TOPICAL ASPIRIN GEL FORMULATION AND ASSESSMENT OF ITS IRRITANCY IN AN *IN VIVO* MODEL

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LIST OF ABBREVIATIONS

AAPS	American Association of Pharmaceutical Scientists
AHN	Acute herpetic neuralgia
FDA	Food and Drug Administration
HPLC	High performance liquid chromatography
ICH	International Conference on Harmonization
LDPI	Laser Doppler perfusion imager
LSC	Lichen simplex chronicus
NMP	N-methyl-2-pyrrolidone
PG	Propylene glycol
PHN	Post herpetic neuralgia
SC	Stratum corneum
TDDS	Transdermal drug delivery system
VAS	Visual analogue scoring

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SUMMARY

***Objectives*:** To formulate a safe, stable, bioadhesive and non-aqueous aspirin gel plaster for treatment of localized pain and itch.

***Methods*:** Aspirin plasters were prepared from copolymer of N-vinylacetamide and sodium acrylate, anhydrous aluminum chloride, propylene glycol (PG) and N-methyl-2-pyrrolidone (NMP) by a casting method. The rheological properties of the polymer-drug matrix gels were studied using oscillatory rheometry. The bioadhesive properties of the plasters were studied using an *in vitro* tensile testing method and an *in vivo* adhesion scoring method on pigs. *In vitro* drug release through synthetic membranes and permeation through pig and human skins were compared using vertical Franz diffusion cells. Accelerated stability testing was carried out to determine the stability of aspirin in the plasters. *In vivo* skin irritation studies of the plasters on pigs were carried out with laser Doppler perfusion imager (LDPI) and visual analogue scoring (VAS) methods. Formulation variables including polymer concentration, ratio of polymer to cross-linking agent and ratio of PG to NMP were studied and optimized with respect to the above mentioned properties.

***Results*:** The rheological studies showed that storage modulus values were higher than loss modulus values, loss tangent values were less than 1 and independent of oscillatory frequency, indicating that all the polymer-drug matrix gels were well cross-linked. The maximum bioadhesion was exhibited by polymer concentration of 9%, polymer to cross-linking agent ratio of 1.5:1 and PG to NMP ratio of 2:1. The drug release and permeation were decreased with increase in amounts of polymer, cross-

linking agent and propylene glycol in the plaster. No significant difference was found between drug permeation through pig skin and human skin. Skin irritation, which was not strong enough to be detected visually, was measured quantitatively with LDPI. The LDPI results were increased with increasing amounts of solvent mixture and cross-linking agent in the formulations. VAS and LDPI results showed enhancement of skin irritation with increase in aspirin content and duration of application of plasters. The accelerated stability testing showed that aspirin was stable in the plasters and no statistically significant changes in physical properties and drug content of the plasters were observed over the period of storage.

***Conclusion*:** The formulation variables showed significant effects on the rheological and bioadhesive properties of the plasters, drug release and permeation, and skin irritation reaction. Increased concentration of polymer, cross-linking agent or propylene glycol resulted in stronger and denser gels and thereby, slower drug release and permeation as well as reduced bioadhesion. The higher proportions of cross-linking agent and solvent caused skin irritation after repeated prolong contact with the skin. It was found that the optimized composition consisting of polymer concentration of 9%, polymer to cross-linking agent ratio of 1.5:1 and PG to NMP ratio of 2:1 is the most suitable polymer-drug matrix gel formulation for a safe and stable aspirin plaster for long term therapeutic use in the treatment of localized itch and pain.

CHAPTER 1

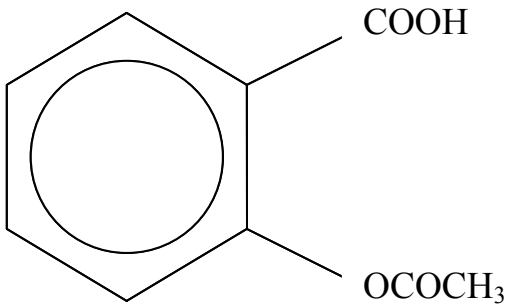
INTRODUCTION

A. Chemical properties of aspirin and its new indications

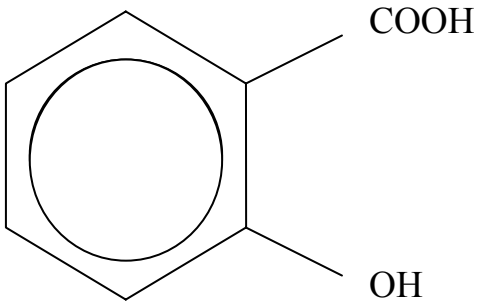
1. Chemical properties of aspirin

Aspirin (*o*-acetylsalicylic acid) is an odorless, colorless or white crystalline powder with a slightly acidic taste. It is slightly soluble in water, freely soluble in alcohol and soluble in ether. It is a weak acid with a pKa of 3.6 and a melting point of 143ºC (British Pharmacopoeia, 2000) (Figure 1a). Aspirin is stable in dry air but in contact with moisture it degrades by hydrolysis to acetic acid and salicylic acid. Although aspirin and salicylates are rapidly absorbed from the stomach and duodenum following oral administration, the fraction of the administered dose reaching the systemic circulation ranges from 50 to 75% (Rowland *et al*., 1972; Pedersen and FitzGerald, 1984) due to their extensive first-pass metabolism. Aspirin with plasma half-life of 14–19 min is rapidly cleared from the plasma. Aspirin is partially hydrolyzed to salicylic acid (Figure 1b) during absorption and is distributed to all body tissues and fluids, including fetal tissues, breast milk and central nervous system.

All salicylates have analgesic, antipyretic, anti-inflammatory and anti-rheumatic effects. They lower elevated body temperature through vasodilatation of peripheral vessels, thus enhancing dissipation of excess heat. The anti-inflammatory and analgesic activity may be mediated through inhibition of the prostaglandin synthetase enzyme complex. Aspirin differs from the other agents in this group in that it more



(a)



(b)

Figure 1. Chemical structures of (a) aspirin and (b) salicylic acid

potently inhibits prostaglandin synthesis, has greater anti-inflammatory effects and irreversibly inhibits platelet aggregation. Single analgesic aspirin dose prolongs bleeding time by inhibiting platelet aggregation (Roth and Majerus, 1975; Roth and Siok, 1978; Roth *et al*., 1978). Because of its broad spectrum of indications, rapid absorption from the upper gastrointestinal tract and extensive distribution throughout the body fluids following oral administration, aspirin is the most widely prescribed drug for the treatment of pain, fever, myocardial infarction, atrial fibrillation, angina, ischemic stroke and coronary artery bypass grafts (Antiplatelet Trialists' Collaboration, 1994; Hennekens, 1997). Oral dosage of 325 to 650 mg every 4 h is needed for minor aches and pains and up to 500 mg every 3 h or 1000 mg every 6 h for some extra strength. Therapeutic salicylate level is 150–300 μg/ml (Martindale, 1996).

However, oral administration of aspirin is limited due to its gastrointestinal toxicity (McAdam *et al*., 1995). Other adverse reactions of aspirin are drug allergy, renal irritation, aggravation of chronic urticaria, fetal malformations in early pregnancy, metabolic acidosis in children and respiratory alkalosis in adults. To avoid these side effects and pre-systemic loss of the drug, alternative routes of administration have been considered. Particularly, percutaneous delivery of aspirin is attractive due to its new indication in the treatment of acute herpetic neuralgia (AHN), post herpetic neuralgia (PHN) and pruritus.

2. New indications of aspirin

Herpes zoster, also known as shingles or zoster, is a viral infection caused by the varicella-zoster virus, which is the virus that causes chickenpox. The symptom of zoster is burning pain, tingling or extreme sensitivity in one area of the skin usually

limited to one side of the body. There may also be fever or headache. The pain may last longer. It is unusual but possible to have pain without blisters or blisters without pain. Treatment for shingles includes oral administration of antiviral drugs, steroids, antidepressants, anticonvulsants and analgesic agents. The severity and duration of an attack of shingles can be significantly reduced by immediate treatment with the antiviral drugs such as acyclovir, valacyclovir and famcyclovir. These drugs may also help stave off the painful aftereffects of shingles, i.e., post herpetic neuralgia. A symptom of post herpetic neuralgia is constant pain or periods of pain that can continue after the skin has healed. It can last for months or even years and is more common in older people.

There is still inadequate knowledge about the mechanisms of neuropathic pain in general, and of herpetic pain in particular. There is a limited range of proven therapies effective in relieving the pain of AHN and PHN. Although a wide variety of treatments have been claimed to be effective in AHN and to prevent and/or control PHN, limited success and/or side effects (e.g., anticholinergic effects of tricyclic antidepressants) can generally be expected after trying to relieve acute herpetic and post herpetic pain. Topical applications of various preparations such as capsaicin, analgesic cream (EMLA), lidocaine cream or patch, aspirin/chloroform mixture and indomethacin cream have been reported to be effective in relieving the pain of AHN and PHN (Yosipovitch *et al*., 2001). De Benedittis *et al.* (1992) studied a double-blind controlled clinical trial with aspirin/diethyl ether mixture in the treatment of acute and post herpetic neuralgia. The successful results from non-controlled study proved that topical application of aspirin in chloroform mixture alleviated pain in post herpetic neuralgia (King, 1993).

Itch, also known as pruritus, is defined as an unpleasant sensation on the skin that provokes the desire to rub or scratch the area to obtain relief. Itch can cause discomfort and frustration. In severe cases, it can lead to disturbed sleep, anxiety and depression. There are numerous causes of pruritus. Basically, they can be classified under the following 4 main headings: (1) skin disease such as eczema, (2) systemic diseases such as chronic renal failure, (3) damage to nerve fibers such as post herpetic itch, and (4) psychiatric itch such as delusions of parasitosis. Itch can also be classified as localized and generalized. Lichen simplex chronicus (LSC) or localized circumscribed neurodermatitis is a troublesome type of itchy dermatosis, characterized by chronic itching and scratching as well as a self-perpetuating scratch-itch cycle. The persistent scratching causes formation of thick and leathery hyper-pigmented skin (excoriation, lichenification) and reduces the effectiveness of the skin as a major protective barrier. Therefore, itch and lichenification constitute its hallmark and seem to be more common in Asians (Marks, 1999; Tianco *et al*., 1991; Yap *et al*., 1994).

The primary treatment of itch is to stop the itch scratch cycle. This may include counseling to become aware of the importance of not scratching, stress management measures or behavior modification. Antihistamines, sedatives or tranquilizers may be needed to reduce itching and stress. However, the treatment of lichen simplex chronicus is a challenge to the dermatologist since topical treatments using high potency steroids were not effective (Marks, 1999). It was reported that topically applied aspirin rapidly decreased histamine induced itch (Yosipovitch *et al*., 1996). Recently, a double-blind crossover placebo-controlled trial was conducted with 29 patients suffering from lichen simplex chronicus to assess the effect of topical administration of aspirin/dichloromethane solution and it was concluded that topical

aspirin/dichloromethane solution might be a practical, safe and efficient method for the treatment of lichen simplex chronicus (Yosipovitch *et al*., 2001). Unfortunately, the formulation of topical aspirin was not stable because of the usage of volatile solvent. A stable topical preparation of aspirin would be an excellent option for the treatment of lichen simplex chronicus and other localized itch. Salicylic acid, the degradation product of aspirin, is also widely used as a topical therapeutic agent for psoriasis, ichthyosis and acne because of its keratolytic action and permeability through healthy skin (Schwarb *et al*., 1999).

B. Percutaneous drug delivery systems

1. Physiology of the skin

The skin is principally composed of two parts, namely the outer epidermis and the inner dermis. The dermis contains capillaries, sebaceous and sweat glands, hair follicles and nerves; the epidermis, on the other hand, is avascular. The epidermis has a multilamellar structure that represents the different stages of cell differentiation. By moving upwards from the proliferative basal layer, the cells change in an ordered fashion from metabolically active and dividing cells to dense, functionally dead and keratinized cells, corneocytes. These keratinized cells are surrounded by multilamellar lipid bilayers and constitute the unique "brick and motar" structure of the outer 10–20 μm of the epidermis, called the stratum corneum. The bricks composed of the keratin rich corneocytes are embedded in a 'mortar' composed of multiple lipid bilayers consisting of ceramides, fatty acids, cholesterol and cholesterol esters. These bilayers form regions of semicrystalline, gel and liquid crystals domains. The intact stratum corneum provides the main barrier to permeability of substances due to its unique 'brick and mortar' structure. The lipophilic nature of the intercellular lipids limits the

permeability of hydrophilic and large molecules of peptide and protein drugs in the skin. Drugs have three potential pathways through the skin, namely intercellular route, transcellular route and appendages route. The skin appendages occupy only 0.1% of the total human skin surface and the contribution of this pathway is usually considered to be small. Drug permeation through the skin is mainly though the intercellular lipid route. The penetration enhancers mainly act on the intercellular lipids (Moser *et al*., 2001) (Figure 2).

2. Types of percutaneous drug delivery systems

Based on the target of action, percutaneous drug delivery systems are divided into topical, regional and transdermal delivery. Topical delivery can be defined as the application of a drug-containing formulation to the skin to directly treat cutaneous disorders or the cutaneous manifestations of a general disease. The delivery systems are placed against the skin to deliver drugs to the local tissues immediately beneath the application site. Active ingredients may or may not require intracutaneous penetration and deposition. Regional delivery system, on the other hand, is placed against the skin to deliver drugs to deep regions in the vicinity of, but still somewhat remote from, the application site. It involves the application of a drug to the skin for the purpose of treating diseases or alleviating disease symptoms in deep tissues beneath the application site. Topical and regional delivery systems are open applications and may experience profound compositional shifts and phase changes during use.

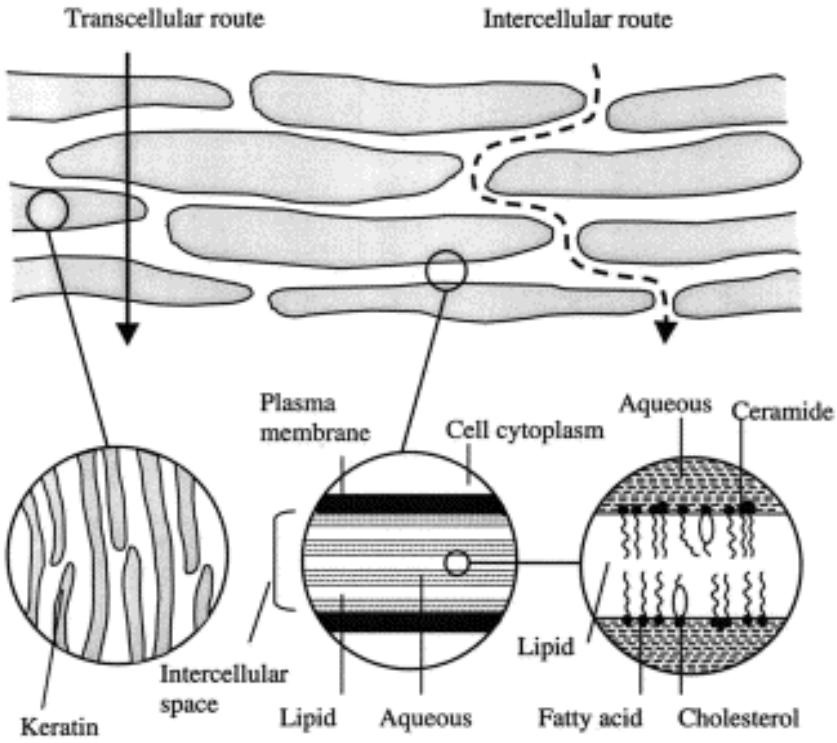


Figure 2. Drug transport through human skin (Moser *et al*., 2001)

The application techniques and the amounts of usage for topical and regional delivery systems are highly specific and they can be applied only on diseased, damaged skin. They are short-acting applications, with drug level in the local tissues related to product efficacy. Systemic absorption is absolutely undesirable, although, some are unavoidable. By removal of system, the application can be interrupted without affecting tissue wear and tear. Only small fraction of total drug is delivered from dermatological formulations. Transdermal delivery system (TDDS) is placed against the skin to deliver drugs to the systemic circulation to mediate pharmacological changes somewhere totally remote from the application site. Therefore it involves the application of a drug to the skin to treat systemic disease and is aimed at achieving systemically active levels of the drug. They are occluded applications with compositions relatively invariant during use. They are applied on a predetermined surface area with a precise dose. However, drug levels in local tissue are related to product efficacy and the levels of drug in the blood are unavoidable which lead to systemic toxicity (Flynn, 1993).

Percutaneous drug delivery systems have several advantages, compared to other routes of administration. There are no gastrointestinal irritation and no hepatic first-pass effect. The administration does not require needles or professional supervision and the treatment can be terminated immediately upon improvement of the disease. Therefore, these delivery systems increase patient acceptance and compliance. Transdermal delivery systems provide a steady maintenance of blood level over a predictable period of time. Compared with other dosage forms, TDDS may not be cost effective. However, some limitations of these delivery systems, such as cosmetic appearance, smell and size have to be improved as they can affect patient acceptance. The size of

the system will be dependent on the amount of drug released from the system and permeated through the skin as well as the blood level needed to elicit the desired pharmacological effect. If the drug is inherently skin sensitizing or irritant, skin irritation and sensitization may be unavoidable. Some people with very active daily life style or those having psychological factors such as fright and aggression may not be able to get a system to adhere to their skin because of excessive sweating or oiliness. Moreover, the usage of topical and regional delivery systems is limited to treat dermal disorders, with the skin as the target organ.

The dosage forms for percutaneous delivery of drugs include powders, solutions, lotions, creams, pastes, gels, ointments, plasters and patches. Gels, plasters and patches are more attractive as percutaneous delivery systems. Gels are semisolid or solid systems consisting of a three-dimensional cross-linked polymer networks in an aqueous or non-aqueous liquid vehicle. Depending on the nature of interaction between adjacent polymer chains, gels may be categorized as chemical and physical systems (LaPorte, 1997). The networks of chemical gels are held together by covalently cross-linked polymer chains. Conversely, physical gels are composed of polymer chains in which interaction between adjacent polymer chains are facilitated by secondary molecular forces (e.g., van der Waal's bonds and hydrogen bonding) (Kavanagh and Ross-Murphy, 1998). Gels can be fabricated in the form of plasters by spreading on a backing material. Plasters are applied to the skin to provide prolonged contact at the application site. Medicated plasters provide effects at the site of application.

3. Considerations in the development of plasters for drug delivery

The ideal plaster for therapeutic percutaneous drug delivery should be strong and flexible in nature and has a smooth and soft surface for ease of contact with the skin surface. It should also have strong bioadhesive properties for prolonged retention on the skin to allow adequate drug release and penetration through the skin to achieve therapeutic effect. The amount of drug at the target site should commensurate with the clinical requirements. The plaster should be able to be removed easily without leaving any residue of drug or other ingredients on the skin upon termination of the treatment. The plaster should not cause any irritation and harmful effect to the skin. The drug should be stable in the formulation during storage. The drug candidate for percutaneous drug delivery should possess suitable chemical properties, such as low dose, low polarity, low melting point, low molecular weight and lipophilicity. Other requirements for a drug suitable for percutaneous delivery are permeability through the skin, no adverse effect on the adhesiveness of the plaster such as skin irritation and allergic reactions, favorable pharmacodynamic and pharmacokinetic properties, relatively broad therapeutic window, low skin metabolism and consistent tissue drug level for continuous administration (Flynn and Stewart, 1988).

C. Characterization of plasters

1. Rheological properties of gels

The clinical and non-clinical performances of polymeric gels are dependent on their mechanical/rheological properties. Therefore, the study of the rheological behavior of polymeric gels is necessary to ascertain their quality and therapeutic efficacy. Rheology is the study of the deformation (strain) of materials under stress applied. If the material regains its original shape and position when the stress is removed, it is

called elastic. If the material fails to do so, it is said to have flowed and is described as viscous. As a simplification, solids are elastic and liquids are viscous. A gel shows both elastic and viscous properties and is viscoelastic. Rheometry is an experimental method to quantify viscoelastic behavior of a gel. The most commonly used methods to study the mechanical and rheological properties of gel systems are flow rheometry, oscillatory rheometry and creep analysis. In an oscillation rheometry, the material is subject to a sinusoidal stress. The rheological behavior is characterized by the dynamic moduli G′ and G″ as a function of frequency, where G′ is the storage (elastic) modulus and G″ the loss (viscous) modulus. The storage modulus is a measure of the energy stored and recovered per cycle of deformation, reflecting the solid-like component of viscoelastic behavior of the material. The loss modulus is a measure of the energy lost per cycle, reflecting the liquid-like component (Ferry, 1980). The loss tangent, tan δ, calculated from the ratio of viscous and elastic moduli, provides a comparative contribution of elastic and viscous components to the gel system.

2. The bioadhesive properties of plasters

Bioadhesion is defined as a state in which two bodies, one or both of them of biological nature, are held together for an extended period of time by interfacial forces (Solomonidou *et al*., 2001). Satisfactory bioadhesion is essential for the successful application of a bioadhesive drug delivery system. It refers to the strength of attachment of the dosage form to the biological tissue. Polymers of varying structures have been shown to exhibit bioadhesive properties. It is generally accepted that polymers with strong anionic charges and a number of carboxyl or hydroxyl groups have good binding potential. A bioadhesive polymer should have sufficient chain flexibility to allow interpenetration into the biological substrate, favorable surface

energy properties to facilitate spreading over the biological surface, and a sufficient macromolecular size to produce an interpenetrating layer and entanglements. Polyacrylic acid polymers have been reported to possess good bioadhesive properties (Smart *et al*., 1984; Park and Robinson, 1984; Peppas and Buri, 1985).

Several techniques for *in vitro* determination of bioadhesion have been reported, which include tensile testing (Park and Robinson, 1985), shear stress testing (Smart *et al*., 1984), adhesion weight method (Smart and Kellaway, 1984), fluorescent probe method (Park and Robinson, 1984), flow channel techniques (Mikos and Peppas, 1986) and texture analysis (Wong *et al*., 1999). The measurement of forces required to detach a bioadhesive dosage form from a biological substrate using a tensile tester is the most direct way to quantify bioadhesive performance. The adhesion of plaster onto the skin surface can be evaluated by applying the plaster on the skin and estimating the adhesion by a visual scoring system developed by Hill Top Research Inc. (Table 1).

Table 1. Assessment of adhesion of plaster by visual analogue scoring

Adhesion score	Description
0	90% adhered (essentially no lifting off the skin)
1	75% to <90% adhered (only some edges lifting off the skin)
2	50% to <75% adhered (less than half of the system lifting off the skin)
3	<50% adhered but not detached (more than half the system lifting off the skin without falling off)
4	patch detached (patch completely lifting off the skin)

D. *In vitro* drug release and skin permeation studies

In vitro techniques are simple, reliable, reproducible and relevant methods to assess release of drug from a dermatological product and permeation through the skin. A major advantage of *in vitro* techniques is that they allow for measurement of permeation through human skin of chemicals too toxic to test ethically in human subjects. Therefore, it is widely used in the assessment of percutaneous absorption of potentially toxic chemicals. Permeation rates and skin metabolism can be measured more accurately in an *in vitro* system because sampling is performed directly beneath the barrier layer (Aungst *et al*., 1986). Skin metabolism can be studied in viable skin without interference from systemic metabolic processes. Finally, permeation measurements are much more easily obtained from diffusion cells than from analysis of biological specimens from clinical studies. The accuracy of *in vitro* measurements depends on the use of proper methodology.

1. Drug release and permeation studies using vertical Franz diffusion cell

Although many different diffusion cells have been used for permeation studies, there are only two basic cell types: one-chambered cell and two-chambered cell. The two-chambered cell has two chambers of equal volume (often from 2 to 10 ml) that are separated by the skin membrane. The one-chambered cell has a chamber beneath the skin but is open to the environment above the skin. The two-chambered cell is useful for studying mechanisms of drug diffusion through the skin and also applicable to the measurement of permeation from drug delivery devices that release drug at an infinite dose and produce a steady-state rate of delivery. The one-chambered cell is useful for the study of permeation of chemicals through the skin and determination of steady-state absorption kinetics. Finite-dose techniques and the design of a static diffusion cell

were described by Franz (1975). A flow-through cell system (Bronaugh and Stewart, 1985) was introduced to automate sample collection from a one-chambered cell. It also facilitates the maintenance of skin viability since the physiological receptor fluid is continually replaced.

An appropriate type of skin or membrane is used for evaluation of *in vitro* percutaneous penetration. There are two types of skin used in the diffusion cell technique, namely human skin and animal skin. Different part of the skin can be used, such as full-thickness skin, epidermis and stratum corneum. Female abdomen is the standard site for skin removal to minimize the variability in the results due to differences in permeability properties between different anatomical sites. Testing has to be repeated with skin from different donors to reduce the variation between individuals in skin permeability. Since animal skin is more readily available than human skin, it is possible to standardize the skin slice regarding anatomic site, age and sex of animal. However, most animals have furry skin and the thickness of the stratum corneum is often thinner than that of human skin. Therefore, the results obtained from *in vitro* animal skin study may not represent the *in vitro* human skin study. It was reported that pig skin has a close physiological similarity to human skin and is mostly recommended because of its availability (Sartorelli *et al*., 2000). In addition, hairless guinea pigs are also recommended to assess relative permeability and to compare *in vitro* and *in vivo* results.

