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นางสาวชลธิชา ศรีแสวง

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FORMULATION DEVELOPMENT AND EVALUATION OF GLUCOSAMINE HYDROCHLORIDE FOAMING SOLUTION FOR TRANSDERMAL DELIVERY

Miss Chonticha Srisawang

A Thesis Submitted in Partial Fulfillment of the Requirements

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CHONTICHA SRISAWANG: FORMULATION DEVELOPMENT AND EVALUATION OF GLUCOSAMINE HYDROCHLORIDE FOAMING SOLUTION FOR TRANSDERMAL DELIVERY. ADVISOR: PHANPHEN WATTANAARSAKIT Ph.D., CO-ADVISOR: ASST. PROF. WALAISIRI MUANGSIRI Ph.D., JITTIMA CHATCHAWANSAISIN Ph.D., 110 pp.

Glucosamine hydrochloride (GS HCl) is an aminomonosaccharide and classified as symptoms-modifying drug in osteoarthritis. This study focused on development of glucosamine foam preparation. The effect of component of foam formulation on the permeation across the pig ear skin *in vitro* were elucidated using Franz-diffusion cell at 32°C for 24 hours and stability of GS HCl foam formulation were determined quanlitatively using validated HPLC method by pre-column derivetization with phenylisothiocyanate (PITC). The percent cumulative amount of permeated drug after 6 hours and the permeability constant (k_p) up to 6 hours of various components of formulation was calculated. The potential of enhancing permeability of the surfactant studied can be ranked as the following, Desyl glucoside (cumulative amount of permeated drug 18.05%, k_p = $2.20x10^{-6}$ cm/s), Caprylyl/Capryl Glucoside (cumulative amount of permeated drug 11.55%, k_p = $1.46x10^{-6}$ cm/s) and Tween 80 (cumulative amount of permeated drug 10.67%, k_p = $1.26x10^{-6}$ cm/s). And GS HCl foam containing desyl glucoside was the highest GS HCl permeation which more than solution (cumulative amount of permeated drug 28.05%, k_p = $3.10x10^{-6}$ cm/s). The formulation with various surfactants prepared from tartrate buffer at pH 3 and sodium metabisulfite as antioxidant and storage at 5°C better stability than others. The presence of small quantity of ethanol in preparations could enhance the drug permeability. The results indicate that there is a possibility to develop a GS HCl transdermal dosage forms.

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ชลธิชา ศรีแสวง: การพัฒนาสูตรตำรับและการประเมินสารละลายกลูโคซามีนไฮโดรคลอไรด์ที่ก่อโฟมเพื่อการนำส่งยาผ่านผิวหนัง (FORMULATION DEVELOPMENT AND EVALUATION OF GLUCOSAMINE HYDROCHLORIDE FOAMING SOLUTION FOR TRANSDERMAL DELIVERY) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: ดร. พรรณเพ็ญ วัฒนาอาษากิจ, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม: ผศ. ร.ต.ท.หญิง ดร.วลัยศิริ ม่วงศิริ, อ.ดร.จิตติมา ชัชวาลย์สายสินธ์, 110 หน้า.

กลูโคซามีนไฮโดรคลอไรด์เป็นสารในกลุ่มอะมิโนโมโนแซคคาไรด์เป็นยาในกลุ่มบรรเทาอาการข้อเสื่อม การศึกษานี้จะทำการพัฒนาสูตรตำรับกลูโคซามีนไฮโดรคลอไรด์ในรูปแบบโฟมโดยการศึกษาผลของส่วนประกอบของสูตรตำรับที่มีผลต่อการซึมผ่านผิวหนังหมูแบบภายนอกกายและความคงตัวของตัวยา การศึกษาการซึมผ่านทำโดยใช้ชุดฟรานซ์ดิฟฟิวชันเซลล์ที่อุณหภูมิ 32 องศาเซลเซียสในช่วงเวลา 24 ชั่วโมงและการวิเคราะห์หาปริมาณตัวยากลูโคซามีนไฮโดรคลอไรด์เพื่อศึกษาปริมาณการซึมผ่านและปริมาณตัวยาที่คงอยู่ด้วยวิธีโครมาโตกราฟีชนิดของเหลวสมรรถนะสูงโดยทำปฏิกิริยากับสารฟีนิลไอโซไธโอไซยาเนตก่อนทำการวิเคราะห์ ทำการคำนวณหาค่าร้อยละของปริมาณสะสมของยาที่ซึมผ่านผิวหนังที่เวลา 24 ชั่วโมงและค่าคงที่ของการซึมผ่านผิวหนังในช่วงเวลา 6 ชั่วโมงของสูตรตำรับต่างๆที่มีความเข้มข้นของตัวยาร้อยละ 10 พบว่าสูตรตำรับที่มีประสิทธิภาพในการช่วยการซึมผ่านของยาเข้าสู่ผิวหนังมากที่สุดเรียงตามลำดับได้แก่ เดซิลกลูโคไซด์ (ปริมาณสะสมของยาที่ซึมผ่านผิวหนังเท่ากับ 18.05% ค่าคงที่การซึมผ่านผิวหนังเท่ากับ $2.20x10^{-6}$ เซ็นติเมตรต่อวินาที) คาพริวิริว/คาพริวกลูโคไซด์ (ปริมาณสะสมของยาที่ซึมผ่านผิวหนังเท่ากับ 11.55% ค่าคงที่การซึมผ่านผิวหนังเท่ากับ $1.46x10^{-6}$ เซ็นติเมตรต่อวินาที) และทวีน 80 (ปริมาณสะสมของยาที่ซึมผ่านผิวหนังเท่ากับ 10.67% ค่าคงที่การซึมผ่านผิวหนังเท่ากับ $1.26x10^{-6}$ เซ็นติเมตรต่อวินาที) และสูตรตำรับที่ประกอบด้วยเดซิลกลูโคไซด์ในรูปแบบโฟมจะมีประสิทธิภาพในการซึมผ่านของยาเข้าสู่ผิวหนังได้ดีมากกว่าในรูปแบบสารละลาย (ปริมาณสะสมของยาที่ซึมผ่านผิวหนังเท่ากับ 28.05% ค่าคงที่การซึมผ่านผิวหนังเท่ากับ $3.10x10^{-6}$ เซ็นติเมตรต่อวินาที) สูตรตำรับที่ประกอบด้วยทาเทรตบัฟเฟอร์พีเอช 3 และโซเดียมเมตาไบซัลไฟว์เป็นสารต้านอนุมูลอิสระเก็บที่อุณหภูมิ 5 องศาเซลเซียสมีความคงตัวมากที่สุด ในสูตรตำรับที่มีเอทานอลปริมาณน้อยสามารถเพิ่มการซึมผ่านของยาสู่ผิวหนังได้จากผลการทดลองพบว่ามีความเป็นไปได้ที่จะนำส่งยากลูโคซามีนไฮโดรคลอไรด์ผ่านผิวหนัง

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สาขาวิชา เภสัชอุตสาหกรรม ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

ปีการศึกษา 255 5 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม .....

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LIST OF ABBREVIATIONS

°c	=	degree Celsius
cm	=	centimeter
et al	=	et alii, „and other“
g	=	gram
GS HCl	=	glucosamine hydrochloride
h	=	hour
kg	=	kilogram
k_p	=	permeability constant
µg	=	microgram
µl	=	microliter
mg	=	milligram
min	=	minute
ml	=	milliliter
PBS	=	phosphate buffer saline
PITC	=	phenyl isothiocyanate
pH	=	the negative logarithm of the hydrogen concentration
s	=	second
w/v	=	weight by volume

CHAPTER I

INTRODUCTION

Osteoarthitis (OA) is the most common joint disease. Incidence and prevalence of the disease is closely related to patient‟s age. OA causes chronic disability, making a significant concern to public health. OA, a degenerative disease of the joints, is caused by a decrease in cartilage matrix production, which leads to roughening and fissuring of the cartilage, follows by changes of the underlying bones. Common treatments include physical therapies, such as exercises, weight reduction and use of pain relievers including acetaminophen and nonsteroidal anti-inflammatory drugs (NSAIDs), COX-2 selective inhibitors (Kanwisher et al, 2005), opioid analgesic, injection of glucocorticoids or hyalulonic injection, and alternative medicine, for example vitamin A, vitamin C, and vitamin E, ginger oil, turmeric oil, omega-3 fatty acids, chondroitin sulfate, and/or glucosamine.

Glucosamine is an aminomonosaccharide, which is a common monosaccharide in the human body. Glucosamine is a fundamental substance for the biosynthesis of macromolecules which are found in many tissues particularly in the cartilage such as glycolipids, glycoprotein, glycosaminoglycan, hyaluronate, and proteoglycans (Zerkak and Dougados, 2004). In clinical trials, glucosamine has been reported to improve symptoms in OA to reduce a progression of joint-space narrowing in patients with knee OA. Therefore, glucosamine is called a symptoms-modifying drug in a treatment of OA. (Piperno et al., 2000).

Commercial available dosage forms containing glucosamine include tablet, capsule, powder, cream, gel, injection, and transdermal patch (The Arthritis and Glucosamine Information Center, 2010: Online). Oral administration of glucosamine gives poor oral bioavailability (F=0.26) due to extensive degradation in the gastrointestinal tract and extensive first-pass metabolism in the liver (Anderson et al., 2004). Common and mild side effects from glucosamine consumption include constipation, diarrhoea, nausea, dyspepsia, excessive gas, abdominal distension, abdominal cramps, headache, and skin rash. Other rare side effects include increases

musculoskeletal pain, urinary tract infection, vertigo, blood pressure fluctuation, and depression (Hughes and Carr, 2002; Regnister et al., 2001). However, glucosamine is considered as a safe compound in a treatment of OA when compared to administration of other drugs such as analgesics and NSAIDs (Chard and Dippe, 2000; Regnister et al., 2001) which can cause epigastric pain, heartburn, diarrhea, and increased blood sugar level in DM patient (Herotin et al., 2004). Glucosamine transdermal administration would be an alternative route to overcome glucosamine poor oral availability problem.

Transdermal administration is shown to be a convenient way with high patient compliance to deliver drug. However, glucosamine is a hydrophilic compound with low skin permeability. Permeation rate of glucosamine sulfate through rat skin was reported to be 13.27 mcq/cm^2/h (Kanwischer et al., 2005). In another study, glucosamine sulfate was shown to permeate through mice skin but the permeability of the compound was too low to shown any therapeutic effects (Lynk Bio Tech, 2002: Online).

Biological barrier are an important issue in transdermal administration. Ingeneral generally agreed that the stratum corneum, the outermost layer of the skin, is the barrier to the passage of drugs across the epidermis. The methods employed for modifying the barrier properties of the stratum corneum to enhance drug penetration through the skin can be divided as physical enhancement technives such as iontophoresis, and chemical enhancement such as organic solvents, fatty acids, and alcohols (Chan, 2005). These chemical enhancers should be safe and suitable for formulations. One of the chemical enhancer, that attractive and often used as an enhancer to increase drug permeation is ethanol.

Foam formulation was shown to have advantages effects over conventional dosage forms in enhancing skin permeation. Foam formulation of betamethasone valerate gave rise to a higher bioavailability without toxicity associated with the absorped dose (Feldman et al., 2001). A release rate of betamethasone benzoate foam formulation was higher than that of cream formulation (Berry and Woodford, 1977). Foam has an attractive appearance and is easy to apply on the skin. Therefore, foam formulation may be a good alternative preparation for OA patients who have to apply

the product without high mechanical shearing force over an inflamed area (Huang et al., 2005)

This study focused on development of glucosamine foam preparation. The effect of component of foam formulation on the permeation across the pig ear skin and stability of formulation were of interest. Effects of non-ionic surfactants, pH, and other components on physical stability of glucosamine were investigated. Results of this study suggested the possibility of initial steps to developing foam preparation of glucosamine as a commercial product.

CHAPTER II

LITERATURE REVIEW

1. Glucosamine hydrochloride

Glucosamine hydrochloride or 2-Amino-2-deoxy-D-glucopyranose hydrochloride ($C_6H_{13}NO_5HCl$) is a odorless white powder with a molecular weight of 215.63 g/mol Figure (2-1). Solubility of glucosamine Hcl in water is 0.1 g/ml. Glucosamine has a pKa of 7.75.

Physicochemical properties of glucosamine hydrochloride

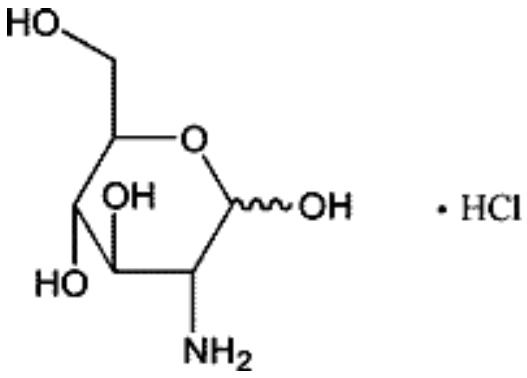


Figure 2-1 Chemical structure of glucosamine hydrochloride

Mechanism of action

Glucosamine is an aminomonosaccharide derived from chitin in crustacean shells. Glucosamine inhibits IL-1-induced COX-2 and PGE_2 synthesis, inhibit NF-ĸB binding activity, and reduce releasing of cytokine indicating that these compounds may have inflammatory properties (Largo et al., 2003).

Glucosamine is available in various forms, including glucosamine hydrochloride, glucosamine sulfate, and N-acetyl-D-glucosamine (Laurie and Nixon, 2006).

Pharmacokinetic

The pharmacokinetic parameters of glucosamine are summarized in Table 2-1. Although orally administered glucosamine is absorbed from intestinal mucosa over 90% but absolute bioavailability is only 20% because of extensive first pass metabolism (Anderson et al., 2004).

Table 2-1 Pharmacokinetic parameter of glucosamine (Anderson et al., 2004)

Parameter	Glucosamine
Oral bioavailability	20%
Plasma $t_{1/2}$	58h
Plasma protein binding	90%
Excretion	In the urine 40% In the feces 2%

Side effect

The common adverse effects on the gastrointestinal tract include epigastric pain, heartburn, and diarrhea (Henrotin, Sanchez and Baligand, 2004).Since glucosamine is derived from shellfish people who are allergic to shellfish can develop an allergic reaction to glucosamine (The Arthritis and Glucosamine Information center, 2005).

Therapeutic uses

Orally administration of 1,500 mg glucosamine per day is effective in alleviating the OA symptoms and improving joint function (Henrotin et al., 2004). A long term study (1-3 years) has shown that the patients with the less severe knee OA, will prolong a disease progression (Bruyere et al., 2003). Another benefit of glucosamine is wound healing effect by enhancement of hyaluronic acid production (McCarty, 1996).

Administration

The recommended oral starting dose is two 250 mg capsules taken three times a day, or a single daily dose of 1884 mg for at least six weeks. The recommended duration of the therapy is three months. Glucosamine should be taken at least 15 minutes before meal. The oral administration can be usefully combined with the intramuscular administration to accelerate and improve the therapeutic effects. The commercial products of glucosamine are shown in Figure 2-2, 2-3 and 2-4.

Figure 2-2 Commercial oral products of glucosamine (The Arthritis and Glucosamine Information Center, 2010: Online)

Figure 2-3 Commercial transdermal products form of glucosamine (The Arthritis and Glucosamine Information Center, 2010: Online).

Figure 2-4 Commercial topical products of glucosamine (The Arthritis and Glucosamine Information Center, 2010: Online).

2. Transdermal drug delivery system

A transdermal drug product is intended to deliver the drug systemically to treat or prevent disorder in location distant from the site of topical application. Drug released from the transdermal drug delivery system is absorbed through the various skin layers, the stratum corneum, epidermis and dermis in to blood circulation and transported to target tissue to achieve therapeutic effect (Shah, 1994). Now the transdermal drug product in the markets are in many dosage forms such as solution, gel, cream, ointment, emulsion, microemulsion, liposome and transdermal patch.

Advantage of transdermal delivery

Skin became popular as a potential site for systemic drug delivery because it could (1) avoid drug degradation due to gastric and intestinal enzymes (2) to avoid hepatic first-pass metabolism and (3) to enable absorption control (Walters and Roberts, 2002).

Drug delivered orally way encounter the first-pass metabolism. Furthermore, some drugs are acid labile and unstable in the acidic environment of the stomach, and some drugs, such as NSAIDS, can cause gastrointestinal bleeding through irritation. The transdermal route can overcome these problems. The non-invasive method of delivery received.

2.1 Structure and function of skin

The skin acts as a two-way barrier, preventing the ingress of foreign molecules and the egress of endogenous substances. Skin is essentially composed of two major layers: an outer, unvascularized epitherial layer (the epidermis), and an inner layer (the dermis).

The epidermis

The epidermis composes of 5 stratums. The stratum corneum is the outermost layer of the skin with approximately 10-20 μm thick. It is nonviable epidermis and consists of 15-25 flattened, stacked, hexagonal, and cornified cells embedded in a mortar of intercellular lipid. Each cells approximately 40 μm in diameter and 0.5 μm thick. The thickness varies, according to areas of the body associated with frequent direct and substantial physical interaction with the physical forces. The stratum cornuem barrier properties may be partly related to its very high density (1.4 g/cm^3 in the dry state), its low hydration of 15-20%, compared with the usual 70% for the body, and its low surface area for solute transport (Walters and Roberts, 2002).

The dermis

The dermis, critical component of the body, not only provides the nutritive, immune, and other support systems for the epidermis, through a thin papillary later adjacent to the epidermis, but also plays a role in temperature, pressure, and pain regulation. The dermis is a coarse reticular layer with about 0.1-0.5 cm thick and consists of mainly collagenuos fiber sparse fibroblasts present in the dermis produce the connective tissue composing of collagen, laminin, fibronectin, and vitronectin. (Walters and Roberts, 2002).

The subcutis

The innermost layer of the skin is the subcutaneous tissue or hypodermis. The hypodermis acts as a heat insulator, a shock absorber, and an energy storage region. This layer is a network of fat cells arranged in lobules and linked to the dermis by interconnecting collagen and elastic fibers. As well as fat cells (possibly 50% of the body‟s fat), the other main cells in the hypodermis are fibroblasts and adipocytes can be stimulated by the accumulation of interstitial and lymphatic fluid within the skin and subcutaneous tissue (Walters and Roberts, 2002).

Skin appendages

There are four skin appendages: the hair follicles with their associated sebaceous glands, eccrine sweat glands, apocrine sweat glands, and the nails. Each appendage has a different function (Walters and Roberts, 2002).

2.2 Transport pathway through the stratum corneum

The major limitation to transdermal delivery is the skin itself, and the major barrier to penetration of matter is provided of matter is provided by a superfacial layer of the skin, the stratum corneum and its compact structure (Suhonen, Bowstra and Urtti, 1999).

Transport across the stratum corneum barrier might occur by any combination of the three pathways as shown in figure 2-5 (1) a transcellular pathway (2) a paracellular pathway or (3) an appendageal pathway.

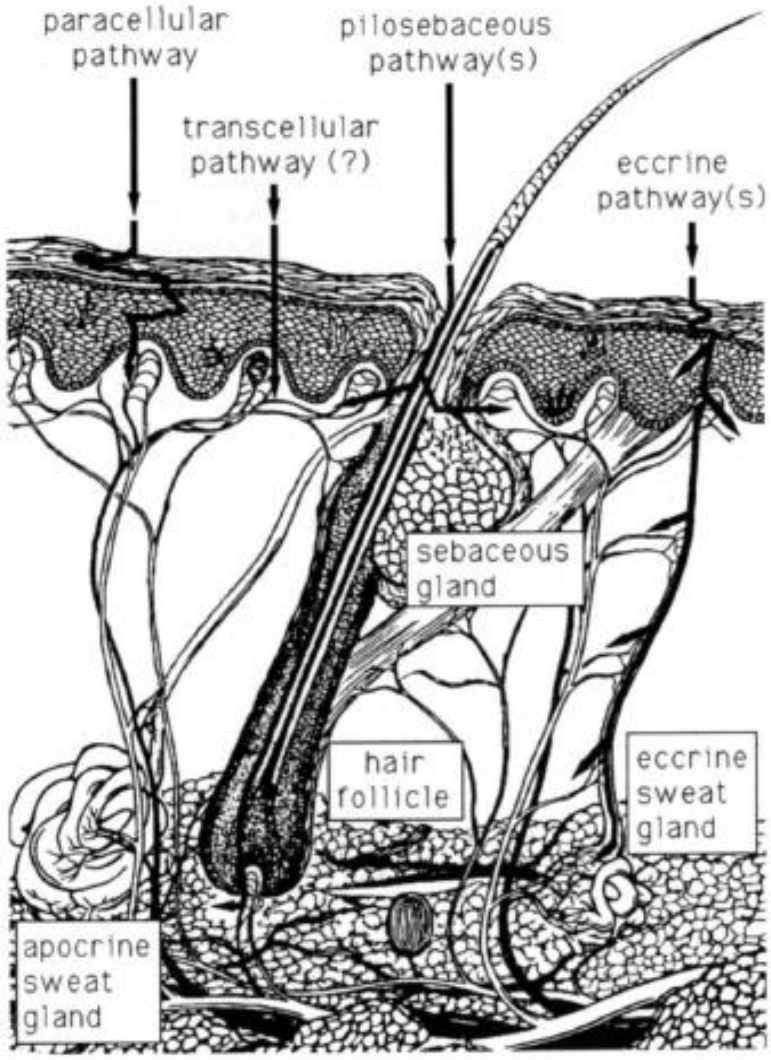


Figure 2-5 Potential pathways of permeation through human skin (Cullander, 1992)

Transcellular pathway includes passive transport of small molecules, active transport of ionic and polar compounds, and endocytosis and transcytosis of macromolecules (Hsieh, 1994).

Paracellular pathway is the transport of molecules through tight junctions. Ions, peptides and proteins were reported to be absorbed via this pathway (Hsieh, 1994).

While hydrophilic solutes employ appendageal pathway, the transport through pore, or shunt pathways, the transport through hair follicle and sweet ducts (Mitragotri, 2003).

2.3 Permeation enhancement

Permeation enhancers are divided into three groups according to the physicochemical properties of molecules to be absorped: polar, nonpolar, and polar/nonpolar molecules. Polar permeation enhancers cause conformational change of the membrane protein. Nonpolar permeation enhancers alter rigidity of the bilayer lipid structure. The binary enhancer (amphoteric molecules) cause both conformational change of the membrane protein and rigidity alterlation of lipid binary structure. Therefore, a critical step in development of transdermal drug delivery is employing of permeation enhancer in the preparation is that drug concentration that reaches the target site is in sufficient quantities to achieve its desired therapeutic effect. Techniques to improve drug penetration through the skin are classified as physical and/or chemical techniques. (Shah, 1994).

2.3.1 Permeation enhancement by physical techniques

There are several methods classified as physical enhancement techniques such as iontophoresis, sonoporation, and microneedles (Thong, Zhai and Maibach, 2005)

The iontophoresis and ultrasound (so called phonophoresis or sonophoresis) techniques have been reported to successfully deliver various therapeutic agents through skin. Major disadvantageous of these is the technique itself that receive professional technical skills in order to deliver drug to patient properly. In addition, quality of the device such as electrode, uniformity of current distribution are also key success factors of these methods (Shah, 1994).

Microneedle arrays, can deliver drug through the stratum corneum and to the skin capillaries. However, the limitation of this method is requirement of professional technical skills as mentioned earlier (Thong, Zhai and Maibach, 2005).

2.3.2 Permeation enhancement by chemical techniques

Permeation enhancement by chemical techniques employs chemical enhancers. Such as organic solvents, fatty acids and alcohols, detergents and surfactants (table 2-2).

Table 2-2 A partial list of some chemical enhancers (Thong, Zhai and Maibach, 2005)

Enhancer	Type
• Ethanol	organic solvent
• Ethyl acetate	organic solvent
• Sodium lauryl sulfate	surfactant
• Isopropylmyristate	surfactant
• Lauryl alcohol (also, lauric acid and lauryl lactate)	organic solvent
• DMSO (dimethyl sulfoxide)	organic solvent
• 1-Dodecylaza- cycloheptane-2-one (Azone®)	surfactant
• Polysorbates	surfactant
• Propylene glycol (PG)	surfactant

A proper permeation enhancer must significantly enhance drug penetration through the epidermis without severe irritatation or skin damage. These chemicals should be safe and nontoxic, pharmacologically and chemically inert, chemically stable, nonirritating, and nonallergic. In addition, the skin tissue should revert to its normal integrity and barrier properties upon removal of the chemical.

DMSO, an organic solvent, is a great enhancer because it is a powerful solvent. However, DMSO is shown to do harm to the stratum corneum by alteration of biochemical and structural integrity of the skin (Shah, 1997).

Ethanol is another compound often used as an enhancer/cosolvent. Ethanol is employed in numerous prescription and over-the-counter drug products. Some topical formulations contain ethanol up to 90%.

