Comparison of Skin Penetration of Niacinamide between Local and International Brands

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"A thesis report, submitted to the Department of Pharmacy, East West University, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy"

DECLARATION BY THE CANDIDATE

I, Afifa Binta Saifuddin, hereby declare that the dissertation entitled "Comparison of Skin Penetration of Niacinamide between Local and International Brands", submitted by me to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Honors) with original research work carried out by me under the core supervision and guidance of Tilka Fannana, Senior lecturer and co-supervision of Dr. Tasnuva Haque, Assistant Professor, Department of Pharmacy, East West University, Dhaka.

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CERTIFICATE BY THE SUPERVISOR

This is to certify that the dissertation entitled "Comparison of Skin Penetration of Niacinamide between Local and International Brands", submitted to the Department of pharmacy, East West University in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy was carried out by Afifa Binta Saifuddin (ID: 2014-1-70-061) under our guidance and supervision and that no part of the research has been submitted for any other degree. We further certify that all the sources of information and laboratory facilities availed of in this connection is duly acknowledged.

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ENDORSMENT BY THE CHAIRPERSON

This is to certify that the dissertation entitled "Comparison of Skin Penetration of Niacinamide between Local and International Brands" is a bona fide research work done by Afifa Binta Saifuddin (ID: 2014-1-70-061) under our guidance and core supervision of Tilka Fannana, Senior Lecturer and co-supervision of Dr. Tasnuva Haque, Assistant Professor, Department of Pharmacy, East West University, Dhaka.

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ABSTRACT

The purpose of this research study was to compare skin penetration of niacinamide in two different day cream formulations. One of the formulations was from an international brand and the other was a local brand. Niacinamide is also known as vitamin B_3 and is a common ingredient in cosmetic formulations. To conduct the study, tape stripping protocol was developed. Sequential tape stripping was performed on human volunteers applying the formulations on their volar forearms. The tape strips were then extracted with solvent (methanol:water 20:80) and analyzed in UV Spectrophotometer at 262nm. There was no significant difference in cumulative SC (stratum corneum) removed from the two formulation applying sites. Statistically significant difference was observed in the amounts of niacinamide penetrated in the SC between the international brand and local brand, ($p<0.05$). However, when male and female group was compared in niacinamide penetration from the two brands of cream formulation, no difference was found ($p>0.05$). Therefore, niacinamide penetrated the SC in significantly higher quantities in case of international brand which is also a market leader worldwide. Modification in formulation of the local face cream is needed to improve the penetration of niacinamide in to the skin.

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CHAPTER 1
INTRODUCTION

1. Introduction

The purpose of this research is to compare difference of skin penetration of niacinamide (NIA) between a local cosmetic formulation of Bangladesh and an international formulation. A basic knowledge on the following factors is needed to understand skin penetration of a compound.

1.1 An overview on skin anatomy

Skin is the largest organ in our body and it is the main part of the integumentary system. Its average weight is about 4-5 kilograms. The skins main function is to protect the interior of the human body from the external environment. It also has a role in the metabolism of vitamin D amongst other things. It makes up 12-15% of body weight and has an entire surface area between 1-2 meters. Skin is the first barrier against infectious disease and prevents fluid loss from organs, which allows the body to maintain homeostasis. The skin is such an important organ that even moderate burns on more than 30% of the skin can be life-threatening due to fluid loss and infection (Kolarsick et al., 2011).

The primary functions of the integumentary system include:

- Maintain internal temperature (sweating & shivering)
- Excrete excess fluids and waste
- Receive of pressure, pain, heat, and cold
- Produce and secrete melatonin & vitamin D
- Protect the body from infection
- Maintain fluid balance (Kolarsick et al., 2011).

Human body consists of two types of skin, one is thick skin another is thin skin. Thick skin is there in the soles of foot and palms of hands which are much thicker and less elastic than thin skin and also hairless.