The full-thickness skin is the skin with the subcutaneous fat removed. The stratum corneum controls the rate of penetration for most compounds. After passing the stratum corneum, hydrophilic compounds will pass through the hydrophilic dermis

while lipophilic substances will be retained (at least partly) in the dermis. Therefore, full-thickness skin is not suitable for investigation of percutaneous penetration of lipophilic compounds (Sartorelli *et al*., 2000). However, for lipophilic drugs, an estimation of skin absorption can be achieved by measuring the content of the test substance in the skin (besides the content in the receptor fluid and surface wash). Isolated epidermis could be prepared by different heat separation technique using a water bath or microwave oven. The advantage of using isolated epidermis is that the penetration of both lipophilic and hydrophilic compounds can be investigated. The disadvantage is that the separation procedure may disturb the permeation properties of the skin. Dermatomed skin is a skin slice (200–400 μm) cut from the full-thickness skin using a dermatome. The dermatomed layer obtained includes the epidermis and some dermal tissue. Instead of isolated epidermis, dermatomed skin can be used to investigate the penetration of lipophilic and hydrophilic compounds. The cutting may disturb the permeation properties and it is important to ensure that the skin slice is free of dermis. Stratum corneum (SC) is prepared by incubating the human epidermal membranes with SC side upwards in 0.5% (w/v) sodium bicarbonate solution containing 0.1% (w/v) trypsin, at 37±1°C for 3 h. The SC is then removed, thoroughly washed and dried in a vacuum desiccator. After 24 h, the SC is dipped in acetone solution for 20 s to remove sebaceous lipids and dried again (Kligman and Christophers, 1963 and Kumar *et al*., 1989).

The selection of the receptor fluid is important to create *in vitro* conditions that can adequately simulate the *in vivo* situation. The normal saline or an isotonic buffer solution could be used for measuring the absorption of water-soluble compounds. Some chemicals are metabolized significantly during the percutaneous absorption

process (Kao *et al*., 1984). The viability of skin can be maintained for 24 h in a flow-through diffusion cell using a physiological buffer as the receptor fluid (Collier *et al*., 1989). Drugs with water solubility of less than approximately 10 mg/l would present a potential problem in a diffusion cell with a standard aqueous receptor fluid (Bronaugh and Stewart, 1985). Thus, nonionic surfactants or non-aqueous solvents like ethanol, propylene glycol and polyethylene glycol 400 may be incorporated into the aqueous receptor fluids to increase the drug solubility in the receptor medium. The most effective receptor fluid that could be used without apparent damage to the skin was found to be the nonionic surfactant, polyethylene glycol 20 oleyl ether (Williams, 1991). The concentration of the drug that accumulates in the receptor fluid is measured in the permeation study. This is commonly expressed as the cumulative amount of drug in the receptor fluid. When the rate of penetration through the skin reaches a pseudo steady-state, the flux of the drug through the skin can be obtained from the slope of the linear portion of the curve of the cumulative amount of drug in the receptor versus time.

Based on acceptable *in vitro*/*in vivo* correlations, *in vitro* method is used as a predictor of *in vivo* performance. In *in vitro* drug release study, the vertical Franz diffusion cell system is used to assess the drug release characteristics from topical formulations through a commercially available mixed cellulose/cellulose nitrate synthetic membrane. Application of a synthetic membrane avoids certain experimental issues associated with the use of excised tissue but it also reduces meaningful extrapolation to an *in vivo* setting. Aliquot samples from the aqueous receptor phase can be analyzed for drug content by high performance liquid chromatography (HPLC) or other analytical methods. A plot of the amount of drug released per unit area ($\mu g/cm^2$)

against square root of time may yield a straight line, the slope of which represents the release rate, also defined as the steady-state flux. This release rate is formulation-specific and can be used to monitor batch-to-batch uniformity (Shah *et al*., 1992 and 1993). The difference in slope between two batches of the same formulation or between two formulations relates to differences in their relative ability to release the drug across the synthetic membrane under the chosen experimental conditions. The release rate of the drug from a topical formulation largely depends on manufacturing variables, which influence the quality of the preparation (Shah *et al*., 1992). For these reasons, *in vitro* release studies using a synthetic membrane can be employed as a quality control measure to assess batch-to-batch uniformity.

2. Guidelines for *in vitro* percutaneous studies to establish release and permeation rates of drug products (FDA and AAPS, 1987)

Human skin should be used in the form of dermatomed sections or epidermal sections. In comparative studies, skin samples from the same body site should be utilized. If the stored skin is utilized, the conditions of harvesting and storage should be described. The effect of storage also should be ascertained. If volatility of the receptor medium is a problem, a quantitative accounting of cell design must be made. The receptor medium should provide an effective sink for the penetrant. In most studies, an isotonic solution buffered to pH 7.4 is a suitable and preferred receptor fluid. With hydrophobic compounds, studies should be done either by: (1) using a lipophilic receptor fluid that has no effect on the skin membrane or (2) using an isotonic solution containing an appropriate concentration of solubilizer for the hydrophobic compound. The surface temperature of the skin should be maintained at 32°C. The lag times and steady state fluxes should be set for kinetic analysis. Whenever possible the drug

content in both the tissue and receptor at the end of the experiment should be noted and the total mass balance, which includes measurement of the residual drug in the skin, should be determined. The maximum rate and the time to achieve the maximum rate can be determined and compared across different formulations. The apparent steady-state flux (slope) should be reported (Sartorelli *et al*., 2000).

E. Stability testing

When a new drug product is being formulated, it is desirable to determine the stability of the drug entity in the drug product so that a shelf-life or expiration date may be assigned to the product. The chemical stability of a drug in the desired dosage form is of great importance since therapeutic problems may result from poor drug stability. The net result of drug instability is that the patient does not receive the proper dose of the active drug entity and therefore, the full therapeutic effect of the drug is not realized. Additionally, drug decomposition may yield toxic by-products, which endanger the patient. The shelf-life is the length of time required for the product potency to be reduced to some percentage of its original value. For most products, this is the T_{90} or time at which the product retains 90% of its original potency.

Stability of a product is the time interval specified by the manufacturer within which the characteristics of the product remain. The change of these characteristics as the product ages is usually called degradation. The degradation rate can be influenced by environmental variables such as temperature, humidity, oxygen and light. The formulation variables also have effects on the stability of the drug in the product. Products degrade faster when they are subjected to elevated temperature and humidity conditions, which have been used in stability testing for assessment of the shelf-life of

products. Usually two types of stability testing are used in practice, i.e., long-term stability testing and accelerated stability testing. In the long-term stability testing, products are stored in normal storage conditions and monitored for a period of time. Whereas in the accelerated stability testing, products are subjected to elevated stress conditions and the characteristics of the products are monitored for a period of time. The stability of the product at normal storage conditions is predicted from the degradation rates at the stressed conditions. This accelerated stability testing is designed to increase the degradation rate of the drug product to obtain information more quickly, allowing for rapid screening for stable and safe formulations. The current International Conference on Harmonization (ICH) guidelines recommend long-term stability testing at 25°C/60% RH and accelerated stability testing at 40°C/75% RH, or if significant change has taken place at this condition, then 30°C/60% RH can be used as an accelerated condition. Visual inspection of changes in the physical form is performed and the drug and its degradation products are assayed using appropriate analytical methods such as HPLC technique.

F. Skin irritation studies

Skin irritation is identified as a non-immunologic local inflammatory reaction, characterized by erythema, edema or corrosion, following single or repeated application of a chemical substance to the cutaneous site. The conditions of exposure influencing the clinical response to chemical irritants may be divided as follows: (1) extrinsic factors, which influence the ability of a chemical substance to penetrate the skin barrier and produce an inflammatory reaction, and (2) intrinsic (constitutional) factors, which influence an individual's capacity to react with an inflammatory response.

Irritation testing is conducted for several reasons. A single contact with some chemicals may result in acute inflammation and in some cases skin necrosis at the application site (Wilhelm and Maibach, 1990). Necrosis induced by chemicals is called corrosive. Animal tests mandated by regulatory agencies are routinely used to screen materials for their capability to produce acute irritation and corrosion. Chemicals that do not produce acute irritation from a single application may produce inflammation following repeated application to the skin, which is often described as cumulative irritation. Cumulative irritation is most often evaluated in humans. The tests for predicting skin irritation include **c**onventional patch test, cumulative irritation test, chamber scarification test, soap chamber test and immersion technique.

Patch testing is a well-established method for risk assessment and is frequently used to determine the irritation potential of topical agents. It involves application of test material under occlusive conditions and irritation is observed visually and assessed according to a visual analogue score (VAS) (Vowels *et al*., 1995). The Hill Top Research Inc. has developed a patch testing system using the Hill Top chamber, which incorporates a 0.2 ml of test sample. The test sites are examined in standard lighting conditions and the assessment of skin irritation is made only after application of the patch according to the visual scoring system (Table 2). This system has been identified as a preferred patch testing system on the basis of a combination of test sensitivity**,** test material volume and commercial availability**.**

Many advanced bioengineering devices have been used to measure skin irritation. Laser Doppler velocimetry has attracted great interest in skin irritation study. Laser Doppler velocimetry is an optical technique for estimation of microcirculation, based

on the Doppler principle. When the laser beam from a 632-nm helium-neon laser source is directed toward the tissue, reflection, transmission, absorption and scattering occur. Laser light backscattered from moving particles, such as red cells, is shifted in frequency according to the Doppler principle, while radiation backscattered from non-moving structures remains at the same frequency. Thus, increased skin blood flow, which has been shown to be related to skin irritation, can be measured. Recently, Laser Doppler perfusion imager (LDPI) for two-dimensional mapping of blood flow in exposed tissue was developed (Wardell *et al*., 1993). It is based on similar principles as laser Doppler velocimetry but makes it possible for quick assessment of superficial blood perfusion and eliminates the unavoidable skin touch of the conventional flow meter that may affect the measurement. It is a useful objective tool complementing visual assessment of skin erythema and has been used to study *in vivo* irritation.

Table 2. Visual scoring system for skin irritation (Mills *et al.*, 1998)

Score	Irritation
I	*Dermal response*
0	No evidence of irritation
1	Minimal erythema, barely perceptible
2	Definite erythema, readily visible; minimal edema or minimal papular response
3	Erythema, edema
4	Definite edema
5	Erythema, edema, and papules
6	Vesicular eruption
7	Strong erosion spreading beyond test site
II	*Other effects:*
A	Slight glazed appearance
B	Marked glazing
C	Glazing with peeling and cracking
D	Glazing with fissures
E	Film of dried serous exudates covering all or part of the patch site
F	Small petechial erosions and/or scabs

CHAPTER 2

LITERATURE REVIEW

A. Studies on therapeutic action and adverse effects of aspirin

1. New indications of oral aspirin

Apart from its common usage as an analgesic and anti-inflammatory drug, several studies have proven that aspirin can be used in antiplatelet therapy. Aspirin in doses of 50, 100, 250, and 1000 mg daily suppressed thromboxane formation and significantly inhibited platelet function (Martindale, 1996). A number of clinical trials have been done with the use of aspirin in both primary and secondary prevention of myocardial infarction and stroke (Antiplatelet Trialists' Collaboration, 1994; Hennekens, 1997). Experimental studies in animals and observational studies in humans indicated that regular use of aspirin might decrease the risk of colorectal adenomas, which are the precursors to most colorectal cancers. These findings were further proven by conducting a randomized, double-blind trial on a total number of 517 patients. The results from the studies showed that one or more adenomas were found in 17% of patients in the aspirin group and 27% of patients in the placebo group ($p<0.05$). It was concluded that daily use of aspirin was associated with a significant reduction in the incidence of colorectal adenomas in patients with previous colorectal cancer (Sandler *et al.*, 2003).

2. New indications of topical aspirin

The afferent C-fibres subserving itch of cutaneous origin respond to histamine, acetylcholine and other pruritogens except mechanical stimuli. Therefore, H_1-

antihistamines are effective in treatment of insect bite reactions and most forms of urticaria. However, itch in systemic disease and in most dermatoses do not respond to low-sedative H_1-antihistamines (Twycross *et al*., 2003). Although it has been reported that oral aspirin does not relieve itch (Daly and Shuster, 1986) and it can increase histamine induced itch (Hagermark, 1973), many studies have found that topical application of aspirin can be used for treatment of localized itch and pain. Aspirin is an inhibitor of cyclo-oxygenase enzyme which results in the inhibition of the biosynthesis of prostaglandins. Prostaglandins cause hyperalgesia by sensitizing the small nerve fiber endings to various mediators. These small nerve fibers transmit pain and itch mainly by C fibers. Therefore, prostaglandins play an important role in pain and itch (Lovell *et al.*, 1976). Aspirin inhibits the effect of prostaglandins on these nerve endings (Cashman and McAnulty, 1991 and McCormack and Brune, 1991), and thus, topical application of aspirin can be used for treatment of localized itch and pain. In a single blind study of sixteen volunteers, topical application of aspirin/dichloromethane solution significantly reduced the duration and magnitude of histamine induced itch ($p<0.05$) (Yosipovitch *et al*., 1997). However, in another study, topical aspirin reduced skin irritation induced by sodium lauryl sulphate but did not reduce histamine induced itch in the model used (Thomsen *et al.*, 2001).A clinical double-blind crossover placebo trial on 29 patients with lichen simplex chronicus of at least 3 months duration showed that LSC responded well to topical aspirin/dichloromethane solution. Therefore, this treatment was suggested to be a practical, safe and efficient treatment for lichen simplex chronicus (Yosipovitch *et al*., 2001). A new topical treatment for acute herpetic neuralgia and post herpetic neuralgia with aspirin/diethyl ether mixture was proven to be highly efficient treatment for both diseases from the results of an open-label study and a double-blind controlled clinical trial (De Benedittis *et al*.,

1992). Short-Form McGill Pain Questionnaire was used to study the pain patterns and relationships in 42 patients with herpes zoster and post herpetic neuralgia before and after topical application of aspirin in chloroform. All patients from this study reported that pain decreased promptly after treatment, with maximum relief at 20 to 30 min and lasting 2 to 4 h (King, 1993). The effect of oral aspirin has been compared with topical aspirin/chloroform solution in patients with acute herpetic neuralgia (King, 1988). Topical aspirin provided significantly higher pain relief than oral aspirin in all the patients. Three studies have reported that topical aspirin in diethyl ether provided good to excellent pain relief, whereas oral aspirin provided poor pain relief. Kassirer and King (1988) reported that three patients with post herpetic neuralgia were treated effectively with aspirin dissolved in a moisturizer (Vaseline Intensive Care Lotion). This study was further studied by Balakrishnan *et al.* (2001) to prove that topical aspirin in moisturizer was clearly superior to oral aspirin in relieving the pain of AHN. Compared to oral administration, higher levels of aspirin in skin but negligible blood levels were observed in topical administration. It was also reported that the aspirin concentration in the skin was much higher than that in the blood after topical application (Bareggi *et al.*, 1998).

3. Adverse effects of oral aspirin

There are many studies reporting side effects of oral administration of aspirin. 787 reports of adverse reactions to aspirin were sent to the committee on safety of medicines. These included 95 reports of blood disorders and 53 reports of analgesic nephropathy associated with the use of preparations containing aspirin, phenacetin and codeine. Others adverse effects of aspirin reported were aplastic anaemia, haemolytic anaemia, thrombocytopenia, hyperglycaemia and glycosuria, deafness, exacerbated

anginal attacks, hepatotoxicity in patients with rheumatoid arthritis, systemic lupus erythematosus or similar disorders, toxic epidermal necrolysis, and Reye's syndrome (Martindale, 1996). A report from The Dutch TIA Trial Study group, 1991 showed that aspirin with dose as low as 30 and 75 mg/day caused serious gastrointestinal bleeding. In patients with peptic ulcer and related diseases and those on warfarin, aspirin is contraindicated because of its gastrointestinal toxicity and aggravation of gastrointestinal bleeding (Shorr *et al*., 1993 and Turpie *et al*., 1993). It was proven that inhibition of gut cyclooxygenase enzyme by aspirin is the underlying mechanism of gastrointestinal toxicity (Walt, 1992 and Masferrer *et al*., 1993) and gastrointestinal bleeding was aggravated because of the gastrointestinal tract and platelets share a common isoform of the cyclooxygenase enzyme (Smith, 1992).

According to the above reports on side effects of oral aspirin, it is unsafe to use oral aspirin for a prolonged period of time. Therefore, a safer route of administration of aspirin should be considered. Topical administration has more advantages than oral administration, not only in the way of less side effects but also greater therapeutic effect. Moreover, aspirin has physical and chemical properties that are favorable for percutaneous absorption, i.e. weak acid with a pKa value of 3.6, low melting point of 143°C, low molecular weight of 180.2 and lipophilicity (British Pharmacopoeia 2000). Aspirin elicits its therapeutic activity with relatively low therapeutic dose of 30 mg/day (Patrignani *et al.*, 1982) and is well tolerated by the skin. It was reported that transdermal administration of sodium salicylate, aspirin, and other non steroidal anti-inflammatory drugs by iontophoresis has been widely used in the treatment of musculoskeletal illnesses (Lark and Gangarosa, 1990; Saggini *et al*., 1996; Demirtas and Oner, 1998; Rosenstein, 1999). However, one case of adverse reactions from

transdermal administration of lysine acetylsalicylate by anodic iontophoresis, such as generalized cutaneous eruption, with small, nonconfluent erythematous-pomphoid lesions and pruritus, lip angioedema, nasal obstruction, and a sense of constriction in the chest, was reported (Macchia *et al*., 2002). Therefore, the formulation of a safe and stable topical aspirin should be studied to achieve the effective therapeutic usage.

B. Formulation of percutaneous drug delivery systems

A liposome-gel formulation containing 1% (w/w) hydrocortisone was prepared to study the targeted and sustained delivery of hydrocortisone to normal and stratum corneum-removed skin without enhanced skin absorption (Kim *et al.*, 1997). The study showed that percutaneous absorption of hydrocortisone across the stratum corneum-removed skin was significantly faster than that across the normal skin, suggesting that the SC behaved as a penetration barrier to the liposome-bound drug. It was found that the liposome gel reduced the skin absorption of hydrocortisone, compared with the conventional ointment formulation. The amount of hydrocortisone absorbed by the SC-removed skin from the liposome gel after 8 h was less than one-third of that from the conventional ointment. In addition, higher and sustained skin concentrations of hydrocortisone were achieved for the liposome-gel as compared to the ointment. After 4 h, the plasma concentration of hydrocortisone obtained from the liposome-gel was only one-fourth of that from the ointment ($p<0.01$). It was assumed that interaction of hydrocortisone with phosphatidylcholine, a component of the liposomes and skin, might retard the diffusion of the drug in the skin. However, the short retention time of the gel formulation prevented sufficient delivery of drug for therapeutic activity. Davis *et al.* (1997) patented supersaturated topical compositions of two miscible non-aqueous and non-volatile solvents for topical application. A composition of the

invention consisted of two distinct phases, which were mixed prior to the application on the skin surface. Although it was suitable for delivery of water-sensitive drugs, it was not easy for patient to prepare as a device and it was not bioadhesive to stick on the body surface well. Murdock *et al.* (2002) invented a non-occlusive, bioadhesive topical systems for transdermal administration of an amine compound to relieve pain. The topical systems were formulated in creams or gels form comprising active substance dispersed in solvent. Therefore, crystallization of drug by vaporization of solvent and degradation of drug upon exposure to the air were not avoided. Another study reported that a hydroalcoholic gel formulation with hydroxypropylcellulose was more suitable for piroxicam than an oil-in-water cream formulation (Rafiee-Tehrani and Mehramizi, 2000). This study proved that an effective amount of drug was released from the aqueous gel formulation. However, it was not suitable for water sensitive drugs like aspirin and morphine.

The anti-thrombotic action of aspirin on 19 healthy, male and female volunteers was examined by using two aspirin patch systems without (type A) and with (type B) limonene as a permeation enhancer (McAdam *et al*., 1995). Both type A and type B patches had the same surface area of 50 cm^2. The amount of aspirin was 84 mg in type A patch and 120 mg in type B patch. Daily application of two type A patches (total surface area of 100 cm^2 and total aspirin of 168 mg) for 14 days showed that 85% reduction in serum thromboxane B_2 (TXB_2) in six male subjects and 32% reduction in serum TXB_2 in four female subjects on day 14. Analysis of the residual drug in the patch showed that each patch delivered 18±3 mg of aspirin on day 1 and 17±4 mg of aspirin on day 14, with no difference between males and females. Daily application of

a single patch B for 21 days resulted in 60% suppression of serum TXB_2 on day 14 and 84% suppression on day 21 in nine male subjects. The results of analysis of the applied patches showed that patch B delivered 33±3 mg of aspirin daily. It was reported that no plasma aspirin was detected, whereas plasma salicylate was 157±38 ng/ml and 133±20 ng/ml on day 14 with patch A and patch B, respectively. Hydrolysis of aspirin to the inactive product, salicylic acid, was reported in three subjects. All volunteers exhibited mild and self-limiting skin reactions characterized by varying degrees of erythema and pruritus, more likely on the site of repeated application. Therefore, these patches are not safe for clinical use although they are therapeutically effective.

C. Evaluation of physical properties of plasters

1. Rheological properties of gels

Polymeric gels have been used as platforms for drug delivery, primarily as a result of the wide range of physicochemical properties offered by such systems (Jones *et al*., 1996 and 1997). Manipulations of the type and concentration of polymer used, the state of the polymer, the formation of gel networks and pH of the formulation are strategies that may be successfully used to engineer a defined product performance (Woolfson *et al*., 2001). As a result, several studies have examined the mechanical and rheological properties of gel systems using traditional rheological methods, e.g., flow rheometry, oscillatory rheometry and creep analysis. The rheological properties of poly(acrylic acid) gels have been characterized by dielectric spectroscopy and oscillatory rheometry in several studies (Barry, 1974; Craig *et al*., 1994). The rheological properties of cellulose ethers and of polymer gel networks have been described (Doelker, 1987 and Jones *et al*., 1997, 1998). Several studies on rheological (viscoelastic properties) and textural characteristics (hardness, compressibility,

adhesiveness and cohesiveness) of bioadhesive, semi-solid, polymeric systems containing active substances (drugs) by textural analysis and flow rheometry were reported by Jones *et al.* (1996, 1997, 1998, 2002, 2003). They used oscillatory rheometry and texture analyzer to examine the mechanical properties of bioadhesive polymeric gel for topical drug delivery. The relationship of polymer concentration and viscoelastic/mechanical properties of gel was reported. In oscillatory analysis, higher polymer concentrations increased the storage modulus (G′), the loss modulus (G″) and the dynamic viscosity (η′), yet decreased the loss tangent (tan δ). The relationships between G′ or G″ and frequency were observed to level off at higher frequencies, which is indicative of polymer chain entanglement and network formation. Increased viscosity of formulation product led to increased hardness, compressibility and work of syringeability of the formulation. Another study reported the viscoelastic properties of polymeric systems in a mixture of pharmaceutical solvents. The addition of water to nonaqueous Carbopol 934P solution caused the transformation of the low viscosity solution to a gel with significant elastic behavior, due to physical interaction and entanglement of the polymer segments with solvents such as propylene glycol, glycerol and water (Chu *et al*., 1992).

2. Bioadhesive properties

Bioadhesive polymers, as components of drug delivery systems, enable adhesion of the systems to skin surfaces and thus may facilitate drug delivery to defined sites. Presently, there is no universal test method for bioadhesion measurement and some results of bioadhesion studies reported in the literature appeared to be contradictory (Smart *et al*., 1984; Jones *et al*., 1996; Gandhi *et al*., 1994; Jones *et al*., 1997; Chary *et al*., 1999 ; Solomonidou *et al*., 2001). The results were dependent on the methods used.

Tensile testing is a simple method to study the adhesive properties of formulations. It involves the determination of the detachment force required to overcome the adhesive bond between the formulation and a substrate. It was reported that films based on polyvinylpyrrolidone were generally more mucoadhesive than corresponding formulations based on polyvinyl alcohol. The optimal mucoadhesive polymer concentration range was generally found to be between 2 and 10% (w/w). Higher polymer concentrations did not further enhance the mucoadhesive properties, and in some cases even decreased mucoadhesion. Tensile testing was used to determine the mucoadhesive properties of three structurally similar polyacrylic acid polymers that differ in the extent of cross-linking, namely Carbopol 974P, Carbopol 971P and Noveon AA-1 (polycarbophil). The hydration time was found to affect the mucoadhesive performance. The adhesiveness decreased rapidly when certain levels of hydration were achieved. The clinical performance of topical products is directly related to their hardness, compressibility, adhesiveness and cohesiveness (Solomonidou *et al*., 2001). Lin *et al.* (1993) have described the relationship between the viscoelastic properties of hydroxypropylmethylcellulose gels and their clinical performance. Tamburic and Craig (1995) also reported the relationship between adhesiveness of polymeric formulations and loss tangent. Therefore, in the development of formulations for topical application, the consideration should be given to the viscoelastic properties to ensure optimization of product performance. The adhesion properties of formulations were also evaluated by an *in vivo* method that employed a visual scoring system. The *in vivo* adhesiveness of two types of glyceryl trinitrate transdermal patch (Epinitril: EPI-10 and ND-10) was assessed to find a compromise between good and lasting adhesion and easiness of removal (Santoro *et al*., 2000). A slightly partial detachment of the border of the patch or formation of

creases occurred in most applications of EPI-10 and ND-10. Complete detachments or migrations were not observed. The difference of adhesion between EPI-10 and ND-10 was not significant. Due to inter-individual differences of the skin, a total and lasting adhesion in all subjects was seldom achieved.

The clinical and non-clinical performances of the polymer gels are dependent on their viscoelastic and bioadhesive properties. The cross-linked and viscoelastic gel ascertains the stability and durability of the plaster and also increases the bioadhesion of the plaster. Therefore, it is useful as a percutaneous delivery system to prolong drug residence time at the site of application and allows an enhancement in absorption of the drug.

D. *In vitro* drug release and skin permeation studies

The diffusion cell method is the standard method for *in vitro* percutaneous permeation studies. The vertical Franz diffusion cell is most commonly used in almost all of the *in vitro* studies on percutaneous absorption of drugs. A buffer solution is normally used for hydrophilic compounds (Skelly *et al*., 1987) and solubilizing additive is used for lipophilic compounds (Moser *et al*., 2001) for the receptor compartment of the diffusion cell.