Ethanol has a relatively low incidence of topical reactions such as contact dermatitis and skin irritation, erythema on human subjects could be observed after application of preparation containing low concentration of ethanol on volunteers who are allergic to ethanol. Ethanol was believed to enhance skin permeation by both changes the conformation of membrane protein and lipid extraction from the bilayer structure. Both mechanisms result in an increase of solute diffusivity.

However dilute ethanolic solutions was primarily reported to increase drug solubility in the epidermis rather than increasing in drug diffusivity. The octanol/water partition coefficient of solutes can be used to predict effectiveness of ethanol as permeation enhancer of that particular solutes (Yum et al., 1994).

Ethanol has been reported to increase the flux of many drugs through skin such as ibuprofen, flubiprofen, indomethacin, isosorbide dinitrate, cyclobarbital, didanosine and fluoxetine (Parikh and Ghosh, 2005).

Surfactants

Another group of well-known chemical enhancer is the surfactants. The surfactants can be classified into several categories based on their physicochemical properties: (1) nonionic surfactants, (2) ionic surfactants include; cationic surfactant, anionic surfactants and zwittering ionic surfactant.

Denaturation of protein in the keratin may contribute to enhanced permeability of the stratum corneum. It is known that ionic surfactant can cause epidermal protein denaturation resulting in an opening of the proteinaceous polar pathway. However, extent of protein denaturation by these surfactant does not well correlate with an increase in epidermis permeability.

Ionic surfactant especially anionic surfactants cause swelling of the stratum corneum. Although, the damage is reversible, resulting in an increase of skin permeability. The extent of this damage was time and concentration dependent. (Yum

et al., 1994). SLS is an example of a powerful irritant. Non-ionic surfactants are considered as safe (Williams and Barry, 2004). It has been reported that, microemulsion of nonionic surfactant, tween 80, could effectively enhance the permeability of GS HCl (Punlapa, 2007).

The glucosides are non-ionic and non-toxic surfactants with antimicrobial activity and biodegradability. These mild and biodegradable agents are well tolerated by even the most sensitive skins. They are derived from natural raw materials (coconut and sugar). They are widely used as biocompatible detergents in foods, detergents, and in the pharmaceutical industry.

2.4 In vitro study of percutaneous absorption

Most common methods for evaluation of *in vitro* skin penetration is a use of diffusion cells. The major advantage of *in vitro* investigations is that the experimental condition can be controlled precisely, such that only variable are the skin and the test material. Although a potential disadvantage is that little information on the metabolism, distribution and effects of blood flow on permeation can not be obtained.

Diffusion cell design

In vitro systems range in complexity from a simple two-compartment „static" diffusion cell to multijacketed "flow-though" cells. Excised skin is always mounted as a barrier between donor chamber and a receptor chamber, and the amount of compound permeating from the donor to the receptor sides is determined as a function of time. Efficient mixing of the receptor phase (and sometimes the donor phase) is essential. Neither of these process should interfere with diffusion of the permeant. Sampling from the bulk liquid rather than the side arm, and accurate replenishment after sampling, are important practical considerations. It is essential that air bubbles are not introduced below the membrane during sampling.

Two design of static diffusion cells are upright ("Franz") or side-by-side type (Fig 2-6) receptor chamber volumes and surface areas of exposed membranes should be accurately measured, and precise values should be employed in subsequent

calculations, the side-by-side cells are useful for the determination of flux from saturated solutions or determination of absorption of the permeant in the gaseous state. The upright cells are useful for studying absorption from formulations spread on the membrane. The donor compartments can be capped to provide occlusive conditions, or left open, according to the objectives of the particular study.

Flow-through cells are useful when the permeant has a very low solubility in the receptor medium. Sink conditions are obtained as the fluid is continually replaced. However, the dilution produced by the continuous flow may compromise analytical method problems especially when the analytical sensitivity is low.

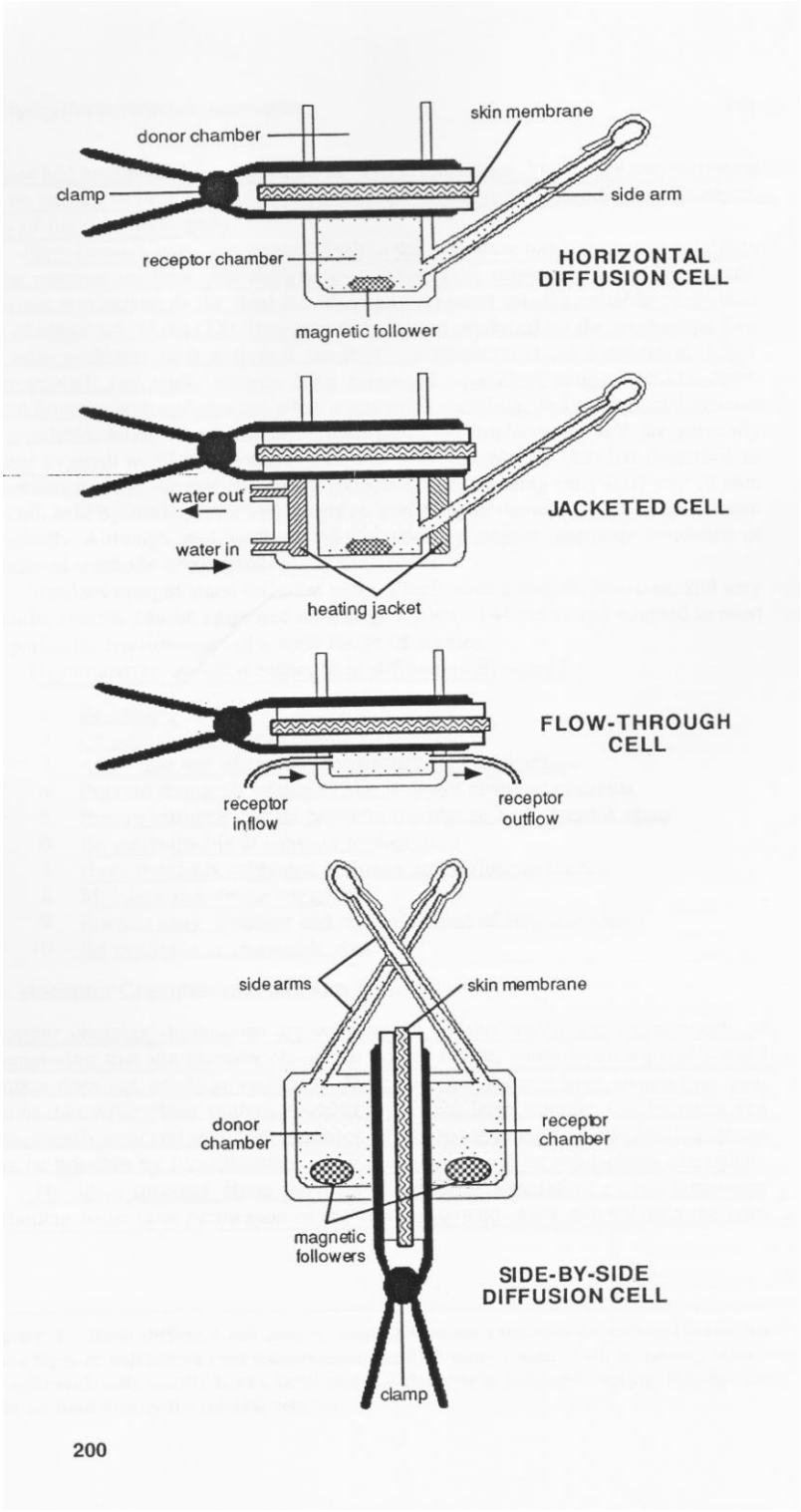


Figure 2-6 Basic diffusion cell designs (Brain, Walters and Watkins, 2002).

Receptor Chamber and Medium

A large receptor volume is necessary to ensure sink conditions. In general, the highest concentration of the permeant in the receptor fluid should less than approximately 10 % of its saturation solubility. High concentration of permeant present in the receptor medium lead to lower permeation rate. Large samples can be taken and subsequently concentrated., or by solid-phase extraction. The most commonly used receptor fluid is pH 7.4 phosphate buffer saline (PBS). (Brain, Walters and Watkinson, 2002).

Animal models for human skin for *in vitro* percutaneous absorption studies.

A major potential variant in the design of *in vitro* diffusion study is of type of skin membrane. The skin membrane generally used in *in vitro* permeation study include human skin membrane, animal skin form rats, mice, rabbits, guinea pigs, pigs and snakes and synthetic or artificial membrane such as cellulose acetate (Huong et al, 2009).

Skin membranes from pigs or miniature pigs have been reported to be a good animal model (Bronaugh et al., 1987). Thickness of the stratum corneum of pigs and rats is comparable to that of humans (16.8±0.7 μm). Rat skin has approximately 289 hair follicles/cm^2, while pig and human have around 11 hair follicles/cm^2. Therefore, pig skin is a better skin model in the in vitro permeation study than rat skin.

Pig ear skin was selected to be used in this study because it was extensively employed in dermatological research including testing of transdermal system, or skin permeability. The stratum superfacial dermis of the pig ear skin had a very homogeneous and compact construction similar to that of human (Meyer et al, 2007).

The permeation process

The skin is a heterogeneous membrane. However, simple diffusion laws can be used to describe the percutaneous absorption process. Since transdermal delivery involves the application of a device over a long period of time, it is generally assumed that steady-state conditions have been reached. Therefore, the Fick"s first law is the most relevant theory under such conditions. The second law describes non-steady state diffusion and can be used to analyze lag times and release rates from matrix type transdermal patches.

The most quoted from of Fick"s first law of diffusion describes steady-state diffusion through a membrane:

$$J = \frac{KD}{h} \times \left(C_O - C_i\right) \tag{1}$$

Where J is the flux per unit area, K is the stratum corneum-formulation partition coefficient of the drug, and D is its diffusion coefficient in the stratum corneum of path length h; c_0 is the concentration of drug applied to the skin surface, and C_i is the concentration inside the skin. In most practical, $C_O >> C_i$, and Eq. (1) simplifies to

$$J = K_P C_i \quad (2)$$

Where kp (=KD/h) is the permeability coefficient, which has units of velocity (often quoted as cm h^{-1}). It is a heterogeneous rate constant and encodes both partition and diffusional characteristics.

The input rate of the drug into the systemic circulation, from a patch of area A, is therefore given by the product

$$\text{Input rate} = A \times k_p \times c_0 \quad (3)$$

The output or elimination rate from the systemic circulation equals the clarance (Cl) multiplied by the plasma concentration at steady state ($c_{p.ss}$)

$$\text{Output rate} = Cl \times c_{p.ss} \quad (4)$$

Hence Eqs. (3) and (4) may be combined to predict the drug‟s plasma concentration following transdermal delivery:

$$c_{p.ss} = \frac{Ak_p c_o}{Cl} \quad (5)$$

Therefore, the plasma concentration at steady state depends directly on the area of the device, the skin permeability coefficient, and the applied concentration and is inversely related to drug‟s clearance. These parameters can be estimated from basic physicochemical properties, which are typically measured during pre-formulation (Hadgraft and Guy, 2003).

3. Foam delivery systems

Foam is defined as a dispersion of gas in a liquid or a solid, whereas the volume fraction of gas in the foam is in a range of 0.5 to 0.9. Foams can be classified into 2 types, liquid and solid foams. Solid foams can be generated when the liquid phase is changed into gel or solid phase after foam formation.

Foams are considered as a dosage form. The European Pharmacopoeia contains a monograph (1105) called "Medicated Foams" European Pharmacopoeia defines foam as "formulation, consisting of a large amount of gas dispersed in a liquid phase". Foams can be produced by mechanical means or by supersaturation of the liquid phase with gas. Liquid can be supersaturated with gas either by dissolving gas under pressure or by gas formation in situ. Most formulations on the pharmaceutical market are aerosol foams. Airspray® Pump foam dispensers create propellant-free foams (Arzhavitina and Steckel, 2010).

Foams for dermal drug delivery have some advantages compared to the traditional vehicles for treatment of topical disorders such as ointment, creams, lotions, gels or solutions. Foam formulations are generally easier to apply, lessdense, and spread more easily compared with other topical dosage forms. This is a major advantage when applying a medicament to highly inflamed skin (Prudon et al., 2003). Moreover, there is evidence that patients prefer foams over other vehicles leading to an increase in compliance.

In order to evaluate foam performance, foam expansion and foam liquid stability tests were performed in a cylinder and calculated using the following equation (Arzhavitina and Steckel, 2010);

$$Foam\,\exp ansion: FE(\%) = \frac{V(foam) - V(formulation)}{V(formulation)} \times 100\% \qquad (6)$$

where V (foam) is volume of produced foam (ml); V (formulation) is volume of formulation to produce V (foam) (ml).

The higher the FE the more foamable is the formulation.

$$Foam liquid stability : FLS(\%) = \frac{V(liquid\ 30\,\min)}{V(\ formulation)} x100\% \quad (7)$$

where V (liquid 30 min) is volume of liquid drained after 30 min.
The lower the FLS the more stable is the produced foam.

CHAPTER III

MATERIAL AND METHOD

Materials

Drug-

Glucosamine Hydrochloride USP (Lot no BCBD5943V, supplied by Sigma-Aldrich, Singapore)

Standard Glucosamine Hydrochloride 99% assay, (Lot no. 125K0024, supplied by Sigma-Aldrich, Singapore)

Chemical-

Acetonitrile (Matl. number 10071743, Burdick & Jackson, B&J ACS HPLC Certified solvent, SK chemicals, Korea)

Butylated hydroxyanisole (BHA) (Lot no. K31605762, Aketong Chemical Company (1985), Bangkok, Thailand)

Caprylyl/capryl glucoside (Lot no. TF92331, Adinop.co.th, Bangkok, Thailand)

Citric acid (Lot no.50520, supplied by Sigma-Aldrich, Singapore)

Desyl glucoside (Batch no. CE11050021, Thai Sanguanwat Chemical Company, Bangkok, Thailand)

Ethyl alcohol (Absolute alcohol AR quality, Lot no. 7C111110112, Chachoengsao, Thailand)

Ethylenediaminetetraacetic acid (EDTA) (Batch no. 0903328, Ajax Finechem Pty Ltd, New South Wales, Australia)

Lauryl glucoside (Lot no. CE91870016, The East Asiatic (Thailand) Public Company)

Methanol (Matl. number 10071753, Burdick & Jackson, B&J ACS HPLC Certified solvent, SK chemicals, Korea)

Methyl paraben (Lot no. 20090708, Aketong Chemical Company (1985), Bangkok, Thailand)

n-hexane (Lot no. 05010072, Lapscan, Dublin, Ireland)

Paracetamol powder USP 24 (Batch no. 0907006, Aketong Chemical Company (1985), Bangkok, Thailand)

Phenyl isothiocyanate (PITC) (Lot no. 1110115, Fluka, Tokyo, Japan)

Polyoxyethelene 20 sorbitan monooleate (Tween 80) (Lot no. 392141/1, Fluka, Buchs, Switzerland)

Potassium Dihydrogen orthophosphate (Batch no.AF401428, Ajax Finechem Pty Ltd, New South Wales, Australia)

Propylene glycol (Lot no. E132080208, Srichand United Dispensary Co., Ltd, Bangkok, Thailand)

Propyl paraben (Lot no. 20090710, Aketong Chemical Company (1985), Bangkok, Thailand)

Sodium cittrate (Batch no. 0911213, Asia Pacific Specialty Chemical Limited, New South Wales, Australia)

Sodium metabisulfite (Batch no. F2K105, Ajax Finechem Pty Ltd, New South Wales, Australia)

Sodium tartrate (Batch no. 0912479, Ajax Finechem Pty Ltd, New South Wales, Australia)

Tartaric acid (Lot no. 53156812-1, supplied by Apex chemical, Bangkok, Thailand)

Ultrapure water

Membrane-

Pig ear skin was purchased from a fresh market in Nonthaburi, Thailand.

Equipment-

Analytical balance (A200S, Sartorius Analytical, Sciencetific Promotion, Switzerland)

High performance liquid chromatography machine (Shimadzu, binary pump: LC-10AB, autosample: SIL-20A HT, Detector: SPD-20A, Japan)

HPLC column (Aquasil 18, Thermo Hypersil, UK)

Vortex mixer (VELP cod. F 20220170, Scientifica, Europe)

Water bath (28L/8/SH/C, Polyscience co., Ltd., USA)

Method

1. High-Performance Liquid Chromatographic Technique (HPLC) for glucosamine hydrochloride analysis by pre-column PITC derivatization (Pulapa, 2007).

Glucosamine Hydrochloride (GS HCl) standard solutions were prepared from an aqueous stock solution to make concentrations of 1, 30, 60, 100 and 140 µg/ml. Paracetamol, an internal standard, were prepared from a methanolic stock solution to produce a solution with a concentration of 0.3 mg/ml. Phenyl isothiocyanate (PITC) were freshly prepared in methanol at 100 µg/ml prior to the derivatisation step.

Derivatisation procedure

Four hundred microlitre of standard solutions or sample were transferred into a centrifugal tube. Then, 250 µl of 0.1 M sodium acetate and 200 µl of methanol were added, The mixture were shaken and left for 15 minutes before and addition of 250 µl of PITC methanolic solution. This solution was then vortexed for 30 seconds before being placed in a water bath at 80 °C for 30 minutes. The samples were then cooled down to the room temperature. Then, 100 µl of the internal standard and 200 µl of n-hexane were added. This solution was vortexed for 1 minute. Then the lower part of this solution was removed for analysis by HPLC.

Instrumentation and chromatographic conditions

Separation of phenylthiocarbonyl-glucosamine adducts and paracetamol internal standard from other compounds in the formulation was achieved on a reverse-phase column 250x4.6 mm internal diameter (Aquasil C18) using a high performance liquid chromatography system. Sample of 20 µl were injected. Analysis was carried out using acetonitrile: water: phosphoric acid (100:900:1 v/v/v) as a mobile phase

delivered at 1.5 ml/min. The UV detector was operated at 245 nm. Every injections, the column was flushed with 100 % acetonitrile.

1.1 Validation for the quantitative determination of GS HCl by HPLC

The parameters evaluated to ensure the acceptability of the performance of the selected analytical method were specificity, linearity, precision, and accuracy.

1.1.1 Specificity

Under the selected conditions, the peaks of other components must not interfere with the peak of GS HCl and the internal standard. The validation was made by comparing the chromatrogram of GS HCl, in internal standard, and other components.

1.1.2 Linearity

Linearity was studied by preparing standard solutions in the solvent system (linearity of system) and placebo (linearity of method) at different concentration levels such as 1, 30, 60, 100, and 140 µg/ml. Linearity regression analysis of the absorbance versus the corresponding concentration were performed, and the coefficient of the determination was calculated.

1.1.3 Precision

Within run precision

The within run precision was determined by analyzing in six replicates (n=6) for one concentration at 60 µg/ml of GS HCl in solvent system, prepared solution on the same day. The percent coefficient of variation (%CV) of six assay values was calculated.

Between run precision

The between run precision was determined by analyzing in six replicates (n=6) for one concentration at 60 µg/ml of GS HCl in solvent system, prepared solution for two days. The percent coefficient of variation (%CV) of six assay values was calculated.

1.1.4 Accuracy

The accuracy was determined by analyzing in three replicates (n=3) for each of three concentrations at 1, 60, and 140 µg/ml of GS HCl in solvent system. The percent recovery of glucosamine for each concentration was calculated.

2 Solubility study of GS HCl

Solubility of GS HCl was determined by dissolving an excess of GS HCl in 2 ml of defined solvents (water, ethanol 10 %, or PBS pH7.4). The dispersion was kept stirring using a shaker at ambient temperature up to 94 hours. The supernatant was withdrawn and filtered through filter paper 0.45 micron prior to analyzed for dissolved GS HCl by HPLC method as described in section 1.

3 Preliminary study of GS HCl foam formulation development

Glucosamine foaming solution was prepared by dissolving paraben concentrate and surfactant in ethanol and dissolving GS HCl in buffer. The aqueous phase was then slowly added to the ethanolic mixture. The mixture was adjusted to 100 ml in volumetric flask with water.

Factors affecting stability of glucosamine formulation including types of non-ionic surfactants (decyl glucoside (DG), lauryl glucoside (LG), and caprylyl/capryl glucoside (CCSP)), surfactant concentrations, pH values in a range of 3-6.5, and buffer systems (citrate buffer (CT), tartrate buffer (TT) and phosphate buffer (PP)) were studied (Table 3-1). Stability study of the foam formulation was conducted at room

temperature and at 40°C for 7 days. Physical stability of the formulations was evaluated by organoleptic test; i.e., color change, odor, and feeling. In order to evaluate foam performance, foam expansion and foam liquid stability tests were performed using a cylinder method. The foam was discharged into a glass cylinder. Initial volume of produced foam, volume of formulation to produce, and volume of liquid drained after defined time intervals were recorded and calculated using the following equation (6) and (7) that were described earlier. Formulations with physical stabilities, good feeling, and suitable foam performance were selected for further studies.

4 Development of GS HCl foam formulation

Glucosamine foaming solution was prepared by dissolving 10g GS HCl in 0.25 M citrate buffer or in 0.25 M tartrate buffer in the presence of 0.5% sodium metabisulfite, 0.01% BHA or 0.05% EDTA (table 3-1). The ethanolic mixture was prepared by mixing 1.72 g paraben concentrate with 1% DG, CCSP and Tween 80 in alcohol USP. The aqueous phase was then slowly added to the ethanolic mixture. The mixture has been adjusted to 100 mL in volumetric flash with water.

The formulations were kept in tight container at 40 $\pm$ 2°C and 30 $\pm$ 2°C in the dark or under uv light (279 lux) at room temperature for 7 days. Physical stability of the formulations was evaluated by organoleptic test; clarity, color, odor and feeling and pH measurement.

Table 3-1 Formula of glucosamine hydrochloride foaming solution

	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}	F_{11}	F_{12}	F_{13}	F_{14}	F_{15}	F_{16}	F_{17}	F_{18}	F_{19}	F_{20}	F_{21}
GS HCl (g)	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Surfactant																					
LG	•	•	•	•	•	•	•														
DG								•	•	•	•	•	•	•							
CCSP															•	•	•	•	•	•	•
Buffer																					
CT pH 3	•							•							•						
CT pH 4		•							•							•					
CT pH 5			•							•							•				
TT pH 3				•							•							•			
TT pH 4					•							•							•		
TT pH 5						•							•							•	
PP pH 6.5							•							•							•
Others																					
Ethanol 10%	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Paraben conc	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•

F, Code of formulation

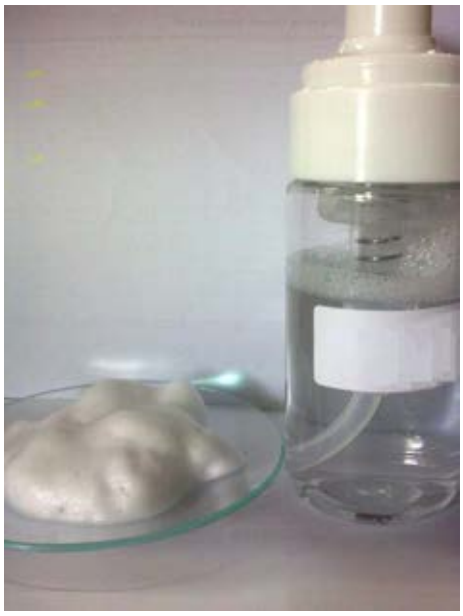


Figure 3-1 Apparatus to generate foam

In this study, types of surfactant were investigated for the effect of formulation components on permeation of GS HCl across pig skin and stability. It has been reported that, Tween 80 microemulsion was enhancing permeability of the GS HCl across pig skin (Punlapa, 2550).

Therefore the formulations selected from preliminary study and Tween 80 were selected for improving their stability by adding antioxidant and/or chelating agent.

Table 3-2 Formulation of glucosamine hydrochloride foaming solution for study of antioxidant and chelating agent

Formulation	Citrate buffer (pH 3)			Tartrate buffer (pH 3)		
	Sodium metabisulfite	BHA	EDTA	Sodium metabisulfite	BHA	EDTA
DG_1	／					
DG_2		／				
DG_3			／			
DG_4				／		
DG_5					／	
DG_6						／
$CCSP_1$	／					
$CCSP_2$		／				
$CCSP_3$			／			
$CCSP_4$				／		
$CCSP_5$					／	
$CCSP_6$						／
Tween 80_1	／					
Tween 80_2		／				
Tween 80_3			／			
Tween 80_4				／		
Tween 80_5					／	
Tween 80_6						／

5 Stability study

The optimized formulations were kept in tight container with temperature controlled at 40 ± 2°C, 75±5% RH; 30 ± 2°C, 75±5% RH and 5 ± 3°C for 3 months. 10% of GS HCl in DG solution in the absence of ethanol and 10% of GS HCl in water were kept in tight container at 5 ± 3°C as controls. All of formulations were also prepared in triplicate. At appropriate times, samples were taken and evaluated for its

physical and chemical stabilities. The physical stabilities were determined as mentioned in 4. The chemical stability was evaluated by determination of GS HCl content using HPLC method as described in 1.