The skin is separated into three main layers called the epidermis, dermis, and hypodermis. There is a rich blood supply in the integumentary system but the vessels do not supply blood to the epidermis rather than blood vessels arise from the dermis, supply blood in the dermis and only to the base of the epidermis. Top or middle of the epidermis do not get any blood at all.

Appendages of the skin

Hair: the visible part of hair above the skin is called hair shaft and the part under the skin consists of hair root and hair follicle.

Sebaceous gland: Connected to the hair follicle there is sebaceous gland which secretes oil to lubricate and waterproof the skin and hair.

Sweat gland: There is also eccrine gland around the dermis area which extends up to the epidermis. These eccrine glands are basically sweat glands that secret sweat.

Arrector pili muscle: There are muscles that connect hair root to the epidermis, causes the pulling of hair e.g. goose bumps. They are known as arrector pili muscles.

Nerve ending: There are also nerves in the dermis layer which brings information from the brain and also send information to it. These nerves include hair follicle receptor for the hair, lamellae corpuscle (which is a mechanoreceptor sensitive to pressure and vibration). There are other three nerve endings that branch out all the way to the basal layer of epidermis. These free nerve endings include pain receptor.

The Layers of the Skin

Epidermis

The epidermis is the outermost layer of the skin. Epidermis is avascular and consists of keratinised sheet of squamous epithelium. There are a few layers, called strata that make up the epidermis. All nutrients for the living cells of the epidermis diffuse from the basement membrane of the dermis below it. From the bottom layer to the outermost layer the strata include:

- Stratum basale – a layer of single cells that lays on the basement membrane of the dermis. It is the very bottom layer of epidermis where there are actively dividing keratinocytes. These actively dividing cells move up and push the dead cells out. They are actively dividing because they get the blood supply but as they move up they lose the blood supply and become dead cells. Other cells that are found in stratum basale are: *melanocytes*-responsible for producing melanin which gives the color of skin, *tactile* cells- nerve cells for sensing things.

- Stratum spinosum –Above the stratum basale there is another layer called stratum spinosum composed of keratinocytes which are connected by desmosomes (cell junction) and are tightly packed. There are *dendritic cells* found in the spinosum layer which are part of the immune system.
- Stratum granulosum –Above the stratum spinosum there is stratum granulosum where layer of keratinocytes are there with the organelles slowly being destroyed due to the absence of blood supply. As these move up to the top layer the keratinocytes are already dead. Eventually, the keratin protein produced will make up the majority of the dead cells in the next two layers.
- Stratum lucidum – This layer of dead keratinized cells is only found in areas where skin is thick, such as the soles of the feet, and is not found in thin skin areas, such as the forearm.
- Stratum corneum –The very top layer of epidermis is called stratum corneum which consists of 20-30 layers of dead keratinocytes. When skin is rubbed, flakes of these dead keratin cells are sloughed off.

Dermis

The dermis is the layer below the epidermis. The dermis is primarily made up of connective tissue layers and proteins including collagen, elastin, and reticular fibers. The arrangement of these fibers allow for the dermis to be extremely elastic and flexible. Dermis can be further divided into the papillary layer and the reticular layer.

Papillary layer is important in forming one's own unique fingerprint because it contains dermal papillae.

Reticular layer allows for blood vessels, glands, hair follicles, and nerves to be embedded in the dermis. The two main glands embedded in the skin include the sweat and sebaceous glands.

Hair and nail growth begin in the dermis. Highly keratinized epithelial cells are arranged to make up hair and nails.

