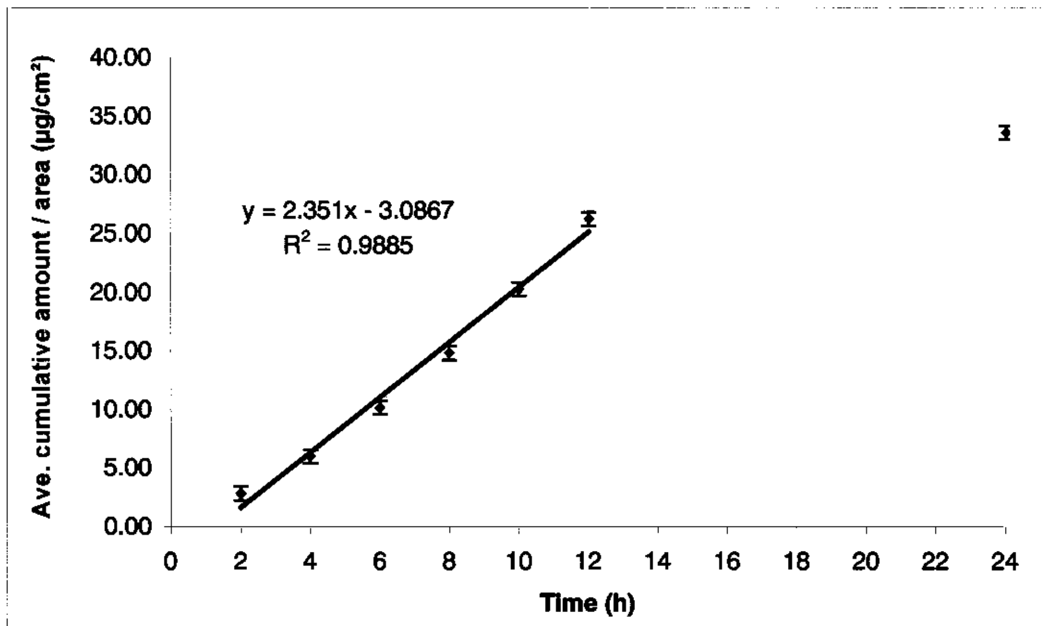


Figure A.5: Average cumulative amount of isopropyl acetylsalicylate penetrated through the skin as a function of time.

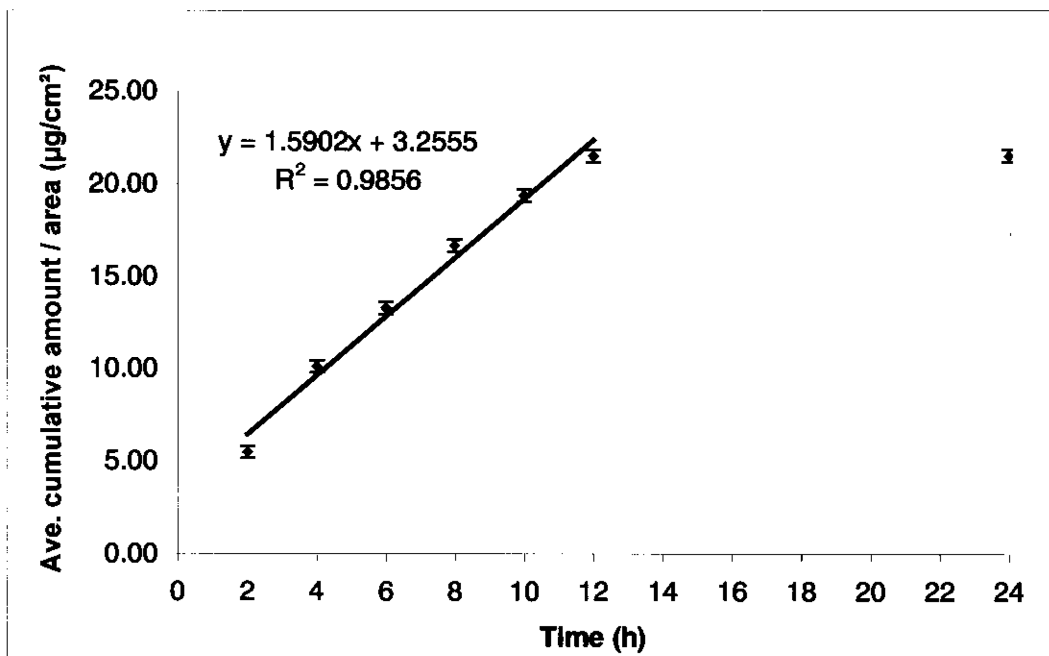


Figure A.6: Average cumulative amount of butyl acetylsalicylate penetrated through the skin as a function of time.