6 *In vitro* permeation study through pig ear skin

The *in vitro* permeation study was used as a tool for determining the most suitable system, which could provide the highest permeability coefficient and flux of GS HCl through skin.

Fresh pig-ears were collected from a local market in Nonthaburi, Thailand. Pig-ear skins were cleaned with water and prepared by heating them in a water bath at 60 °C for 45 seconds. Then the whole skin was removed carefully from the underlying cartilage. The skin was cleaned and rinsed with the PBS pH 7.4. The skin specimen was then cut into the size of 4x4 cm^2 wrapped in aluminium foil and stored at -20 °c for up to 7 days (Dick and Scott, 1992). The frozen skin was thawed to room temperature prior to use.

The *in vitro* permeation study of GS HCl formulation system across pig ear skin was conducted using franz-diffusion cells (Fig 3-2). The experiment was carried out in six replicates for each formulation. The diffusion cell possessed an available diffusion area of 2.72 cm^2. The excised skin was mounted between the donor and the receptor compartment. Two milliliters each formulation, were pipetted and applied to pig skin membrane. The receptor compartment of diffusion cell was filled with 14 ml of PBS pH 7.4. The receptor medium was maintained at a constant temperature of 37°C and stirred by a magnetic stirrer at speed of 900 rpm. One milliliter of receptor medium was collected at 0, 1, 3, 6, 12, 18 and 24 hours and 1 ml of the fresh medium was replaced. The samples were then analyzed for GS HCl by HPLC as mentioned in 1.

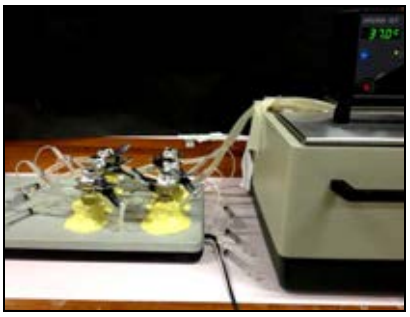


Figure 3-2 Modified Franz-diffusion cells for *In vitro* permeation studies

The permeated amount of GS HCl was calculated by multiplying GS HCl concentration with the receptor volume. For each skin specimen, drug permeated was plotted against time. The permeability coefficient (kp, cm/sec) of GS HCl was calculated from equation (Mashru et al., 2005).

$$K_P = \frac{J_{SS}}{c_d} = \frac{1}{A} \cdot \frac{\Delta Q}{\Delta t} \cdot \frac{1}{cd} \quad (8)$$

Where A is the effective area (cm^2)

c_d is the saturated solubility of Glucosamine in formulation (µg/ml)

Q is the cumulative mass of Glucosamine that passes through the membrane in time t (µg)

t is time (second)

After *in vitro* permeation studies, the membrances were observed under an invert microscope. Fresh pig-ear skin treated in the same membrane as mentioned above was used as a control.

7 Statistical analysis

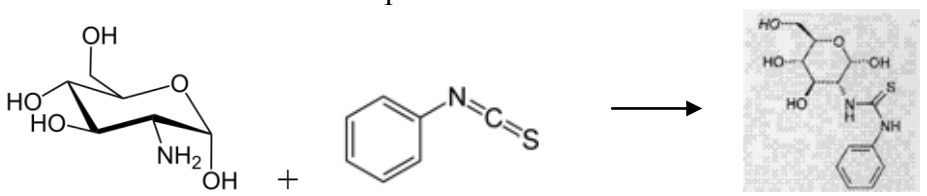
Data analysis with ANOVA or T-test at p-value of 0.05

CHAPTER IV

RESULTS AND DISSCUSSION

1. High-Performance Liquid Chromatographic technique for drug analysis (Pulapa, 2007)

The maximum UV wavelength of GS HCl is relatively low at 190 nm. Preliminary studies reported that composition of the formula could interfere with the absorbance of GS HCl at this wavelength.Therefore, the derivatized product of GS HCl and phenyisothiocyanate (PITC), phenylthiocarbonyl-glucosamine (Fig4-1), with a maximum UV absorption wavelength at 245 nm was made in order to avoid the interference of the formulation compositions.



Glucosamine PITC phenylthiocarbonyl-glucosamine

Figure 4-1 Glucosamine and phenylisothiocyanate reaction

1.1 Analytical method validation

GS HCl was analyzed by HPLC technique employing the pre-column derivatization with PITC. The analysis method validation parameters such as specificity, linearity, precision, and accuracy according to International Conference on Harmonisation (ICH) guidelines. Figure 4-2 shows chromatogram of GS HCl standard and paracetamol in water after derivatization. The chromatogram showed two major peaks with a retention time of 4.6 and 5.8 minute, corresponding to GS HCl and paracetamol, respectively. The chromatogram of GS HCl and paracetamol in the present of other inactive ingredients (Figure 4-3) and chromatogram of non-drug containing formulation system (Figure 4-4), showed that GS HCl peak was not interfered by other compounds in the formulations. The method was shown to possess a linearity in a concentration between 1 to 140 µg/ml with r^2 of 0.9999 (Figure 4-5). Percentage of recovery and %RSD were reported to be in a range of 99.69 % to 102.78 % and less than 0.73 %RSD. The validation results are given in APPENDIX A.

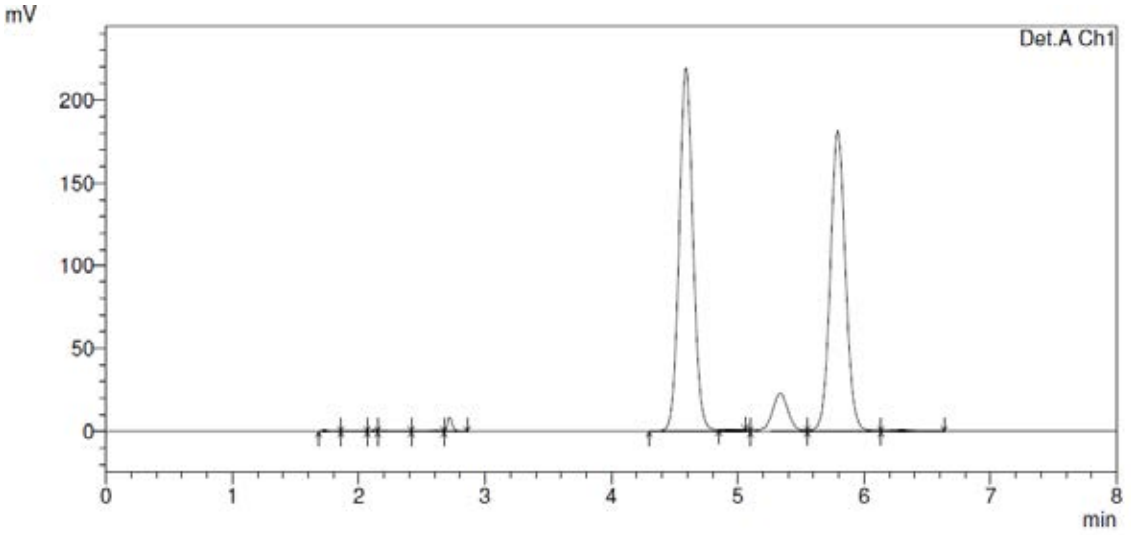


Figure 4-2 Typical HPLC chromatogram of GS HCl and paracetamol (internal standard) in water, with retention time of 4.6 minute and 5.8 minute, respectively.

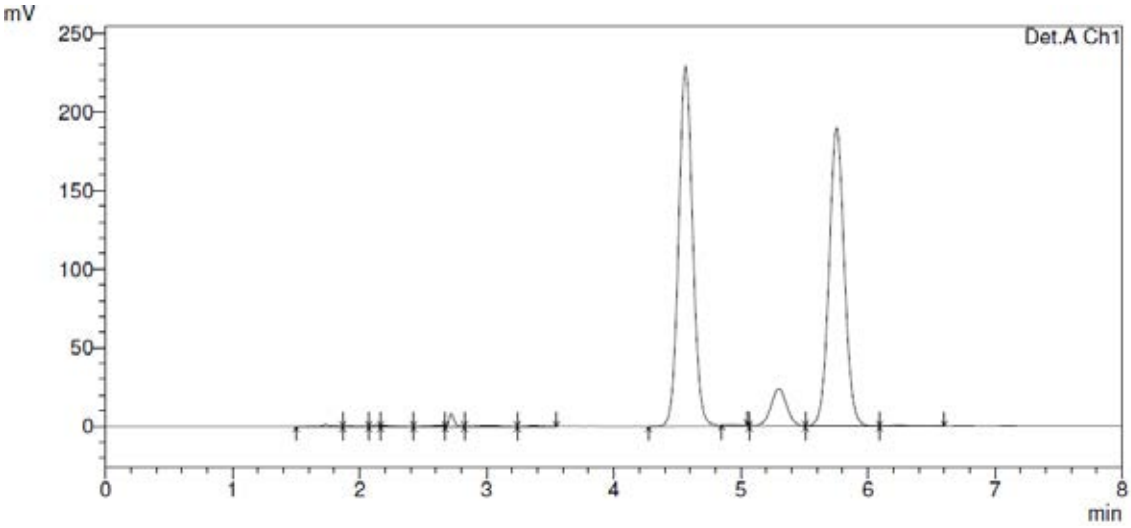


Figure 4-3 Typical HPLC chromatogram of GS HCl and paracetamol (internal standard) in formulation.

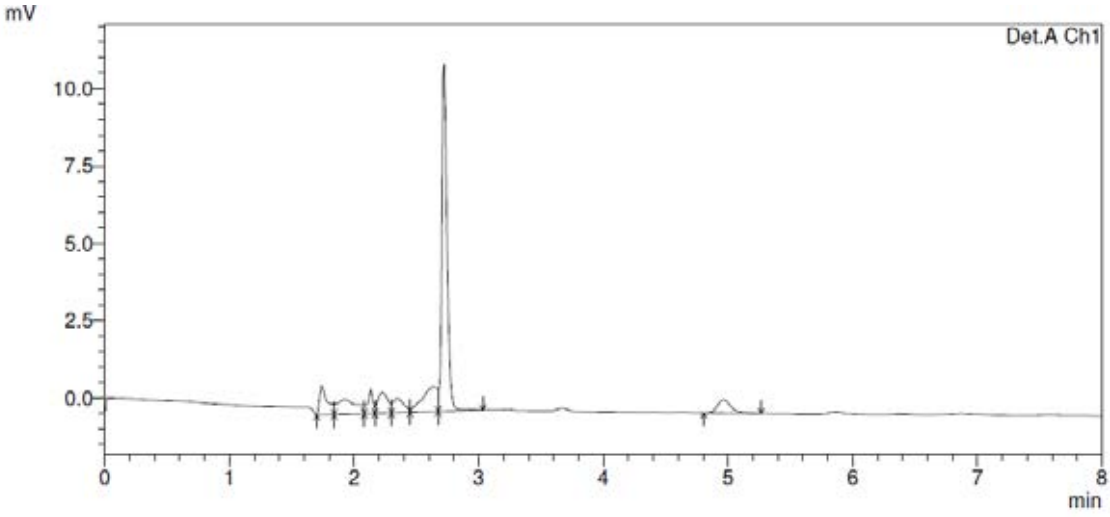


Figure 4-4 Typical HPLC chromatogram of non-drug containing formulation system

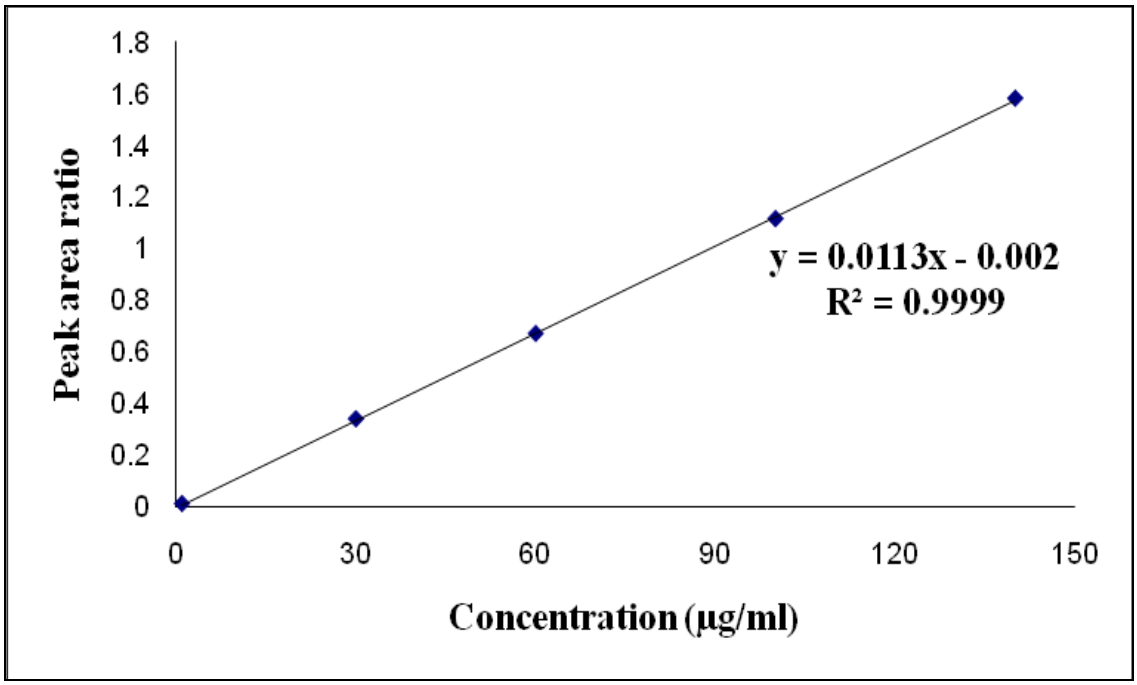


Figure 4-5 Linearity of system

2. Solubility study of GS HCl

In this study, the solubility of GS HCl at room temperature were determined and shown in Table 4-1. According to USP XXXV, solubility of GS HCl in all solvents is defined as very soluble (parts of solvent required for 1 part of solute less than 1 g/ml). The solubility characteristics of GS HCl confirm again that it is a very hydrophilic compound, and can be easily dissolved in water, ethanol 10% and PBS 7.4. The obtained GS HCl solubility information would be employed to estimate maximum formulation loading dose and to control sink conditions for receptor medium in the *in vitro* permeation study of GS HCl.

Table 4-1 Solubility of GS HCl in various solvents at ambient temperature

Solvent	GS HCl solubility (mg/ml)	Solubility [a]
Water	107.65	Very soluble
Ethanol 10%	100.16	Very soluble
PBS 7.4	100.30	Very soluble

[a] From the United States Pharmacopeia XXXV

3. Preliminary study of GS HCl foam formulation development

3.1 Foam formulation

Concentrations of each surfactant in water were varied in order to determine appropriate concentration of surfactants that produced suitable appearance of foam and texture of foam after application. Formulations containing DG and CCSP gave clear solotions, while formulation containing LG gave rise to cloudy mixtures. In order to evaluate foam performance, foam expansion and foam liquid stability studies were performed using a cylinder method %FE and %FVS were calculated using the equation (6) and (7), respectively that described in literature review and reported in

Table 4-2. The results showed that the density of foam was increased as the concentration of non-ionic surfactant was increased. The formulations with surfactant concentration of 0.75% w/v did not show good appearance of foam. The percent foam expansion (%FE) was increased as the concentration of non-ionic surfactant was increased. Preparation containing CCSP gave the highest %FE, followed by those containing DG and LG. While, %FLS was not different from each other, foam brokedown to solution within 3 minutes and did not related with surfactant concentration. Thus, %FLS was not used as a selection criteria. A surfactant concentration of 1% w/v was selected for future study because of this concentration was the lowest concentration of surfactant for produced suitable appearance of foam in order to avoid potential problem due to surfactant induced skin irritation.

Table 4-2 10% glucosamine hydrochloride foam performance of formulations in various surfactants and concentration of surfactants

Formulation	Concentration of surfactant	V(foam)	V(liquid 30min)	V(formulation)	%FE	%FLS
DG_1	10	150	5	5	2,900	100
DG_2	5	100	5	5	1,900	100
DG_3	1	50	5	5	900	100
DG_4	0.75	35	5	5	600	100
LG_1	10	145	5	5	2,800	100
LG_2	5	100	5	5	1,900	100
LG_3	1	45	5	5	800	100
LG_4	0.75	30	5	5	500	100
$CCSP_1$	10	180	5	5	3,500	100
$CCSP_2$	5	152	5	5	2,940	100
$CCSP_3$	1	58	5	5	1,060	100
$CCSP_4$	0.75	50	5	5	600	100

3.2 Effect of pH, types of surfactant, and types of buffer on physical stability of 10% glucosamine foam formulation

Evaluation of the physical stability by organoleptic test; i.e., color change, odor, and feeling, and pH. Formulations F_1-F_{21} were kept in tight containers at room temperature or 40 $\pm$ 2°C at 7 days and were evaluated for its physical stability in order to study the effect of pH, type of surfactants, and type of buffers on stability of GS HCl. Formulations containing LG gave rise to cloudy mixtures, while formulations containing DG or CCSP gave clear solutions (figure 4-6). Therefore, formulations containing DG or CCSP were selected for further studies. Preparations containing CCSP gave strong characteristic odor due to distinctive odor of the surfactant, followed by those containing DG. And the formulations containing CCSP gave better feeling after application than those containing DG. Freshly prepared glucosamine formulation gave clear solution and turned to dark brown solution over time. Physical instability of glucosamine preparation was easily observed (figure 4-7– 4-10).

(A)

(B)

(C)

Figure 4-6 GS HCl formulation freshly prepared with DG (A) LG (B) CCSP (C)

The browning color is caused by the carbonyl-amine reaction known as Millard reaction. Glucosamine is an reducing sugar. Therefore, the primary amine attached to the sugar backbone of glucosamine could react with the carbonyl carbon of the reduced form of another glucosamine molecule. An unionized form of the primary amine is the reactive form. Therefore, glucosamine undergoes millard reaction at a faster rate when formulation pH value is in a range of 3.0-6.5 where most of primary amine is unionized.

In this research, pH values of the formulation were varied in a range of 3-6.5 in order to avoid skin irritation. In addition, normal human skin surface has an acid (pH < 7), thus, formulations containing various surfactants with appropriate buffer solutions of pH 3.0 (0.25 M citrate buffer(CT), 0.25 M tartrate buffer(TT)), pH 4.0 (0.25 M citrate buffer, 0.25 M tartrate buffer), pH 5.0 (0.25 M citrate buffer, 0.25 M tartrate buffer) and pH 6.5 (0.3 M phosphate buffer(PP)) were prepared. Acetate buffer (pH 3.0-5.0) was not chosen in this study because of the strong characteristic odor of acetate buffer.

The results showed that, physical in stability of glucosamine formulation was obviously observed as the pH was increased. Glucosamine preparations were the most stable at pH 3 (Figure 4-7– 4-10). The pH values of the formulation were slowly decreased over 7 days when buffer systems of the preparation were CT pH 4.0-5.0, TT pH 4.0-5.0 and PP pH 6.5 (Table 4-3 and Figure 4-11), The uncontrollable pH value of the formulations were speculated to be due to a low buffer capacity of 0.25M buffer system. A higher buffer concentration resulted in bad feeling, left salt residue after application. The color of formulations at pH 3 was not significantly changed. Therefore, the formulations containing DG and CCSP at pH 3 (0.25 M CT and 0.25 M TT) were selected for investigate in the future study.

Glucosamine sulfate showed highest stability at pH 5.00 in 0.05 M acetate buffer. The second most stability of glucosamine sulfate was at pH 2.97 in 0.05 M acetate buffer (Kanwischer et al., 2005), the inconsistent results between this study was differenced from results of the study by Kanwischer et al was due to difference in glucosamine salt form and also different type of buffers in formulations. In this study, glucosamine hydrochloride was selected as active ingredient in formulations. According to the earlier published study, changing in salt form of glucosamine can

demonstrate in different physical properties; pH in aqueous solution of sulfate and hydrochloride salt are 3.5-5.0 and 3.0-5.0, respectively. Moreover, choosing the suitable buffer system will extend the stability of formulation so the type buffer is vital. From Kanwischer et al. study, acetate buffer was used however in this experiment was not selected acetate buffer since this buffer has strong odor, so do not suitable for topical formulation. Due to the fact that, was not performed the stability study in acetate buffer therefore this experiment did not show the most stable pH in acetate buffer and which type of sulfate or hydrochloride salt were more stable in such buffer system.

(A)

(B)

(C)

(D)

(E)

(F)

Figure 4-7 GS HCl formulation F_8 at day 0 (A), F_8 after an incubation at 40°C for 7 days (B), F_9 at day 0(C), F_9 after an incubation at 40°C for 7 days (D), F_{10} at day 0(E), F_{10} after an incubation at 40°C for 7 days (F)

(A)

(B)

(C)

(D)

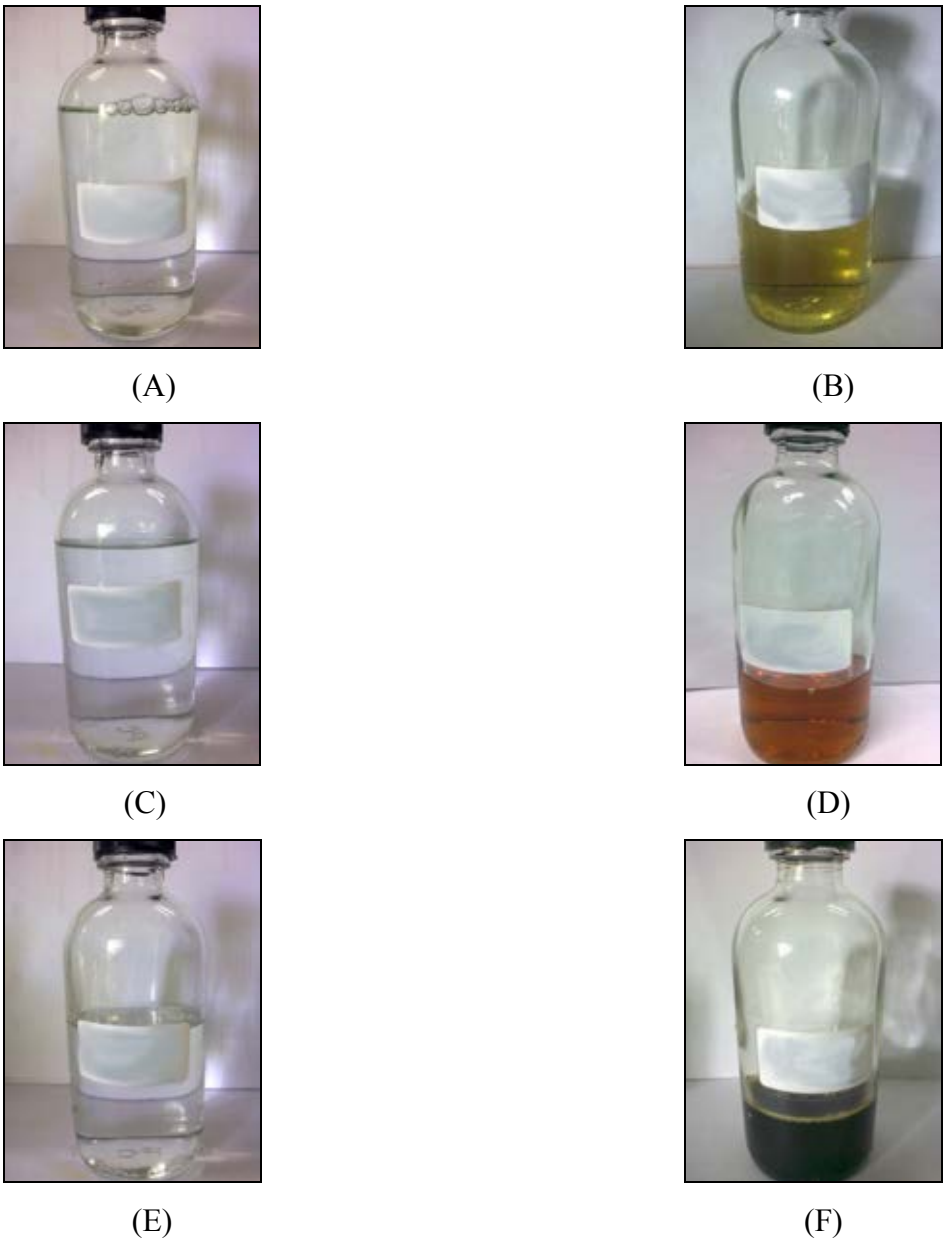
(E)

(F)

(G)

(H)

Figure 4-8 GS HCl formulation F_{11} at day 0 (A), F_{11} after an incubation at 40°C for 7 days (B), F_{12} at day 0 (C), F_{12} after an incubation at 40°C for 7 days (D), F_{13} at day 0(E), F_{13} after an incubation at 40°C for 7 days (F) F_{14} at day 0 (G), F_{14} after an incubation at 40°C for 7 days (H)