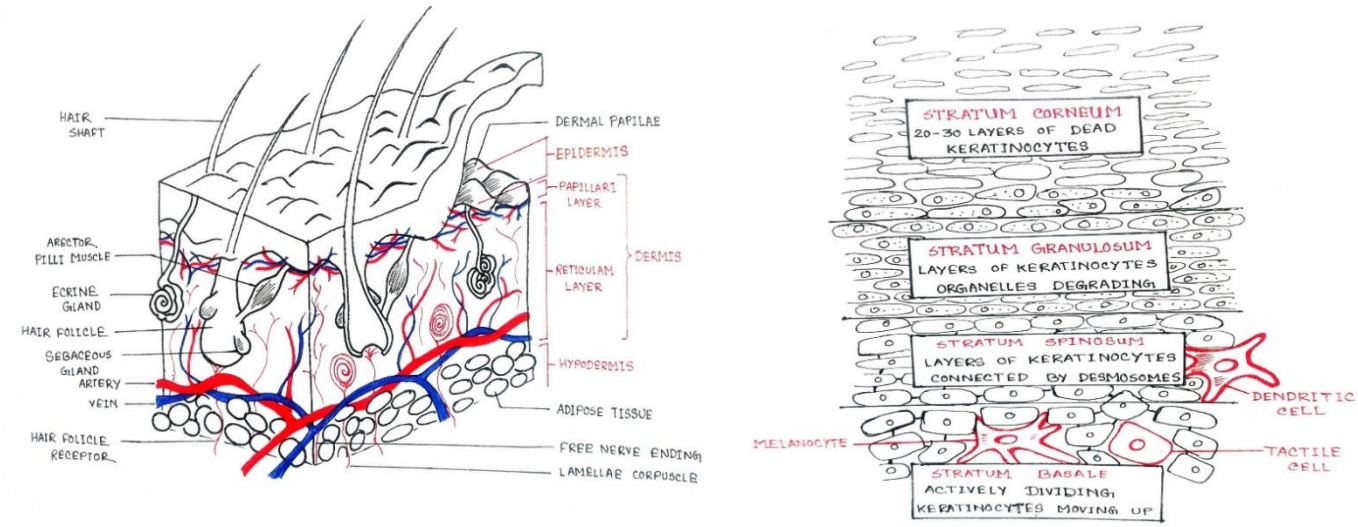


Figure no 1.1: Layers of skin

Hypodermis

The hypodermis is the bottommost layer of skin, located under the dermis. It is also known as the subcutaneous layer. Hypodermis consists of adipose tissue which serves as body's fat storage and takes part in thermoregulation (Kolarsick et al., 2011).

1.2 Criteria of a suitable candidate to penetrate the skin

Chemical absorption through the stratum corneum occurs by a passive process in which the chemical diffuses through this dead skin barrier. Human skin has many functions and its most apparent is that of a defense organ, both physical and biological. Penetration from outside into the body of any compound is primarily prevented by the corneal layer of the epidermis. This outer layer is just a few micrometers thick, but effectively forms a barrier that. Although absorption is not only dependent on penetration, but also on other variables such as skin metabolism, insufficient release from the carrier, partitioning in an unwanted reservoir, without penetration nothing happens. Essentially, the corneal layer consists of apoptotic keratinocytes that have transformed themselves into keratin-rich, lipoprotein-containing envelopes and lipid bilayers with hydrophilic regions in between. Estimates of the amount of chemicals absorbed through the skin can be assumed that the chemicals passively diffuse through dead skin barrier and are then carried into the body by the blood flow supplied to the dermis. A number of

conditions can affect the rate at which chemicals penetrate the skin. The potential of a chemical to penetrate the skin can be ranked based upon the following issues:

- Hydrophilicity or lipophilicity of the compound is an important fact. Since the stratum corneum is hydrophobic in nature it will allow the penetration of lipid soluble molecules more readily than water-soluble compounds. Most compounds pass the epidermal barrier through the intercellular route.
- Water-soluble molecules may penetrate through the openings of sweat glands and hair follicles. The total surface of these openings amounts to 0.1% of the total skin surface area, which is insignificant.
- Strong lipophilic compounds are however hampered by the hydrophilic regions in the bilayer.
- Optimal absorption will occur for molecules that are small, essentially having molecular weight below 500 dalton and also having low melting point (Bos & Meinardi, 2000).
- Frequency and duration of skin contact, the area of skin in contact with the chemical and the concentration of the chemical can also lead to the extent of skin penetration.
- The likely retention time of the material on the skin (e.g., highly volatile or dry powder materials are not likely to remain in contact with the skin, whereas materials with a higher molecular weight and sticky materials will remain in contact with the skin and thus be available for dermal exposure).
- Skin penetration may be increased under conditions of high humidity. When temperatures are elevated, sweating may contribute to increased skin penetration.
- Physically damaged skin or skin damaged from chemical irritation or sensitization or sunburn generally absorbs chemicals at a much greater rate than intact skin.
- If a chemical is in contact while wearing gloves or other protective clothing, the substance becomes trapped against the skin, leading to a much higher rate of permeability than with uncovered skin.

A common indicator of dermal absorption potential is the relative solubility of a material in octanol and water, often called the octanol-water partition coefficient ($K_{o/w}$). This partition coefficient is often expressed in the logarithmic form as Log $K_{o/w}$. Chemicals with a log $K_{o/w}$ between -0.5 and + 3.0 are the most likely to penetrate the skin. Chemicals must have some degree of lipid (fat) solubility to absorb into the stratum corneum. To penetrate into the layer of

skin, they must have some degree of solubility in water.(OSHA Technical Manual (OTM), 2012.)

Cosmetic ingredient penetration into the skin

If a cosmetic ingredient is to arrive at its proper location in the skin, it must be soluble in the type of tissue where it will reside. Skin cells membranes are composed of lipid soluble materials. The interior of these skin cells is mostly water and to remain there, a substance must be water soluble. This makes delivery of cosmetic ingredients quite complex because water soluble substances will not pass through the border of the cell to the inside where they exert their desired activity. The cosmetic product must therefore be configured with the proper amount of oily substances and watery substances to pass through cell membranes to the interior of the cell. Furthermore, only certain types of fats with specific chemical designs will pass through the borders of skin cells and/or other skin structures. This further illustrates the complexity of effective cosmeceutical design. Much cosmetic formula is not based on good knowledge of biochemistry and skin physiology. For example, it is common to be advised never to put alcohol on skin. While it is true that excessive use of highly volatile, quickly evaporating alcohols can cause skin dryness, a variety of special alcohols act as penetration enhancers and antimicrobials in cosmetics (Dehaven, 2014).

Alcohols which occurs naturally also facilitates skin penetration. For example, menthol, derived from the species *Mentha* gives the mint family a typical taste and smell. Menthol functions as a transdermal delivery agent, taking desirable molecules across the skin barrier to where they are used. It is highly lipid soluble (dissolves in oil) and therefore crosses the skin barrier. It has been proven to assist the movement of many molecules, including medicines, from the surface of the stratum corneum (outer skin layer) to the internal layers of the skin.

Size of the particle in the cosmetic preparation relates dramatically to its ability to penetrate skin and cause an effect. Some cosmetics containing collagen claim that they increase the collagen content of skin. Collagen, being a very large molecule, is unable to penetrate skin even when combined with penetration enhancers, although it can be used as a moisturizing agent. Elastin, on the other hand, is a much smaller molecule and when placed on the skin, can penetrate the skin and has been found later to be incorporated into connective tissue bundles.

Sometimes, combining ingredients in a certain way improves the desired result or otherwise changes their individual action. When kojic acid is used in combination with arbutin glycoside or glycolic acid, the improvement in hyper-pigmentation increases by as much as 60% compared to using either ingredient alone. The chemical activity of the preparation to inhibit tyrosinase, an enzyme used in producing melanin, increases as much as 13% by combining these ingredients in the proper way.

Applying a cosmetic in a certain way may also change its activity. For example, increased time of application usually leads to higher activity. Occlusion (covering the product with plastic or a medical hydrogel) usually increases penetration (Dehaven, 2014).