The human skin is the best and most relevant skin model for *in vitro* percutaneous absorption studies (Sekkat and Guy, 2001). Skins of animals, such as mouse, rat, guinea pig, rabbit and pig, have been used as a model skin because the availability of human skin is limited. The pig skin most resembles the human skin in histological and biochemical properties (Gray and Yardley, 1975; Meyer *et al*., 1978; Klain *et al*.,

1979). The epidermis from the pig ear skin was considered a good model for the study of percutaneous absorption of drugs and the effect of penetration enhancers on skin (Kligman and Christophers, 1963; Chambin *et al*., 1993; Hoeck *et al*., 1997; Bhatia and Singh, 1997; Levang *et al*., 1999). A few studies have proven that the permeability of drugs through pig skin is similar to that through human skin (Roberts and Mueller, 1990; Dick and Scott, 1992; Pendlington *et al*., 1998). Other animal skin models show higher skin permeation rates than that of human skin models. The excised human breast skin showed lower penetration rate than skin from other anatomical sites (Harada *et al*., 1993). After comparing four animal skin membranes (mouse, rat, rabbit, pig) and human skin, Wu *et al.* (1997) proved that the excised human breast skin was the least permeable of all the skin type and it had no significant difference in permeation compared with pig skin.

The permeation of lipophilic drugs is significantly and artificially impeded *in vitro* when the skin membrane includes a significant part of the dermis. Therefore, the epidermis is used for lipophilic drugs (Hawkins and Reifenrath, 1986; Kemppainen *et al*., 1991; Reifenrath *et al*., 1991; Tata *et al*., 1994). For polar drugs of high aqueous solubility, the stratum corneum barrier is completely rate limited; hence, stripped skin (skin without stratum corneum) is recommended (Scott *et al*., 1992). Full-thickness human cadaver skin was used for *in vitro* skin permeation study of naloxone and examination of the effects of various penetration enhancers on skin (Aungst *et al*., 1986). The other studies using the viable skin (epidermis plus dermis) and stripped skin (stratum corneum removed skin) proved that percutaneous absorption of hydrocortisone across stripped skin was significantly faster than that across normal

viable skin, suggesting that stratum corneum behaves as a penetration barrier for the liposome gel formulation (Kim *et al*., 1997).

Several studies have proven that different types of penetration enhancers had different effects on *in vitro* percutaneous absorption of active substances. The most commonly used enhancers are ethanol, propylene glycol, fatty acids, fatty alcohols, aliphatic esters, surfactants, sulfoxides, amides and N-methyl-2-pyrrolidone (NMP). Propylene glycol, ethanol and N-methyl-2-pyrrolidone increase drug solubility in the skin and enhance skin permeation. The stratum corneum uptake of propylene glycol led to higher solubility of ibuprofen in the skin (Irwin *et al.*, 1990) and increased skin permeation of metronidazole (Wotton *et al.*, 1985). Bhatia and Singh (1997) proved that the higher permeability of luteinizing hormone-releasing hormone (LHRH) through pig epidermis was observed with NMP and isopropyl myristate. However, Park *et al.* (2001) found that only fatty alcohols enhanced the skin permeation of captopril and no significant enhancing effect was produced by dimethyl sulfoxide, NMP and oleic acid. *In vitro* release of drug from a topical product might also be used to predict *in vivo* performance. A rank order correlation between *in vitro* release rate and *in vivo* blanching intensity has been observed for two brands of betamethasone valerate cream in healthy subjects (Shah *et al*., 1992). A similar rank order correlation has been observed for two brands of hydrocortisone cream between *in vitro* release through a synthetic membrane and drug concentration in the stratum corneum. The correlation with skin blanching in healthy subjects was also shown in this study (Caron *et al*., 1990)

E. Stability of aspirin

Aspirin is a moisture sensitive drug that breaks down to give acetic and salicylic acids. The degradation of aspirin has been generally considered to be due to a hydrolytic reaction in the presence of water. It also decomposes in polyethylene glycol by a transesterification process. Therefore, its stability is often the initial concern in formulation development.

Spancake *et al.* (1991) and Garrett (1957) extensively studied the hydrolysis of aspirin in aqueous solutions. The effect of water on the rate of degradation of aspirin was greater in methoxypolyethylene glycol than in polyethylene glycol. A small effect of added water on the degradation of aspirin in polyethylene glycol suggested that aspirin molecules might be entrapped in polyethylene glycol in the presence of water, reducing a molecular contact between water and aspirin. The greater effect of water on degradation of aspirin in methoxypolyethylene glycol might be due to the fact that in methoxypolyethylene glycol a transesterification reaction was partly blocked and when water was added the unreacted aspirin became available for hydrolysis. It was concluded that stability of aspirin might be achieved in polyhydric alcohols by blocking the free hydroxyl group in aspirin molecule. Citric acid tended to slow down the decomposition process even in the presence of added water (Whitworth *et al*., 1973). Whitworth and Asker (1973) also reported that aspirin demonstrated the greatest stability in decaglycerol octaoleate and the lowest stability in decaglycerol tetraoleate at 4, 26 and 45°C. They proved that the hydroxyl value and the viscosity of the polyglycerol ester influenced the stability of aspirin. Excipients can have effects on aspirin stability under accelerated stability conditions. Cunningham and Scattergood (2001) examined the effect of Starch 1500, a partially pregelatinized starch, in

combination with microcrystalline cellulose and two hydrophilic superdisintegrants on the stability of aspirin tablets. Starch 1500 has a lower propensity for moisture absorption than either croscarmellose sodium or sodium starch glycolate. Thus, Starch 1500 inhibited water activity within the formulation and retarding moisture interaction with aspirin. It was found that Starch 1500 reduced or eliminated the deleterious effects of other excipients in the study.

Accelerated stability studies are a common approach for predicting the long-term stability of pharmaceutical formulations. The chemical and physical stability of nifedipine sustained release dosage forms prepared with Gelucire® 53/10 was investigated by using accelerated stability study. This study suggested that moisture-resistant packaging was useful for the acceptable shelf-life of this type of dosage form (Remunan *et al*., 1992). Another accelerated stability study showed that the solid state of contraceptive steroid, Nestorone (in powder form or incorporated into silastic implants) did not undergo detectable degradation even under severe experimental conditions (Ahmed *et al*., 1995). Gleditsch and Waaler (2001) evaluated the shelf life of sodium metabisulphite stabilized morphine injections in plastic ampoules at temperatures ranging from 50 to 80°C. Proniuk *et al.* (2002) suggested that non-aqueous solvents such as glycerin and Transcutol P should be utilized in the development of a topical formulation for stability of the active ingredients. Although many studies proved that accelerated stability studies predict the long term stability study for preformulation study of pharmaceutical products, Nakamura *et al.* (2002) found that accelerated stability testing failed to predict the stability of minodronic acid liquid formulation stored in SiO_2 treated glass ampoules. It was due to the complex formation between the drug and aluminum ions leached from the glass of regular

ampoules. This complex formation was exothermic reaction and could not be observed at elevated temperatures. Therefore, the accelerated stability testing failed to predict the stability of minodronic acid injection liquid.

F. Skin irritation studies

1. Patch testing and VAS assessment of skin irritation

Many consumer product companies developing topical medications are interested in assessing skin irritation before further development of the final product. Patch testing is frequently used to determine the irritation potential of topical agents. Mills *et al.* (1998) conducted two randomized clinical trials on 21 and 18 subjects respectively to assess the irritation potential of commercially available tretinoin formulations, compared with tretinoin formulations containing polyolprepolymer-2. The study employed a standard Hill Top occlusive chamber (25 mm diameter) and a visual scoring system for grading irritation.

Cumulative irritation is most often evaluated in humans, however, a variety of exaggerated exposure tests have been employed in animals. The modifications of the method described by Draize *et al*. (1944a, 1944b) are used to evaluate primary irritation and corrosion in animal tests. Haeberlin (1959) reported the use of a guinea pig model to demonstrate heightened irritability of the skin due to irritant dermatitis to 40% croton oil. However, this observation differed from that seen in humans, in that a more extensive or chronic dermatitis did not further heighten the susceptibility to irritation (Roper and Jones, 1985). Another study used rabbit to determine the relative irritancy of three cosmetic samples of known human irritancy. In this study, the visual estimation of irritation was based on the Uttley and van Abbe's method (1973).

Allergic and toxic contact reactions in guinea pigs have been studied with the naked eye assessment of erythema and oedema (Anderson and Staberg, 1985). Another study reported that sodium lauryl sulphate induced skin irritation on guinea pigs was studied with naked eye assessment method (Anderson *et al*., 1986). Although VAS method is common and useful technique to assess skin irritation, it has some disadvantages such as inter-individual variance among examiner's assessments and qualitative detection of visible skin irritation.

2. Laser Doppler perfusion imager assessment of skin irritation

The objective, non-invasive and quantitative assessment technique is desirable to complement the VAS method. This is made possible with laser Doppler perfusion scanning technology (Serup and Jemec, 1995). Laser Doppler velocimetry has been widely used to investigate vascular disorders and physiopathological changes in skin microcirculation. It is a continuous and non-invasive recording of microvascular tissue perfusion, which enables simultaneous measurement of average velocity and concentration of blood cells in the illuminated tissue volume. Such tissue perfusion measurements typically show large variations due to the high spatial resolution of this technique, even at adjacent sites. Therefore, it might be useful to represent perfusion as an image rather than as a single value recorded at one point (Issachar *et al*., 1998). This study was further studied by Sorensen *et al.* (1996) on two patients with reflex sympathetic dystrophy. The technique has been used to scan superficial blood perfusion in patch test reactions (Fischer and Bjarnason, 1996; Quinn *et al*., 1993; Mattsson *et al*., 1997; Wardell *et al*., 1996; Bjarnason *et al*., 1999; Fullerton *et al*., 2001 & 2002), as well as in other applications (Harrison *et al*., 1993; Mayrovitz and Carta, 1996; Wang *et al*., 1997; Fullerton *et al*., 1995; Mannor *et al*., 1996). Bjarnason *et al.* (1999) supported the claim that the laser Doppler perfusion scanning technology

is valuable for non-invasive objective assessment of patch test reactions. Laser Doppler imaging was found to be an important new method for characterization and grading of the inflammatory response of single exposure irritant reactions (Fullerton *et al*., 2002). However, Noon *et al.* (1996) concluded that laser Doppler instruments, including the novel scanning perfusion imager, did not detect glucocorticoid-induced skin blanching, perhaps because it reflected venular rather than arteriolar vasoconstriction.

The correlation between laser Doppler perfusion imaging and visual scoring of patch test sites was studied in subjects with experimentally induced allergic and irritant contact reactions. It was found that LDPI correlates with VAS in contact allergic reactions, but not in irritant reactions (Goon *et al*., 2004).

G. Summary

In summary, topical application of aspirin is an attractive mode of delivery due to its indications for treatment of itch and pain and its advantages of less systemic adverse effects on the gastrointestinal tract. Although different types of formulations for topical aspirin have been studied, none of them was successful due to instability and skin irritation of aspirin. It is important to develop a strong bioadhesive topical formulation to achieve a safe, stable and therapeutic topical application of aspirin. Measurement of viscoelastic properties of aspirin gel by rheometer, measurement of detachment force of aspirin plasters by tensile tester and animal adhesion test are basic methods to assess the physical properties and bioadhesiveness of aspirin plasters. Different types of bioadhesive polymers and chemical penetration enhancers play essential roles in drug release and skin permeation of drug. *In vitro* drug release study and skin permeation

through pig skin and human skin using vertical diffusion cells give good estimation of amount of drug released and permeated through skin *in vivo*. Accelerated stability testing is a convenient method to assess the shelf-life of final product in short period. Quantitative assessment of skin irritation using non-invasive techniques such as LDPI can be a useful objective tool complementing qualitative assessment of skin erythema by VAS.

CHAPTER 3

AIMS AND OBJECTIVES

The aim of this study is to develop a safe, stable, bioadhesive and non-aqueous aspirin gel plaster for the treatment of localized pain and itch.

Hence, the objectives of this study were:

1. To formulate a non-aqueous, bioadhesive aspirin gel plaster.
2. To study physical properties of the polymer-drug matrix gels using rheometer and tensile tester.
3. To study bioadhesion of the plaster in pigs.
4. To study *in vitro* drug release through synthetic membranes using Franz diffusion cells.
5. To study *in vitro* skin permeation using pig and human skins.
6. To study stability of aspirin plaster using accelerated stability testing.
7. To study *in vivo* skin irritation of the plaster using visual analogue scoring method and laser Doppler perfusion imager technique in pigs.

CHAPTER 4

EXPERIMENTAL METHODS

A. Materials

Copolymer of N-vinylacetamide and sodium acrylate (PNVA, VIAC GE-167, Lot No 820505) was supplied by SHOWA DENKO K.K (Japan). Aluminum chloride anhydrous ($AlCl_3$) was obtained from Fluka Chemika (Germany). Aspirin (purity 99.5% to 100%, BP grade) and salicylic acid (purity 99%, BP grade) were purchased from Sigma-Aldrich Pte, Ltd, Singapore. N-methyl-2-pyrrolidinone (Pharmasolve®, NMP) was supplied by International Specialty Product (ISP, USA). Propylene glycol (PG) was obtained from BDH Laboratory (UK). Absolute alcohol (ethanol) and methanol (analytical grade) were procured from Fisher Scientific (UK). Acetonitrile (HPLC grade) was obtained from EM Sciences, MERCK (Germany). Potassium dihydrogenphosphate salt was obtained from Sigma Chemical (USA).

B. Formulation of aspirin plasters

The gel was made by using PNVA polymer as a matrix material and aluminum chloride as a cross-linking agent. Specific amounts of polymer and aspirin were completely dissolved in a mixture of NMP, PG and ethanol. The final solution was poured into a plastic petri dish of 85 mm diameter and allowed to stand overnight to remove air bubbles. The required amount of aluminum chloride in a mixture of ethanol and glycerin, at a weight ratio of 20 to 1, was gently added onto the above mixture on the following day. The final mixture in the petri dish was dried at room temperature for

2 days to evaporate the ethanol and then left in a desiccator for a week to avoid moisture. The cross-linked gel was transferred onto a non-woven cotton wool backing layer and protected by a plastic release liner. Three formulation variables were studied and optimized, including polymer concentration, polymer to cross-linking agent ratio and PG to NMP ratio (Table 3). The amount of aspirin-containing casting solution in each petri dish was about 3.4 to 4.4 g and the amount of aspirin per square centimeter of plaster was approximately 1.4 mg.The aspirin plasters with different amounts of aspirin (200, 300 and 400mg) were also formulated with polymer concentration of 9%, polymer to cross-linking agent ratio of 1.5:1 and PG to NMP ratio of 2:1 to assess the effect of aspirin itself on skin irritation.

C. Measurement of rheological properties of gels

Oscillatory rheological measurements were carried out using a rheometer (RheoStress® 1 Haake, Germany) with a cone-plate sensor system of diameter 35 mm and cone angle of 1º. Samples were applied to the lower plate and allowed to equilibrate to the measurement temperature of 25°C. The linear viscoelastic regions for the gels were determined by torque sweep studies from 0.5 to 500 Pa at a frequency of 10 Hz. A torque value representative of the linear viscoelastic region was chosen for the frequency sweep analysis. The oscillatory measurements were performed over a frequency range of 1 to 10 Hz at a stress of 10 Pa at 25°C. Rheological parameters including the elastic modulus (G′), viscous modulus (G″) and loss tangent (tan δ) were obtained respectively. Each datum point was the mean of at least three measurements.

Table 3. Compositions of polymer-drug matrix gels

Formulation variables	Amount of ingredients in polymer-drug matrix gels (g)					
	PNVA	$AlCl_3$	PG	NMP	Glycerin	Aspirin
Polymer concentration						
5%	0.17	0.11	2.08	1.06	0.70	0.10
7%	0.23	0.15	2.05	1.02	0.70	0.10
9%	0.30	0.20	2.00	1.00	0.70	0.10
11%	0.36	0.24	1.95	1.00	0.70	0.10
13%	0.43	0.29	1.91	0.96	0.70	0.10
PNVA to $AlCl_3$ ratio						
2.5:1	0.29	0.12	2.00	1.00	0.70	0.10
2:1	0.29	0.15	2.00	1.00	0.70	0.10
1.5:1	0.30	0.20	2.00	1.00	0.70	0.10
1:1	0.29	0.29	2.00	1.00	0.70	0.10
1:1.5	0.29	0.45	2.00	1.00	0.70	0.10
PG to NMP ratio						
1:2	0.30	0.2	1.00	2.00	0.70	0.10
1:1	0.30	0.2	1.50	1.50	0.70	0.10
2:1	0.30	0.2	2.00	1.00	0.70	0.10
3:1	0.30	0.2	2.25	0.75	0.70	0.10
4:1	0.30	0.2	2.4	0.60	0.70	0.10

D. Bioadhesion studies

1. Tensile testing for bioadhesion

The bioadhesive properties of the plasters were investigated by using a tensile tester (Shimadzu, EZ tester, Japan) in the tensile mode. The plaster was cut into strips of 1.5×1.5 cm size. The strip for tensile testing was adhered to the lower stationary compression plate of the tensile tester with double-sided adhesive tapes. The model substrate, a silicone elastomer, which was used to simulate the skin in terms of bioadhesion, was mounted onto the upper plate. The upper plate with the substrate attached was then lowered to the surface of the plaster and allowed to remain in contact with the plaster for 30 s under load of 10 N. It was then raised at a speed of 100 mm/min to detach the substrate from the plaster. The detachment forces, which represented the bioadhesive properties of the plasters in this study, were recorded. Six strips were evaluated for each plaster and the results averaged.

2. Bioadhesion study in pigs

The bioadhesive properties of the plasters in pigs were evaluated by a scoring method (Hill Top Research, Inc., East Brunswick, USA). Yorkshire pigs (female, 11-15 weeks old and 25-35 kg) were used in this study. The dorsal area of pigs, under general anaesthesia (intramuscular injection of Atrosite 0.07 mg/kg and Ketapex 20 mg/kg), was clipped free of hair one day prior to the commencement of the test. Care was taken to avoid abrading the skin. Each pig was housed in individual cage. Different formulations of aspirin plaster were applied on the dorsal area of the pigs for 8 h. An adhesion score was assigned according to the percentage of the plaster adhered to the skin (Table 1). Three determinations were made for each formulation and the results averaged.

E. Drug assay by HPLC

Aspirin and salicylic acid were assayed by using an HPLC system (Thermo-separation Products USA), consisting of gradient pumps (Spectra system P4000), a vacuum membrane degasser (SCM1000), photo diode array detector (Spectra system SN4000) and autosampler (Spectra system AS3000). Separation of aspirin and salicylic acid was performed using a column (Hypersil BDS-C18, 4.6×100 mm, 3 μm, Agilent Technologies, USA) and a guard column (Hypersil ODS, 4.0×20 mm, 5 μm, Agilent technologies, USA) at 40°C. The mobile phase consisted of 70% v/v phosphate buffer (0.02 M KH_2PO_4 in water adjusted to pH 2.6 with 0.1 M 85% orthophosphoric acid solution) and 30% v/v acetonitrile. The flow rate was set at 1 ml/min. Aspirin and salicylic acid were detected at 230 nm. The retention times of aspirin and salicylic acid were 3.2 and 4.1 min respectively.

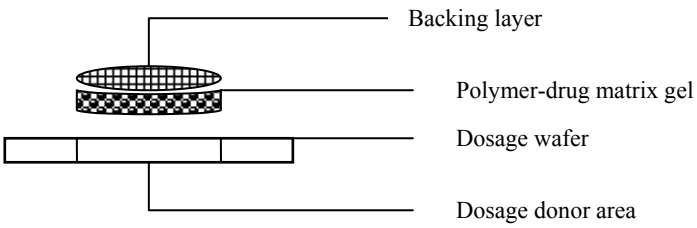
Aspirin and salicylic acid solutions in mobile phase were freshly prepared before injection into the column. Five concentrations of aspirin and salicylic acid ranging from 1 to 5 μg/ml were prepared on a daily basis by dilution of the stock solutions (100 μg/ml) with the mobile phase, respectively. The peak area of each compound was plotted against the concentration to obtain the calibration curves. Accuracy and precision were evaluated with values obtained from analyses of five standard samples for intra-day and inter-day variation. Accuracy was calculated as percentage of the concentrations measured versus the known concentrations. Precision was determined as the coefficient of variation (C.V.).

F. Determination of drug content of the plasters

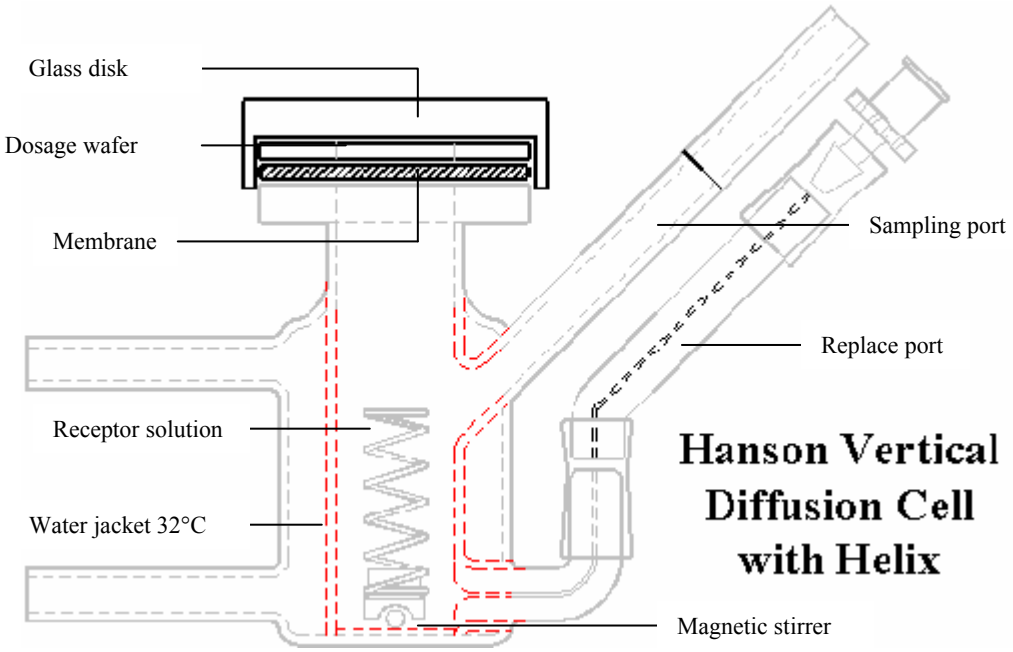
Aspirin plaster was cut into square pieces of 1 cm^2 size and dissolved in 5 ml of pH 7.4 phosphate buffer for 1 h. 50 μl of aliquot was diluted with 700 μl of mobile phase and assayed with HPLC. The aspirin content was calculated from the total amount of aspirin divided by the area of plaster. The uniformity of the drug in the plaster was also determined by assaying the drug content from five different areas of the plaster and the results were averaged.

G. Drug release and permeation studies

Vertical Franz diffusion cell system (MicroettePlus system, Hanson Research, USA) was used in the drug release and permeation studies (Figure 3). The dosage wafer loaded with aspirin plaster was assembled on the synthetic membrane, a regenerated cellulose membrane (Spectrum Laboratories Inc, USA) with pore size of 0.45 μm. This dosage wafer with synthetic membrane, facing downwards, was mounted onto the receptor compartment. The receptor compartment was filled with 7 ml of pH 7.4 phosphate buffer solution at 32°C which was kept stirring at 100 rpm by helix mixer over the course of the experiment. The area of the plaster available for drug release was 1.8 cm^2. At predetermined time intervals over a period of 12 h, 1.5 ml of samples were withdrawn from the receptor compartment with replacement of an equal volume of fresh pH 7.4 phosphate buffer solution to maintain constant volume. The samples obtained were analyzed by HPLC and cumulative amounts of drug released or permeated were determined. All the experiments were repeated at least six times and the results were averaged.



(a)



(b)

Figure 3. Diagram of (a) application of aspirin plaster into the dosage wafer and (b) the vertical diffusion cell used in MicroettePlus system

The vertical Franz diffusion cell system (MicroettePlus system, Hanson Research, USA) was also used for the skin permeation studies. The test procedure was similar to that of the drug release studies, except that the epidermis from pig abdominal skin and human abdominal skin were used. Six epidermis pieces were used for each formulation of the aspirin plaster. The pigs (Yorkshire, female, 25–35 kg, 11–15 weeks old) used to harvest abdominal skin were sacrificed and the skins obtained within half an hour. The human abdominal skin was obtained from patients undergoing liposuction operation (male and female, 35–65 years, Chinese). The consent of the patients was taken to use their disposed skin in this study. The human epidermis was removed by using heat separation technique. The human epidermis was taken after the whole skin had been soaked in water at 60°C for 2 min and rinsed with normal saline solution. It was then rinsed several times with distilled water until all sodium chloride was removed and stored at –80°C until use. Prior to use, the epidermis was hydrated by soaking in pH 7.4 phosphate buffer for 30 min.

The aspirin plaster with polymer concentration of 9%, polymer to cross-linking agent ratio of 1.5:1 and PG to NMP ratio of 2:1 was used to compare the amount of aspirin permeated through 3 different barriers: synthetic membrane, pig skin and human skin. Six samples were used for each barrier. The amount of aspirin permeated through 3 barriers was obtained as same procedure mentioned in drug release and permeation studies.

H. Accelerated stability testing

The aspirin plasters were stored in a climatic chamber (KBF 240WTB Binder Labortechnik, Germany) at an elevated temperature of 40°C and relative humidity of

75% for 4 weeks. Samples were then removed and assayed for aspirin and salicylic acid using HPLC according to the procedure described previously (Section G).