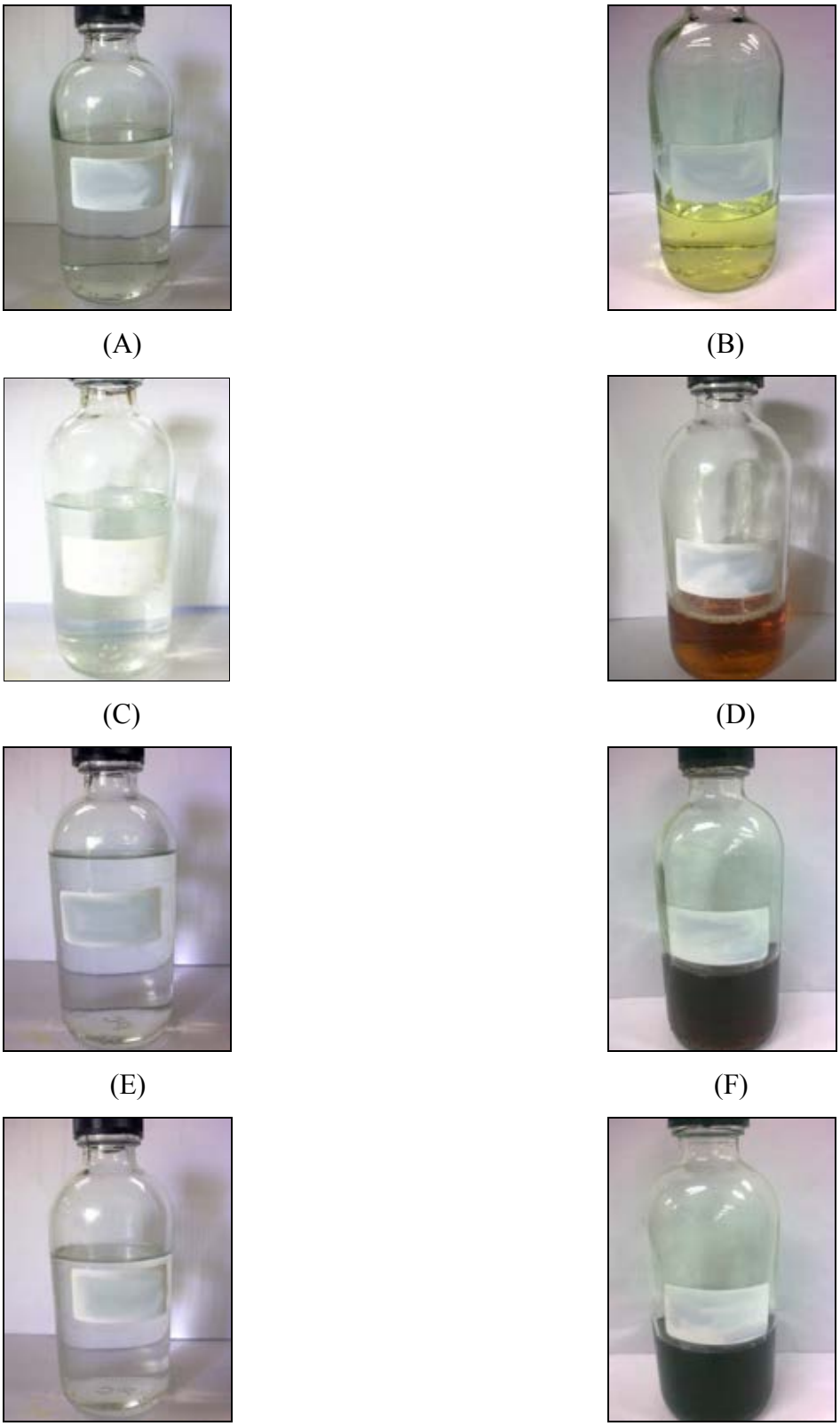


(A) (B)

(C) (D)

(E) (F)

Figure 4-9 GS HCl formulation F_{15} at day 0 (A), F_{15} after an incubation at 40°C for 7 days (B), F_{16} at day 0 (C), F_{16} after an incubation at 40°C for 7 days (D), F_{17} at day 0(E), F_{17} after an incubation at 40°C for 7 days (F)



(A) (B)

(C) (D)

(E) (F)

(G) (H)

Figure 4-10 GS HCl formulation F_{18} at day 0 (A), F_{18} after an incubation at 40°C for 7 days (B), F_{19} at day 0 (C), F_{19} after an incubation at 40°C for 7 days (D), F_{20}

at day 0(E), F_{20} after an incubation at 40°C for 7 days (F) F_{21} at day 0 (G), F_{21} after an incubation at 40°C for 7 days (H)

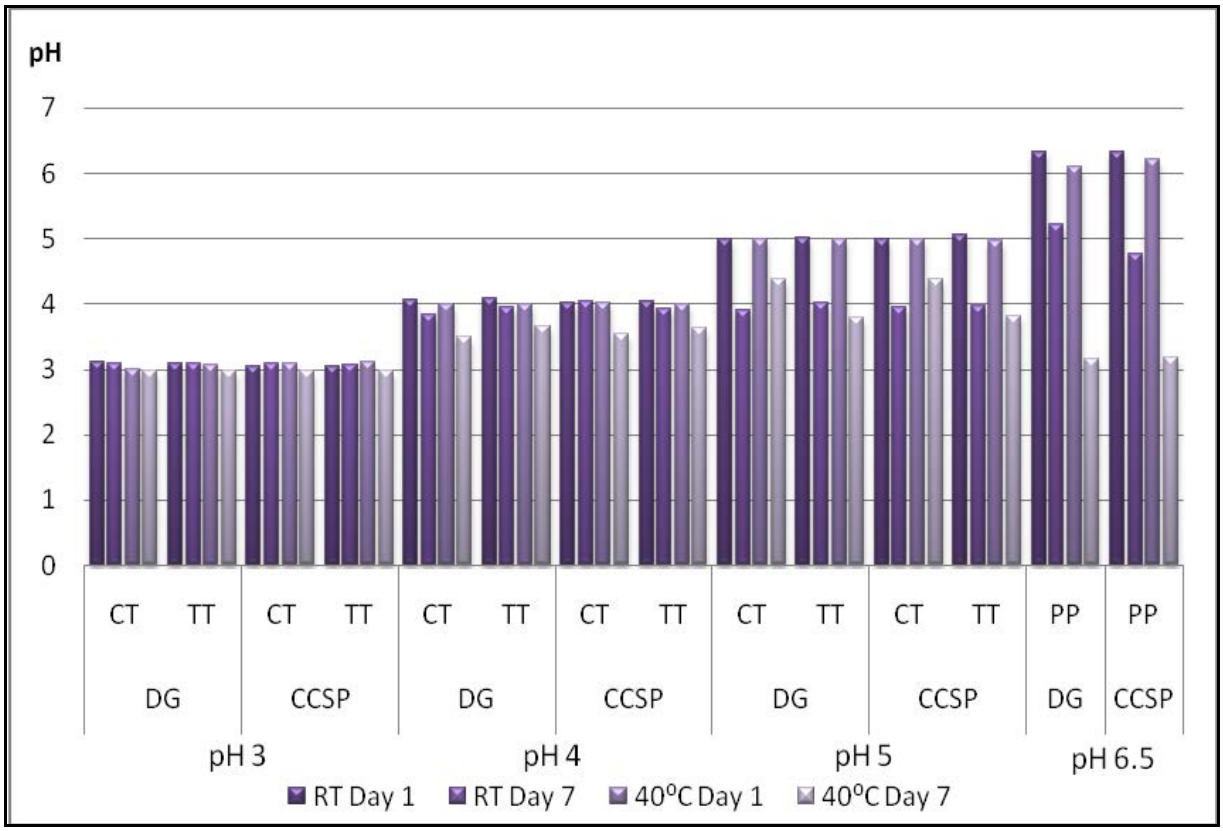


Figure 4-11 pH values of glucosamine foam preparation at RT and 40°C (n=1)

Table 4-3 pH values and extent of color change of formulations containing DG and CCSP (F_8-F_{21}) storage under accelerated condition at 40 ºC for 7 days.

Formulation	Buffer	Physical properties 0 day		Physical properties 7 days	
		Color*	pH	Color*	pH
F_8	CT	0	3.00	+3	2.98
F_9	CT	0	4.00	+8	3.45
F_{10}	CT	0	5.00	+18	4.38
F_{11}	TT	0	3.07	+2	2.99
F_{12}	TT	0	3.99	+8	3.65
F_{13}	TT	0	4.99	+10	3.80
F_{14}	PP	0	6.50	+18	3.15
F_{15}	CT	0	3.01	+4	2.94
F_{16}	CT	0	4.01	+9	3.54
F_{17}	CT	0	5.00	+17	4.37
F_{18}	TT	0	3.01	+3	2.99
F_{19}	TT	0	4.00	+8	3.63
F_{20}	TT	0	4.99	+15	3.82
F_{21}	PP	0	6.51	+17	3.17

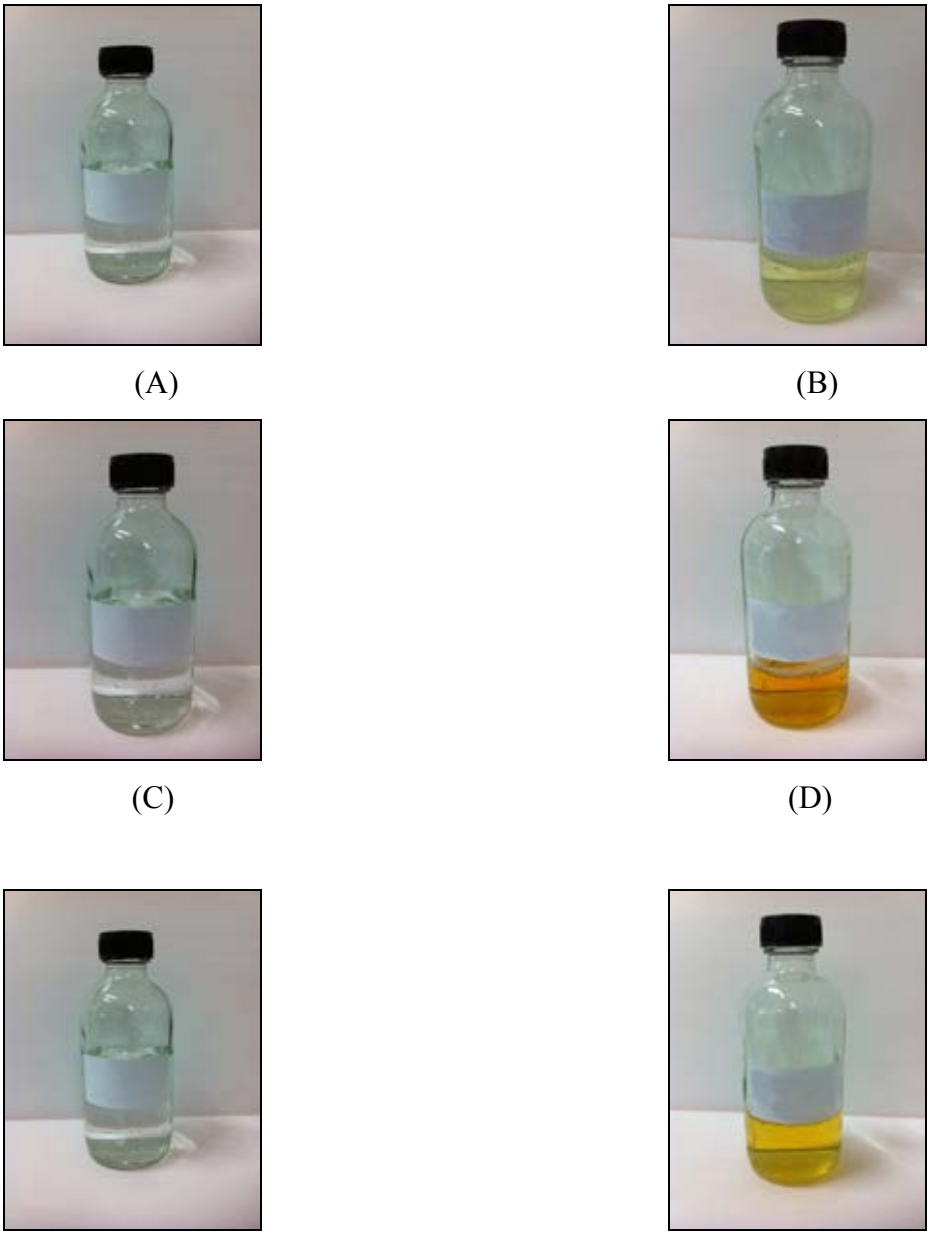
* (+) level change of color, (-) level of turbid, (S) separate

4. Preparation of glucosamine hydrochloride foam formulation

To study the effect of antioxidant and chelating agent on stability of GS HCl formulation were studied by comparing formulations containing 3 groups of antioxidants, can be grouped according to their mechanism of action. BHA is an example of true antioxidants which inhibit chain reactions by reacting with free radicals in the auto oxidation chain reaction. Sodium metabisulfite is a representative of reducing agents which are oxidized at a faster rate than the drug molecule. EDTA, a chelating agent act as s synergist, that enhances the stability by froming complex with heavy metal ions which often catalyse auto oxidations. Buffer effects on stability of GS HCl formulation was also investigated by varying buffer system at pH 3.0 using 0.25 M citrate buffer or 0.25 M tartrate buffer.

The formulations were kept in tight container with temperature controlled at 30 $\pm$ 2°C and 40 $\pm$ 2°C at 7 days and evaluation of the physical stability by appearance, viscosity, and pH. The results show that, all of formulations change in color was observed from colorless to yellow or brown after preparation at 30 °C and 40 °C for 7 days. Color of formulations containing sodium metabisulfite were changed in the smallest extent (Figure 4-12 - 4-17). Sodium metabisulfite is a water soluble reducing agent which is more affective in acidic conditions. Therefore, sodium metabisulfite which is present in the same aqueous phase as GS HCl does result in a maximum antioxidant effect. BHA is insoluble in water but freely soluble in ethanol. Thus the effectiveness of BHA as an antioxidant for GS HCl is less than that of sodium metabisulfite. EDTA was the least effective antioxidant for GS HCl probably due to the fact that oxidation by heavy metal catalyst is not the major degradation pathway of GS HCl. In addition, tartrate buffer seemed to be able to better control pH of the formulation than citrate buffer (Table 4-4).

Therefore formulations containing sodium metabisulfite and TT were selected for further studies.



(A) (B)

(C) (D)

(E) (F)

Figure 4-12 GS HCl formulation in citrate buffer pH 3 DG $Na_2S_2O_5$ 1 at 0 day (A), $Na_2S_2O_5$ 1 after storage at 40°C for 7 days (B) BHA at 0 day (C) BHA after storage at 40°C for 7 days (D) EDTA at 0 day (E) EDTA after storage at 40°C for 7 days (F)

(A)

(B)

(C)

(D)

(E)

(F)

Figure 4-13 GS HCl formulation in tartrate buffer pH 3 DG $Na_2S_2O_5$ 1 at 0 day (A), $Na_2S_2O_5$ 1 after storage at 40°C for 7 days (B) BHA at 0 day (C) BHA after storage at 40°C for 7 days (D) EDTA at 0 day (E) EDTA after storage at 40°C for 7 days (F)

(A)

(B)

(C)

(D)

(E)

(F)

Figure 4-14 GS HCl formulation in citrate buffer pH 3 CCSP $Na_2S_2O_5$ 1 at 0 day (A), $Na_2S_2O_5$ 1 after storage at 40°C for 7 days (B) BHA at 0 day (C) BHA after storage at 40°C for 7 days (D) EDTA at 0 day (E) EDTA after storage at 40°C for 7 days (F)

(A)

(B)

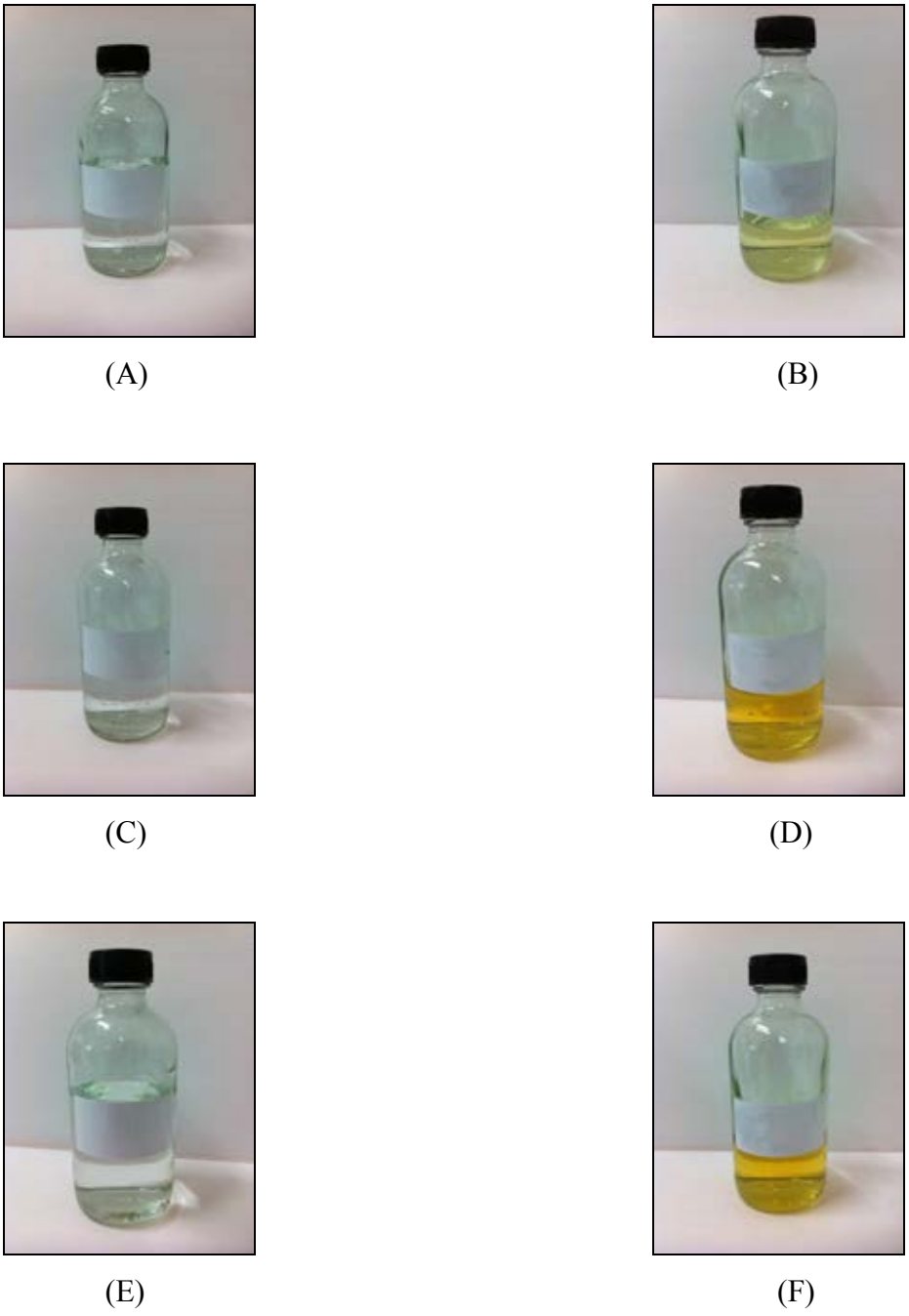
(C)

(D)

(E)

(F)

Figure 4-15 GS HCl formulation in tartrate buffer pH 3 CCSP $Na_2S_2O_5$ 1 at 0 day (A), $Na_2S_2O_5$ 1 after storage at 40°C for 7 days (B) BHA at 0 day (C) BHA after storage at 40°C for 7 days (D) EDTA at 0 day (E) EDTA after storage at 40°C for 7 days (F)



(A) (B)

(C) (D)

(E) (F)

Figure 4-16 GS HCl formulation in citrate buffer pH 3 Tween80 $Na_2S_2O_5$ 1 at 0 day (A), $Na_2S_2O_5$ 1 after storage at 40°C for 7 days (B) BHA at 0 day (C) BHA after storage at 40°C for 7 days (D) EDTA at 0 day (E) EDTA after storage at 40°C for 7 days (F)

(A)

(B)

(C)

(D)

(E)

(F)

Figure 4-17 GS HCl formulation in tartrate buffer pH 3 Tween80 $Na_2S_2O_5$ 1 at 0 day (A), $Na_2S_2O_5$ 1 after storage at 40°C for 7 days (B) BHA at 0 day (C) BHA after storage at 40°C for 7 days (D) EDTA at 0 day (E) EDTA after storage at 40°C for 7 days (F)

Table 4-4 pH values and extent of color change of formulations containing DG CCSP and Tween 80 in the presence of antioxidants ($Na_2S_2O_5$ or BHA) or chelating agent (EDTA) after storage at 30 and 40°C for 7 days.

Formulation	Buffer pH=3	Antioxidant and chelating agent	0 day		7 days			
			pH	Apperance *	pH		Apperance*	
					30°C	40°C	30°C	40°C
DG	CT	$Na_2S_2O_5$	3.01	0	2.81	2.53	+1	+2
DG	CT	BHA	3.03	0	3.01	2.94	+3	+5
DG	CT	EDTA	2.97	0	2.80	2.66	+2	+4
DG	TT	$Na_2S_2O_5$	3.00	0	2.99	2.68	+1	+2
DG	TT	BHA	2.99	0	2.98	2.88	+1	+4
DG	TT	EDTA	2.99	0	2.99	2.79	+2	+5
CCSP	CT	$Na_2S_2O_5$	3.03	0	2.94	2.74	+1	+2
CCSP	CT	BHA	2.99	0	2.82	2.70	+1	+4
CCSP	CT	EDTA	2.99	0	2.82	2.69	+2	+4
CCSP	TT	$Na_2S_2O_5$	3.03	0	3.0	2.98	+1	+2
CCSP	TT	BHA	3.01	0	3.01	3.00	+1	+4
CCSP	TT	EDTA	3.01	0	3.02	2.99	+1	+4
Tween 80	CT	$Na_2S_2O_5$	3.0	0	2.89	2.65	+1	+2
Tween 80	CT	BHA	2.99	0	2.94	2.82	+2	+5
Tween 80	CT	EDTA	2.95	0	2.83	2.75	+3	+5
Tween 80	TT	$Na_2S_2O_5$	3.06	0	3.01	2.93	+1	+2
Tween 80	TT	BHA	3.06	0	2.94	2.63	+1	+4
Tween 80	TT	EDTA	3.05	0	2.90	2.75	+2	+5

* (+) level change of color

5. Stability study

There formulations containing DG, CCSP or Tween 80 as foaming agents in TT pH3 were selected for further investigation of its stability at 5°C, 30°C and 40°C, in persence of 0.1 g/ml sodium metabisulfite.

All freshly prepared GS HCl foaming solutions were clear and colorless solutions. After storage at 30°C and 40°C for 1 month, color of formulations turned yellow. Formulations storage at 5°C reached clear and colorless up to 3 months (fig 4-18) After 3 months of storage at 30°C and 40°C, pH values of all formulations decreased by about 1 pH unit while pH values of the formulation storage at 5°C were content (Table 4-5)


(A)


(B)


(C)


(D)


(E)


(F)


(G)


(H)


(I)

Figure 4-18 GS HCl formulations in the presence of sodium metabisilfite and TT after 3 months of storage of the formulation containing DG after storage at 5°C (A), 30°C (B), 40°C (C). CCSP after storage at 5°C (D), 30°C (E), 40°C (F). Tween80 after storage at 5°C (G), 30°C (H), 40°C (I).

Table 4-5 The changing in physical properties of GS HCl formulations containing DG, CCSP or Tween 80 and 0.1 g/ml sodium metabisulfite as an antioxidant after 3 months at 5 °C, 30 °C and 40 °C (n=3)

Formulation	Physical properties 0 day		Temp (°C)	Physical properties 90 days	
	Color*	pH		Color*	pH
DG	0	3.00	5	0	3.00±0.00
			30	+4	2.20±0.01
			40	+5	2.00±0.00
CCSP	0	3.00	5	0	3.00±0.00
			30	+4	2.22±0.01
			40	+5	2.01±0.01
Tween 80	0	3.00	5	0	3.00±0.00
			30	+4	2.21±0.01
			40	+5	2.02±0.00

* (+) level change of color

Light exposure did not cause any physical instability to the GS HCl foam formulations. Color of preparations kept in the dark and under light was not significantly different from each other.

(A)

(B)

Figure 4-19 GS HCl formulation prepared with DG and sodium metabisulfite tartrate buffer pH 3 under light (A) in the dark (B) after 7 days of storage

As mentioned earlier, the browning color of the formulations was due to the carbonyl-amine reaction, millard reaction. Mechanism of millard reaction is devided into three stages.