1.3 Types of skin penetration

The main mechanism of human skin penetration by a permeant is diffusion. There are three major pathways by which a permeant or solute can diffuse through the skin:

The transapendageal route: permeation through the transappendageal route means permeation via the hair follicles, sebaceous and sweat glands which are collectively called ‘shunt’ routes. However, the transappendageal route is not considered to be a significant pathway for drug permeation because sweat glands and hair follicles occupy only 0.1% of the total surface area of human skin. In addition, permeation of drugs is limited in sweat glands and sebaceous glands. In sweat glands sweat travels in the opposite direction of the perrmeant. Sebaceous gland contains lipid-rich sebum which only allows permeation of hydrophobic molecules. However the transappendageal route can be important for ions and large polar molecules which do not readily penetrate stratum corneum.

The permeation of a permeant across the stratum corneum by intracellular and intercellular routes is called the transepidermal pathway. Considering the ‘brick and mortar’ structure of the stratum corneum, the intracellular permeation occurs in a direct line through the layers of corneocytes and its surrounding intercellular lipid matrix. When permeation occurs in a tortuous way via the intercellular lipid matrix, it is called intercellular pathway.

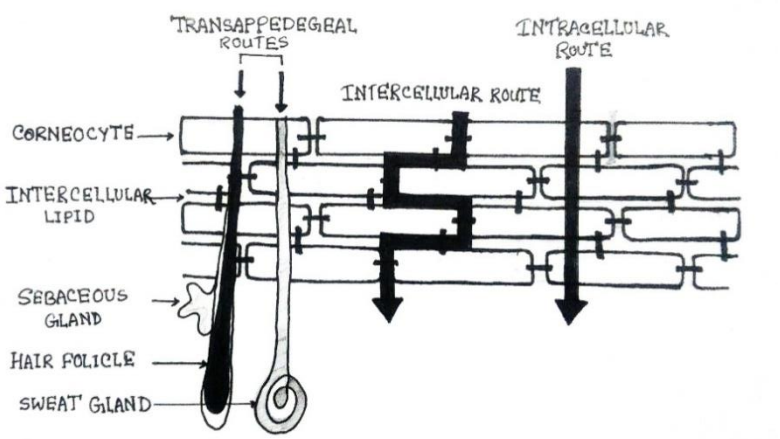


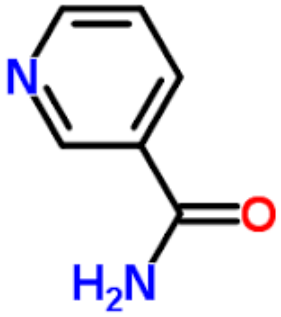
Figure no 1.3: Skin permeation pathways

A number of research studies showed that the intercellular route is the predominant pathway for permeation or diffusion of most drugs or cosmetics across human skin. To diffuse through the intracellular route, the penetrant has to undergo a series of partitioning and diffusion stages in and out of the relatively hydrophilic corneocytes, lipid envelope surrounding the corneocytes and the intercellular matrix. However, in the intercellular routes the penetrant has to cross tortuous intercellular matrix consisting of alternating structures of bilayers (containing both aqueous and lipid domain). In this route of permeation a penetrant travels a 50 times longer path compared with total thickness of the stratum corneum layer. A permeant also has to undergo sequential partition and diffusion through the aqueous and lipid domains of intercellular matrix (Bos & Meinardi, 2000).

1.4 Niacinamide

Niacinamide is a water-soluble B group vitamin

Chemical name - Pyridine-3-carboxylic acid amide. Empirical formula $C_6H_6N_2O$



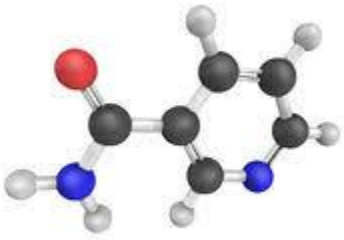


Table 1.4: Physical and chemical properties of NIA

Color	practically white
Form	crystalline powder
Odor	almost odorless, with bitter taste
Molecular mass	122.13 g/mol
Density	1.36 g/cm^3 (20 °C)
Bulk density	~ 0.6 g/cm^3
Solubility	~ 16'000 mg/l, n-octanol, ~ 660'000 mg/l, ethanol 96 %, 691'000 mg/l, water (20 °C)
Partition coefficient	log $P_{o/w}$ -0.38 (octanol/water 20 °C)
pH value	6.0 to 7.5 (5 % aqueous solution)
Melting temperature	128 to 131 °C
Boiling temperature	224 °C
Therapeutic daily dose (adults)	300-1000 mg