I. Skin irritation studies

The dorsal area of healthy female Yorkshire pigs (11−15 weeks old), under general anaesthesia (intramuscular injection of Atrosite 0.07 mg/kg and Ketapex 20 mg/kg body weight of pig), were clipped free of hair one day prior to the commencement of the test. Care was taken to avoid abrading the skin. Three determinations on three different pigs were made for each formulation. Each pig was housed in individual cage. Test plasters with different formulations were applied on dorsal area of pig with 2.5 cm distance and placebo plasters were applied parallel to their corresponding test plasters. The test and placebo plasters were replaced with the new plasters of the same formulations on a 2-day interval basis. All test sites were covered with surgical gauze. The pigs were housed at room temperature 20±2°C and relative humidity of 30 to 70% for 14 days. On the day of measurement, the pigs were allowed to be accommodated to temperature and humidity of the environment in the test room (20±2°C, 30 to 70% R.H.) before the plasters removed. The condition of the skin at the center of application site was then evaluated by the visual scoring method and the cutaneous blood flow of the skin were measured by laser Doppler perfusion imager (Lisca Development AB, Sweden) on day 0 before application of the plasters and 15 minutes after removal of the plasters on day 2, day 4, day 6, day 8, day 12 and day 14. 3 results reading of VAS and LDPI of 3 pigs for each formulation were averaged and compared among different polymer concentrations, different polymer to cross-linking agent ratios, different PG to NMP ratios and also among different days of assessment by

using two-way ANOVA. The results were compared between placebo plasters and their corresponding aspirin plasters by using Student's paired t-test. The plasters with aspirin concentrations of 200, 300 and 400 mg were applied for 14 days as same as above mentioned procedure and assessed the skin irritation with VAS and LDPI methods.

Visual analogue scale was used to assess skin erythema with visual assessment scores ranging from 0 (no irritation) to 7 (strong reaction). The test sites were examined in standard lighting conditions and given a score by a trained independent observer who was unaware of the order of type of plaster applied.

In LDPI testing, the test areas were assessed using a laser head aperture at a distance of 17 cm from the center of the site of plaster application. A back ground threshold of 6.1 V, high resolution, an amplification factor of 1 and an angle as close to 90° as possible between the LDPI laser beam and the center of the plaster were set. A total number of 256 test sites (16×16 mm) were scanned. The mean flux was calculated using LDI 2.5 software. Mean LDPI values were expressed as a percentage of voltage. Biological zero (LDPI values measured on normal healthy skin of dorsal area of pigs on day 0) was subtracted from all readings.

J. Data processing and analysis

The following steps were carried out in data processing and analysis.

1. Before the analysis of the data, a thorough check of the experimental data was done to avoid any mistakes.

2. Throughout this study, regression equation and regression coefficient were used.
3. Two-way ANOVA and general linear model were used to analyze the relationship of formulation factors, such as polymer concentration, polymer to cross-linking agent ratio and PG to NMP ratio, on drug release and permeation, viscoelastic properties, detachment force, bioadhesion scores, VAS and LDPI results.
4. Student's paired t-test was used to assess the differences of LDPI and VAS between aspirin plasters and control plasters.
5. The Pearson's correlation coefficients were calculated to assess the relationship among different formulation variables and viscoelastic properties, drug release, drug permeation and skin irritation.
6. The level of statistical significance was set at < 0.05.
7. The results were analyzed using SPSS-PC version 10.0 (SPSS 10.0, 2000).

CHAPTER 5

RESULTS

A. Formulation of aspirin plasters

Figure 4 shows the structure of the aspirin plasters. It consisted of three layers, namely, a backing layer, a polymer-drug matrix gel and a release liner. The backing layer and release liner had thickness of 0.03 and 0.13 mm, respectively. The thickness of the gel varied from 0.23 to 0.54 mm, depending on the formulation. Drug was dissolved evenly in the polymer matrix gel. The weight/area ratios of the plasters prepared from the same formulation demonstrated low standard deviation values, indicating good reproducibility in the production of aspirin plasters.

The polymer concentration, polymer to cross-linking agent ratio and PG to NMP ratio had different effects on the physical properties of the polymer-drug matrix gels. The physical properties of these polymer-drug matrix gels are summarized in Table 4. All polymer-drug matrix gels obtained from different formulations were transparent gels. With increasing polymer concentration, the polymer-drug matrix gels formed became harder and less bioadhesive. The formulation of 5% polymer produced soft and liquid-like polymer-drug matrix gel. When it came in contact with skin, the moderately wet and viscous surface left viscous gel on the site of application. The polymer-drug matrix gel with 7% polymer concentration was soft and semisolid. Its slightly wet and viscous surface left semisolid gel pieces on the site of application. The formulation of 9%

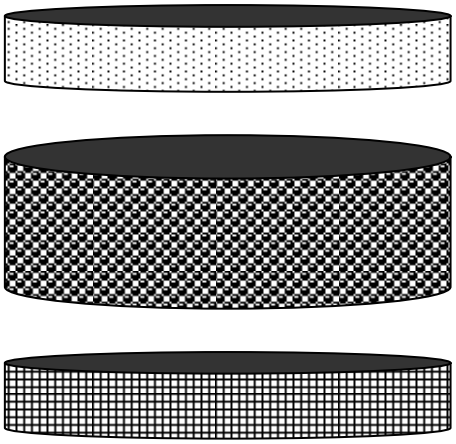


Figure 4. The structure of the aspirin plasters

Table 4. Physical properties of polymer-drug matrix gels

Formulation variables	Texture	State	Type of Surface	Comment
PNVA				
5%	soft	liquid	moderately wet & viscous	liquid gel left on skin after removal of plaster
7%	soft	semi-solid	slightly wet & viscous	left semisolid gel on skin after removal of plaster
9%	soft & elastic	solid	dry & smooth	left no remnants on skin after removal of plaster
11%	firm	solid	dry & slightly rough	adhere to the skin for few hours
13%	hard	solid	very dry & rough	adhere to the skin with applied pressure for few hours
PNVA to $AlCl_3$ ratio				
2.5:1	soft	glue semi-solid	smooth sticky	left sticky gel on skin after removal of plaster
2:1	soft	semi-solid	slightly sticky	semisolid gel left on skin after removal of plaster
1.5:1	soft & elastic	solid	dry & smooth	left no remnants on skin after removal of plaster
1:1	firm	solid	dry	adhere to skin for few hours
1:1.5	hard	solid	very dry	adhere to skin for few hours
PG to NMP ratio				
1:2	soft	solid	smooth sticky	left sticky gel on skin after removal of plaster
1:1	soft	solid	slightly sticky	left sticky gel on skin after removal of plaster
2:1	soft & elastic	solid	dry & smooth	left no remnants on skin after removal of plaster
3:1	soft and viscous	solid	viscous	left viscous gel on skin after removal of plaster
4:1	soft and viscous	solid	viscous	left viscous gel on skin after removal of plaster

polymer produced soft, elastic and solid-like polymer-drug matrix gel. Its smooth and dry surface left no residues on the skin upon removal from the site of application. The.polymer-drug matrix gel formulated with 11% polymer concentration was firm and solid which showed slightly rough and dry surface that adhered on the skin surface for few hours. The formulation of 13% polymer formed a hard and solid polymer-drug matrix gel with moderately rough and very dry surface which adhered on the skin surface with applied pressure for few hours.

Increasing the ratio of polymer to cross-linking agent decreased the cross-linking density of the polymer-drug matrix gel and it led to the formation of a more sticky gel. With polymer to cross-linking agent ratio of 2.5:1, glue like semisolid polymer-drug matrix gel was formed. The surface of the gel matrix was smooth but sticky and a sticky gel was often left on the skin after removal of the plaster. At a ratio of 2:1, the semisolid polymer-drug matrix gel showed a smooth and slightly sticky surface. After removal, the plaster left behind semisolid gel pieces at the site of application. The formulation with a polymer to cross-linking agent ratio of 1.5:1 formed a soft and elastic solid polymer-drug matrix gel which had a smooth and dry surface. It could be removed from the skin without leaving behind any debris. At a ratio of 1:1, the solid polymer-drug matrix gel formed firm and dry surface which made plaster adhered to the skin for few hours. The polymer to cross-linking agent ratio of 1:1.5 gave hard solid polymer-drug matrix gel and formed plaster with very dry and less adhesive surface to skin.

The PG to NMP ratio also influenced the physical properties of the gels. A solid polymer-drug matrix gel with smooth sticky surface was formed with PG to NMP

ratios of 1:2 and 1:1 and left sticky gel on skin after removal of plaster. The solid polymer-drug matrix gel with PG to NMP ratio of 2:1 showed smooth and dry surface and did not leave any debris behind upon removal of the plasters. The formulations with PG to NMP ratios of 3:1 and 4:1 produced solid polymer-drug matrix gel with viscous surface. Although these plasters were viscous and adhered strongly to the skin, they left viscous gel on the skin after removal of the plasters. In summary, formulation with polymer concentration of 9%, polymer to cross-linking agent ratio of 1.5:1 and PG to NMP ratio of 2:1 seemed to show acceptable physical properties.

B. Evaluation of rheological properties of polymer-drug matrix gels andbioadhesive properties of plasters

1. Measurement of rheological behavior of gels

1.1 The effect of polymer concentration

All the formulations with different polymer concentrations showed that G′ values were higher than G″ values and loss tangent values were less than 1. Moreover, loss tangent values were not influenced by the frequency of oscillation, indicating that all the gels were cross-linked gels and the network of the cross-linked gels would not change with oscillation frequency. The lowest value of loss tangent was obtained from the gel with the highest polymer concentration of 13% and the highest value of loss tangent was obtained from the gel with 5% of polymer (Figure 5). The loss tangent was negatively and significantly correlated with the concentration of polymer used (Pearson's correlation, $p<0.05$).

1.2 The effect of polymer to cross-linking agent ratio

All the values of G′ were higher than those of G″ and the values of loss tangent were less than 1 for all the formulations with different ratios of polymer to cross-linking

agent. The loss tangent values were relatively independent of the frequency of oscillation. The lowest loss tangent was obtained from the strongest cross-linked gel formulation with polymer to cross-linking agent ratio of 1:1.5 and the highest loss tangent was obtained from the weakest cross-linked gel formulation with polymer to cross-linking agent ratio of 2.5:1 (Figure 6). The loss tangent was negatively and significantly correlated with the amount of cross-linking agent in the ratio of polymer to cross-linking agent (Pearson's correlation, $p<0.05$).

1.3 The effect of PG to NMP ratio

The G′ values obtained from the plasters with different ratios of PG to NMP were higher than those of G″. The values of loss tangent were less than 1 and were not influenced by the frequency of oscillation. The lowest value of loss tangent was shown with the highest PG to NMP ratio of 4:1 and the highest loss tangent was seen with the lowest PG to NMP ratio of 1:2 (Figure 7). The loss tangent was negatively and significantly correlated with the PG (Pearson's correlation, $p<0.05$). This indicated that the loss tangent was decreased with increase in the proportion of PG in the plaster formulations. In summary, the values of G′ were higher than the values of G″ for all formulations, indicating that all gels showed elastic behaviors. The loss tangent values were less than 1 and were independent of oscillation frequency, indicating that all the gels had elastic, cross-linked network structures. The formulation variables studied exerted different effects on the rheological/mechanical properties of the plasters as shown in the changes of loss tangent values with the compositions.

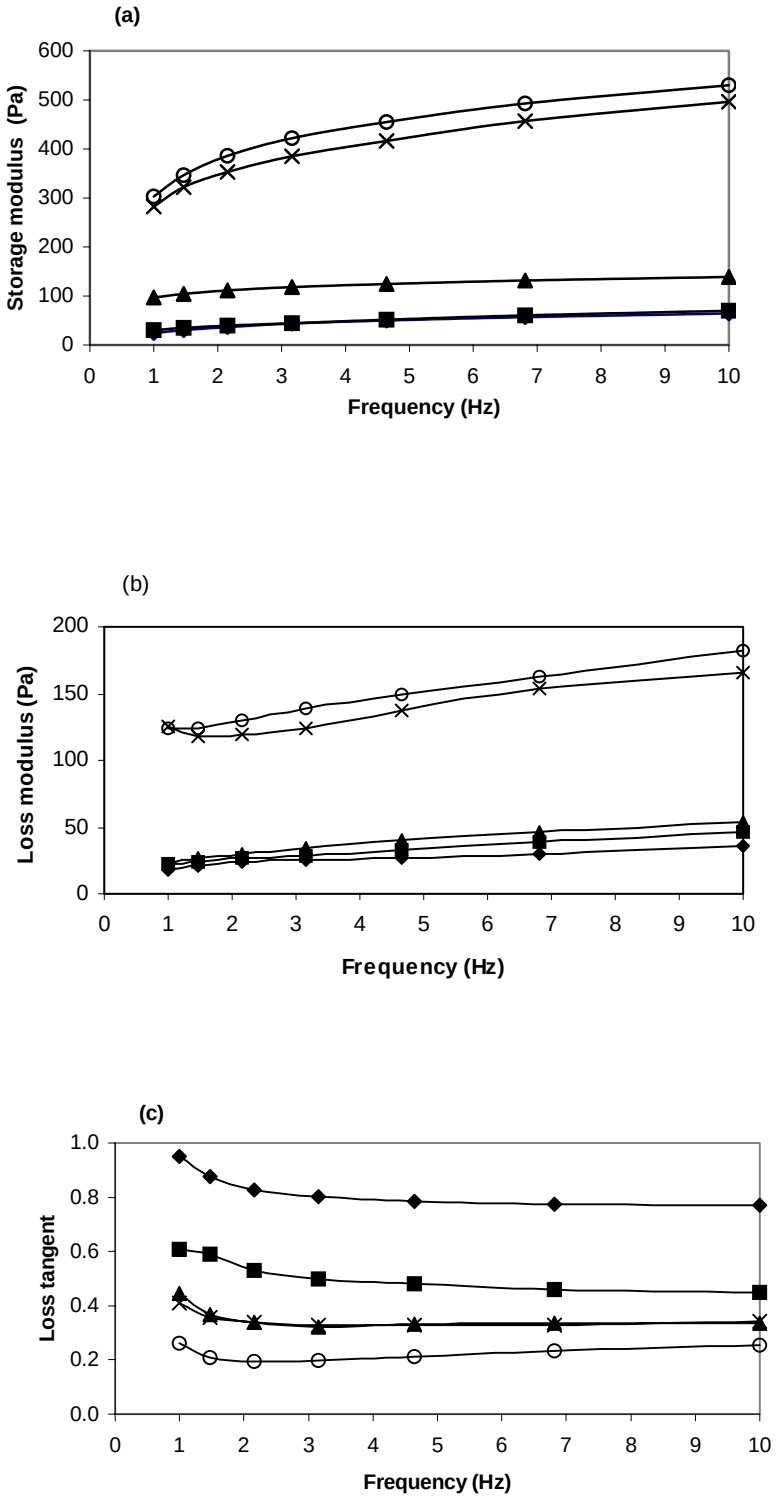


Figure 5. Comparison of dynamic moduli and loss tangent values among the plasters with polymer concentrations of 5% (◆), 7% (■), 9% (▲), 11% (x) and 13% (○)

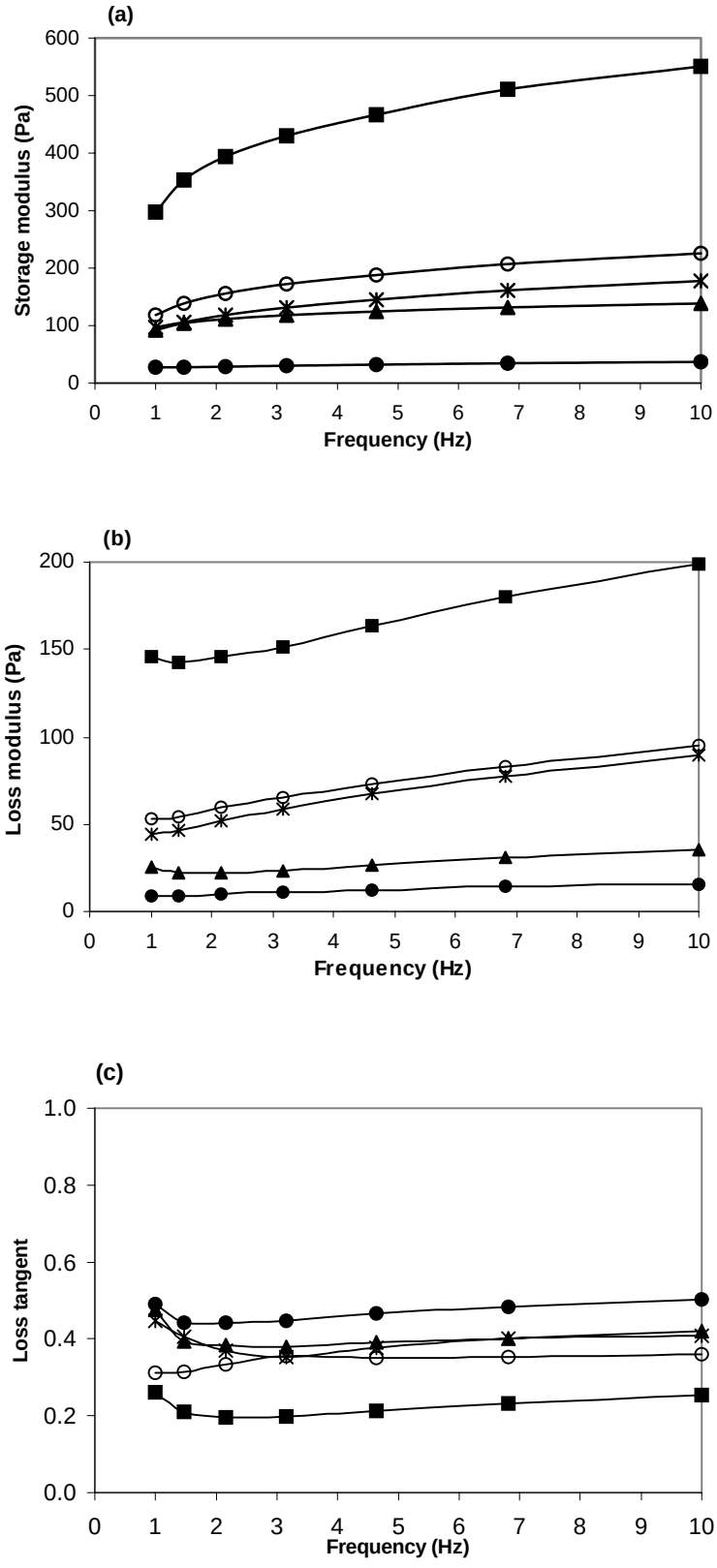


Figure 6. Comparison of dynamic moduli and loss tangent values among the plasters with polymer to cross-linking agent ratios of 2.5:1 (●), 2:1 (▲), 1.5:1 (∗), 1:1 (∘) and 1:1.5 (■)

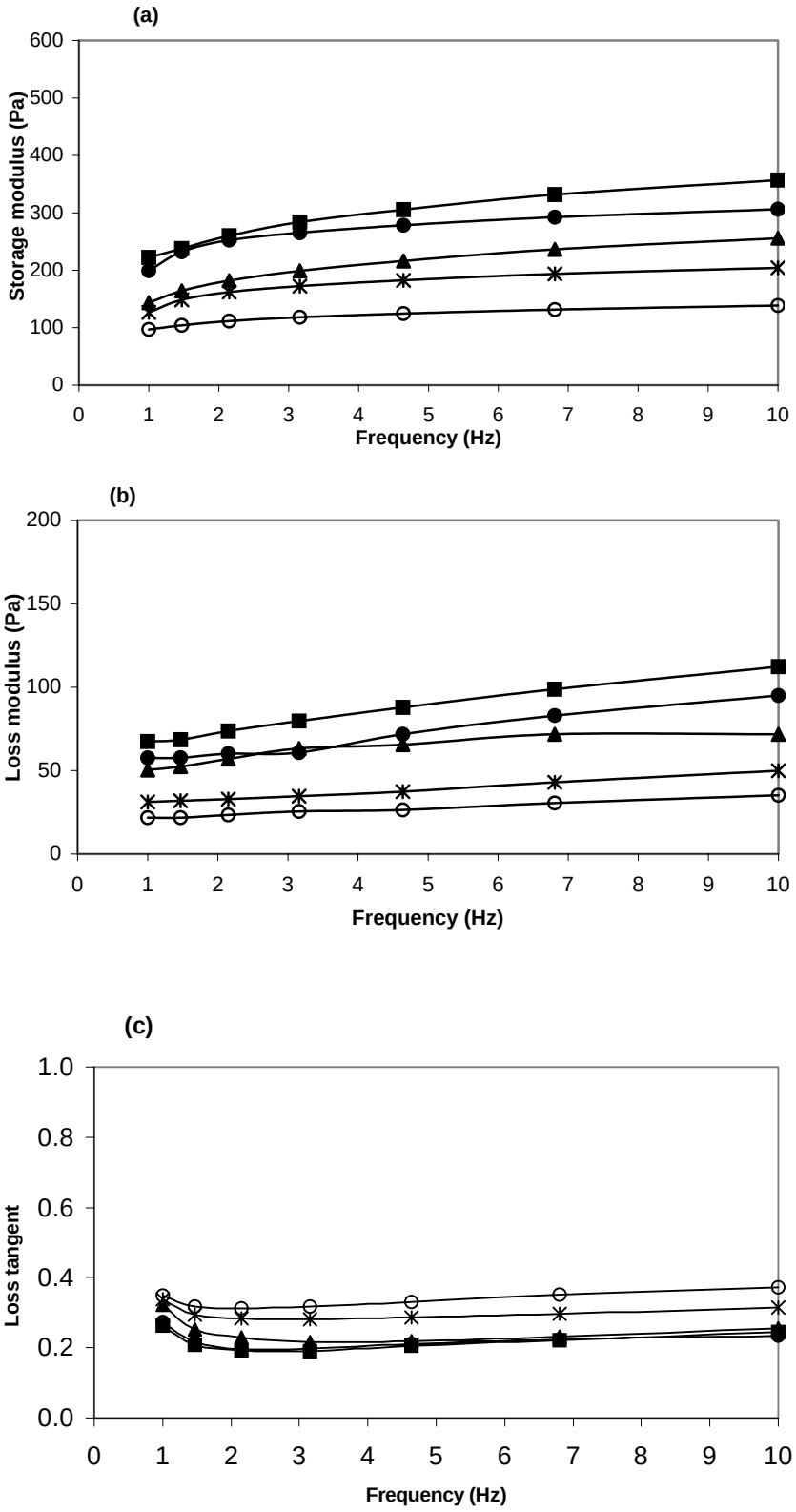


Figure 7. Comparison of dynamic moduli and loss tangent values among the plasters with PG to NMP ratios of 1:2 (∘), 1.5:1 (∗), 2:1 (▲), 3:1(●) and 4:1 (■)

2. Tensile testing for *in vitro* bioadhesion

Among the plasters with different polymer concentrations, the highest detachment force was obtained from the plaster containing 9% of polymer. Plasters with the other polymer concentrations showed lower detachment forces (Figure 8a).

For the plasters with varying ratios of polymer to cross-linking agent, the highest detachment force was seen with the plaster with the polymer to cross-linking agent ratio of 1.5:1. At other ratios, the plasters showed lower detachment forces (Figure 8b).

Among the plasters with various ratios of PG to NMP, the highest detachment force was shown by the plaster with ratio of 2:1 and the plasters with other ratios showed lower detachment forces (Figure 8c).

3. Adhesion study of aspirin plasters in pigs

Table 5 shows the adhesion scores among different variables of the formulations on different days of assessment. It was found that plaster formulated with 9% polymer showed 90% adhesion to the skin. This was due to the bioadhesive nature of the polymer, which was employed in an appropriate amount for adhesion onto the skin for at least 8 h. At 11% and 13% polymer concentrations, % surface adhesion was decreased to 75% and 50% respectively. The optimal ratio of polymer to cross-linking agent was found to be 1.5:1 as it produced the maximum % surface adhesion (90%). Increasing proportion of polymer (2.5:1 and 2:1) gave lower % of surface adhesion (<50% and <75%) and increasing proportion of cross-linking agent (1:1 and 1:2) gave <90% and <75% of surface adhesion. It was found that an appropriate proportion of PG to NMP was necessary for maximum adhesion of the plaster on the skin.