1. Initial stage, caused by (A) sugar-amine condensation and (B) Amodori rearrangement, exhibited no color changing.
2. Intermediate stage, caused by (C) sugar dehydration, exhibited no color changing or yellowing.
3. Final stage, caused by (F) adol condensation and (G) aldehyde-amine polymerization to formation of heterocyclic nitrogen compound exhibited color changing as shown in Figure 4-20.

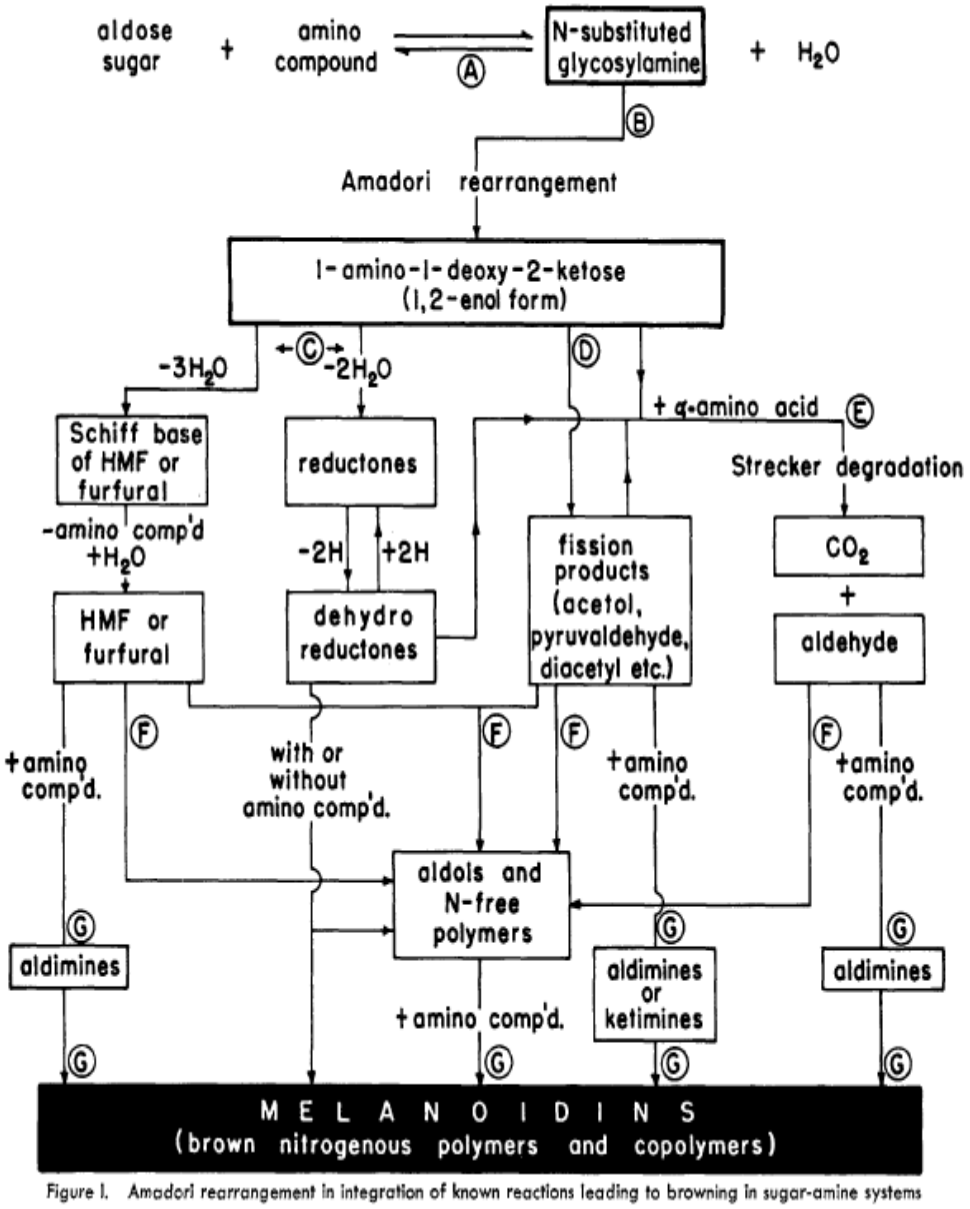


Figure I. Amadori rearrangement in integration of known reactions leading to browning in sugar-amine systems

Figure 4-20 The browning in sugar-amine system (obtained from Hodge, 1953)

Concentration-time profiles of GS HCl remaining in each formulation after storage at 5°C, 30°C and 40°C for 3 months were constructed (fig 4-21 to 4-23). Degradation of glucosamine depended on storage temperature. figure 4.21, 4.22 and 4.23 shows that GS HCl in formulation containing DG, CCSP and Tween 80, respectively, degraded more than 35 percent after 3 months of storage at 30 °C and 40 °C. While concentration of GS HCl formulation in formulation containing DG and CCSP remained unchanged after 3 months of storage at 5 °C but formulation in Tween 80 remained less than 100 percent label because of it was precipitated after 28 days. The extent of GS HCl degradation was in an order of 40°C >30°C >5°C, However, the extent of GS HCl degradation was not a function of surfactant type, (figure 4.24 to 4.26).

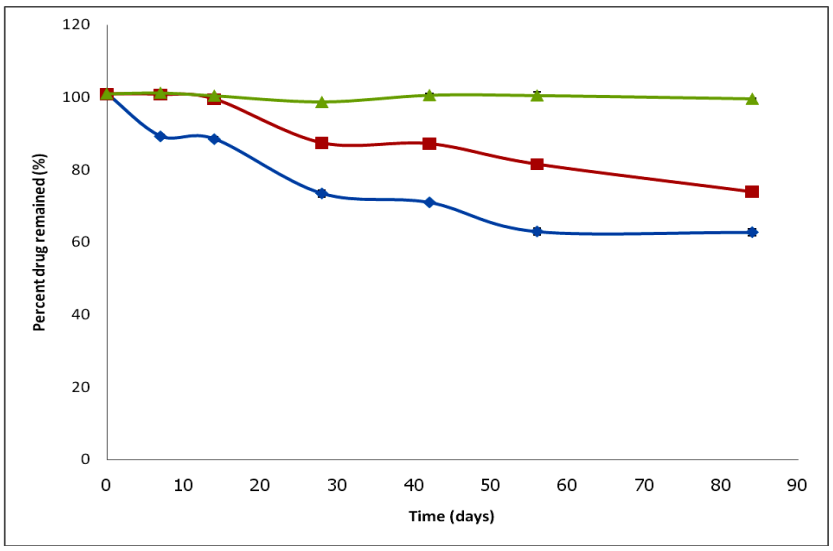


Figure 4-21 Concentration-time profiles of GS HCl in the presence of DG versus time after storage at 5°C (▲), 30°C (■) and 40 °C(◆) (n=3)

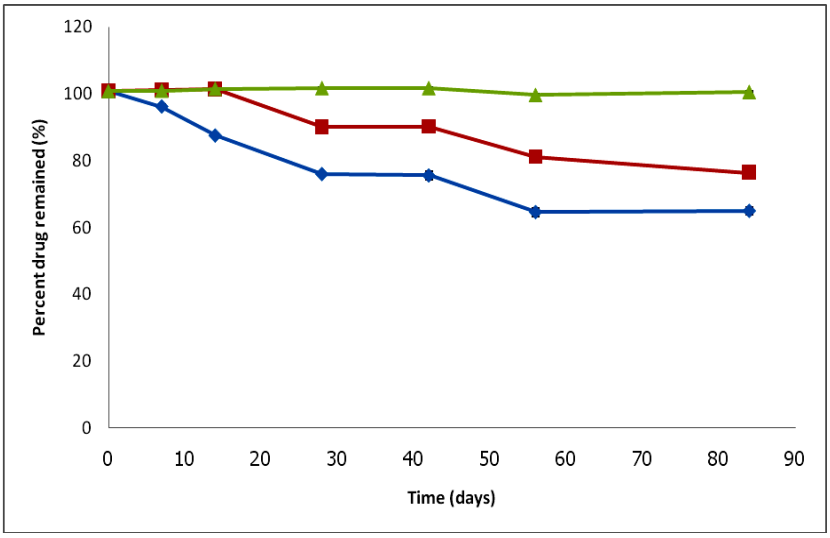


Figure 4-22 Concentration-time profiles of GS HCl in the presence of CCSP versus time after storage at 5°C (▲), 30°C (■) and 40 °C (◆) (n=3)

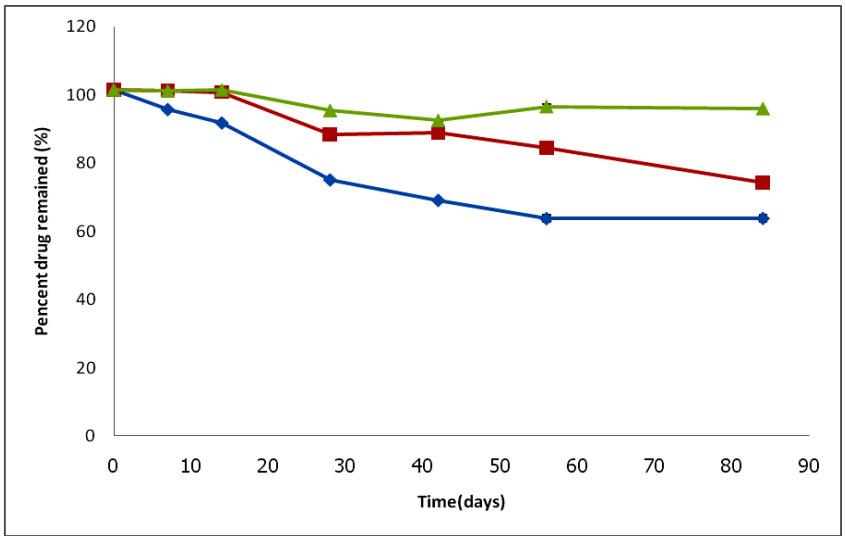


Figure 4-23 Concentration-time profiles of GS HCl in the presence of Tween 80 versus time after storage at 5°C (▲), 30°C (■) and 40 °C (◆) (n=3)

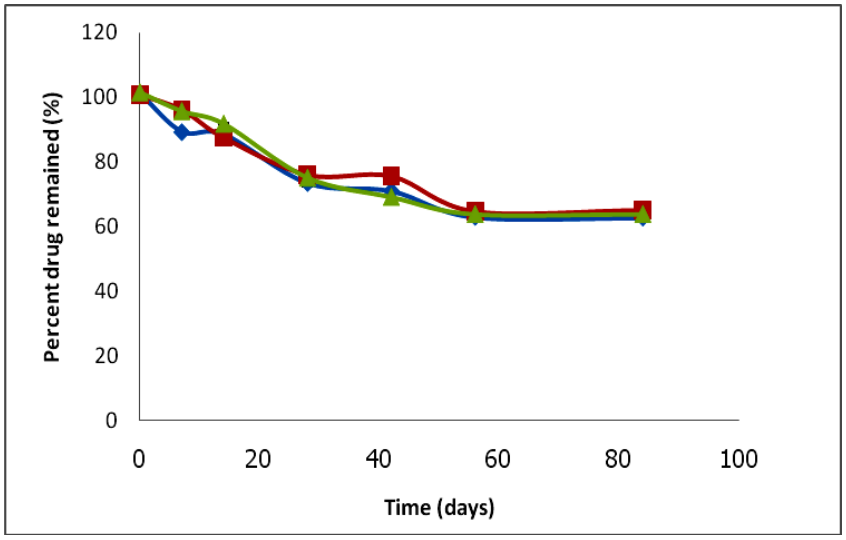


Figure 4-24 Plots of percent label amount of GS HCl formulation in DG, CCSP and Tween 80 versus time of storage at 40 °C TWEEN80 (▲), CCSP (■) and DG (◆)

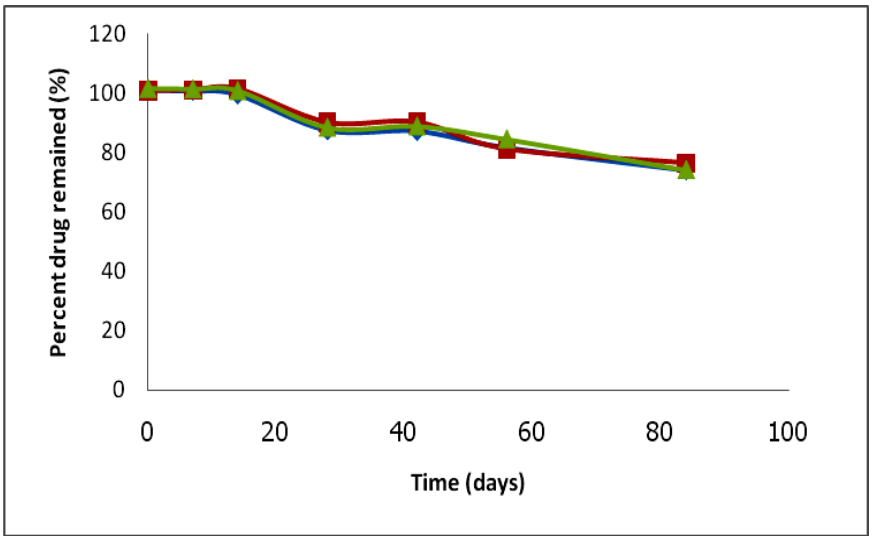


Figure 4-25 Plots of percent label amount of GS HCl formulation in DG, CCSP and Tween 80 versus time of storage at 30 °C TWEEN80 (▲), CCSP (■) and DG (◆)

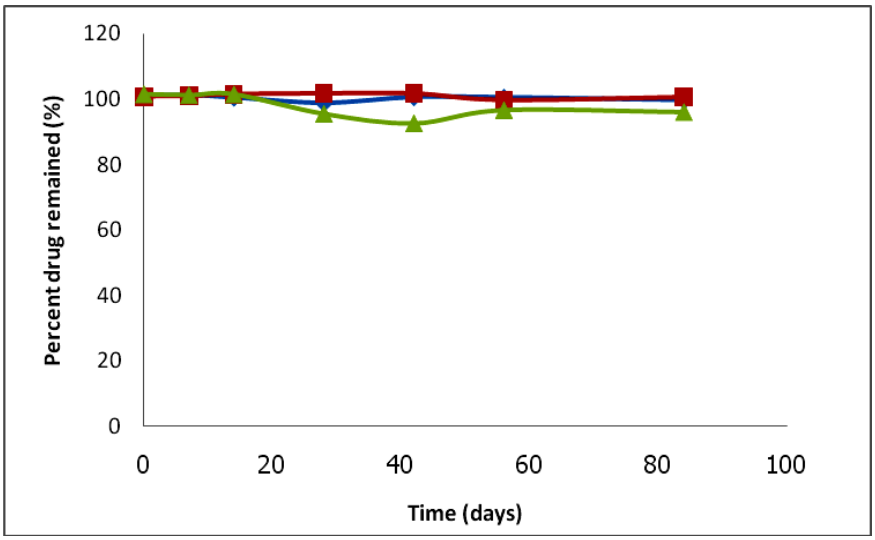


Figure 4-26 Plots of percent label amount of GS HCl formulation in DG, CCSP and Tween 80 versus time of storage at 5 °C TWEEN80 (▲), CCSP (■) and DG (◆)

First order plots of GS HCl in the presence of DG, CCSP or Tween 80 were plotted (Figure 4.27, 4.28 and 4.29), respectively. The non-linearity of these plots was dut to either non-first order reaction of GS HCl degradation under the experimeantal conditions or complication of degradation of GS HCl. Degradation scheme of millard reaction is very complicate and can result in non-linear first order plot. However, in this study, the non-linear first order plots were believed to be due to uncontrollable pH value of the formulations. Therefore, the pseudo-first order conditions were not obtained.

Results from this study suggested that storage conditions for GS HCl foam formulations should be kept in a refrigerator at 4-8 ºC. Further stability study on drug product should be carried out in orders to determine is shelf-life.

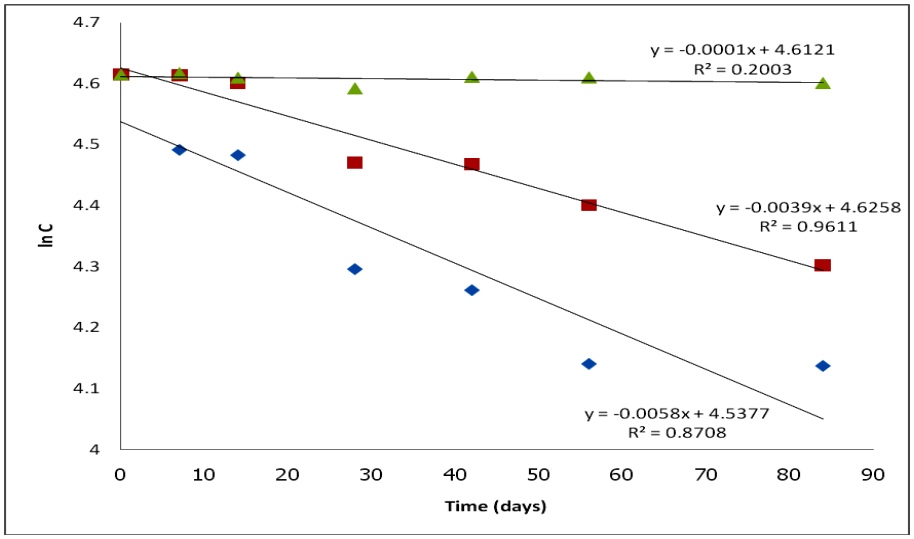


Figure 4-27 Plots of logarithm of concentration of GS HCl in DG versus times at 5ºC, 30ºC and 40 ºC 5ºC (▲), 30ºC (■) and 40 ºC (◆)

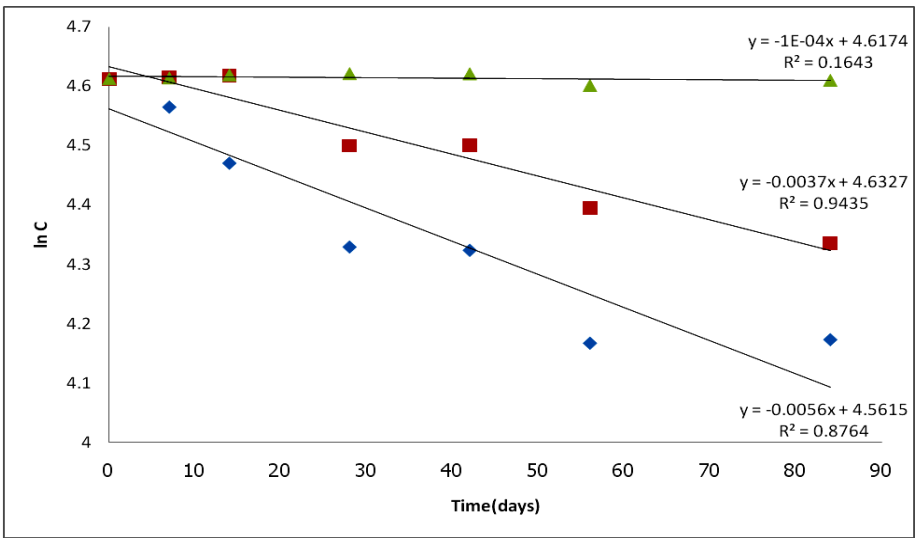


Figure 4-28 Plots of logarithm of concentration of GS HCl in CCSP versus times at 5ºC, 30ºC and 40 ºC 5ºC (▲), 30ºC (■) and 40 ºC (◆)

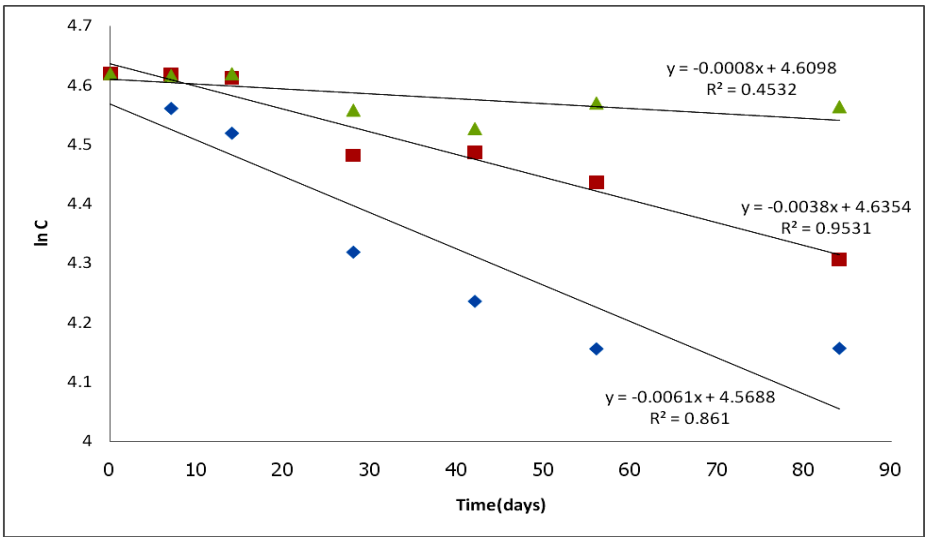


Figure 4-29 Plots of logarithm of concentration of GS HCl in Tween 80 versus times at 5ºC, 30ºC and 40 ºC 5ºC (▲), 30ºC (■) and 40 ºC (◆)

6. *In vitro* permeation study through pig ear skin

The *in vitro* permeation study was selected as a tool for determining the most suitable formulation for delivery GS HCl through pig skin. The *in vitro* permeation study of GS HCl formulation system across pig ear skin was conducted by Franz-diffusion cell.

One of the important factors of the *in vitro* permeation experiment is the sink condition. The ideal receptor phase provides an accurate simulation of the conditions pertaining to *in vivo* permeation of the test compound. As a general rule the concentration of the permeant in the receptor fluid should not be allowed to exceed approximately 10 % of saturation solubility. Excessive receptor-phase concentration can lead to a decrease in the rate of absorption, which may result in an underestimate of bioavailability.

In this study, the PBS 7.4 was used as receptor medium, GS HCl solubility in PBS 7.4 was 100.30 mg/ml Therefore, the maximum concentration of GS HCl in the receptor chamber should be less than 10 mg/ml.

At predetermined time points, 1 ml of receptor medium was withdrawn and analyzed for GS HCl by HPLC. Concentration of GS HCl at each time point was less than 10mg/ml. In other words, the sink conditions were obtained. Accomulated amount of GS HCl permeated through pig‟s ear skin were plotted versus time as shown in (fig 4-31 to 4-35).

Effect of surfactant type

The effect of surfactant type on permeability of the drug was studied by preparation 10% GS HCl foam formulation in the presence of DG, CCSP or Tween 80 using a formulation contain 10% of GS HCl in 10% ethanol as a control. The percent cumulative permeation at 6 hours of GS HCl in DG, CCSP, Tween 80 and ethanol 10% and water were 18.05%, 11.55%, 10.67%, 9.54% and 7.81%, respectively, with a lag time of 1 hours(figure 4-31). The GS HCl in DG was significantly highest of GS HCl permeation at every time in release study at p-value of 0.05. The surfactant had a significant effect on GS HCl permeation.