Long exposure or over ingestion may cause vasodilation, skin dryness, skin rashes, abdominal cramps, diarrhoea, nausea, vomiting, liver problems and excessive pigmentation ("Niacinamide USP FCC Material Safety Data Sheet," 2001).

Cosmetic value of niacinamide

Niacinamide is a form of vitamin B_3 that has certain characteristics which made it a very good ingredient to be incorporated in topical cosmetic formulations.

Vitamin B_3 serves as a precursor to a family of endogenous enzyme co-factors specifically nicotinamide adenine dinucleotide (NAD), its phosphorylated derivative (NADP), and their reduced forms (NADH, NADPH) which have antioxidant properties. These co-factors are involved in many enzymatic reactions in the skin, and thus have potential to influence many skin processes. This precursor role of vitamin B_3 may thus be the mechanistic basis for the diversity of clinical effects observed for a material such as niacinamide. Topical niacinamide has the following effects:

- Niacinamide inhibits sebum production, specifically affecting the content of triglycerides and fatty acids. This may contribute to the observed reduction in skin pore size and thus improved skin texture (a component of texture being enlarged pores).
- Niacinamide increases epidermal production of skin barrier lipids (e.g. ceramides) and also skin barrier layer proteins and their precursors (keratin, involucrin, filaggrin), leading to the observed enhancement of barrier function as determined by reduced transepidermal water loss. This improved barrier also increases skin resistance to environmental insult from damaging agents such as surfactant and solvent, leading to less irritation, inflammation, and skin redness (e.g. facial red blotchiness). Because inflammation is involved in development of skin aging problems, the barrier improvement may contribute to the anti-aging effects of topical niacinamide. The anti-inflammatory and sebum reduction effects of niacinamide likely contribute to the anti-acne effect reported for this material.
- Niacinamide has anti-inflammatory properties (e.g. inhibition of inflammatory cytokines).

- Niacinamide increases production of collagen which may contribute to the observed reduction in the appearance of skin wrinkling.
- Niacinamide reduces the production of excess dermal GAGs (glycosaminoglycans). In cell culture testing as 0.5 mmol niacinamide reduces excess GAG production by 15%.
- Niacinamide inhibits melanosome transfer from melanocytes to keratinocytes, leading to reduction in skin hyperpigmentation (e.g. hyperpigmented spots).
- Niacinamide inhibits skin yellowing. A contributing factor to yellowing is protein oxidation (glycation, Maillard reaction) which is a spontaneous oxidative reaction between protein and sugar, resulting in cross-linked proteins that are yellow–brown in color. These products accumulate in matrix components such as collagen that have long biological half–lives. Niacinamide has antiglycation effects, likely because of its conversion to the antioxidant (NADPH).

Topical niacinamide also improves other aspects of aging skin, such as reduction in sebaceous lipids (oil control) and pore size, which likely contribute at least in part to improved skin texture. Additionally, niacinamide improves skin elastic properties (Draelos, 2016).

1.5 Cosmetic excipients or chemical penetration enhancers aiding skin penetration of a compound

Excipients

Excipients are a group of heterogeneous materials, ranging from simple, well-known inorganic substances to new, more complex, and functionally highly specific materials, such as bio-macromolecules, which significantly differ in origin, physical, and chemical characteristics. Excipients are substances other than active pharmaceutical ingredients in finished pharmaceutical dosage forms. Almost all drug dosage forms include some kind of excipient to guarantee the dosage, stability, and bioavailability. Currently, approximately 1000 excipients of more than 40 functional categories are used in marketed pharmaceutical products.