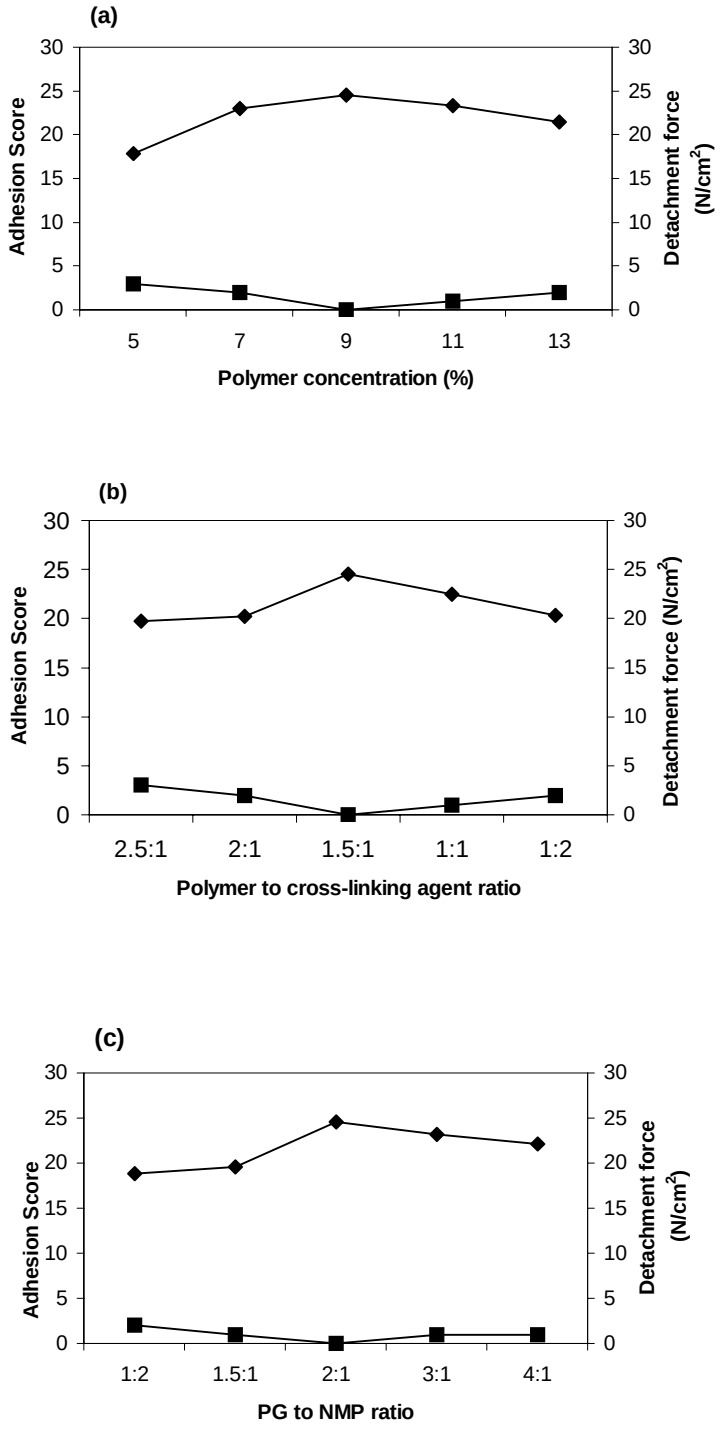


Figure 8. Comparison of adhesion of plasters in terms of detachment force (◆) obtained from *in vitro* tensile testing and adhesion scores (■) obtained from *in vivo* visual analogue scale measurements

Table 5. Comparison of adhesion scores among different types of formulation on different days of assessment[a]

Formulation variables	Day of assessment						
	0	2	4	6	8	12	14
Polymer concentration							
5%	3	3	3	3	3	3	3
7%	2	2	2	2	2	2	2
9%	0	0	0	0	0	0	0
11%	1	1	1	1	1	1	1
13%	2	2	2	2	2	2	2
Polymer to cross-linking agent ratio							
2.5:1	3	3	3	3	3	3	3
2:1	2	2	2	2	2	2	2
1.5:1	0	0	0	0	0	0	0
1:1	1	1	1	1	1	1	1
1:2	2	2	2	2	2	2	2
PG to NMP ratio							
1:2	2	2	2	2	2	2	2
1.5:1	1	1	1	1	1	1	1
2:1	0	0	0	0	0	0	0
3:1	1	1	1	1	1	1	1
4:1	1	1	1	1	1	1	1

[a]Adhesion of plasters was scored by visual scoring scale method:

0 = 90% adhered (essentially no lifting off the skin)
1 = 75% to < 90% adhered (some edges only lifting off the skin)
2 = 50% to < 75% adhered (less than half of the system lifting off the skin)
3 = <50% adhered but not detached (more than half the system lifting off the skin without falling off)
4 = patch detached (patch completely detached from the skin)

The higher proportions of PG (3:1 and 4:1) showed <90% adhesion of the plasters. In higher NMP proportions (1:2 and 1:1.5), surface adhesion were lowered to <75% and <90%. The results obtained from *in vitro* bioadhesion studies of plasters with different polymer concentrations, polymer to cross-linking agent ratio and PG to NMP ratio were consistent with those obtained from the *in vivo* adhesion studies (Figure 8). In conclusion, the plaster formulated with 9% polymer, polymer to cross-linking agent ratio of 1.5:1 and PG to NMP ratio of 2:1 had the greatest bioadhesive properties.

C. HPLC assay

The mobile phase was chosen after several trials with methanol, acetonitrile and phosphate buffer in various proportions and at different pH values. A mobile phase consisting of 70% phosphate buffer of pH 2.6 and 30% acetonitrile (v/v) was selected to achieve complete separation and maximum sensitivity. Flow rates between 0.5 and 1.5 ml/min were studied. A flow rate of 1.0 ml/min gave the optimal signal-to-noise ratio with a reasonable separation time. Using reversed-phase C_{18} column, the retention times for aspirin and salicylic acid were found to be 3.1 and 4.2 min respectively. The maximum absorption of aspirin and salicylic acid occurred at 230 nm and this wavelength was chosen for the analysis.

Five different concentrations of aspirin and salicylic acid were assayed using HPLC for plotting the standard curves of aspirin and salicylic acid (Figures 9). Linear plots were obtained for concentrations of 1–5 μg/ml of aspirin and salicylic acid by linear regression analysis. The standard error of slope and intercept of aspirin were 286.46 and 94.62. The standard error of slope and intercept of salicylic acid were 1206.64 and 398.54. The precision of the method (intra-day and inter-day variations of

determinations) was checked by repeating three times of each concentration of aspirin and salicylic acid. As shown in Table 6, the intra-and inter-day variations for aspirin and salicylic acid were less than 3%, indicating that the analytical method developed was accurate and precise for aspirin and salicylic acid. The limit of detection was calculated at signal to noise ratio of 3:1 and was 0.05 μg/ml for both aspirin and salicylic acid. The limit of quantitation was calculated at signal to noise ratio of 10:1. It was 0.1 μg/ml for aspirin and 1 μg/ml for salicylic acid.

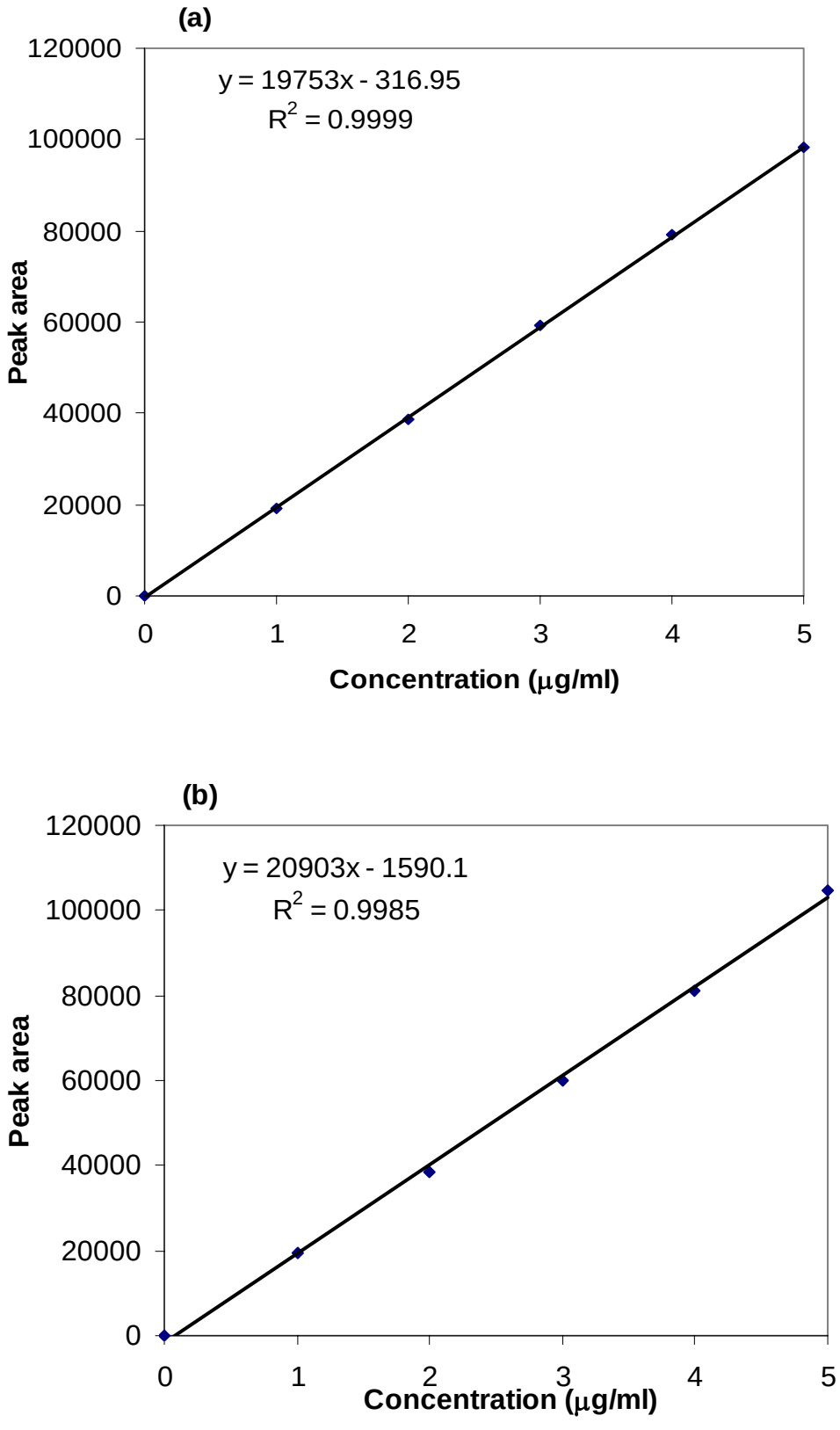


Figure 9. Standard calibration curves of (a) aspirin and (b) salicylic acid

Table 6. Validation of HPLC method

Theoretical concentration (μg/ml)	Aspirin		Salicylic acid	
	Measured concentration* (μg/ml)	C.V. (%)	Measured concentration* (μg/ml)	C.V. (%)
Inter-day variation				
1	1.01 ± 0.02	0.79	1.02 ± 0.08	0.82
2	2.05 ± 0.04	1.62	1.96 ± 0.10	1.50
3	2.99 ± 0.07	1.84	3.02 ± 0.14	2.00
4	3.92 ± 0.09	1.98	3.89 ± 0.14	1.80
5	5.14 ± 0.03	0.28	5.03 ± 0.12	0.95
Intra-day variation				
1	1.05 ± 0.03	2.20	1.02 ± 0.09	1.19
2	2.03 ± 0.04	1.20	1.97 ± 0.11	2.01
3	2.96 ± 0.03	0.40	3.06 ± 0.10	0.88
4	3.93 ± 0.11	2.40	3.89 ± 0.09	0.35
5	5.16 ± 0.09	1.40	5.04 ± 0.13	1.09

*Mean ± S.D. (n=3)

D. *In vitro* drug release and skin permeation studies

1. *In vitro* release of aspirin

1.1 The effect of polymer concentration

The effect of polymer concentration on *in vitro* drug release is shown in Figure 10. The highest release rate of aspirin was achieved with 5% polymer concentration. The lowest release rate of aspirin was achieved from the highest polymer concentration of 13%. The amounts of drug released from the plasters with different polymer concentrations were significantly different (two-way ANOVA; $p<0.05$) and the amounts of drug released at different sampling times were also significantly different (two-way ANOVA; $p<0.05$).

All the release profiles were linear when plotted against the square root of time (Figure 11), indicating that drug release was determined by diffusion mechanism and the synthetic membrane did not pose as a permeation barrier to aspirin and salicylic acid. The release rates obtained from the slopes of the best fit lines were plotted against polymer concentration (Figure 12). Release rate decreased with increasing polymer concentration ($p<0.05$).

1.2 The effect of polymer to cross-linking agent ratio

The effect of ratio of polymer to cross-linking agent on the release of aspirin from the plasters is shown in Figure 13. The highest release of aspirin was achieved from the plaster with the least cross-linkage between polymer and cross-linking agent. The lower the proportion of cross-linking agent used, the higher was the drug release. The fraction released was significantly different (two-way ANOVA; $p<0.05$) among the

plasters with different ratios of polymer to cross-linking agent and at the different sampling times.

The release profiles showed linear relationships with the square root of time (Figure 14). The release rates obtained from the slopes of these linear curves were plotted against the ratio of polymer to cross-linking agent (Figure 15). Release rate decreased with increasing proportion of cross-linking agent (Pearson's correlation, $p<0.05$).

1.3 The effect of PG to NMP ratio

As shown in Figure 16, the higher release rates of aspirin were achieved from the plaster with PG to NMP ratios of 1:2 and 1:1. The lowest release rate of aspirin was observed for the plaster with ratio of 4:1. The fraction released was significantly different among the plasters with different ratios of PG to NMP (except 1:2 and 1:1) and significantly different at different sampling times (two-way ANOVA; $p<0.05$). The released fraction of aspirin from the plaster with PG to NMP ratio of 1:1 is comparable with that of ratio of 1:2, showing that the higher proportion of NMP in the ratio of PG to NMP had no effect on the release rate of aspirin.

When the drug release was plotted against the square root of time, a linear relationship was obtained (Figure 17). The release rates obtained from the slopes of the linear curves were plotted against the ratio of PG to NMP (Figure 18). Release rate decreased with increasing proportion of PG used in the plaster formulation (Pearson's correlation, $p<0.05$). Table 7 shows the results obtained from Pearson's correlation, indicating that drug release decreased with an increase in polymer concentration, proportion of cross-linking agent and proportion of PG (Pearson's correlation, $p<0.05$). Among them, polymer concentration had the strongest effect on the drug release.

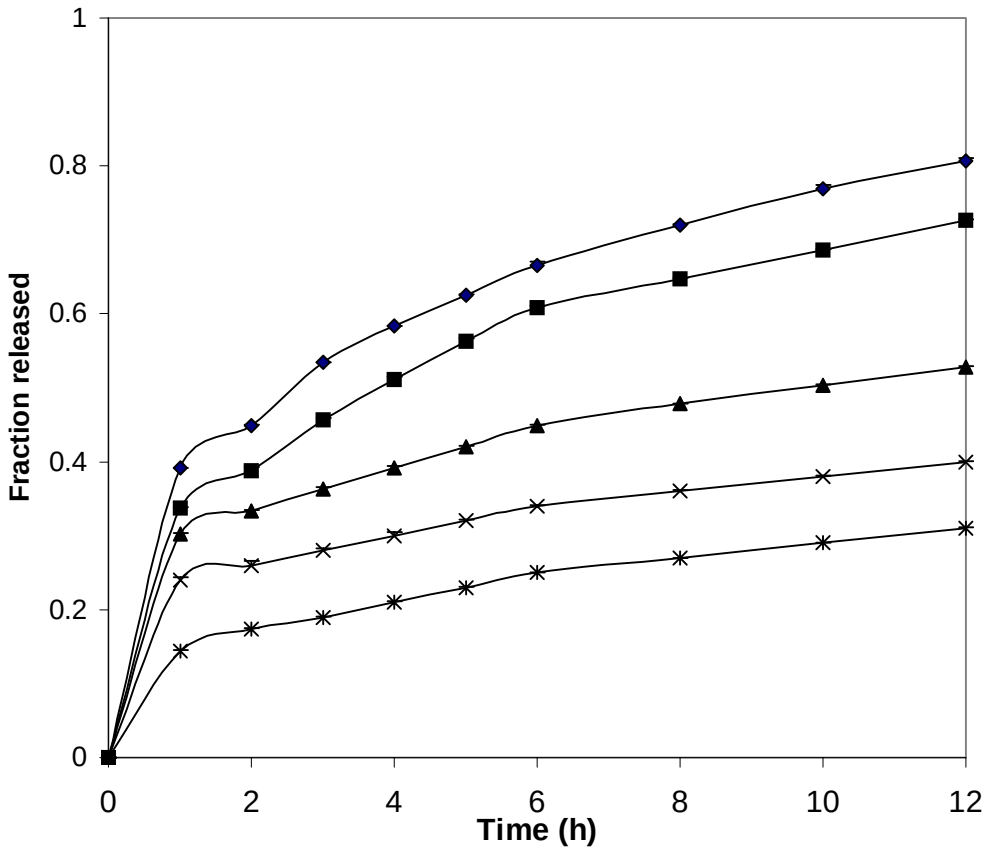


Figure 10. The effect of polymer concentrations of 5% (◆), 7% (■), 9% (▲), 11% (×) and 13% (∗) on drug release from the plasters through synthetic membrane

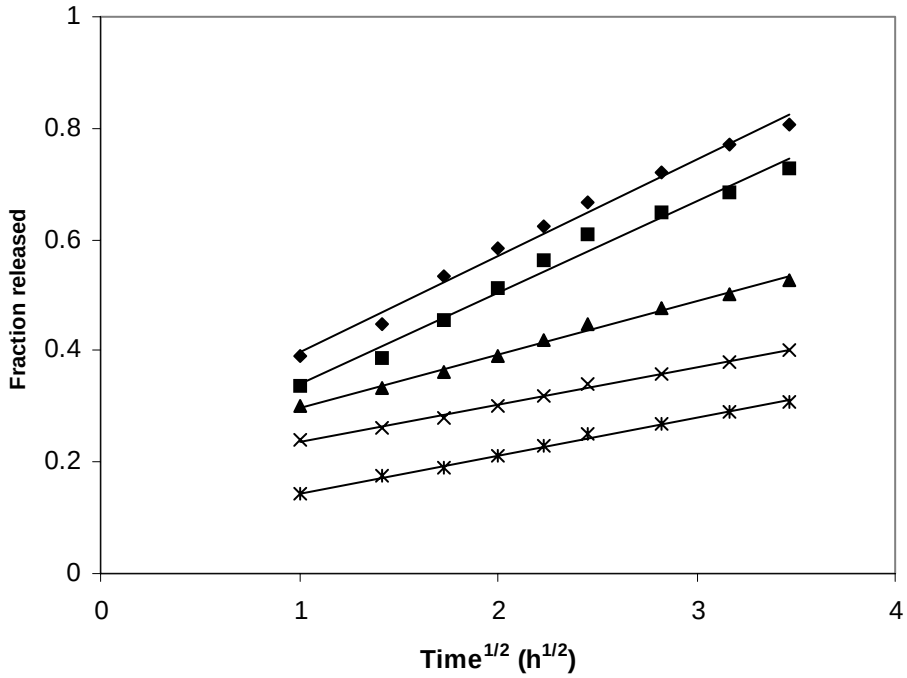


Figure 11. The correlation of fraction of aspirin released from plasters with polymer concentrations of 5% (◆), 7% (■), 9% (▲), 11% (×) and 13% (∗) with square root of time

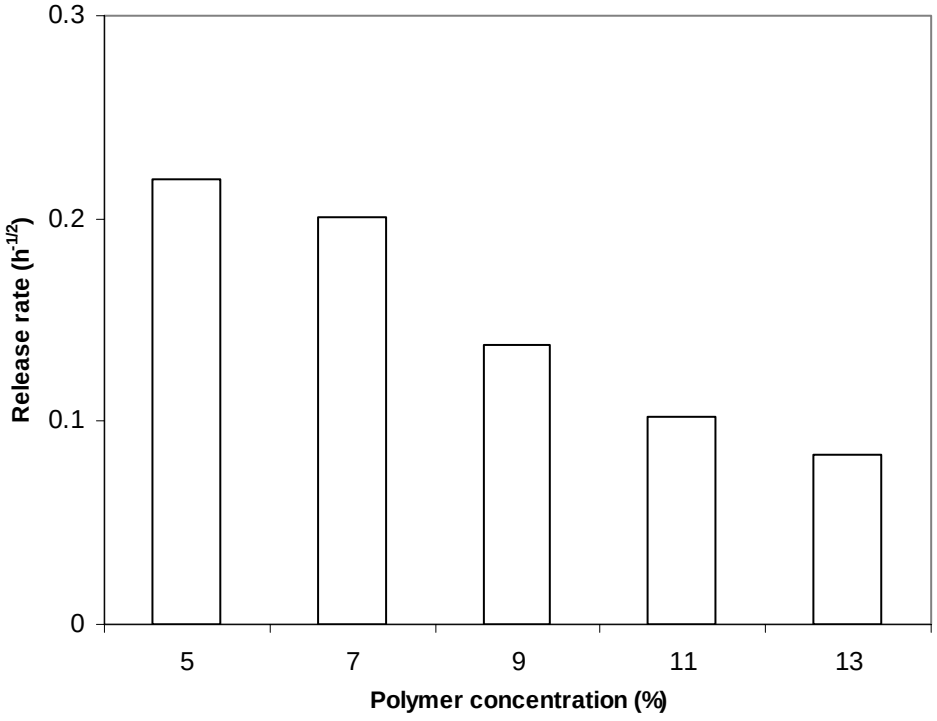


Figure 12. The effect of polymer concentration on release rate of aspirin through synthetic membrane

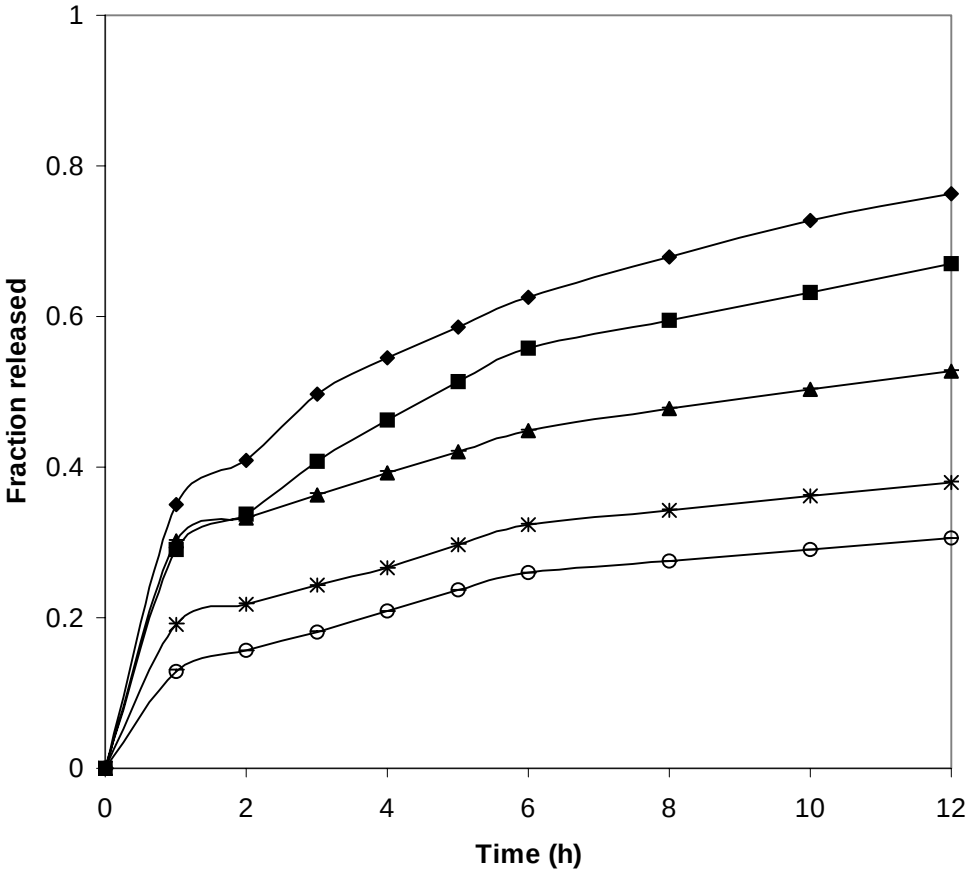


Figure 13. The effect of polymer to cross-linking agent ratios of 2.5:1 (◆), 2:1 (■), 1.5:1 (▲), 1:1 (∗) and 1:1.5 (○) on drug release through synthetic membrane

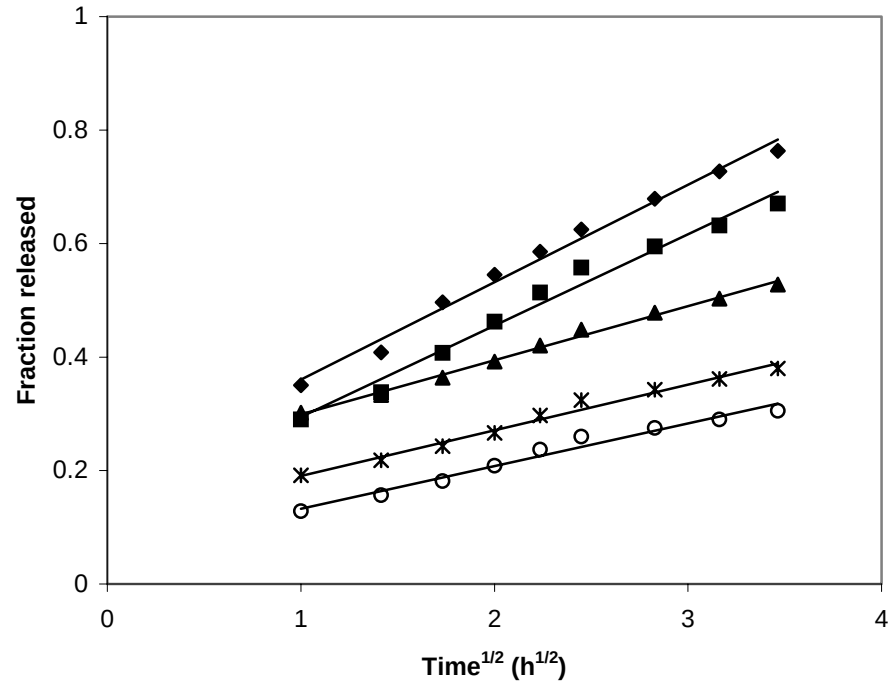


Figure 14. The correlation of fraction of aspirin released from plasters with polymer to cross-linking agent ratios of 2.5:1 (◆), 2:1 (■), 1.5:1 (▲), 1:1 (∗) and 1:1.5 (∘) with square root of time

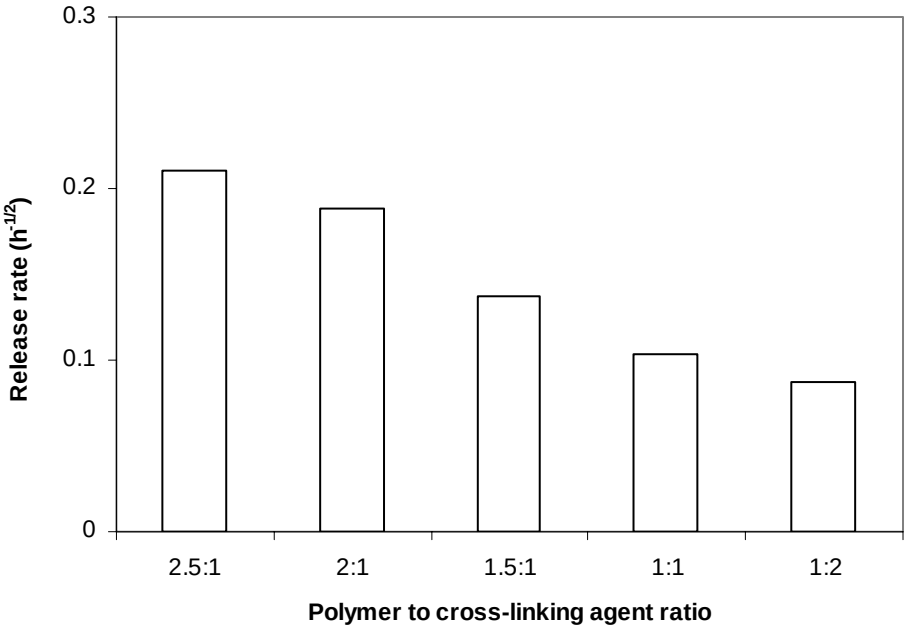


Figure 15. The effect of polymer to cross-linking agent ratio on release rate of aspirin through synthetic membrane