In view of difference potency of each penetration enhancer, DG in combination with ethanol was the best penetration enhancer for this system

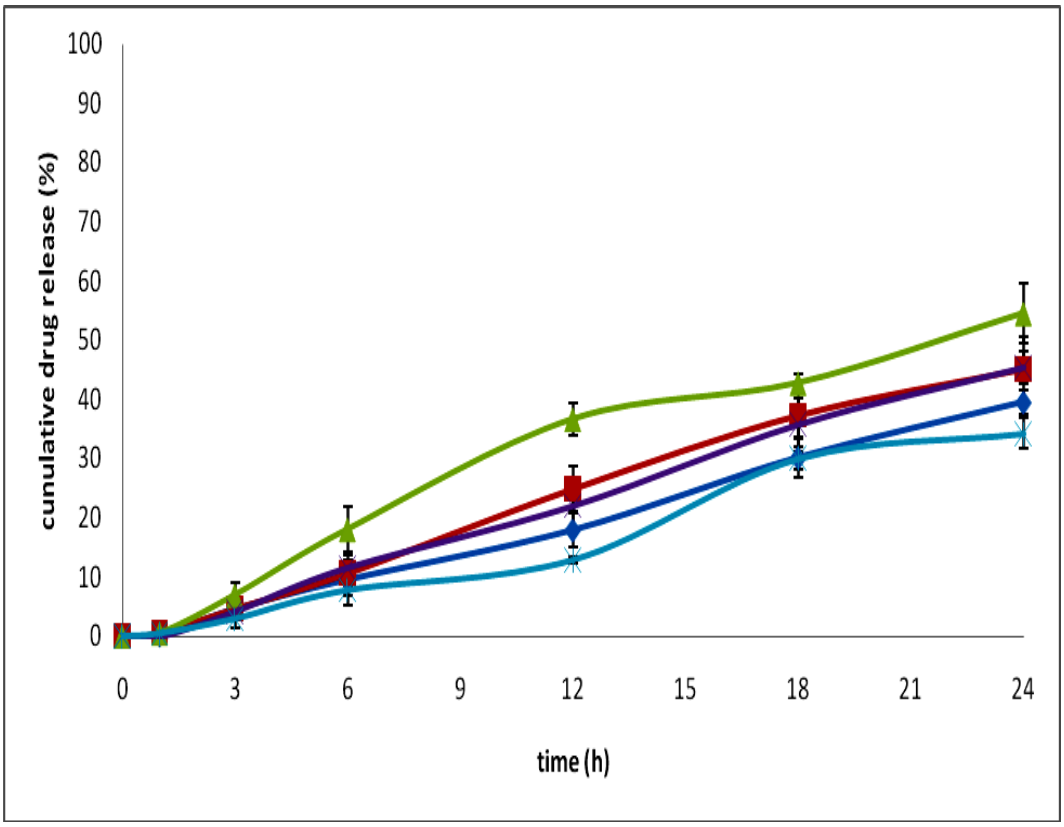


Figure 4-30 Permeation profiles across pig-ear skin of 10% GS HCl in 10% ethanol (◆), water (ж) and in the presence of DG(▲), CCSP(X) or Tween 80 (■) (n=6)

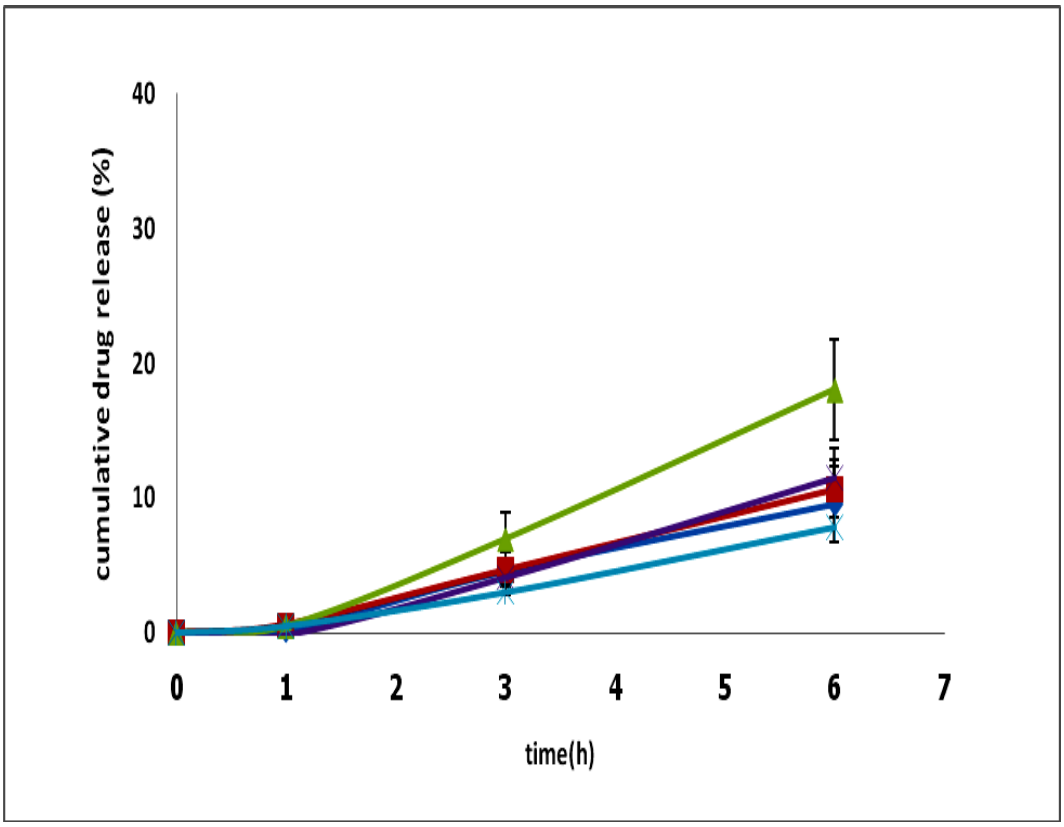


Figure 4-31 Permeation profiles of 10% GS HCl in ethanol 10%, water and 10% GS HCl in various surfactant type in ethanol 10% across pig-ear skin at 32 ºC in 6 hours (n=6) DG(▲), Tween 80(■), CCSP (X), GS HCl in water (ж), GS HCl in ethanol(◆)

Effect of ethanol

The effect of ethanol on drug permeation was shown in figure 4-32, after 6 hours, the permeation of GS HCl in the presence of 10% ethanol (18.05%) was significantly higher than that of GS HCl in the absence of 10% ethanol (10.42%) at 3 and 6 hours at p-value of 0.05. Thus, low concentration of ethanol could enhance the permeation of drug through skin. It has been reported that high concentration of ethanol inversely affected, the permeation of drug, because the high concentration of ethanol could denature proteins on the skin membrane. Ethanol at a concentration higher than 70% causes serious dehydration of the tissue or even denaturation of proteins on the skin (Sznitowska, 1996).

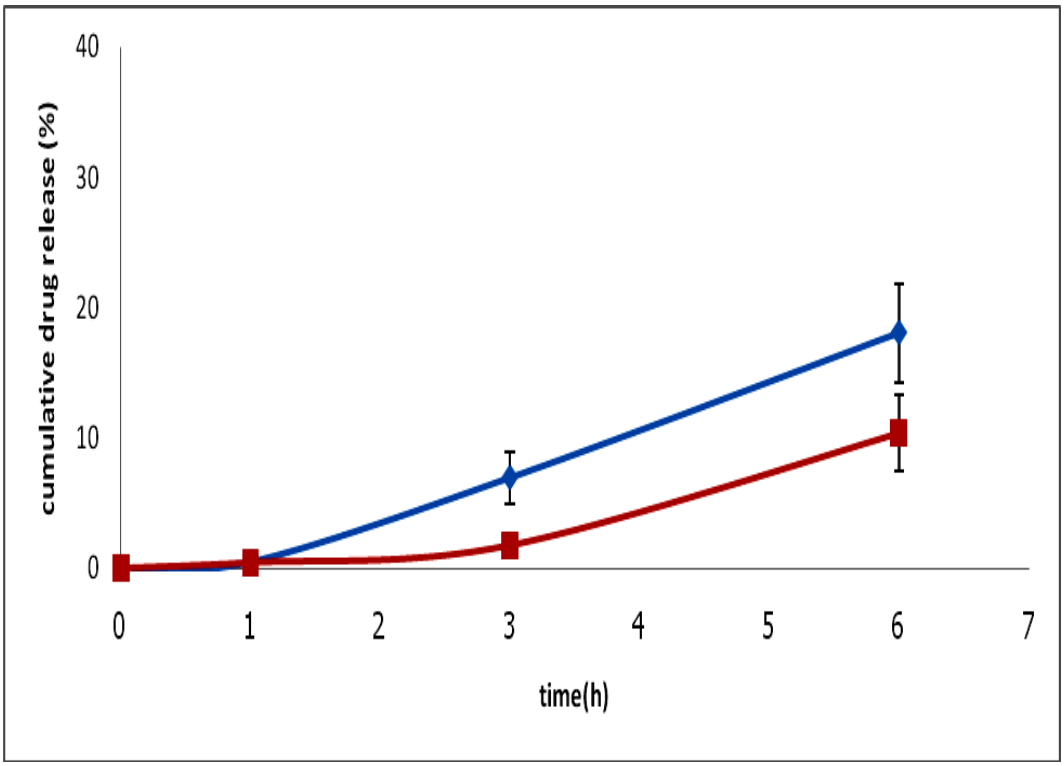


Figure 4-32 Permeation profiles of 10 % GS HCl containing DG and TT pH 3 across pig-ear skin in the presence 10% ethanol (◆) and in the ansence of 10% ethanol (■) (n=6)

Effect of drug concentration in DG foam formulation

The effect of drug concentration (5 and 10%) on the permeation of drug through skin was also studied. Higher concentration of drug loading led to more amount of drug permeated through the skin. 10% of GS HCl was significantly higher than 5% of GS HCl at p-value of 0.05. The percent cumulative permeations are shown in figure 4-33.

When increase amount of drug to 2 times higher than the typical amount did not result in higher absorption through skin in the same proportion suggesting that amount of drug loaded and amount of drug absorption did not directly correlate with each other. Moreover, percent drug loading in the selected formulation could not be higher than 10% because drug concentrate was approaching its solubility limit and resulting in precipitation of GS HCl in the preparation.

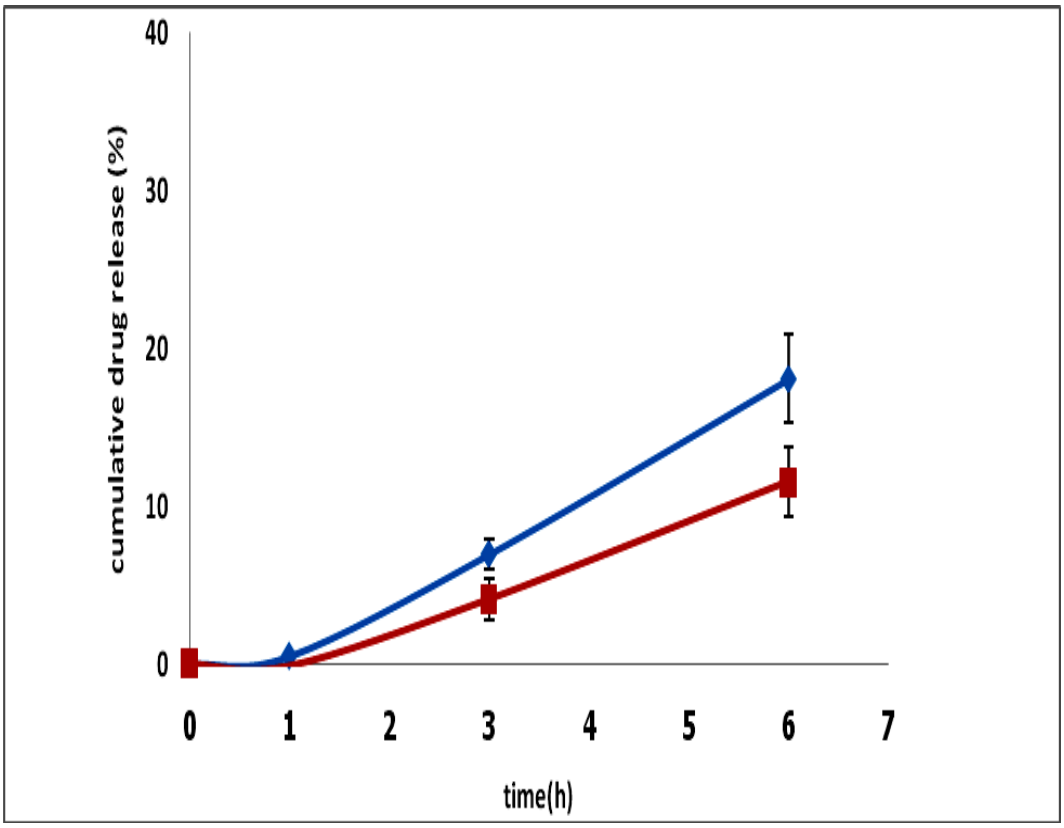


Figure 4-33 Permeation profiles of GS HCl in DG formulation, concentration 5% and 10% GS HCl across pig-ear skin at 32 °C (n=6) (10% GS HCl (◆), 5% GS HCl(■))

Application technique on skin permeation

In this study, 2 different characteristics of the same formulation,i.e. foam and solution, were compared for its skin permeability it was found that foam characteristics could permeate at a greater extent than the solution (figure 4-34). And 10% GS HCl in DG foam formulation was significant higher than in 10% GS HCl in DG solution formulation at p-value of 0.05 at every time in release study. It has been reported that foam formulation was able to deliver a greater amount of the active drug such as, betamethasone valerate, clobetasol propionate, ketoconazole and clindamycin, and increased delivery rate when compared with other formulations, i.e. gel, cream and solution. The studies speculated that the evaporation of the foam vehicle, may

cause the active ingredient to concentrate at the interface, leading to the saturation and then to supersaturation. This supersaturation although generally a transient condition contributing to the enhanced rate of delivery. In addition, supersaturation may result in a pseudo drug reservoir within the stratum corneum (Huang et al., 2005).

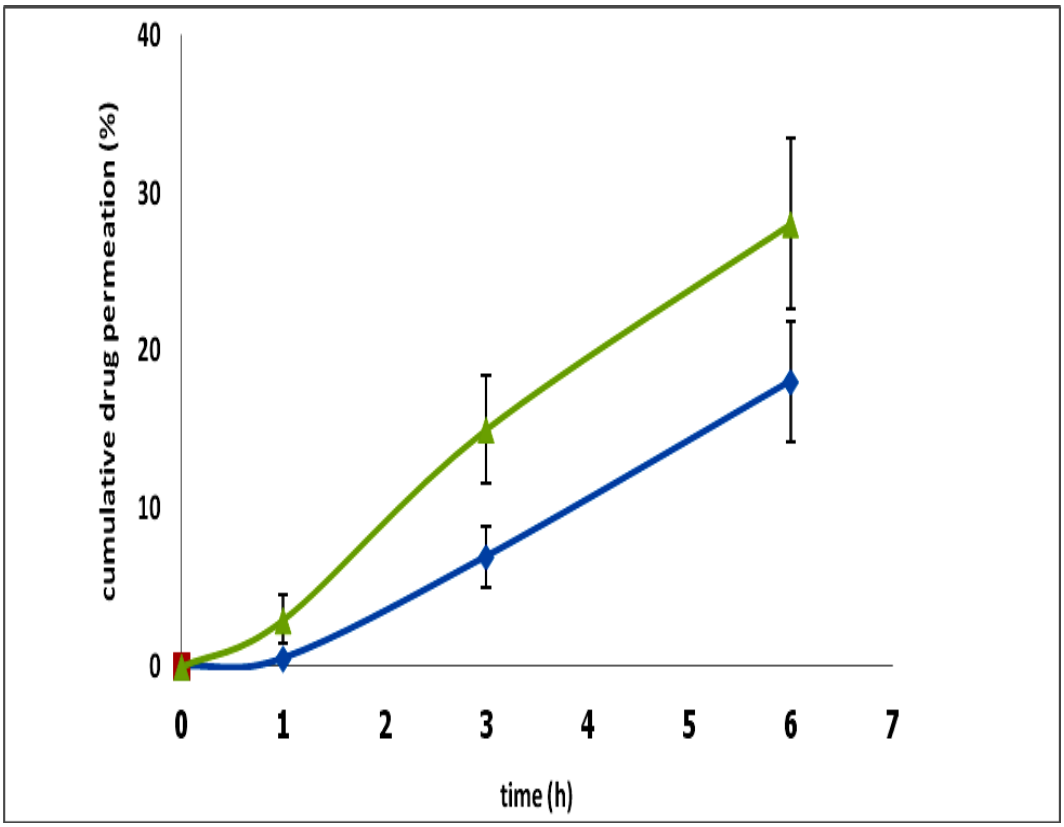


Figure 4-34 Permeation profiles of 10% GS HCl in DG solution formulation and 10% GS HCl in DG foam formulation across pig-ear skin at 32 ºC (n=6) (solution (♦), foam(▲))

Absorption property through skin of formulation made of foam based was better than solution based. Especially in this experiment, in formulation with DG shown higher absorption and formulation that combined surfactant with 10% Ethanol contribute to better absorption than using either surfactant or 10%Ethanol.

In addition the ability to solubilize GS HCl of the formulation was important factor influencing permeation enhancement of this drug. GS HCl is a hydrophilic drug with high water solubility; therefore the possibly pathway of GS HCl is may be favorable to pores.

Transdermal transport through pores plays a dominant role of permeation of hydrophilic solutes. This pathway had been proposed several years ago. The rational

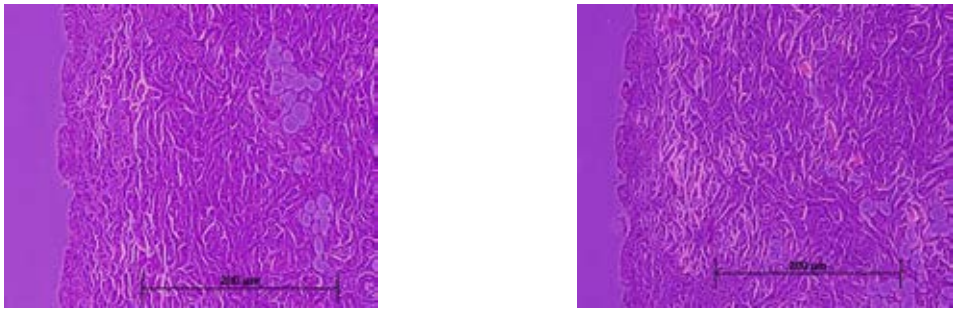
for the existence of transdermal transport through pores in the stratum cutaneous is due to the imperfections in the lipid bilayers. These imperfections cause by defects created by steric constraints placed by the keratinocytes on intercellular lipid bilayers (Mitragotri, 2003). It has been reported that defects in lipid bilayers exist due to lateral phase separation, or osmotic stresses in the bilayer (Cevec and Richardsen, 1999). Several additional argruments could be made to support this posturate. First, it is hard to imagine that flawless lipid bilayers exist over the entire skin area. Second, hydration of SC induces significant swelling (Lars et al., 1997) and fluidization (Alonso et al., 1996) of SC lipid bilayer. This may increase the likelihood of the formation of defects. Third, structure defects are more likely to occur in mixed lipid systems.SC lipid bilayers, which comprise of at least four major components (ceramides, fatty acids, cholesterol and cholesterol sulfate) are likely to exhibit packing defects. Takeing all together imperfections existed in lipid bilayers and may allow permeation of hydrophilic drugs such as glucosamine through skin.

Table 4-6 Permeability constant (kp) and flux of glucosamine hydrochloride 10% and ethanol 10% in various dosage form across pig ear skin

Formulation	Dosage forms	Surfactants	kp (cm/s)	Flux (μg/cm2*h)
1	solution	DG	$2.20x10^{-6}$	792.68
2	solution	CCSP	$1.46x10^{-6}$	527.18
3	solution	Tween 80	$1.26x10^{-6}$	452.88
4	foam	DG	$3.10x10^{-6}$	1116.90

From fig.4-31, cumulative amount of drug permeation within 8 hours were used in evaluation of slopes of the permeation profiles with known effective area of permeation and saturated solubility of GS HCl in receptor medium, permeability constant (k_p) was calculated based on equation (8). Then, fluxes of GS HCl from each formulations were calculated from permeability constants according to equation (1). Finally, permeation constants and fluxes were reported in table 4-6. The foam formulation of GS HCl gave hight values of permeability constant and flux of $3.10x10^{-6}$ cm/s and $0.112x10^{3}$ μg/cm2*h, respectively. So the most suitable drug delivery system through skin for GS HCl was DG foam formulation.

The skin were taken to prepare for viewing under the inverted microscope. The skins were not significantly different from each other and showed a normal appearance as shown in figure. 4-35. Therefore, ingredients in GS HCl foam formulation did not do any harm to the pig-ear skin.



(A) **(B)**

Figure 4-35 Pictures showing the ultrastructure of freshly pig-ear skin (A) and pig-ear skin foam formulation treated group; prepared from DG (B)

CHAPTER V

CONCLUSIONS

In this study, glucosamine hydrochloride (GS HCl) was prepared in various components of formulations such as surfactants, buffer and antioxidants in order to investigate stability of formulations the potential of the permeation enhancement of GS HCl through pig ear skin. In this study of GS HCl was the most stable when the formulation pH value was 3 in the presence of sodium metabisulfite. The recommended storage conditions of this preparation was "storage at 4-8°C"

Invitro permeability study, showed that permeation of GS HCl through pig-ear skin was affective of surfactant type and administration technique in an order of DG foam formulation > DG solution formulation > CCSP solution formulation > TWEEN 80 solution formulation

The presence of 10% ethanol in the formulation, could improve permeability of GS HCl since ethanol at low concentration is a permeation enhancer.

Suggestion for the further study

The development of GS HCl transdermal delivery system should gave more information such as:

1. The *in vitro* and *in vivo* permeation of GS HCl transdermal formation through human skin.

2. Prevention or retardation of millard reaction of GS HCl in the formulation.

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APPENDICES

APPENDIX A

Analysis of glucosamine hydrochloride and HPLC method validation

1.1 High-performance liquid chromatographic technique for drug analysis

Analysis method validation parameter of GS HCl and results of validation process conclude in Table 1A.

Table 1A Analysis method validation parameter of GS HCl and results of validation process

Parameter[a]	Result value	Limited of acceptability
1. Specificity	No other peak interfere	No other peak interfere major peak
2. Linearity - the correlation coefficient (r^2)	0.9999	>0.9995
3. Precision - RSD (%)	0.63	$\leq$2
4. Accuracy - recovery (%)	100.75	98-105

[a] International Conference on Harmonisation, 1996

From the results, it can be acceptable validation parameter (specificity, linearity, precision and accuracy)

1.1.1 Specificity

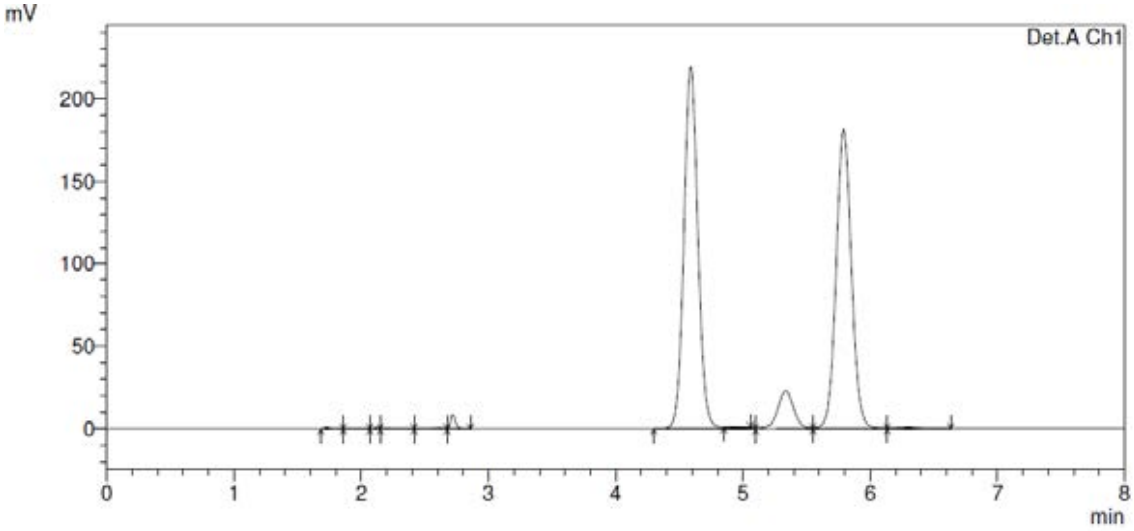

Figure 1A HPLC chromatogram of glucosamine hydrochloride and paracetamol (internal standard) having good resolution, with retention time of 4.6 and 5.8 minute respectively

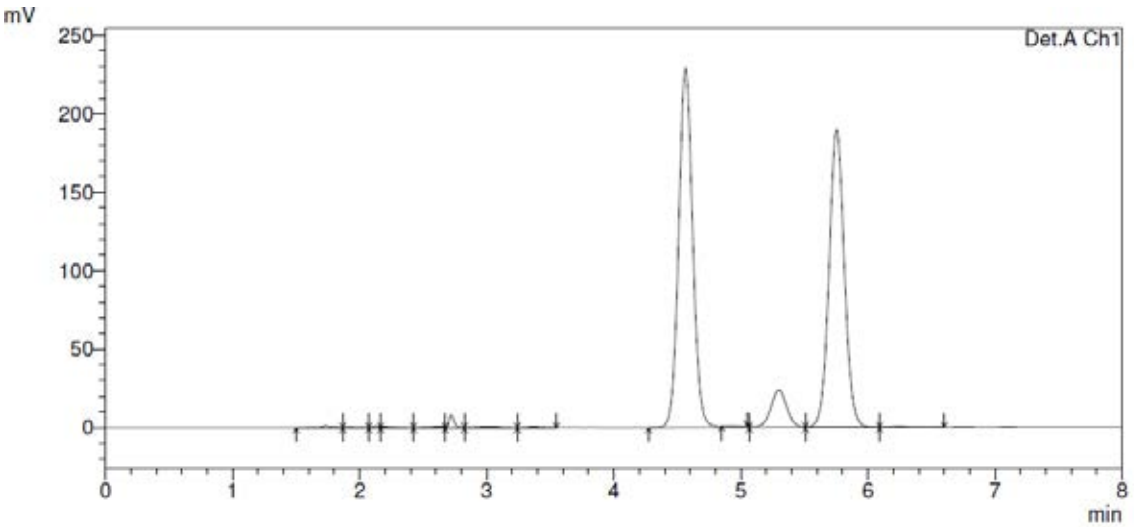

Figure 2A HPLC chromatogram of glucosamine hydrochloride and paracetamol (internal standard), in formulation having good resolution, with retention time of 4.6 minute and 5.8 minute respectively