Excipients range from inert and simple to active and complex substances that can be difficult to characterize. Traditionally, excipients were often structurally simple, biologically inert, and of natural origin, such as corn, wheat, sugar, and minerals. Many more novel and increasingly

complex excipients have been developed as novel drug formulation delivery systems emerge and evolve. The inert and innocuous nature of excipients is no longer a given feature in drug formulations. Many excipients are potential toxicants at high doses in animals, though safe in humans at therapeutic doses, including commonly used excipients such as cyclodextrins, dextrans, and polyethylene glycol (Kerlin & Li, 2013).

In cosmetic formulations a number of excipients are also used. Among the cosmetic excipients chemical penetration enhancer is notable. Modern cosmetic formulations (face cream, body lotions and sunscreen formulations) contain one or more of these chemical penetration enhancer.

Chemical penetration enhancers

Chemical penetration enhancers are agents that partition into and interact with the components of the SC to increase skin permeability in a temporary and reversible manner. Thus, skin penetration enhancers reversibly reduce the barrier properties, i.e. resistance of the SC to drug penetration, and allow drugs to penetrate more readily into the viable skin tissue and in some cases also into the systemic circulation (CPEs increase drug transport across the skin) (Dragicevic, Atkinson, & Maibach, 2015). Chemical penetration enhancers are used frequently in topical formulations and cosmetic formulations as well. Chemical penetration enhancer facilitates delivery of drug or active ingredients through skin efficiently. However, there are a few disadvantages of penetration enhancers as some of these can cause skin irritation.

CPEs form a diverse pool of chemical compounds that can be classified into groups on the basis of how structurally related they are. Penetration enhancers often work well when used together, i.e. they show synergistic effects in enhancing the penetration of the drug into/through the skin (when used together, they exert higher effects than when used alone). Permeation enhancers are conventionally divided into several groups based on their chemical structure rather than the mechanism of action. This is partially due to the difficulty determining a primary or mixed mode of action for many of them. Furthermore, compounds from the same group can exert their effect through different mechanisms. More than 300 substances have been shown to have skin penetration potential and this number is still growing. Most known enhancers fall into the following categories: alcohols (ethanol, pentanol, benzyl alcohol, lauryl alcohol, propylene glycols and glycerol), fatty acids (oleic acid, linoleic acid, valeric acid and lauric acid), amines (diethanolamine and triethanolamine), esters (isopropyl palmitate, isopropyl myristate and ethyl

acetate), amides (1-dodecylazacycloheptane-2-one, urea, dimethyl acetamide, dimethyl formamideand pyrrolidone derivatives), hydrocarbons (alkanes and squalene), surfactants (sodium laureate, cetyltrimethylammonium bromide, Brij and Tween), terpenes (D-limonene, carvone and anise oil), sulfoxides (dimethyl sulfoxide) and phospholipids (lecithine). The importance of water, or hydration of the stratum corneum, is not to be underestimated. A fully hydrated stratum corneum (under occlusion) presents lesser diffusional resistance to xenobiotics than its dehydrated counterpart (Paudel et al., 2010).

Ingredients of a day cream

A day cream or a vanishing cream is chemically an oil in water emulsion consisting of stearic acid, an alkali, a polyol and water. Vanishing creams are also known as stearate creams because it contains stearic acid. The alkali reacts with stearic acid to form an in situ emulsifier. A typical day cream formulation may contain the following ingredients

Table 1.5: common ingredients in a day cream

Ingredients	Amount	Purpose of use
Stearic acid	17-22%	Main constituent of the oil phase of the formulation
Alkali (KOH)	0.5-1.0%	Forms in situ emulsifier upon reacting with stearic acid
Water	~70%	Constituent of the aqueous phase of the formulation
Triethanolamine	~1.5%	Emulsifier
Glycerin	~6.0%	Polyol that acts as humectant and also has moisturizing property
Alcohol	4.0-6.0%	Antiseptic
Cetyl alcohol	~4.5%	Emollient
Cetostearyl alcohol	~1.5%	Thickening agent to provide good texture to the formulation
Parahydroxy benzoates	0.1-0.5%	Preservative
perfume	Quantity sufficient	Additive

The formulation those were considered for this research study are also day cream/ vanishing cream. The ingredients they contain have been listed from the label of their packaging.