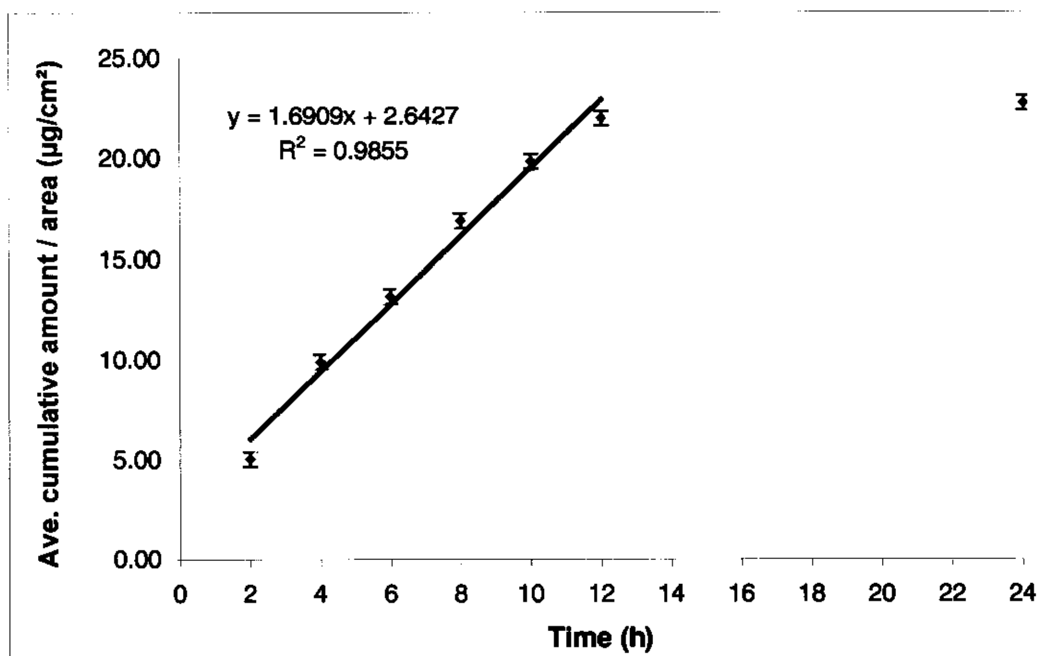


Figure A.7: Average cumulative amount of 1-methylpropyl acetylsalicylate penetrated through the skin as a function of time.

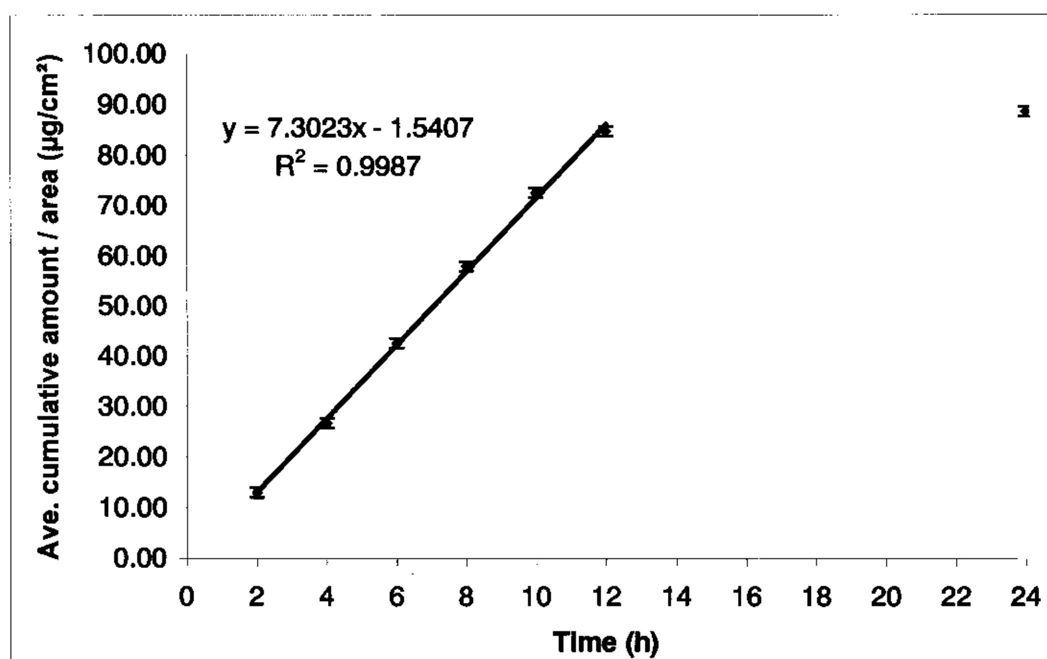


Figure A.8: Average cumulative amount of tert-butyl acetylsalicylate penetrated through the skin as a function of time.

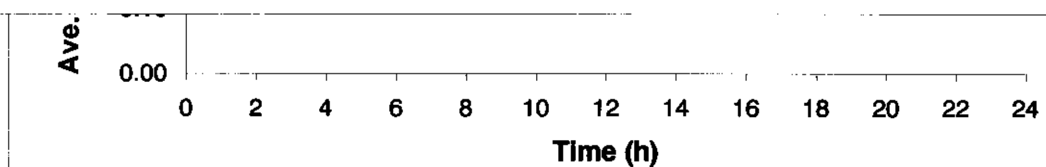


Figure A.10: Average cumulative amount of 1-methylbutyl acetylsalicylate penetrated through the skin as a function of time.