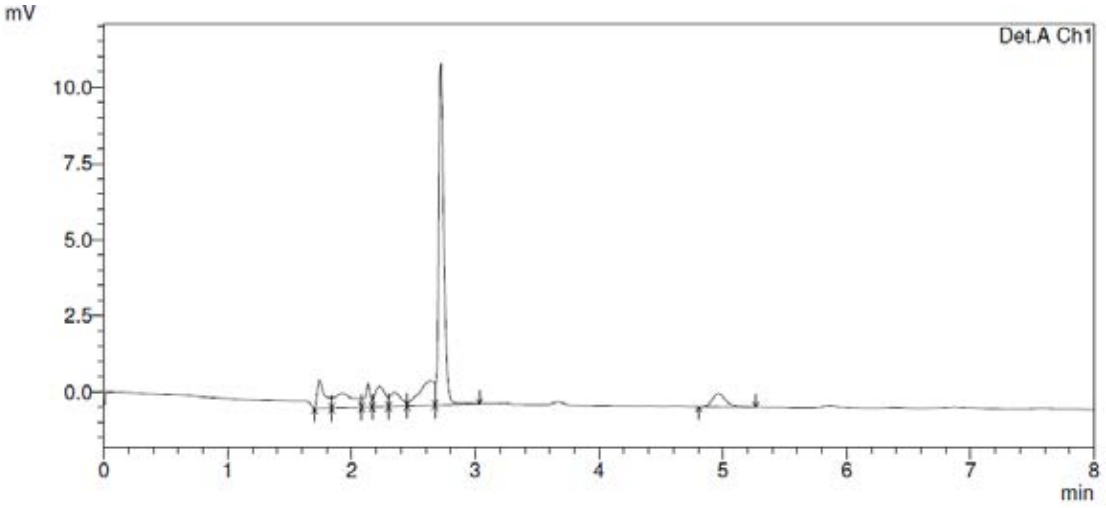

Figure 3A HPLC chromatogram of non-drug containing formulation system having no other peak interfere

1.1.2 Linearity

Table 2A Linearity of glucosamine hydrochloride

GS HCl concentration (mcg/ml)	Peak area ratio				
	Set no. 1	Set no. 2	Set no. 3	average	%CV
1	0.0107	0.0112	0.0110	0.0110	2.0649
30	0.3392	0.3391	0.3395	0.3393	0.0544
60	0.6707	0.6690	0.6707	0.6701	0.1507
100	1.1146	1.1156	1.1138	1.1147	0.0812
140	1.5811	1.5803	1.5826	1.5813	0.0744

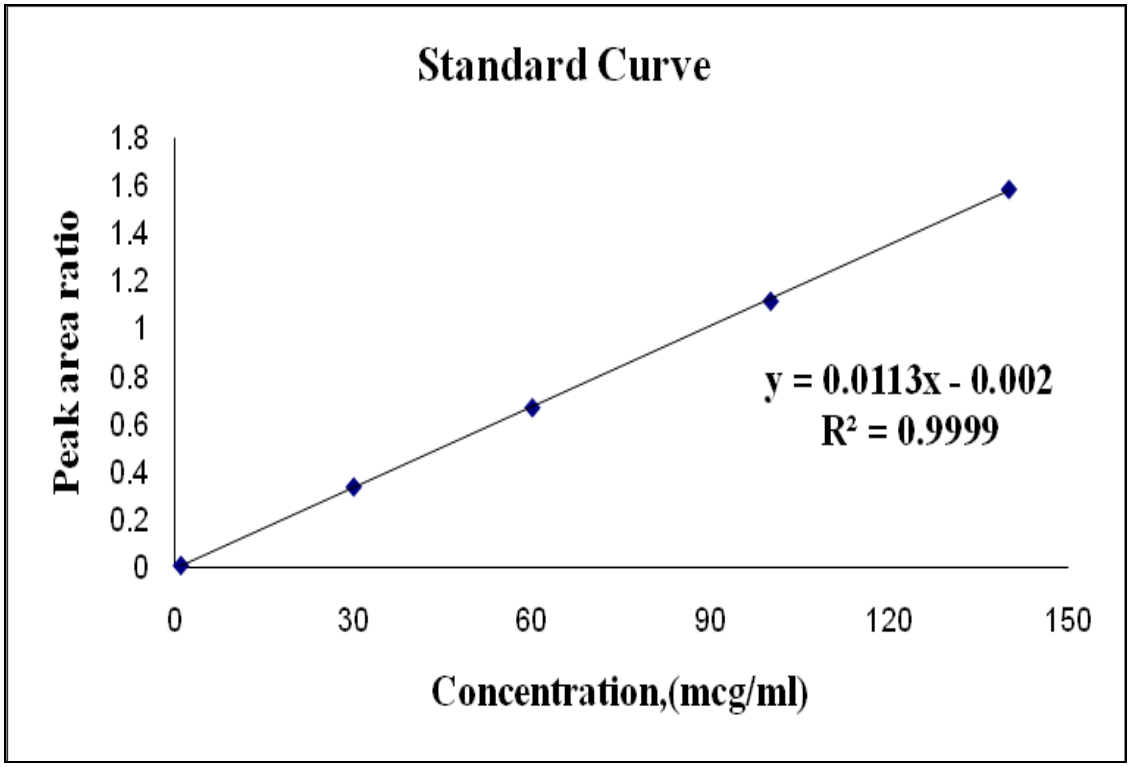

Figure4A The linearity of system

1.1.3 Precision

Table3A Within run precision

GS HCl con. (mcg/ml)	Calculated concentration of GS HCl from calibration curve (mcg/ml)							
	No.1	No.2	No.3	No.4	No.5	No.6	average	%CV
60	61.48	61.33	60.96	61.06	60.51	61.52	61.15	0.62
60	61.00	60.13	61.06	60.70	61.27	61.30	60.91	0.73
60	61.82	62.28	61.72	61.28	61.54	61.83	61.75	0.54

Table4A Between run precision

Day	Calculated concentration of GS HCl from calibration curve (mcg/ml)							
	No.1	No.2	No.3	No.4	No.5	No.6	average	%CV
1	61.00	60.13	61.06	60.70	61.27	61.30	60.91	0.73
2	60.74	60.73	61.04	60.57	60.54	60.52	60.69	0.32
Average between day	60.80							
% CV between day	0.26							

1.1.4 Accuracy

Table5A The analytical recovery of glucosamine hydrochloride

Know concentration (mcg/ml)	Calculated concentration From calibration curve (mcg/ml)	% Recovery
1.01	1.03	101.56
	1.03	102.78
	1.02	100.83
60.6	60.79	100.31
	61.15	100.91
	60.75	100.25
142.24	141.80	99.69
	142.78	100.38
	143.06	100.57
Average		100.75
SD		0.71
% CV		0.71

APPENDIX B

Permeation of glucosamine hydrochloride formulations across pig ear skin

TABLE1B Permeation of glucosamine hydrochloride in DG solution across pig ear skin (GS HCl concentration in donor = 10%)

Calibration curve data

Concentration (μg/ml)	1	30	60	100	140
Peak area ratio	0.01	0.31	0.58	0.95	1.34

$$Y = 0.0095X + 0.01$$

$$R^2 = 0.9997$$

Time (hours)	Cumulative release (%)						Average (%)	SE
	1	2	3	4	5	6		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	0.47	0.76	0.29	0.78	0.48	0.30	0.51	0.21
3	4.41	8.23	7.27	4.74	4.07	9.60	6.39	2.30
6	13.19	14.76	17.97	19.43	19.08	23.85	18.05	3.77
12	35.27	38.47	35.49	32.82	37.35	37.32	36.12	2.02
18	41.93	43.34	41.48	45.63	44.49	42.46	43.22	1.59
24	58.50	54.41	51.33	50.00	58.55	54.40	54.53	3.54

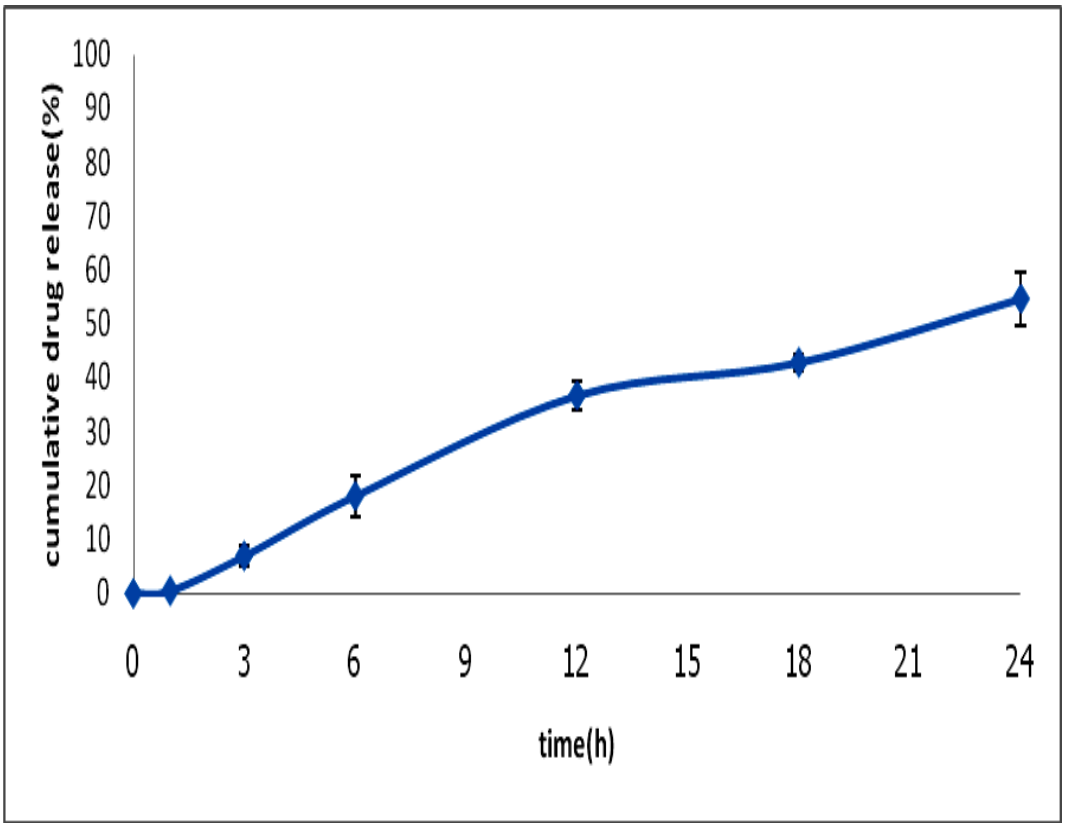

Figure 1B. Permeation profiles of GS HCl in DG formulation

TABLE2B Permeation of glucosamine hydrochloride in CCSP solution across pig ear skin (GS HCl concentration in donor = 10%)

Calibration curve data

Concentration (µg/ml)	1	30	60	100	140
Peak area ratio	0.01	0.31	0.58	0.95	1.34

$$Y = 0.0095X + 0.0111$$

$$R^2 = 0.9997$$

Time (hours)	Cumulative release (%)						Average (%)	SE
	1	2	3	4	5	6		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	-0.43	0.39	-0.43	-0.07	-0.22	0.05	-0.12	0.31
3	3.51	6.44	2.69	4.39	3.48	4.39	4.15	1.29
6	9.61	14.31	8.61	12.89	11.21	12.65	11.55	2.15
12	20.41	26.98	15.71	24.45	20.78	23.63	21.99	3.92
18	34.55	35.39	27.88	40.93	36.73	38.86	35.72	4.49
24	41.99	48.91	44.38	48.69	43.38	44.84	45.36	2.84

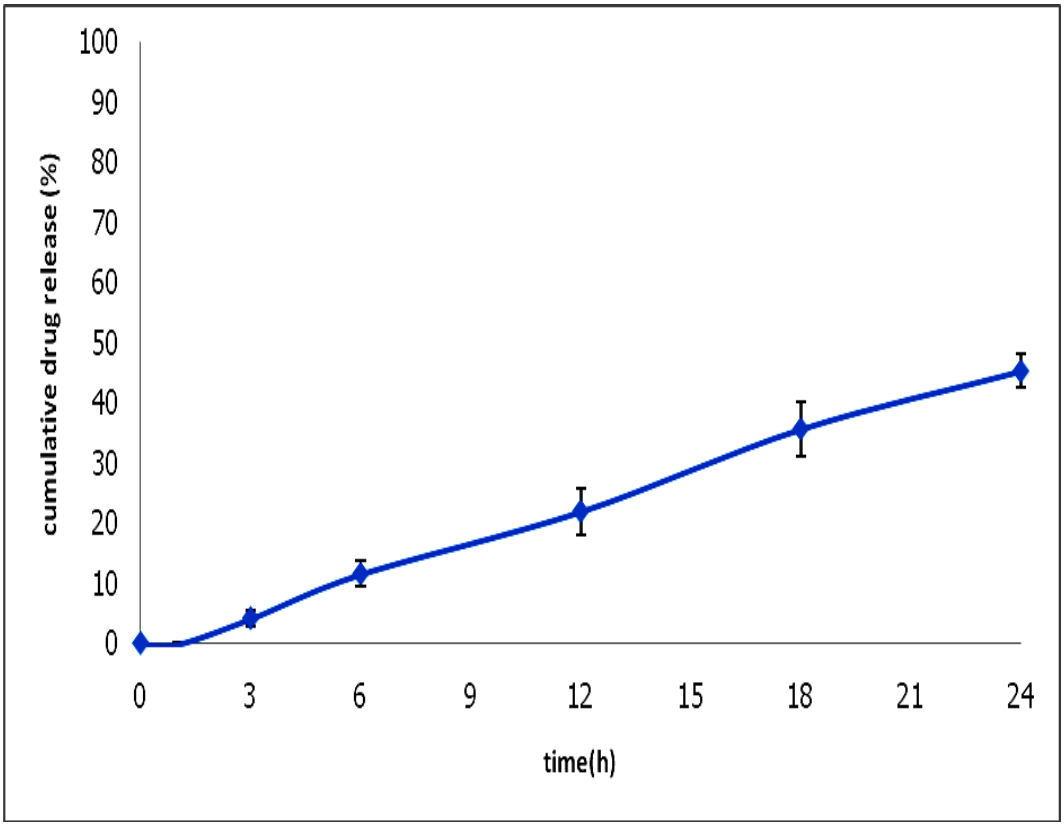

Figure 2B. Permeation profiles of GS HCl in CCSP formulation

TABLE3B Permeation of glucosamine hydrochloride in TWEEN solution across pig ear skin (GS HCl concentration in donor = 10%)

Calibration curve data

Concentration (μg/ml)	1	30	60	100	140
Peak area ratio	0.01	0.31	0.58	0.95	1.34

$$Y = 0.0095X + 0.0086$$

$$R^2 = 0.9997$$

Time (hours)	Cumulative release (%)						Average (%)	SE
	1	2	3	4	5	6		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	0.97	0.24	0.25	0.97	0.24	0.90	0.60	0.39
3	5.11	4.48	3.25	6.82	4.90	3.43	4.67	1.30
6	12.52	7.66	11.63	13.16	9.00	10.02	10.67	2.14
12	26.44	18.75	27.89	28.08	21.14	26.86	24.86	3.93
18	41.91	32.01	35.28	36.26	35.99	42.14	37.26	3.99
24	49.40	46.03	38.78	52.94	43.21	39.93	45.05	5.50

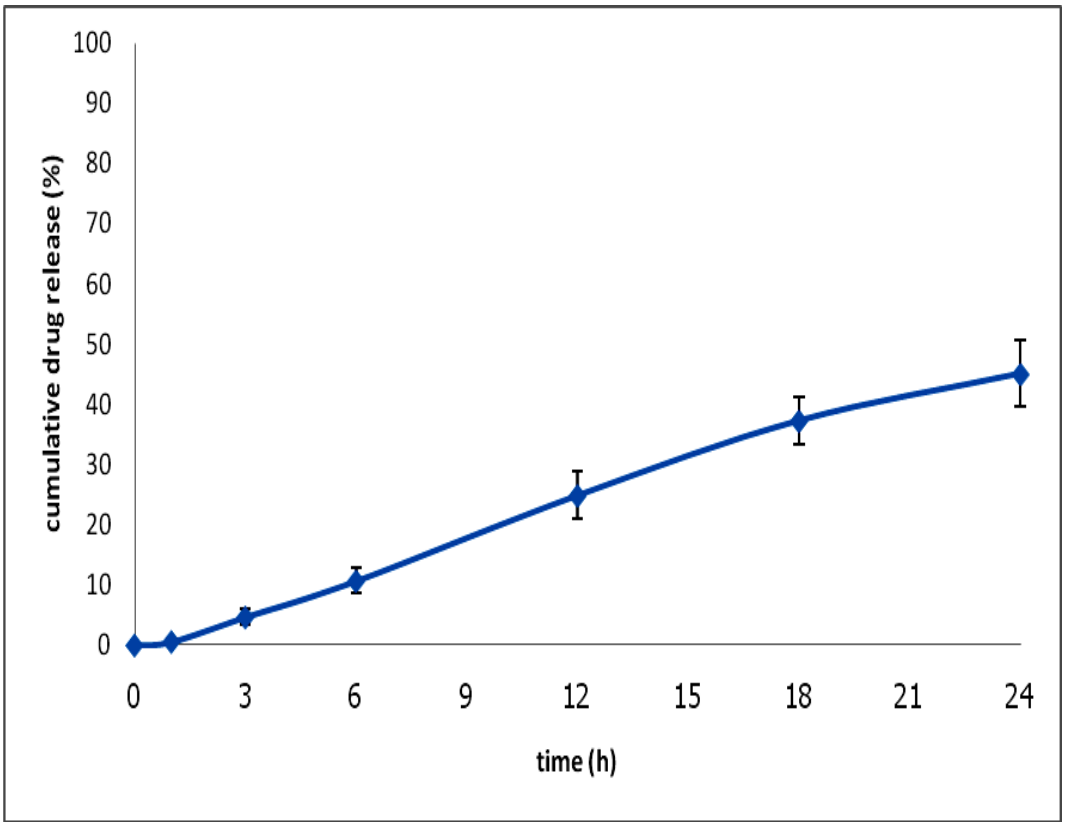

Figure 3B. Permeation profiles of GS HCl in Tween 80 formulation

TABLE4B Permeation of glucosamine hydrochloride in DG solution across pig ear skin (GS HCl concentration in donor = 10% and without ethanol)

Calibration curve data

Concentration (µg/ml)	1	30	60	100	140
Peak area ratio	0.01	0.31	0.57	0.94	1.33

$$Y = 0.0094X + 0.0097$$

$$R^2 = 0.9996$$

Time (hours)	Cumulative release (%)						Average (%)	SE
	1	2	3	4	5	6		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	0.53	0.49	0.70	0.31	0.30	0.30	0.44	0.16
3	1.46	1.47	2.21	1.54	2.43	1.56	1.78	0.43
6	8.23	7.23	13.21	14.79	9.65	9.40	10.42	2.94
12	31.43	21.86	28.05	20.28	23.83	30.12	25.93	4.59
18	31.41	38.51	37.30	41.99	35.48	35.23	36.65	3.56
24	45.14	41.74	42.46	43.24	43.37	45.61	43.59	1.51

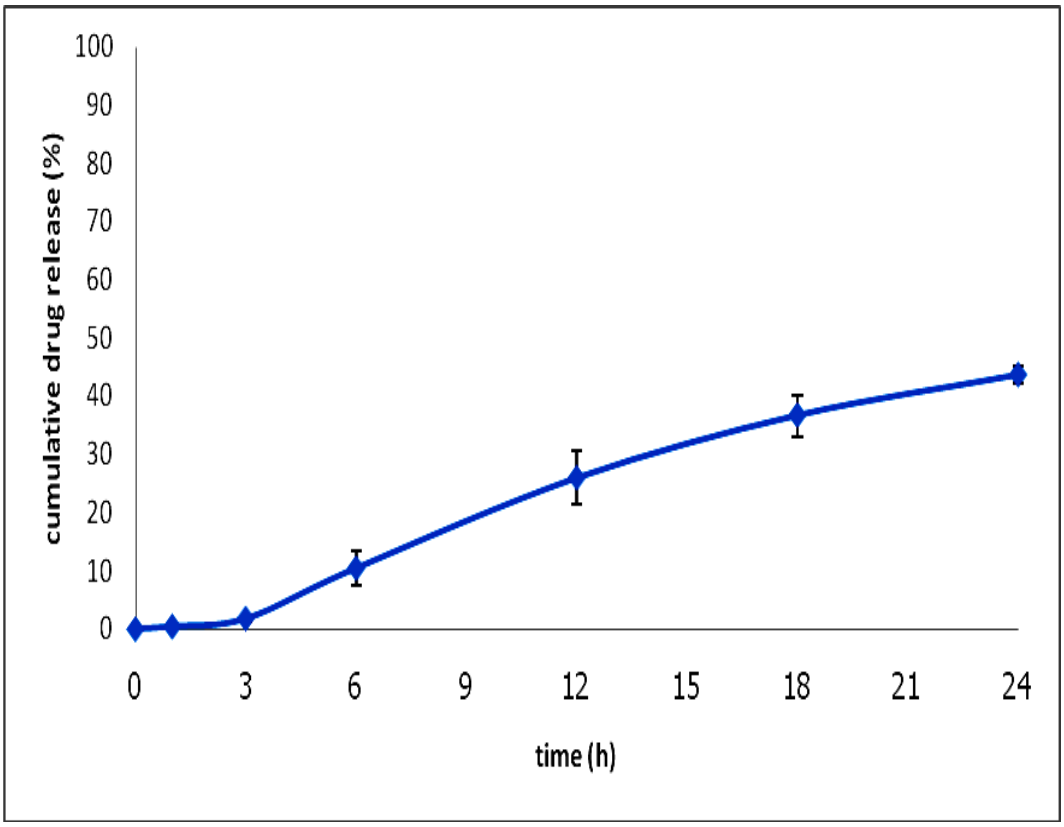

Figure 4B. Permeation profiles of GS HCl in DG formulation without ethanol

TABLE5B Permeation of glucosamine hydrochloride in DG solution across pig ear skin (GS HCl concentration in donor = 5%)

Calibration curve data

Concentration (μg/ml)	1	30	60	100	140
Peak area ratio	0.01	0.31	0.57	0.95	1.34

$$Y = 0.0095X + 0.0086$$

$$R^2 = 0.9996$$

Time (hours)	Cumulative release (%)						Average (%)	SE
	1	2	3	4	5	6		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	-0.40	0.38	-0.41	-0.03	-0.23	0.08	-0.10	0.31
3	3.46	6.42	2.66	4.37	3.44	4.37	4.12	1.30
6	9.59	14.29	8.58	13.01	11.20	12.69	11.56	2.18
12	20.42	18.98	14.21	24.88	20.74	24.02	20.54	3.83
18	38.31	35.58	28.00	30.39	36.86	37.75	34.48	4.27
24	36.60	34.00	35.01	37.79	35.21	36.25	35.81	1.34

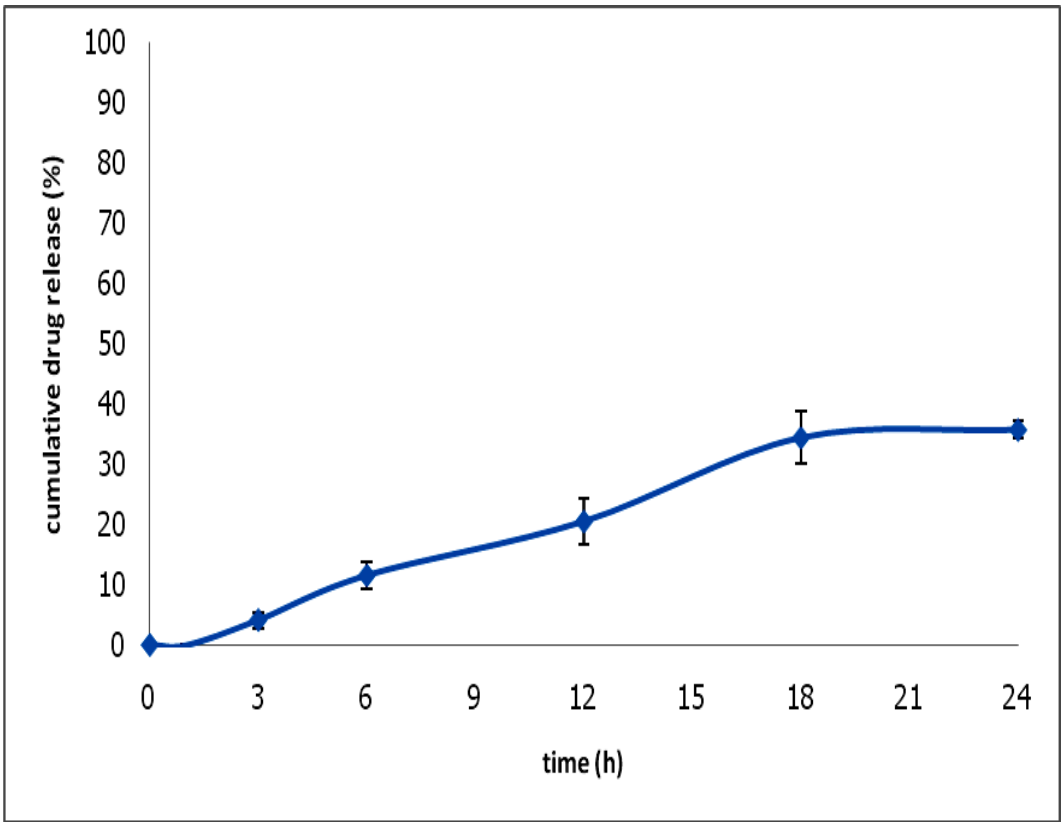

Figure 5B. Permeation profiles of GS HCl in DG formulation (GS HCl concentration in donor = 5%)