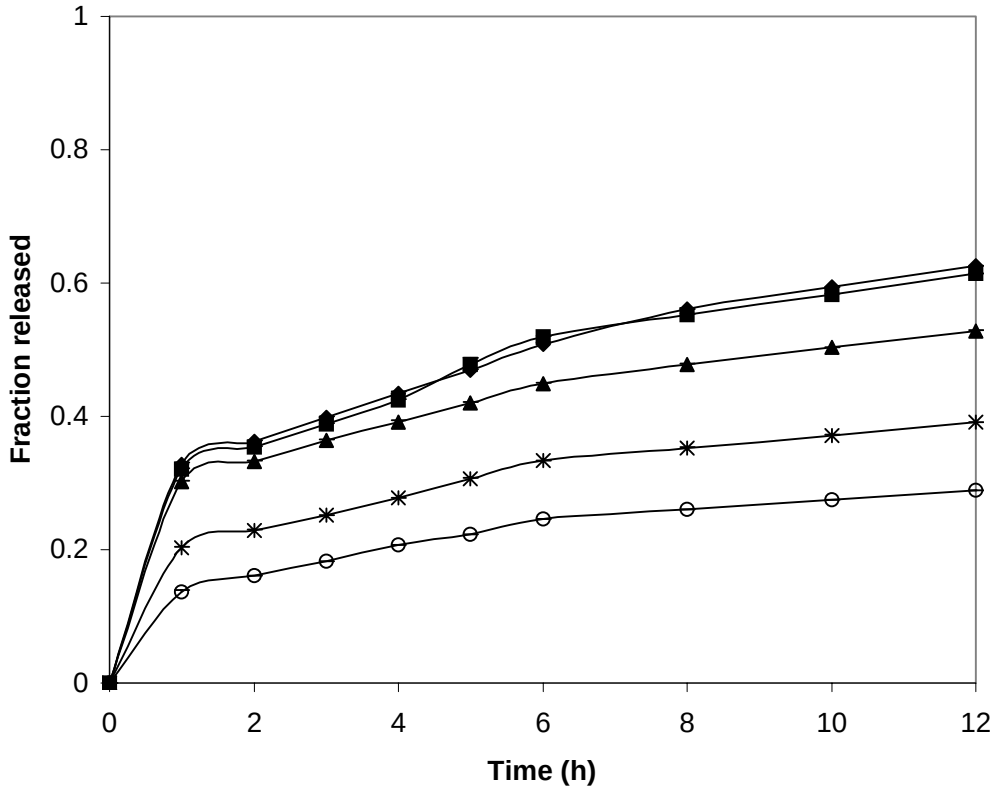


Figure 16. The effect of PG to NMP ratios of 1:2 (◆), 1:1 (■), 2:1 (▲), 3:1 (∗) and 4:1 (∘) on drug release from the plasters through synthetic membrane

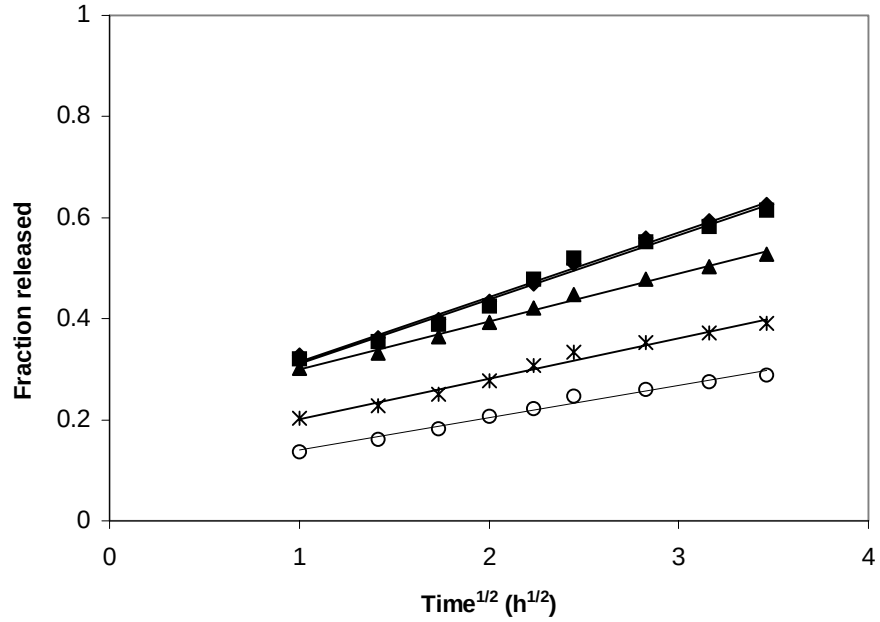


Figure 17. The correlation of fraction of aspirin released from plasters with PG to NMP ratios of 1:2 (◆), 1:1 (■), 2:1 (▲), 3:1 (∗) and 4:1 (○) with square root of time

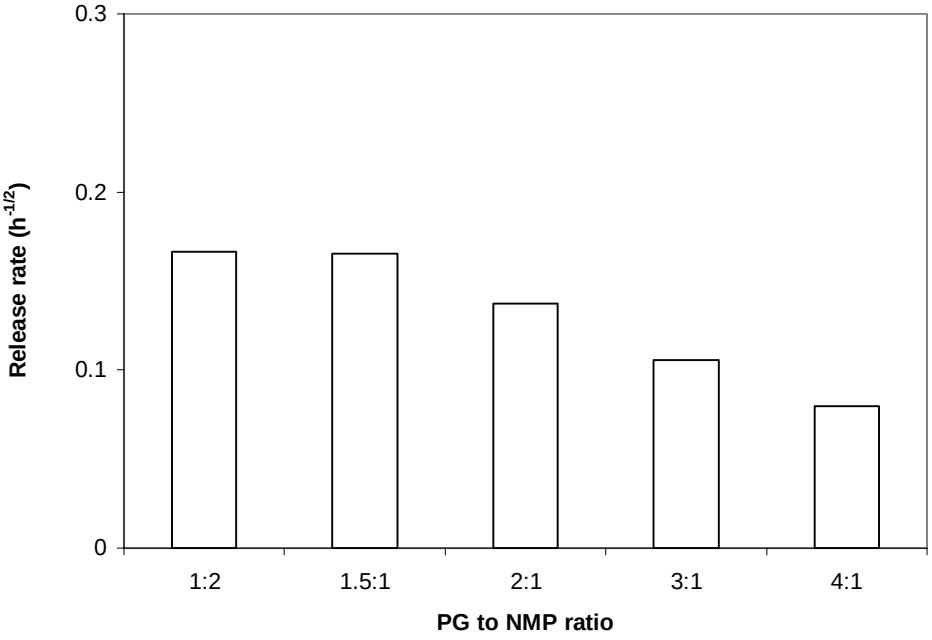


Figure 18. The effect of PG to NMP ratio on release rate of aspirin through synthetic membrane

Table 7. Pearson's correlation of polymer concentration, polymer to cross-linking agent ratio and PG to NMP ratio with drug release through synthetic membrane

Variables	Pearson's correlation	R	p-value
Polymer concentration	-0.59	0.35	<0.05
Polymer to cross-linking agent ratio	-0.57	0.33	<0.05
PG to NMP ratio	-0.52	0.27	<0.05

2. Study of aspirin permeation through pig abdominal skin

2.1 The effect of polymer concentration

Figure 19 shows the effect of polymer concentration on permeation of aspirin through pig skin. The highest permeation rate was shown by the formulation with the lowest concentration of polymer. Cumulative amounts of drug permeated through pig skin from the plasters with different polymer concentrations were significantly different (two-way ANOVA; $p<0.05$) and also significantly different among different sampling times (two-way ANOVA; $p<0.05$). The amounts permeated showed a linear relationship with square root of time (Figure 20) and was negatively and significantly correlated with polymer concentration (Pearson's correlation, $p<0.05$) (Figure 21). It was indicated that the cumulative amount of aspirin permeated through pig skin decreased with increase in polymer concentration.

2.2 The effect of ratio of polymer to cross-linking agent

The effect of ratio of polymer to cross-linking agent on the permeation of aspirin through pig abdominal skin is shown in Figure 22. The highest amount of aspirin was permeated was found for a plaster with the lowest concentration of the cross-linking agent. The cumulative amounts of drug permeated through pig skin for the plasters with different ratios of polymer to cross-linking agent were significantly different at different sampling times (two-way ANOVA; $p<0.05$). The permeation results were linearly related to square root of time (Figure 23) and the permeation rate was negatively and significantly correlated with the proportion of cross-linking agent (Figure 24, Pearson's correlation, $p<0.05$).

2.3 The effect of ratio of PG to NMP

The higher proportion of PG in formulation decreased the amount of aspirin permeated through pig skin (Figure 25). The cumulative drug permeation through pig skin for the plasters with different ratios of PG to NMP was significantly different at different sampling times (two-way ANOVA; $p<0.05$). The cumulative amount of aspirin permeated is linearly related to the square root of time (Figure 26). The permeation rate of aspirin was negatively and significantly correlated with the proportion of PG (Pearson's correlation, $p<0.05$). The permeation rate decreased with increase in proportion of PG (Figure 27).

The cumulative amount of drug permeated through pig skin was negatively and significantly correlated with the polymer concentration, the proportions of cross-linking agent and PG (Pearson's correlation, $p<0.05$). Among them, the ratio of PG to NMP had the greatest effect on the permeation of the drug through pig skin (Table 8) whereas the polymer concentration had the greatest effect on aspirin release through synthetic membrane (Table 7).

3. Comparison of flux of aspirin through different types of barriers

Three types of barriers including synthetic membrane, pig skin and human abdominal skin were used for *in vitro* permeation studies. Figure 28 shows that the cumulative amount of aspirin permeated increased in the following order: synthetic membrane > human skin ≈ pig skin. The pig skin and the human skin showed similar permeability to aspirin, indicating pig skin resembled human skin in terms of drug permeability, particularly for aspirin. This further confirmed that the synthetic membrane did not pose as a permeation barrier in the release studies.

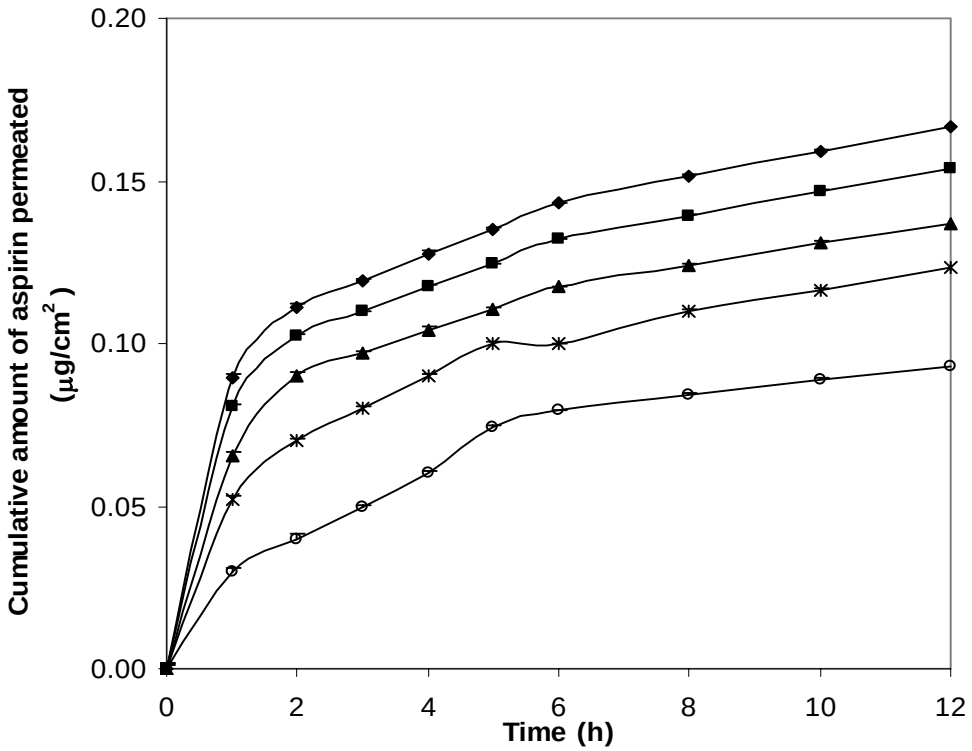


Figure 19. The effect of polymer concentrations of 5% (◆), 7% (■), 9% (▲), 11% (∗) and 13% (∘) on cumulative amount of aspirin permeated through pig skin

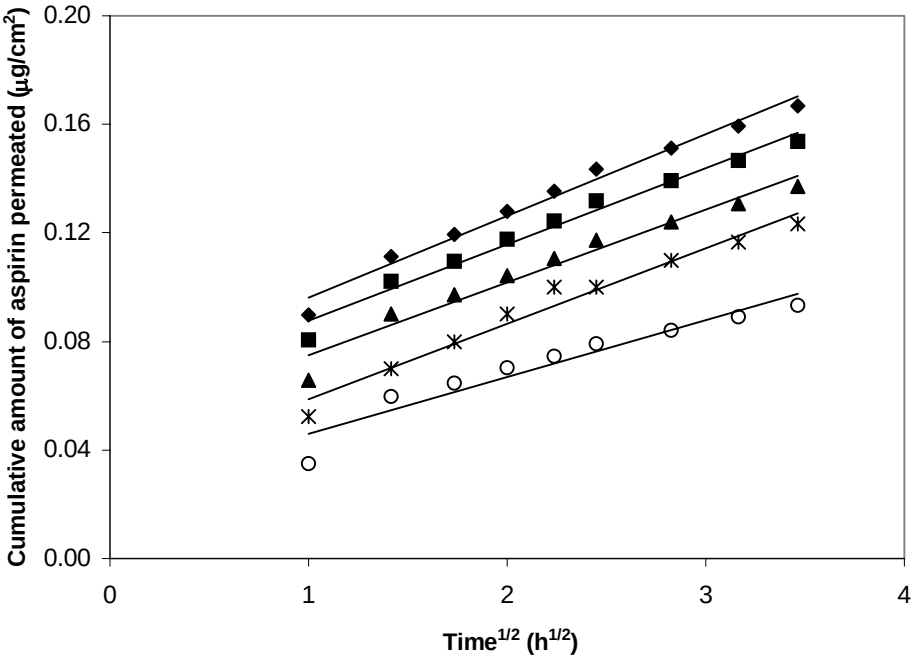


Figure 20. The correlation of cumulative aspirin permeation through pig skin from plasters with polymer concentrations of 5% (◆), 7% (■), 9% (▲), 11% (∗) and 13% (∘) with square root of time

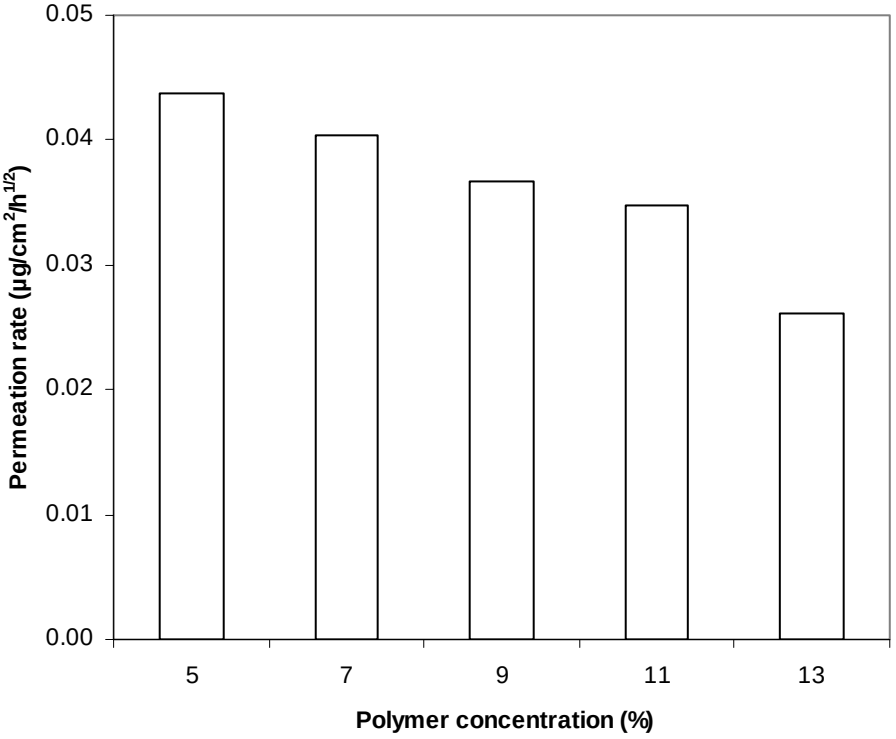


Figure 21. The effect of polymer concentration on permeation rate of aspirin through pig skin

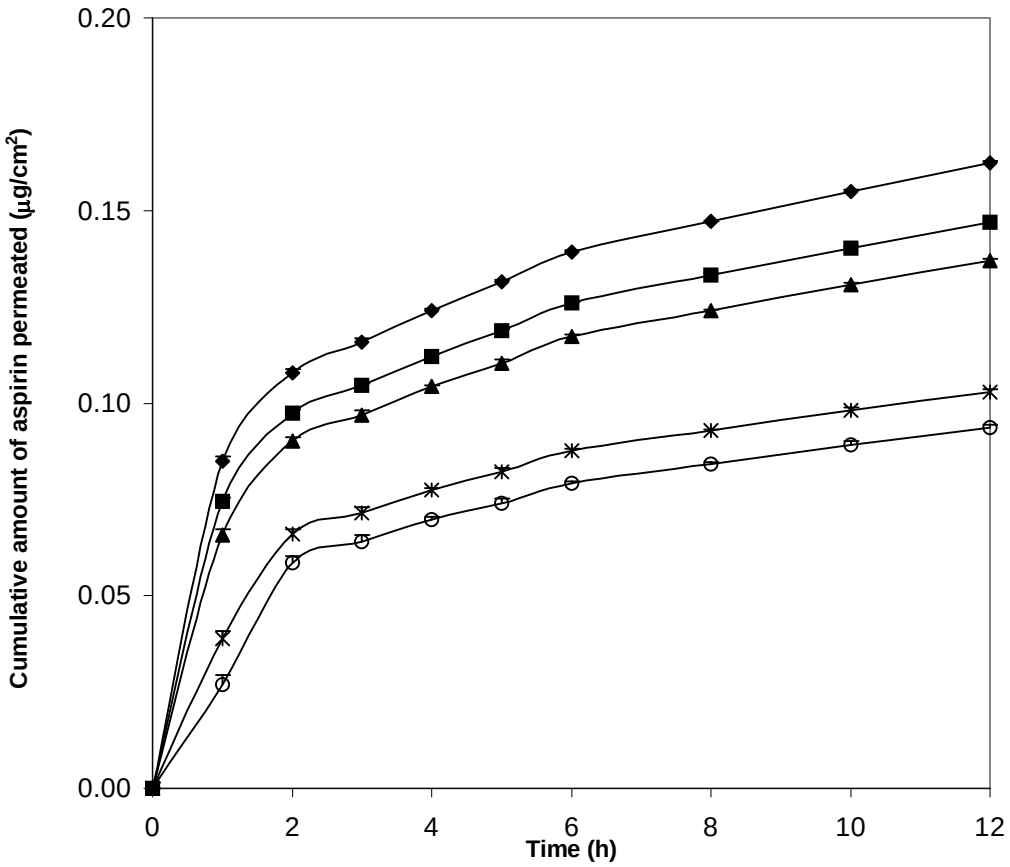


Figure 22. The effect of polymer to cross-linking agent ratios of 2.5:1 (◆), 2:1 (■), 1.5:1 (▲), 1:1 (∗) and 1:1.5 (∘) on cumulative amount of aspirin permeated through pig skin

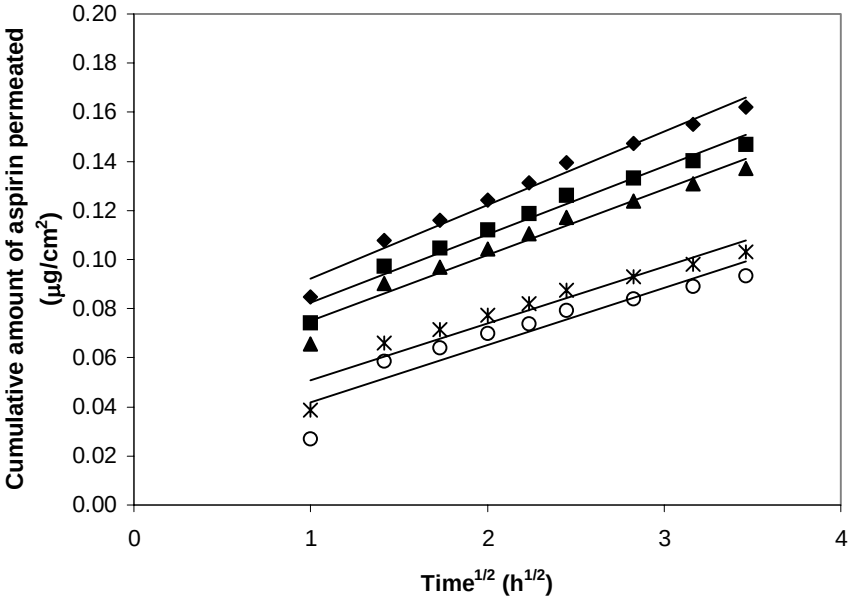


Figure 23. The correlation of cumulative amount of aspirin permeated through pig skin from plasters with polymer to cross-linking agent ratios of 2.5:1 (◆), 2:1 (■), 1.5:1 (▲), 1:1 (∗) and 1:1.5 (∘) with square root of time

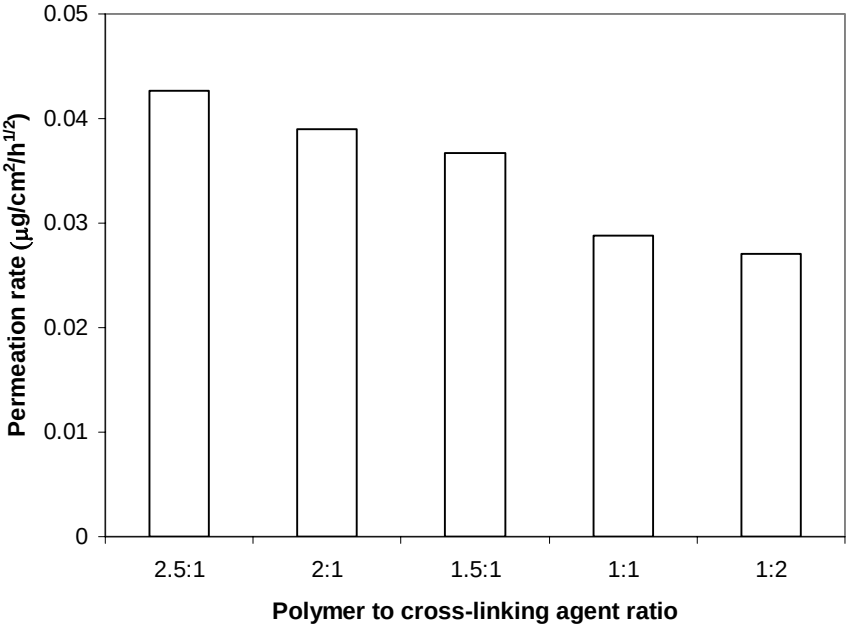


Figure 24. The effect of polymer to cross-linking agent ratio on permeation rate of aspirin through pig skin

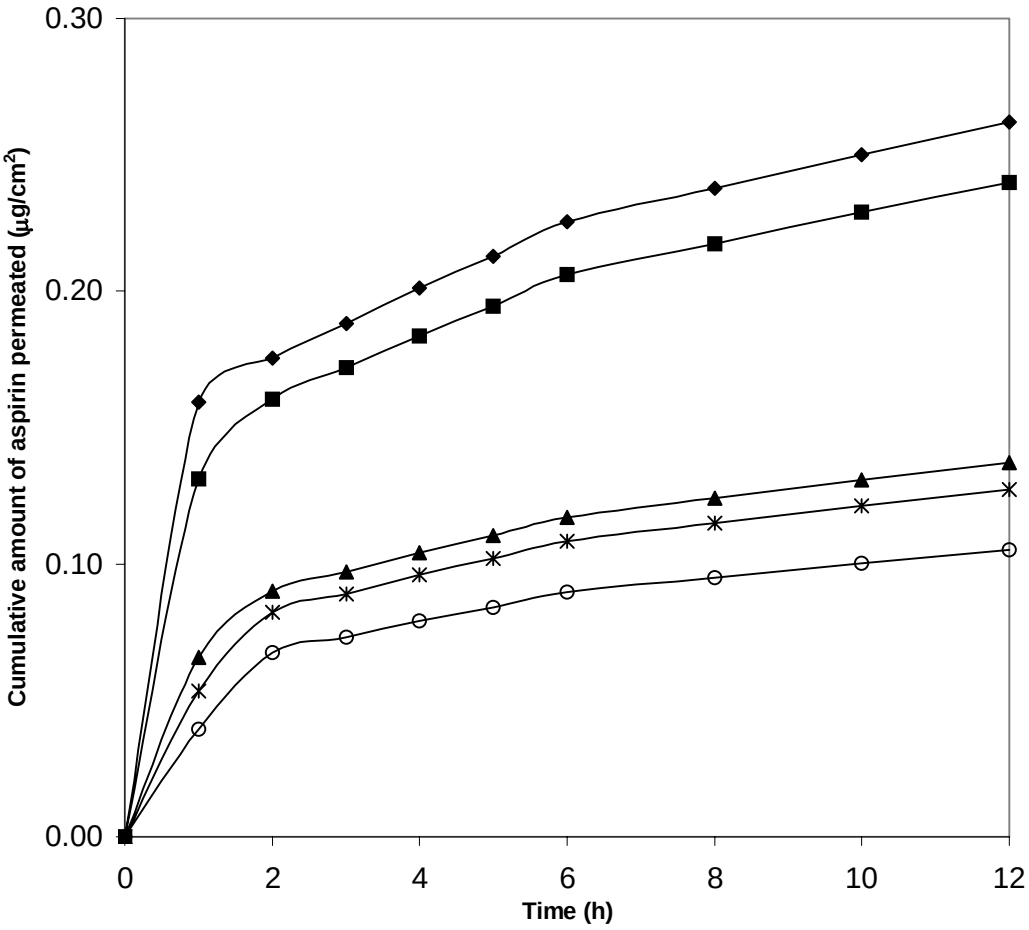


Figure 25. The effect of PG to NMP ratios of 1:2 (◆), 1:1 (■), 2:1 (▲), 3:1 (∗) and 4:1 (∘) on cumulative amount of aspirin permeated through pig skin