TABLE6B Permeation of glucosamine hydrochloride in DG foam across pig ear skin

Calibration curve data

Concentration (µg/ml)	1	30	60	100	140
Peak area ratio	0.01	0.34	0.68	1.12	1.53

$$Y = 0.011X + 0.0113$$

$$R^2 = 0.9997$$

Time (hours)	Cumulative release (%)						Average (%)	SE
	1	2	3	4	5	6		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	3.35	2.39	2.30	1.71	5.88	2.13	2.96	1.53
3	16.20	16.09	19.35	15.31	14.23	8.98	15.03	3.42
6	25.30	18.93	32.89	27.26	30.80	33.11	28.05	5.44
12	22.80	34.15	37.63	27.26	37.86	35.80	32.58	6.17
18	52.97	44.72	48.38	46.29	50.41	35.73	46.42	6.00
24	53.33	33.09	49.06	56.00	50.44	48.31	48.37	8.01

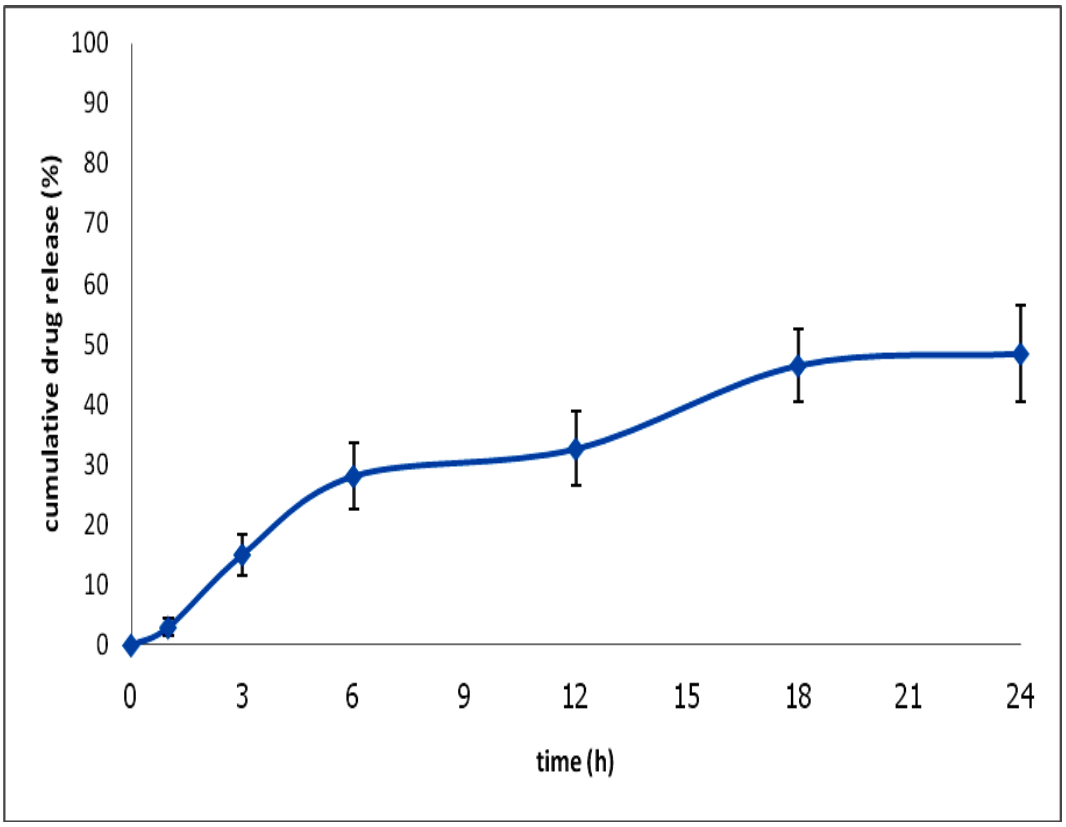

Figure 6B. Permeation profiles of GS HCl in DG foam formulation

TABLE7B Permeation of glucosamine hydrochloride in GS HCl solution in water across pig ear skin (GS HCl concentration in donor = 10%)

Calibration curve data

Concentration (µg/ml)	1	30	60	100	140
Peak area ratio	0.01	0.31	0.57	0.94	1.33

$$Y = 0.0094X + 0.0088$$

$$R^2 = 0.9996$$

Time (hours)	Cumulative release (%)						Average (%)	SE
	1	2	3	4	5	6		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	0.02	2.93	0.00	0.02	0.02	0.02	0.50	1.19
3	1.23	1.17	4.42	3.83	4.37	2.93	2.99	1.49
6	7.61	4.37	7.60	7.61	12.23	7.44	7.81	2.51
12	13.40	12.11	13.29	13.29	12.30	13.28	12.95	0.58
18	28.51	32.52	28.11	31.62	28.10	31.62	30.08	2.05
24	36.88	32.24	31.62	36.50	32.10	36.52	34.31	2.56

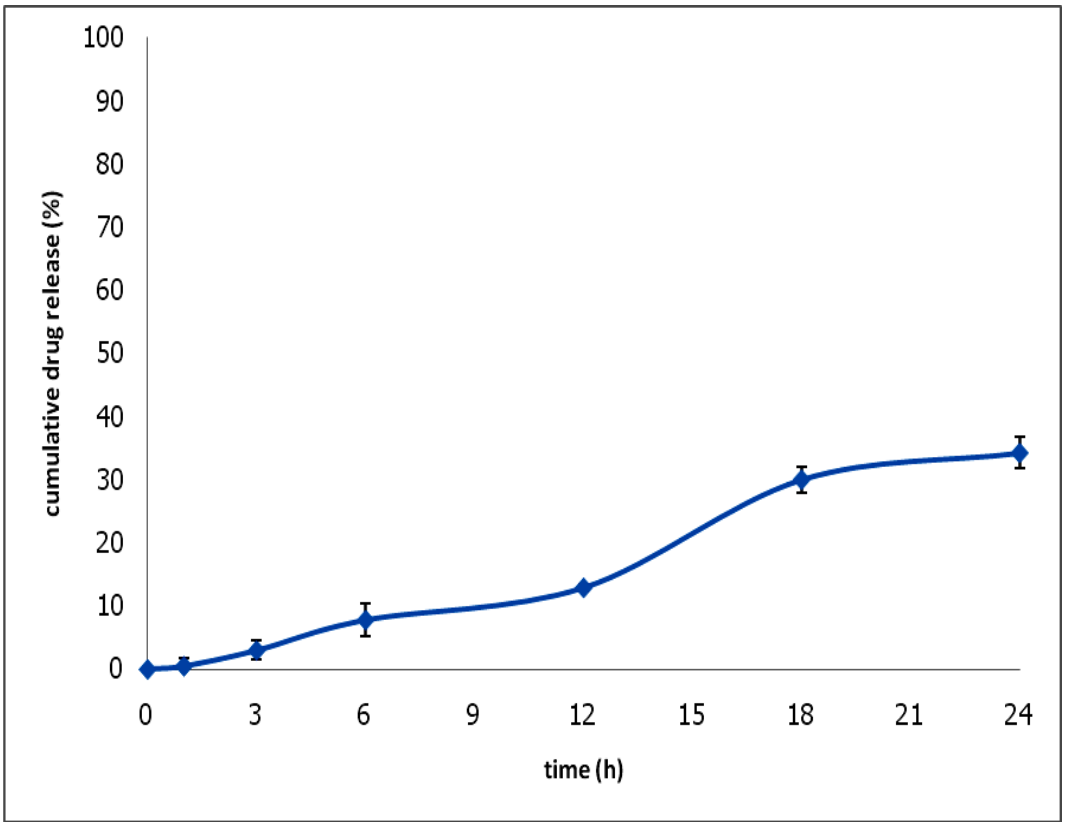

Figure 7B. Permeation profiles of GS HCl in DG formulation in water

TABLE8B Permeation of glucosamine hydrochloride in GS HCl solution in ethanol 10% across pig ear skin (GS HCl concentration in donor = 10%)

Calibration curve data

Concentration (µg/ml)	1	30	60	100	140
Peak area ratio	0.01	0.31	0.58	0.94	1.33

$$Y = 0.0094X + 0.0110$$

$$R^2 = 0.9996$$

Time (hours)	Cumulative release (%)						Average (%)	SE
	1	2	3	4	5	6		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	0.26	0.26	0.24	0.26	0.09	0.09	0.20	0.08
3	3.58	4.54	6.05	3.65	4.96	4.73	4.58	0.91
6	5.02	11.81	8.24	10.62	12.77	8.75	9.54	2.81
12	17.89	18.75	16.34	13.18	21.35	20.14	17.94	2.91
18	26.94	32.05	27.99	31.95	26.95	35.32	30.20	3.43
24	38.42	39.46	39.04	37.12	43.06	40.24	39.56	2.01

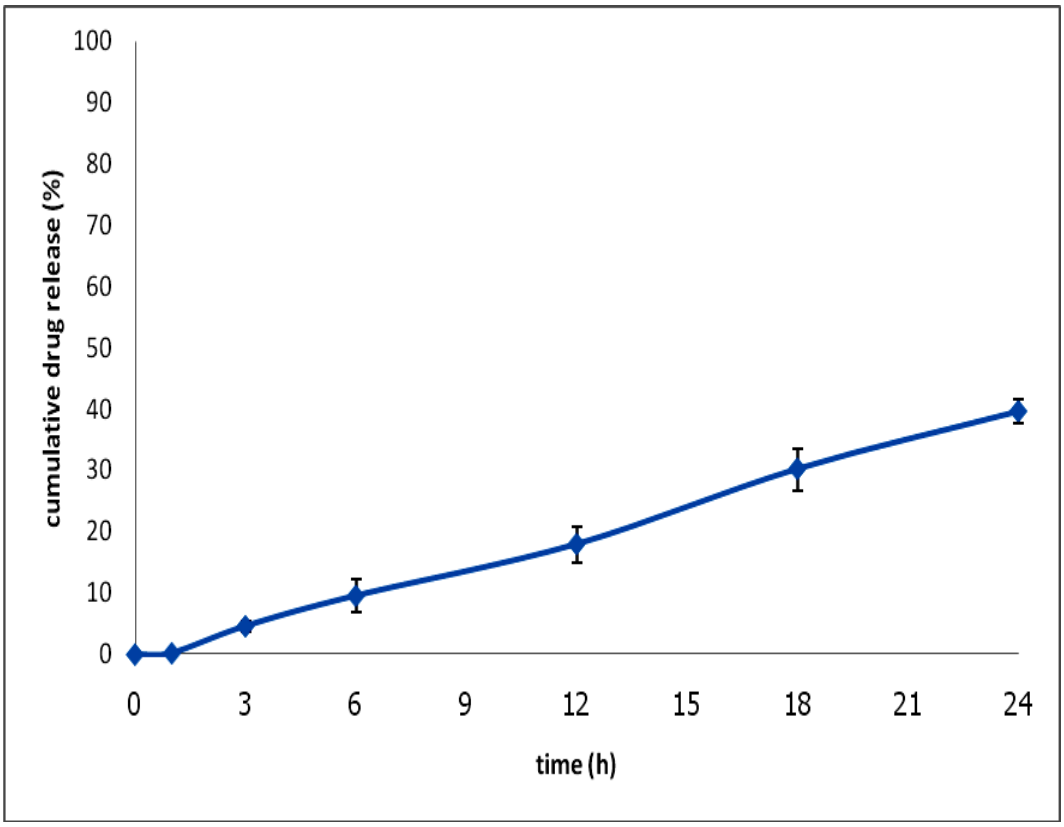

Figure 8B. Permeation profiles of GS HCl in DG formulation in ethanol 10%

APPENDEX C

Stability of glucosamine hydrochloride formulations on 3 months

TABLE 1C Percent drug remained of glucosamine hydrochloride in DG solution after 3 months of storage at 5 ºC

Time (days)	Drug remained (%)			Average (%)	SE
	1	2	3		
0	100.90	101.08	100.89	100.96	0.11
7	100.94	101.64	101.00	101.19	0.39
14	100.16	100.46	100.65	100.43	0.25
28	98.59	98.67	98.86	98.70	0.14
42	101.08	100.11	100.33	100.57	0.51
56	99.88	101.62	99.95	100.48	0.98
84	99.83	99.43	99.50	99.58	0.21

TABLE 2C Percent drug remained of glucosamine hydrochloride in DG solution after 3 months of storage at 30 ºC

Time (days)	Drug remained (%)			Average (%)	SE
	1	2	3		
0	100.90	101.08	100.89	100.96	0.11
7	101.00	101.05	100.35	100.80	0.39
14	99.95	99.11	99.51	99.53	0.42
28	87.38	88.00	86.79	87.39	0.60
42	88.30	86.38	86.77	87.15	1.02
56	81.35	81.37	81.76	81.49	0.23
84	73.80	73.92	73.76	73.83	0.08

TABLE 3C Percent drug remained of glucosamine hydrochloride in DG solution after 3 months of storage at 40 °C

Time (days)	Drug remained (%)			Average (%)	SE
	1	2	3		
0	100.90	101.08	100.89	100.96	0.11
7	88.88	89.83	88.99	89.23	0.52
14	88.67	88.91	87.81	88.46	0.58
28	74.28	72.39	73.55	73.41	0.95
42	70.99	70.98	70.79	70.92	0.12
56	62.54	62.12	63.93	62.86	0.95
84	62.39	61.84	63.73	62.65	0.97

TABLE 4C Percent drug remained of glucosamine hydrochloride in CCSP solution after 3 months of storage at 5 °C

Time (days)	Drug remained (%)			Average (%)	SE
	1	2	3		
0	101.53	100.47	100.22	100.74	0.70
7	101.01	100.70	100.92	100.88	0.16
14	101.32	101.38	101.53	101.41	0.11
28	102.03	101.39	101.49	101.64	0.35
42	101.94	101.22	101.67	101.61	0.36
56	99.15	99.75	99.81	99.57	0.36
84	100.40	100.21	100.79	100.47	0.29

TABLE 5C Percent drug remained of glucosamine hydrochloride in CCSP solution after 3 months of storage at 30 °C

Time (days)	Drug remained (%)			Average (%)	SE
	1	2	3		
0	101.53	100.47	100.22	100.74	0.70
7	101.23	100.83	100.98	101.01	0.20
14	101.61	101.18	101.19	101.33	0.24
28	89.85	90.39	89.83	90.02	0.32
42	90.13	89.68	90.40	90.07	0.37
56	80.84	81.18	81.11	81.04	0.18
84	76.38	76.28	76.32	76.33	0.05

TABLE 6C Percent drug remained of glucosamine hydrochloride in CCSP solution after 3 months of storage at 40 °C

Time (days)	Drug remained (%)			Average (%)	SE
	1	2	3		
0	101.53	100.47	100.22	100.74	0.70
7	96.44	96.57	95.21	96.07	0.75
14	87.71	86.76	87.80	87.42	0.58
28	75.91	75.92	75.91	75.91	0.01
42	75.03	76.83	74.63	75.50	1.71
56	64.26	65.95	63.48	64.56	1.26
84	64.26	66.30	64.27	64.95	1.18

TABLE 7C Percent drug remained of glucosamine hydrochloride in Tween 80 solution after 3 months of storage at 5 ºC

Time (days)	Drug remained (%)			Average (%)	SE
	1	2	3		
0	101.24	101.92	101.38	101.51	0.36
7	101.00	101.16	101.43	101.20	0.22
14	101.63	101.39	101.38	101.46	0.14
28	95.43	95.42	95.40	95.42	0.02
42	92.40	92.51	92.54	92.48	0.08
56	96.26	96.19	97.29	96.58	0.62
84	96.06	95.90	95.94	95.97	0.08

TABLE 8C Percent drug remained of glucosamine hydrochloride in Tween 80 solution after 3 months of storage at 30 ºC

Time (days)	Drug remained (%)			Average (%)	SE
	1	2	3		
0	101.24	101.92	101.38	101.51	0.36
7	101.63	100.99	101.27	101.30	0.32
14	99.42	101.47	101.39	100.76	1.16
28	88.36	88.43	88.42	88.40	0.04
42	88.82	88.79	88.97	88.86	0.10
56	83.79	84.44	85.19	84.47	0.70
84	74.18	74.05	74.30	74.18	0.12

TABLE 9C Percent drug remained of glucosamine hydrochloride in Tween 80 solution after 3 months of storage at 40 °C

Time (days)	Drug remained (%)			Average (%)	SE
	1	2	3		
0	101.24	101.92	101.38	101.51	0.36
7	96.00	95.60	95.46	95.69	0.28
14	91.92	91.88	91.52	91.77	0.22
28	75.03	75.23	75.00	75.09	0.13
42	69.07	69.16	69.08	69.10	0.05
56	63.29	63.11	64.95	63.78	1.01
84	63.58	62.91	65.01	63.83	1.07

TABLE 10C Percent drug remained of glucosamine hydrochloride in GS HCl solution after 3 months of storage at 5 °C

Time (days)	Drug remained (%)			Average (%)	SE
	1	2	3		
0	100.42	100.78	100.73	100.64	0.19
7	100.59	100.60	100.79	100.66	0.11
14	99.97	100.24	100.25	100.15	0.16
28	100.30	100.44	100.86	100.53	0.29
42	100.89	100.89	100.90	100.89	0.01
56	100.85	100.87	100.79	100.84	0.04
84	100.83	100.87	100.85	100.85	0.02

TABLE 11C Percent drug remained of glucosamine hydrochloride in DG solution without ethanol after 3 months of storage at 5 ºC

Time (days)	Drug remained (%)			Average (%)	SE
	1	2	3		
0	100.27	99.55	100.29	100.04	0.42
7	100.69	100.70	100.46	100.62	0.14
14	100.40	100.44	100.60	100.48	0.11
28	100.72	100.48	100.50	100.57	0.13
42	100.57	100.79	100.76	100.71	0.12
56	99.37	99.07	99.72	99.38	0.33
84	100.29	100.40	100.38	100.36	0.06

APPENDIX D

Pig ear skin preparation

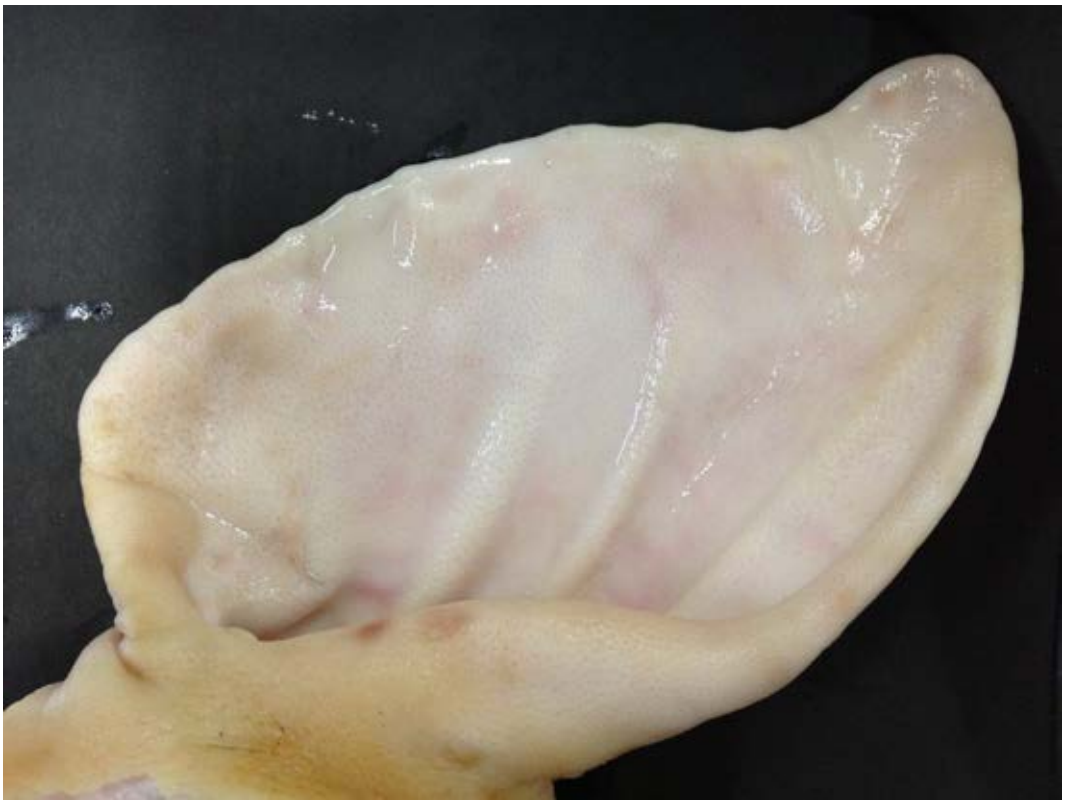

Figure 1D Pig ear obtained from local market in Nonthaburi province of Thailand.

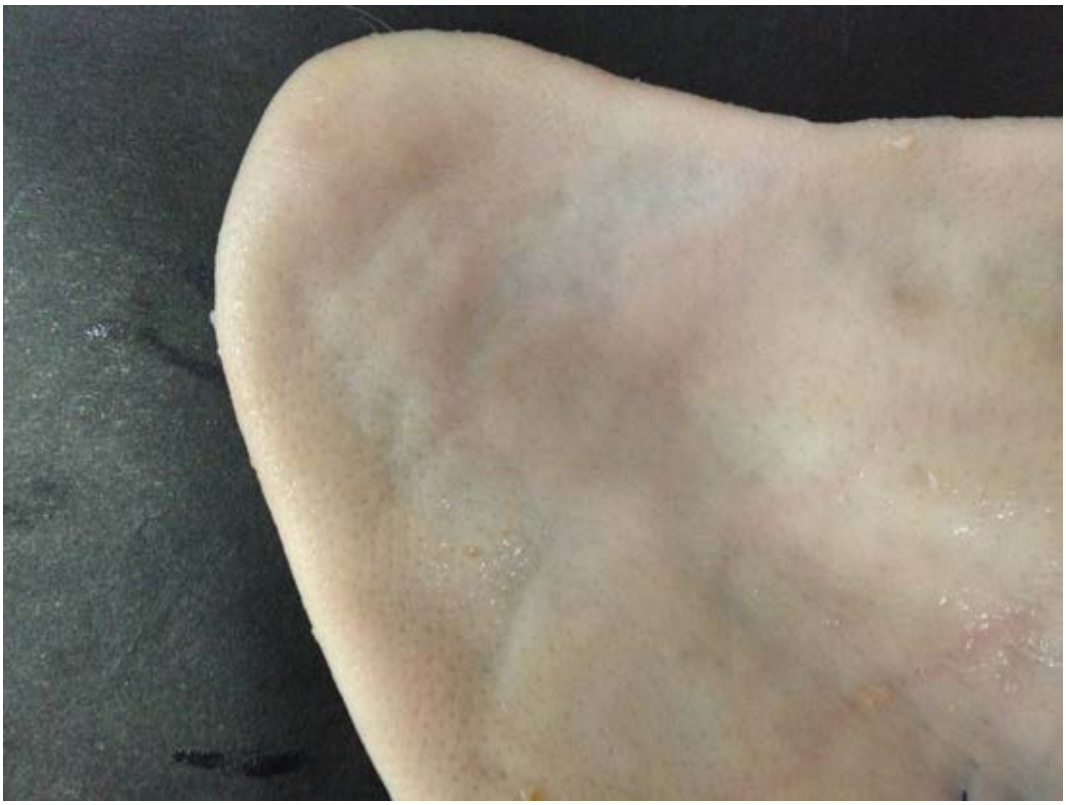

Figure 2D Subcutaneous fat and other extraneous tissues adhering to the dermis werecompletely removed and trimmed if necessary using forceps and scissors

Figure 3D The excised pig ear skin was place between the donor and the receptor compartment of franz-diffusion cell.

VITA

Miss chonticha Srisawang was born on August 5, 1983 in Khon Kaen, Thailand. She obtained her Bachelor degree in Pharmacy from the faculty of Pharmacy, Silpakorn University, Nakornpathom, Thailand.