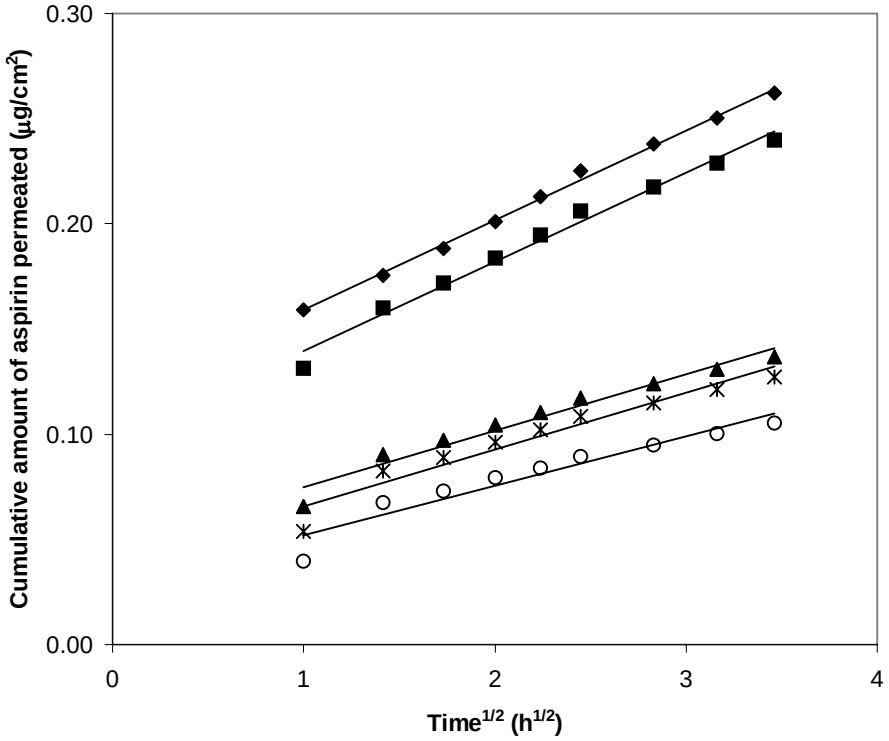


Figure 26. **The correlation of cumulative amount of aspirin permeated through pig skin from plasters with polymer to cross-linking agent ratios of 1:2 (◆), 1:1 (■), 2:1 (▲), 3:1 (∗) and 4:1 (∘) with square root of time**

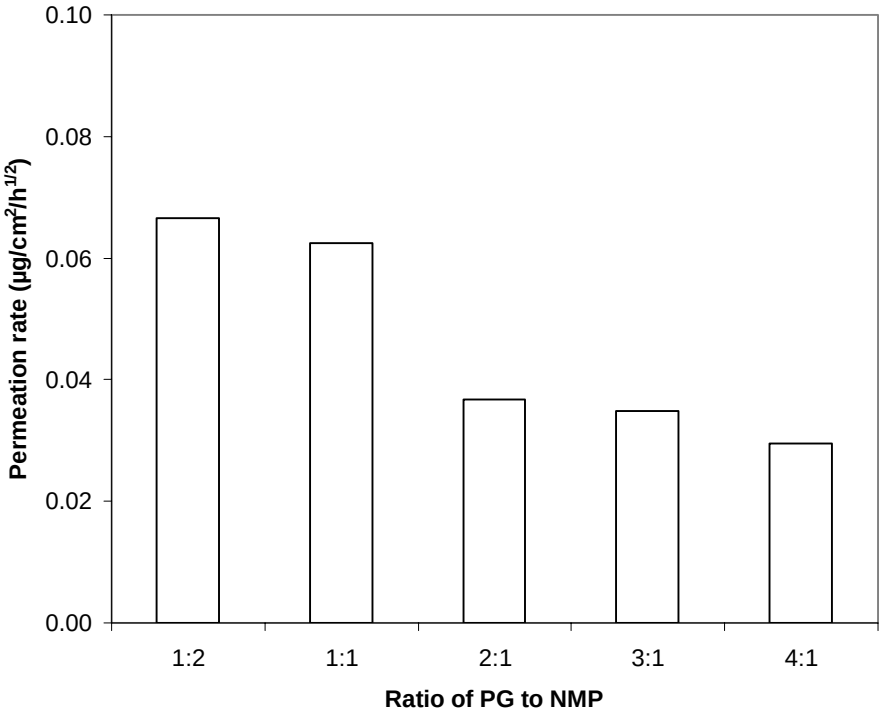


Figure 27. The effect of PG to NMP ratio on permeation rate of aspirin through pig skin

Table 8. Pearson's correlation of polymer concentration, polymer to cross-linking agent ratio and PG to NMP ratio with cumulative drug permeated through pig skin

Type of variables	Pearson's correlation	R	p-value
Polymer concentration	-0.47	0.22	<0.05
Polymer to cross-linking agent ratio	-0.47	0.22	<0.05
PG to NMP ratio	-0.65	0.42	<0.05

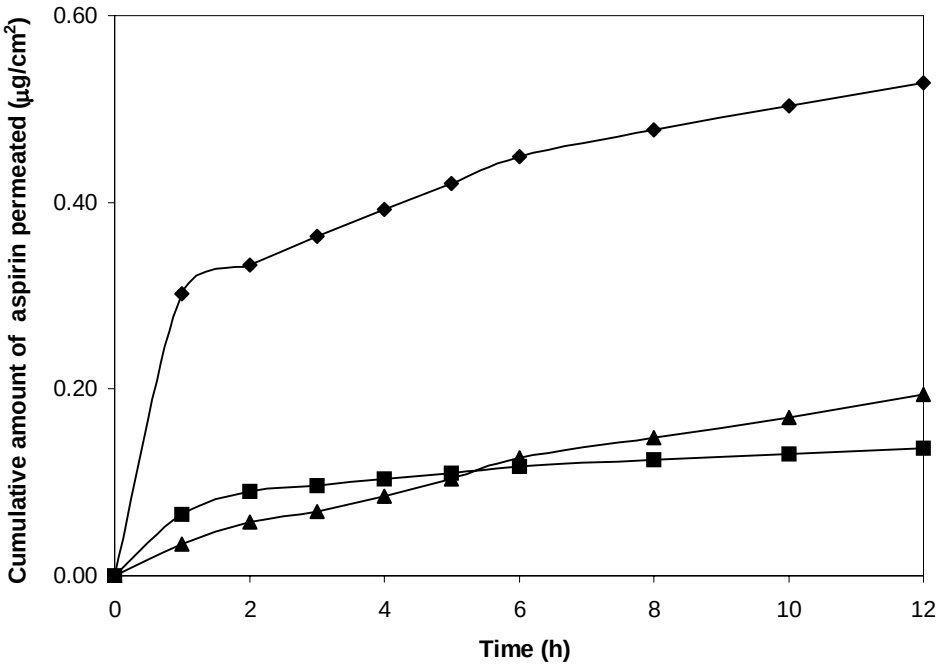


Figure 28. The comparison of aspirin permeation through synthetic membrane (◆), pig skin (■) and human skin (▲) using the aspirin plaster formulated with polymer concentration of 9%, polymer to cross-linking agent ratio of 1.5:1 and PG to NMP ratio of 2:1

E. Accelerated stability testing

Figure 29 shows that there was no significant change in drug content of aspirin plasters after storage in the accelerated stability test conditions, indicating aspirin was stable in the plasters. There were no changes in physical properties of the plasters. Therefore, the aspirin plasters were stable for clinical usage.

F. Skin irritation studies

1. Visual scoring of skin irritation

After application of placebo and aspirin plasters with different formulations for 14 days, the test sites of the skin were assessed visually and scored according to visual analogue scoring system. There was no significant change in visual score before and after application of control and aspirin plasters.

2. Assessment of skin irritation by laser Doppler perfusion imager

2.1. The effect of polymer concentration on skin blood flow

As shown in Figure 30, both placebo plasters and aspirin plasters showed higher skin blood flow results when 5% or 7% of polymer were used, compared to 9%, 11% or 13% of polymer. Skin blood flow results of aspirin plasters and placebo plasters were significantly different for aspirin and placebo plasters with different polymer concentrations and also significantly different on different days of assessment (two-way ANOVA; $p<0.05$). The paired t-test showed that there was a significantly higher skin blood flow due to aspirin plasters than placebo plasters for all polymer concentrations (paired t-test, $p<0.05$).

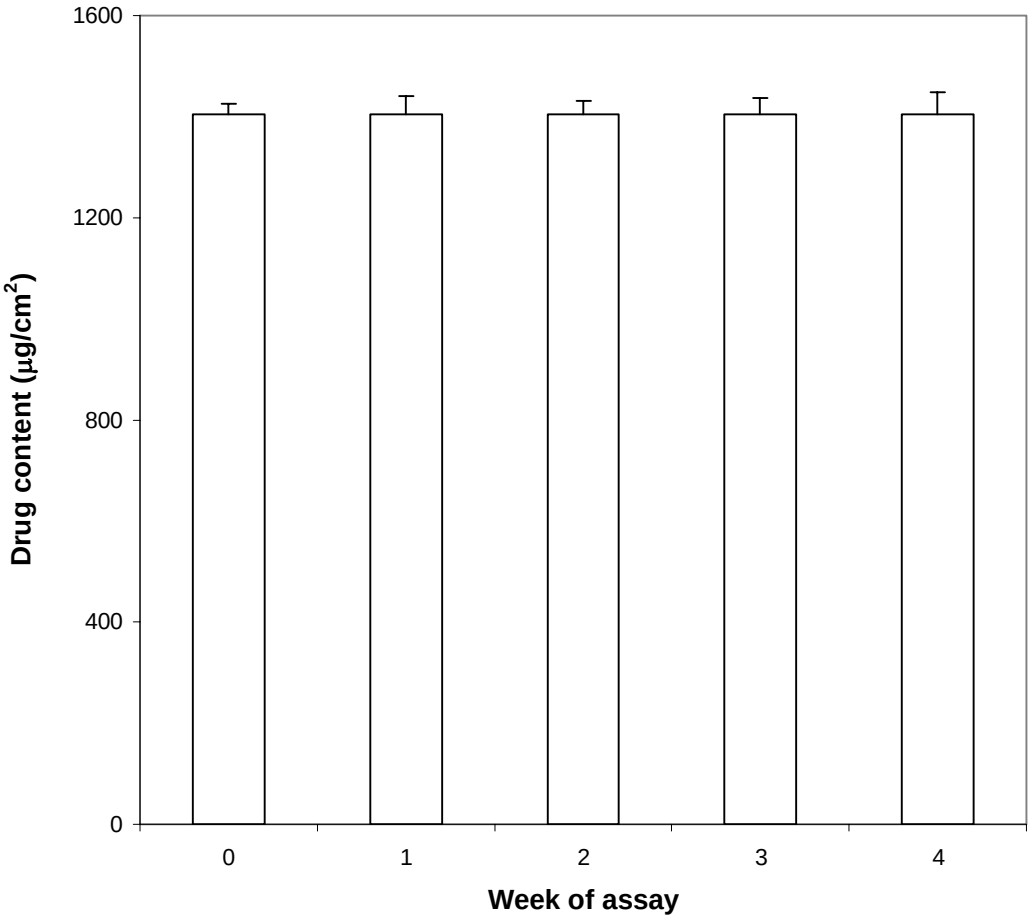


Figure 29. Drug content change in aspirin plasters in the accelerated stability testing

2.2 The effect of polymer to cross-linking agent ratio

Figure 31 shows that the skin blood flow results were higher for the placebo and aspirin plasters with polymer to cross-linking agent ratios 1:2 and 1:1 than those with ratios of 2.5:1, 2:1 and 1.5:1 after 14 days of application. The skin blood flow results for aspirin and placebo plasters were significantly different at different ratio of polymer to cross-linking agent and also significantly different on different days of assessment (two-way ANOVA; $p<0.05$). The skin blood flow results of aspirin plasters were significantly higher than those of the corresponding placebo plasters (paired t-test, $p<0.05$).

2.2 The effect of PG to NMP ratio

Figure 32 shows that the skin blood flow results of both placebo and aspirin plasters were higher for PG to NMP ratios of 1:2, 3:1 and 4:1 than those with ratios of 1.5:1 and 2:1 after 14 days of application of plasters. The skin blood flow results of both aspirin and placebo plasters were significantly different at different ratios and on different days of assessment also (two-way ANOVA, $p<0.05$). The skin blood flow results of placebo plasters were significantly lower than those of the corresponding aspirin plasters (paired t-test, $p<0.05$).

3. Effects of aspirin concentration and duration of application of plasters on skin irritation

Table 9 demonstrates that higher VAS scores were observed with higher aspirin concentrations and longer duration of application of aspirin plasters. The plasters with aspirin concentration of 200 mg showed no irritation on day 0. Minimal erythema was observed on day 2 and day 4. On day 6 and day 8, erythema and minimal edema were

observed. Erythema and edema were detected on day 12 and day 14. For the plasters with aspirin 300 mg, no irritation was seen on day 0. Minimal erythema was observed on day 2 and erythema and minimal edema were detected on day 4. On day 6, 8 and 12, erythema and edema were detected. Definite edema was observed on day 14. The plasters with the highest aspirin concentration of 400 mg showed no irritation on day 0. On day 2, erythema and minimal edema were observed. On day 4, erythema and edema were detected. On day 6 and 8, definite edema was observed and erythema, edema and papules were detected on day 12 and 14. Figure 33 shows that the highest cutaneous blood flow was seen with the plasters with the highest aspirin concentration of 300 mg and the longest duration of application of plasters, day 14.

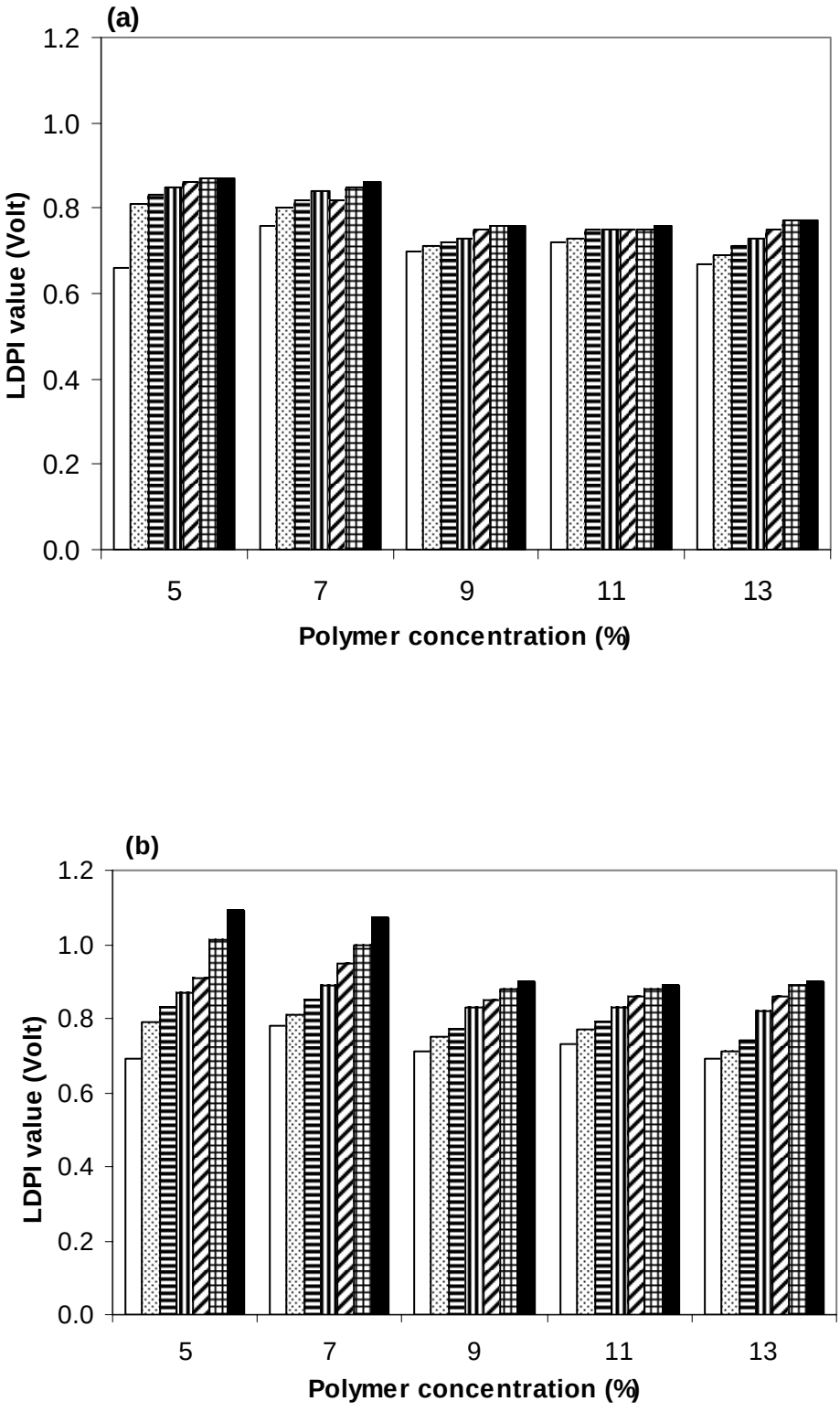


Figure 30. The effect of polymer concentration on LDPI values of (a) placebo plasters and (b) aspirin plasters on different days of assessment: day 0 (□), day 2 (▩), day 4 (▤), day 6 (▥), day 8 (▨), day 12 (▦) and day 14 (■)

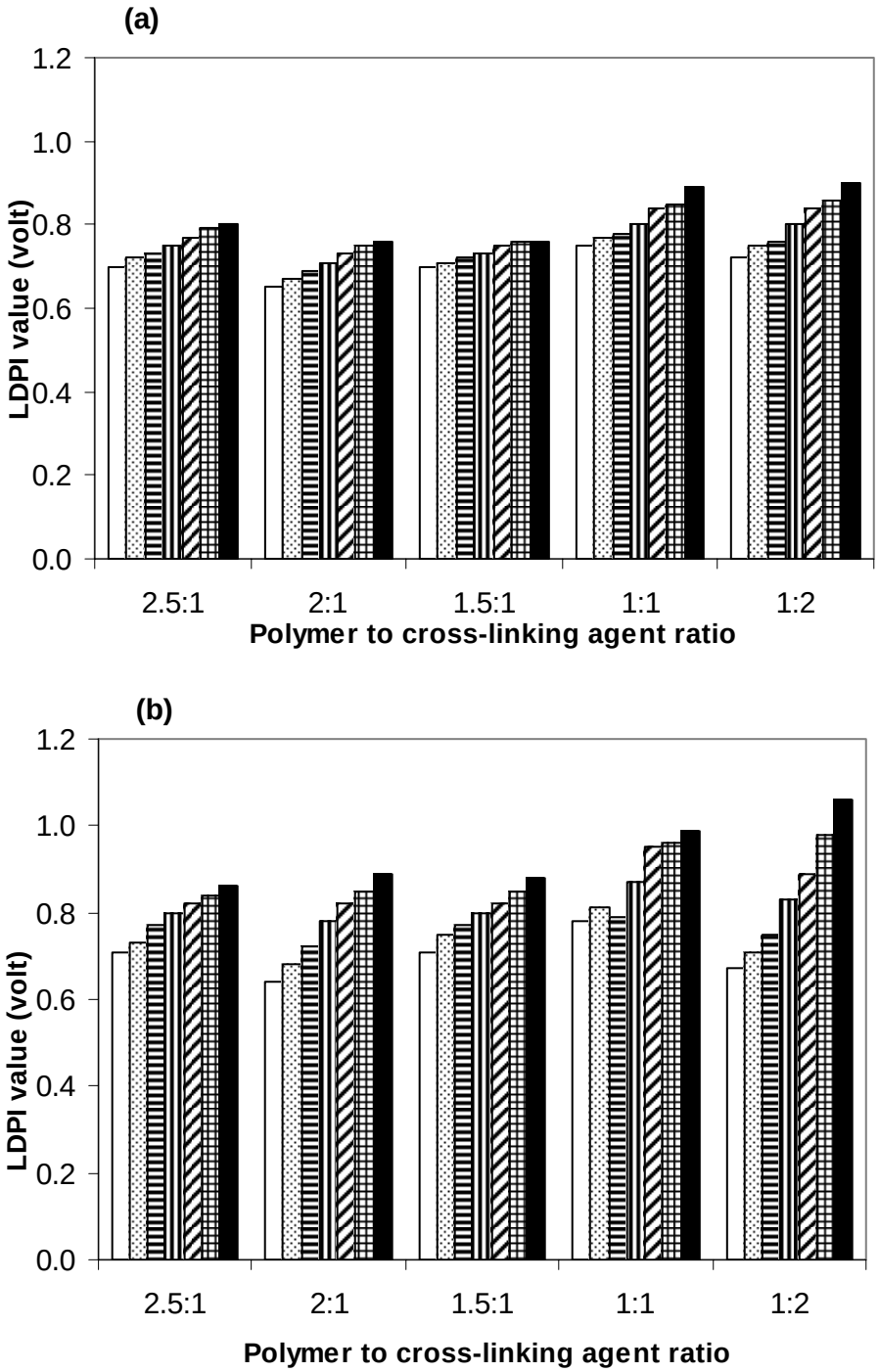


Figure 31. The effect of polymer to cross-linking agent ratio on LDPI values of (a) placebo plasters and (b) aspirin plasters on different days of assessment: day 0 (□), day 2 (▩), day 4 (▤), day 6 (▥), day 8 (▨), day 12 (▦) and day 14 (■)

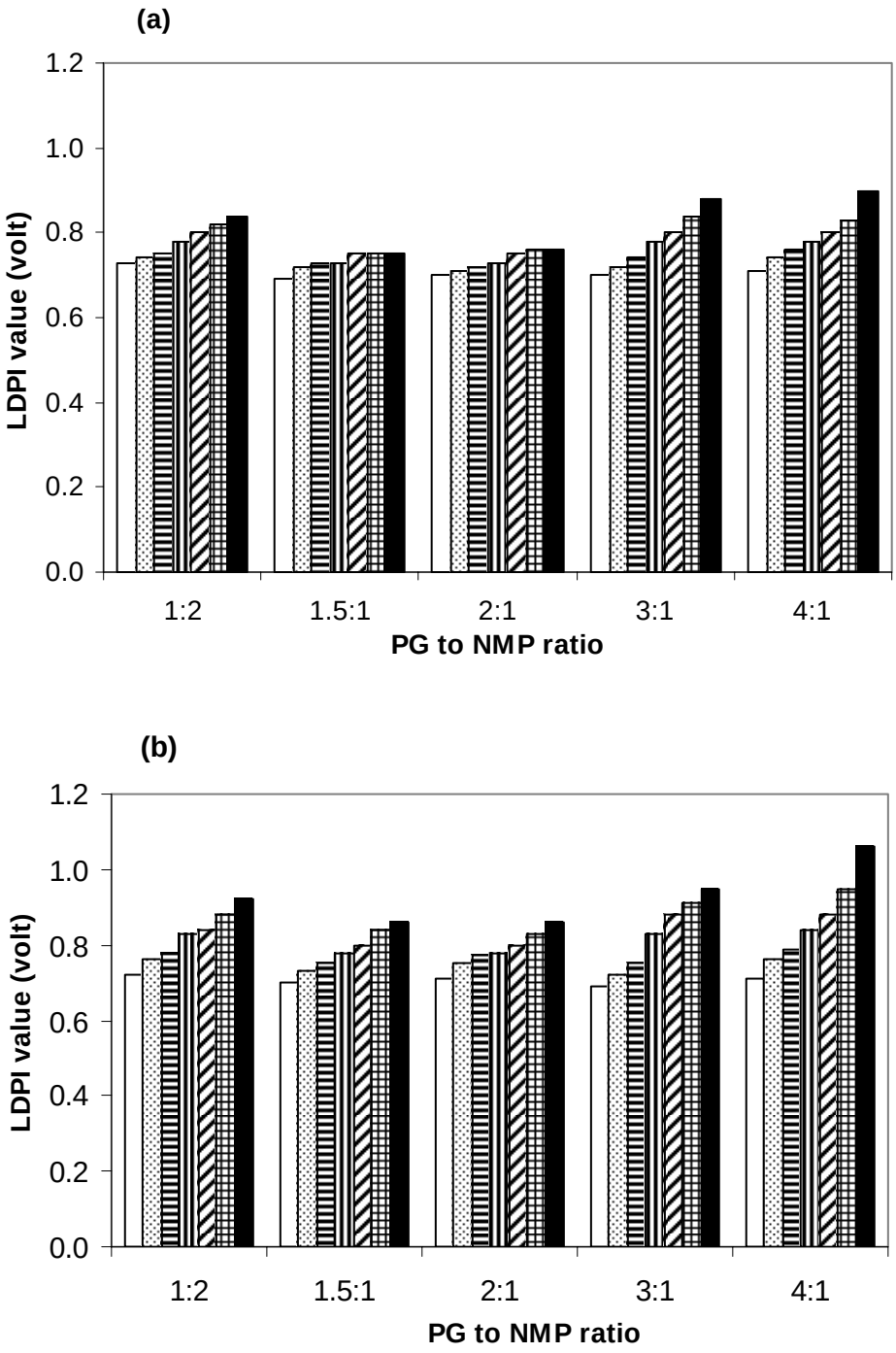


Figure 32. The effect of PG to NMP ratio on LDPI values of (a) placebo plasters and (b) aspirin plasters on different days of assessment: day 0 (□), day 2 (▩), day 4 (▤), day 6 (▥), day 8 (▨), day 12 (▦) and day 14 (■)

Table 9. Visual scores of skin irritation of plasters with aspirin concentrations of 200, 300 and 400 mg on the different days of assessment*

Days of Assessment	Aspirin concentrations		
	200 mg	300 mg	400 mg
0	0	0	0
2	1	1	2
4	1	2	3
6	2	3	4
8	2	3	4
12	3	3	5
14	3	4	5

*0 = No evidence of irritation
1 = Minimal erythema, barely perceptible
2 = Definite erythema, readily visible; minimal edema or minimal papular response
3 = Erythema, edema
4 = Definite edema
5 = Erythema, edema, and papules

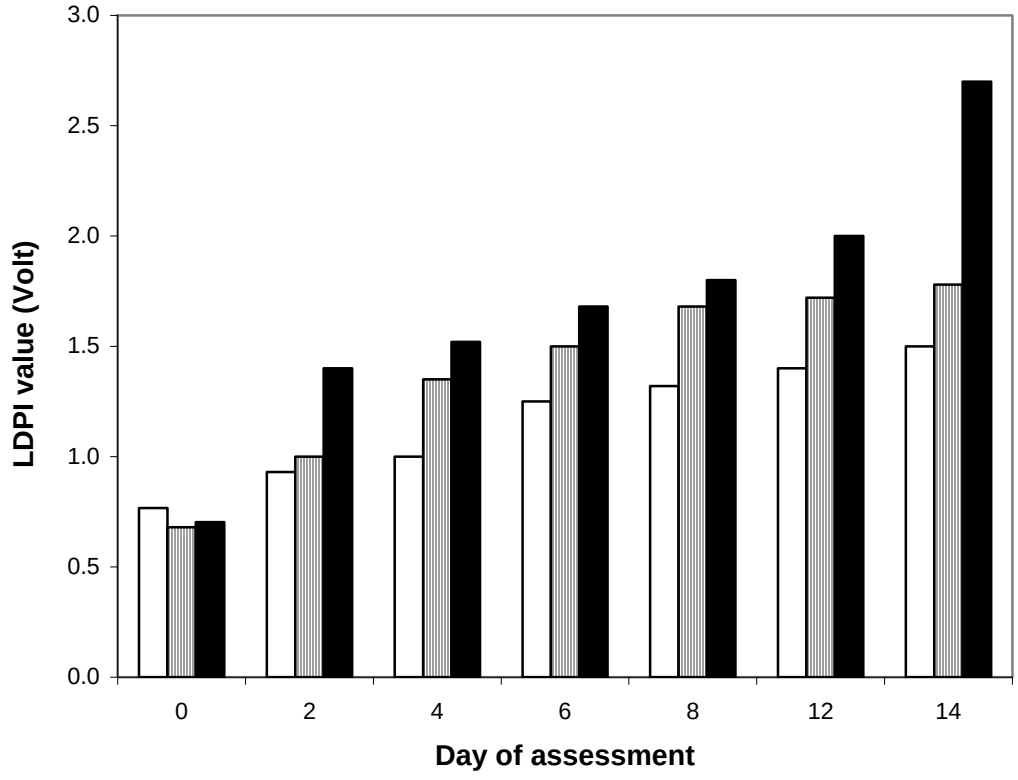


Figure 33. The effect of aspirin concentrations of 200 (□), 300 (▥) and 400 mg (■) and duration of application on LDPI values of plasters formulated with 9% polymer, polymer to cross-linking agent ratio of 1.5:1 and PG to NMP ratio of 2:1

CHAPTER 6

DISCUSSION

A. Formulation of aspirin plaster

Topical application of aspirin preparations is a better alternative to oral administration with respect to the avoidance of unwanted side-effects of aspirin associated with oral administration. The chemical properties of aspirin, such as low polarity, low melting point, low molecular weight and lipophilicity, make it a good candidate for percutaneous delivery. It was reported that aspirin has a good permeability through the skin (McAdam *et al*., 1995). However, overcoming the high water sensitivity of aspirin is a big challenge for the formulator. A non-aqueous system is suitable for preparation of topical aspirin to prevent hydrolysis of aspirin in aqueous medium to inactive degradation products. Although few studies (King, 1993; De Benedittis *et al*., 1992; Kassirer and King, 1988; Balakrishnan *et al*., 2001) with aspirin/solvent solutions have been reported, the use of organic solvents, such as chloroform, diethyl ether and dichloromethane, was not suitable for long-term clinical usage. The aim of the current study was to develop a formulation of non-aqueous bioadhesive plaster for percutaneous delivery of aspirin. A stable and non-irritating plaster was formulated and composed of a monolithic matrix in which aspirin was dissolved. It was supported by a non-woven cotton wool backing layer and covered by a plastic release liner. The release liner was removed just before the application of the plaster on the skin.

It is well known that polyacrylate polymers have good bioadhesive properties and are widely used in the design of transdermal drug delivery systems. However, there are

very few bioadhesive polymers suitable for the development of the non-aqueous plasters. The polymers used for this purpose should meet some requirements, including solubility in water and solvents, gel forming property, bioadhesive property and reaction with cross-linking agent to form a three-dimensional network. According to the preliminary study, the copolymer of N-vinylacetamide and sodium acrylate was found to be suitable for the formulation of aspirin plaster. PNVA exhibits not only non-ionic property of its monomer of N-vinylacetamide but also weak anionic characteristic of its monomer of sodium acrylate. This renders the polymer some typical properties. It is weakly anionic and readily soluble in water, glycerin and various solvents, such as alcohols and aqueous ethanol solution. In the presence of trivalent ions, the polymer is cross-linked and forms an elastic hydrogel. The hydrogel formed is capable of holding a large amount of solvent within the microcavities of its three-dimensional network structure, which is a favorable feature for high drug loading. The polymer molecules in the cross-linked structure still possess their bioadhesive properties but are not as sticky as the uncross-linked counterpart. Moreover, the nonionic property of the polymer makes it resistant to pH changes and the presence of salts. Therefore, its viscosity is resistant to skin acidic pH and to salt from perspiration. In addition, the cross-linked polymer is also resistant to acidic drugs such as aspirin and an excess of aluminum salt during preparation. The hydrophilic nature of this polymer reduces the likelihood of skin irritation. These desirable properties of PNVA make it possible to formulate a bioadhesive non-aqueous delivery system containing a water-sensitive drug at a high dose. Solvents, such as PG, NMP and glycerin, were used in fabricating the aspirin plaster. These solvents are less volatile and aspirin was found to have a good solubility in the solvent mixture. The drug can be entrapped in the matrix network and a high loading of aspirin can be

achieved. Moreover, PG and NMP were reported to be penetration enhancers for some drugs (Aungst *et al*., 1986). Therefore, the combination of PG and NMP was expected to increase the permeation of aspirin through skin.

Non-woven cotton wool was used as a backing layer in this formulation of aspirin plaster. The soft and smooth texture of cotton wool is suitable for application on skin surface and its flexibility also allow easy application on any part of body surface area. It can hold the adhesive polymer matrix tightly even when the plaster is stripped from the skin. A plastic release liner was used for the aspirin plaster. Its transparent appearance made the aspirin plaster cosmetically acceptable. It is easily removed from the plaster prior to application. Therefore it is a suitable release liner for packaging.

B. Physical properties of polymer-drug matrix gels

Different combinations of polymer, cross-linking agent and solvents were studied in an attempt to prepare a suitable plaster for clinical application. Depending on the combination, the plasters showed differences in appearance and texture of polymer-drug matrix gel. Polymer-drug matrix gels consisting of lower polymer concentrations of 5% and 7% were wet and viscous due to the relatively higher content of solvents. Viscous gel residues were left on the skin surface upon removal of plasters. Such plasters are cosmetically unacceptable. Polymer-drug matrix gels with higher polymer concentrations of 11% and 13% were rigid with hard and rough surface and could not adhere to the skin surface for prolonged periods. In the formulation of polymer-drug matrix gels, a lower concentration of the cross-linking agent produced weakly cross-linked gels which appeared sticky and wet, leaving behind sticky residues on the skin surface after removal of the plasters. With higher concentration of the cross-linking

agent, the strongly cross-linked gels made plasters inflexible leading to poor bioadhesion on the skin. When altering the ratio of PG to NMP, a higher proportion of PG resulted in very viscous polymer-drug matrix gel which was cosmetically unacceptable. With a lower proportion of PG, the gel was less viscoelastic and the plaster was less bioadhesive.

1. The rheological properties of aspirin gels

The rheological parameters and the shape of rheograms were directly related to the gel strength of the plasters (Madsen *et al*., 1998). Therefore, the rheological properties of the polymer-drug matrix gels were determined and effects of formulation variables on the gel structure were investigated. The results obtained from the rheological studies showed that G′ values were greater than G″ and loss tangent values were less than 1 over the entire frequency range for all aspirin gel formulations. Loss tangent values were relatively independent of oscillatory frequency in all formulations of aspirin gel. These were consistent with the typical behavior of cross-linked gels, indicating that all the polymer-drug matrix gels had real three-dimensional network structures. However, the gel strength was a function of formulation variables as shown by the changes in the values of loss tangent with formulation variables including polymer concentration, polymer to cross-linking agent ratio and PG to NMP ratio.

The effect of the polymer concentration on the viscoelastic properties of the polymer-drug matrix gels was assessed. It was found that the polymer concentration was negatively and significantly correlated with the loss tangent values (Pearson's correlation, $p<0.05$), indicating that higher polymer concentrations produced stronger cross-linked gels. The formulations with polymer concentrations of 11% and 13%

formed very hard cross-linked gels whereas the formulations with polymer concentrations of 5% and 7% formed weaker cross-linked gels. For formulations with lower concentration of polymer, the higher proportion of solvents accounted for the viscous properties of the gels. There have been several reports of the effects of the concentration of hydrophilic polymers on the viscoelastic properties of pharmaceutical formulations (Jones *et al*., 1996; 1997; 1998 and Talukdar *et al*., 1996) and the increased elasticity was associated with increasing polymer concentration.

The ratio of polymer to cross-linking agent influenced the cross-linking density of the plaster and subsequently the loss tangent values. The amount of cross-linking agent was negatively and significantly correlated with loss tangent values (Pearson's correlation, $p<0.05$). The higher proportion of cross-linking agent in gels with polymer to cross-linking agent ratios of 1:1 and 1:1.5 caused stronger cross-linking between polymer and cross-linking agent and produced stronger gels formed. For polymer to cross-linking agent ratios of 2.5:1 and 2:1, there was insufficient cross-linking agent, resulting in the formation of a weaker cross-linked gel. The optimal ratio of polymer to cross-linking agent was found to be 1.5:1. Therefore, it could be postulated that appropriate proportions of polymer and cross-linking agent were essential for preparing a strong, elastic and cross-linked gel to carry therapeutic amounts of drug.

With respect to the ratio of PG to NMP, the proportion of PG was negatively and significantly correlated with loss tangent values (Pearson's correlation, $p<0.05$). This indicated that loss tangent decreased with increase in the proportion of PG. Therefore, the higher proportion of PG in the solvent mixture formed the more viscoelastic polymer-drug matrix gel and thus increased gel strength. For lower proportion of PG,

the gel was less viscoelastic and it produced weak bioadhesive plaster for skin. Therefore, PG to NMP ratio of 2:1 was the most suitable for the preparation of an acceptable and stable plaster.

2. *In vitro* and *in vivo* bioadhesion studies

The results obtained from the *in vitro* tensile testing and *in vivo* adhesion studies showed that the formulations with 9% polymer concentration exhibited the highest detachment force and strongest bioadhesive properties. At lower polymer concentrations of 5% and 7%, less than 50% and less than 75% adhesion respectively were observed because their resultant gels were rather liquid-like leading to decreased cohesiveness and lack of adhesiveness of the plasters. With higher polymer concentrations of 11% and 13%, the increased polymer concentration caused the stronger and harder gel plasters but adhesiveness of the plasters decreased. Hence, it was concluded that 9% polymer content was optimal for the preparation of strong and bioadhesive plaster.

The maximum bioadhesion was found to be associated with the formulation with polymer to cross-linking agent ratio of 1.5:1. A lower bioadhesion was obtained from the plasters with polymer to cross-linking agent ratios of 2.5:1, 2:1, 1:1 and 1:2. The plasters with ratios of 2.5:1 and 2:1 did not contain enough cross-linking agent for complete interaction with the polymer molecules. Therefore, the cohesiveness as well as adhesiveness of plasters decreased. With increasing the amount of the cross-linking agent in the formulations, the cross-linking density of the gels was increased up to a maximum. Maximal bioadhesion was achieved by the formulation with polymer to cross-linking agent ratio of 1.5:1. At its optimal ratio, polymer molecules were

completely cross-linked and yet possessed some functional groups of the polymer molecules for bioadhesion. Higher amounts of cross-inking agent (1:1 and 1:2) adversely affected the availability of these functional groups, thus bioadhesion decreased. Therefore, an optimal ratio of polymer to cross-linking agent (1.5:1) was important for strong bioadhesion of the plasters.

For PG to NMP ratios of 1:2 and 1:1, the gels formed were solid in nature and bioadhesion strengths were lower. With PG to NMP ratio changing from 3:1 to 4:1, the higher contents of PG increased the viscoelastic properties of the gels but the adhesiveness of plasters was decreased. The best ratio of PG to NMP was found to be 2:1, where the plasters showed the highest detachment force and maximum bioadhesion strength. The results obtained from *in vitro* and *in vivo* adhesion studies showed that a formulation with 9% polymer, polymer to cross-linking agent ratio of 1.5:1 and PG to NMP ratio of 2:1 had ideal bioadhesive property suitable for clinical testing although the plasters prepared did not have the strongest viscoelastic properties. In contrast, the plasters with the strongest viscoelastic property, formulated with polymer concentration of 13%, polymer to cross-linking agent ratio of 1:1.5 and PG to NMP ratio of 4:1 did not have sufficient bioadhesion to be applicable for use in clinical testing.

C. *In vitro* release and permeation studies

1. Release of aspirin from the plasters

The results from the *in vitro* drug release study showed that the highest rate of drug release was from formulation with the lowest polymer concentration while the slowest rate of drug release from the formulation with the highest polymer concentration. The

concentration of the polymer in the formulations was negatively and significantly correlated with the amount of drug released. This was attributed to the denser gel structure with increasing polymer concentration and thus the rates of aspirin release decreased. The plasters consisting of a lower concentration of polymer had loose gel network, therefore the drug was released easily from such type of gels.

The amount of cross-linking agent was negatively and significantly correlated with the drug release rate. This indicated that a higher cross-linking density between polymer and cross-linking agent caused a decrease in rates of drug release from the plasters. When the amount of cross-linking agent was increased to a certain value, the polymer molecules were almost completely cross-linked and strong dense gels were formed. Higher cross-linking density produced smaller cavities in the gel matrices of the plasters and therefore, the release of drug dissolved in the solvents, which were entrapped in the cavity of the cross-linked gel, was slowed down. In contrast, if the amount of polymer was much higher than the amount of cross-linking agent, there was an abundance of the polymer that was not completely cross-linked and a weak gel was formed. Therefore, the drug was easily released from the bigger cavity of the weakly cross-linked gels.

A high concentration of PG in the plasters caused a slow release rate of aspirin. This can be explained by the increased viscoelasticity of the gel containing large amount of PG (Rafee-Tehrani and Mehramizi, 2000; Chi and Jun, 1991 and Larrucea *et al*., 2001). PG was not only a good co-solvent for the drug but it also can act as a penetration enhancer by altering the skin structure. It permeates the skin readily and carries the drug along with it (Squillante *et al*., 1998). Although PG is widely used as a

cosolvent for lipophilic drugs and has the potential to be a skin permeation enhancer, a higher proportion of PG decreased the amount of drug released. Therefore, an appropriate quantity of PG should be used. NMP enhances the permeation of lipophilic drugs by increasing their solubility in the skin (Akhter and Barry, 1985). It was reported that NMP enhanced the permeation of mefanamic acid across rabbit skin (Naito *et al*., 1985). However, an excess amount of NMP might produce dermatitis due to defattening of the epidermis after prolonged or repeated contact with excess amount of NMP (Barry, 1987). Therefore, the amount of NMP to be used in the plasters should be limited to a level that is non-irritating and safe for topical application. The results obtained from our studies showed that PG to NMP ratio of 2:1 was optimal in considering the release and permeation properties as well as the potential irritation.

Overall, the percentage of aspirin released from the plasters was linearly related to the square root of time, indicating that the drug release was controlled by the diffusion of the drug through the tortuous routes made up of the microcavities in the three dimensional gel networks.

2. Permeation studies with pig skins

Although there are significant anatomical and biochemical differences between human and animal skins at cellular levels, animal skins are frequently used for *in vitro* permeation studies. Rat, guinea-pig, snake, pig and mouse skins are commonly used instead of human skin. Among them, pig skin showed the strongest resemblance to human skins (Wu *et al*., 1997). Therefore, in the permeation studies, pig skin was used as permeation barrier. For the optimal formulation, the permeation of aspirin through the excised human skin was also compared to that through the pig skin.

The results obtained from the *in vitro* release studies were consistent and permeation of aspirin through the pig skin decreased with increasing polymer concentration and increasing the cross-linking density of the plaster (Pearson's correlation, $p<0.05$). A higher level of drug release from the plaster resulted in faster permeation rate of aspirin through the pig skin. Increasing the PG to NMP ratio also decreased the permeation of aspirin through the pig skin. However, the change in the ratio of PG to NMP had a greater effect on the permeation of aspirin through the pig skin than through the synthetic membrane. This might be attributed to the enhancement effects of PG and NMP on the skin.

In summary, the results obtained from *in vitro* drug release and permeation studies indicated that plasters formulated from low polymer concentration, low proportion of cross-linking agent and low proportion of PG showed the highest amount of drug released from the plasters and the highest amount of drug permeated through the pig skins. However, the physical properties of these plasters were unsuitable for clinical use. Therefore, plasters formulated with 9% of polymer, polymer to cross-linking agent ratio of 1.5:1 and PG to NMP ratio of 2:1 were chosen for clinical use because of their good physical properties, strong bioadhesiveness and moderate drug release and permeation rates through the pig skins.

3. Comparison of flux of aspirin through various types of barriers

The permeation of aspirin from the optimal plaster through different barriers was compared. The release of aspirin through the synthetic membrane was much higher than its permeation through the pig and human skins (ANOVA, $p<0.05$). The

permeation of aspirin through the pig skin and human skin were not significantly different ($p>0.05$), indicating that the pig skin resembled the human skin. It was reported by other research workers that excised human breast skin had no significant difference compared with the pig skin (Wu *et al*., 1997). Therefore, pig skin is suitable for substituting human skin in *in vitro* skin permeation and *in vivo* irritation studies. The synthetic membrane used was very porous, with pore size of 0.45 μm. It acted as a physical support and did not constitute a permeation barrier compared to the pig and human skins. Therefore, the amount of drug permeated through pig and human skin were significantly lower than that through the synthetic membrane (ANOVA, $p<0.05$).

It was found that aspirin released from the plaster was linearly related to the square root of time. Aspirin has been reported to have good skin permeability (McAdam *et al*., 1995). Therefore, it can be postulated that overall permeation of aspirin through the skins was controlled by the drug released from plaster. The diffusion-controlled system is favorable for development of aspirin plaster in that the permeation and subsequent clinical performance is easily modified by changing the formulation.

D. Accelerated stability testing

The mixture consisting of PG and NMP was used to formulate the non-aqueous gel plaster. After storage under accelerated stability test condition (40°C, 75% RH) for 4 weeks, there were no significant changes in appearance of the plasters and drug content. These findings indicated that aspirin was stable in the non-aqueous solvents, PG and NMP. The non-aqueous environment is the critical factor for preventing water-sensitive drugs like aspirin from hydrolysis.

E. Skin irritation studies

In this study, VAS and laser Doppler perfusion imager were used to assess skin irritation. VAS is a very useful simple method to assess the skin irritation qualitatively by the naked eye and LDPI is an additional tool to assess skin irritation quantitatively by measuring skin blood flow. Therefore, both assessment methods are complementary in the study of skin irritation. The higher cutaneous blood flow was found in site of the skin applied with the plasters formulated with 5% and 7% polymer concentration than that of the site of the skin applied with the plasters formulated with 9%, 11% and 13% of polymers for both aspirin and placebo plasters. The lower concentration of polymer formed semisolid plasters with higher proportion of PG and NMP which caused the skin irritation effect after prolonged contact with skin, leading to increased cutaneous blood flow. For both placebo and aspirin plasters, higher cutaneous blood flow were observed for formulations with polymer to cross-linking agent ratios of 1:1 and 1:2 than those with ratios of 2.5:1, 2:1 and 1.5:1. This was due to the presence of higher proportion of the cross-linking agent, aluminum chloride. Prolonged contact with higher proportion of aluminum chloride caused the skin irritation effect and therefore increased the skin blood flow. Higher cutaneous blood flow was also observed for plasters with PG to NMP ratios of 1:2, 3:1 and 4:1. Prolonged contact of higher proportion of penetration enhancers (either NMP in 1:2 or PG in 3:1 and 4:1) increased dryness of skin and led to increased cutaneous blood flow. Using the VAS method, all the results showed no changes in skin color after application of aspirin and placebo plasters. The increase in the cutaneous blood flow was not sufficient to change the skin surface color. Another reason is the natural pink color of pig skin, which was quite difficult to differentiate between skin irritation and normal skin color. Therefore, LDPI was more sensitive than VAS in the assessment of skin irritation. A recent study

demonstrated that LDPI results did not correlate with VAS in irritant reaction in human (Goon *et al*., 2004).

Aspirin itself also caused skin irritation in pig skin. Using plasters with different concentrations of aspirin (200, 300 and 400 mg), higher values of LDPI and VAS were seen with the higher concentrations of aspirin after application for 14 days. The increase in irritation was a function of the concentration of aspirin and the duration of application of the plaster. Therefore, the aspirin load is limited for the percutaneous delivery device. Duration of application of plaster also played an important role to achieve a safe and effective therapeutic action. The longer the application, the higher the extent of drug penetrated into the skin. Prolonged occlusion of the skin would make it soft and more prone to breakage of the barrier. The surface area of application of plaster also influenced the penetration of the drug. The larger surface area of application of plaster allows the higher amount of drug absorbed, thus leads to higher systemic availability and higher risk of adverse effects. Therefore, proper guidance is necessary for the application of topical plasters to adjust the duration, surface area and concentration of drugs to prevent the unwanted side effects.

F. Limitations of our study

There are some limitations of the study to be mentioned.

1. Measurement of aspirin and salicylic acid levels in the skin after plaster application should be performed to compare with the levels of aspirin and salicylic acid in plasma.
2. A larger sample size will be more sensitive to detect any statistical significant difference in skin irritation on pigs.

3. A clinical trial in healthy human subjects should be done to assess the safety profile and irritancy of the aspirin plasters in humans.
4. After obtaining results in healthy humans it would be of prime importance to evaluate the efficacy and adverse effects of the formulation in patients suffering from itch and painful neuropathies such as post herpetic neuralgia and acute herpes zoster.
5. Other methods of assessment of irritancy which have not been performed in this study such as measure of transepidermal water loss using evaporimeter should be further utilized to assess the irritant effect of long term application of this formulation.

CHAPTER 7

CONCLUSIONS

1. Copolymer of N-vinylacetamide and sodium acrylate (PNVA) has good solubility in various solvents, such as PG, NMP, ethanol, and possesses good bioadhesive property. It can be cross-linked with trivalent ions to form an elastic gel. Hence, it is suitable for formulation of non-aqueous bioadhesive gels.
2. Non-aqueous aspirin gels consisting of PNVA cross-linked with aluminum chloride in a mixture of PG, NMP and glycerin were successfully prepared by a casting method. The resultant polymer-drug matrix gels were soft, flexible, transparent and bioadhesive. The polymer-drug matrix gels consisted of a three-dimensional network where aspirin dissolved in the solvent mixture held in the microcavities of the structure.
3. Formulation variables such as polymer concentrations, polymer to cross-linking agent ratio and PG to NMP ratio greatly influenced the preparation process, physical and bioadhesive properties of the plasters, aspirin release and permeation through pig and human skins, and irritation to pig skin.
4. Increasing polymer concentration increased the plaster mechanical strength but decreased drug release and permeation. Higher proportion of polymer and thus lower proportion of solvents in the plasters reduced irritation on pig skin. Maximum bioadhesion of the plasters was achieved with polymer concentration of 9%.

5. Increasing amount of cross-linking agent also increased the mechanical strength of the plaster but drug release and permeation were decreased. Higher amount of cross-linking agent caused skin irritation after prolonged period of contact with the skin. Polymer to cross-linking agent ratio of 1.5:1 showed the maximum bioadhesion.

6. With increasing amount of PG, the mechanical strength of the plasters increased but drug release and permeation decreased. Irritation of pig skin also increased on prolonged contact with higher proportion of PG or NMP. Maximum bioadhesion of the plasters was produced by PG to NMP ratio of 2:1.

7. Aspirin itself also caused irritation to pig skin, particularly after a long period of application. Therefore, load of aspirin in the plasters and duration of application should be limited.

8. Aspirin was stable in the plasters after storage under accelerated stability test conditions (40°C, 75% RH) for 4 weeks. The non-aqueous environment was crucial for preventing aspirin from hydrolysis.

9. The plasters formulated with 9% of polymer, polymer to cross-linking agent ratio of 1.5:1 and PG to NMP ratio of 2:1 were found to be the most suitable for clinical use because of their good physical properties, strong bioadhesiveness, moderate drug release and permeation through human skins, and no irritation to pig skins.

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