Table 1.6: Ingredients in the international cream

water	triethanolamine,	stearyl alcohol	stearic acid
glycerin	isopropyl isostearate	cetyl alcohol	cetearyl alcohol
niacinamide,	octocrylene	tocopheryl acetate,	C13-14 isoparaffin
ethylhexyl salicylate	panthenol,	benzyl alcohol	PTFE
iodopropynylbutylcarbamate	butyl methoxydibenzoylmethane	phenylbenzimidazole sulfonic acid	triethoxycaprylylsilane
titanium dioxide	behenyl alcohol	polyacrylamide	cetearylglucoside
sucrose polycottonseedate	sodium ascorbyl phosphate	ethylparaben, methylparaben, propylparaben	PEG-100 stearate
carbomer,	disodium EDTA	BHT	zinc oxide
laureth-7	Cl 19140, Cl 6035	*Camellia Sinensis* leaf extract	fragrance

Table 1.7: Ingredients in the local cream

stearic acid	polyethylene glycol	vitamin B3
dimethicone,	disodium EDTA	aqua
tridecyl salicylate	perfume	methyl paraben, propyl paraben

The international day cream formulation contain skin conditioning agents such as niacinamide(NIA), glycerin, C13-14 isoparaffin, panthenol, sucrose polycottonseedate,

emulsifying agents like triethanolamine, cetearylglucoside, PEG-100 stearate, laureth-7, binders such as triethoxycaprylylsilane, polyacrylamide, isopropyl isostearate, antioxidants like BHT, tocopheryl acetate, sodium ascorbyl phosphate. It also contains emulsion stabilizer such as cetearyl alcohol, cetyl alcohol, stearyl alcohol, carbomer and preservatives like iodopropynylbutylcarbamate, ethylparaben, methylparaben, propylparaben, chelating agent disodium EDTA. Titanium dioxide and zinc oxide are opacifyer as wellas sunscreen agent. Ethylhexyl salicylate, octocrylene, butylmethoxy dibenzoylmethane and phenylbenzimidazole sulfonic acid also act as sunscreen agent. Besides some thickening agents and fragrance are there.

The local cream contains skin conditioning agents like tridecyl salicylate, dimethicone and vitamin B_3 (NIA). Polyethylene glycol and stearic acid serve as emulsifier and emollient. Disodium EDTA is used as chelating agent and methyl paraben and propyl paraben are preservatives. Unlike the international cream there are no sunscreen agent in this formulation.

Both the formulations contain some emulsifier, thickening agent, fragrance and preservatives. Although the international cream consists of a number of ingredients that are not present in the local cream. Most of the emulsifiers and preservatives are used in combination so that any possible harmful effect by each ingredient is minimized. The international formulation contains benzyl alcohol which is a chemical that is skin penetration enhancer whereas the local cream contains only one such ingredient (polyethylene glycol) that is not a very efficient penetration enhancer.

1.6 Literature review

Paper-1

Title of the paper: Assessment of niacinamide in vivo skin penetration from a cosmetic moisturizer formula.

Authors name: Zhu C et al.

Journal title: Journal of American Academy of Dermatology

Date of publication: April 2013

A human in vivo study was carried out to compare niacinamide penetration from a new cosmetic moisturizer formula versus the current formula. Niacinamide skin penetration was considerably increased from the new formula. The assessment of niacinamide skin penetration was accomplished via tape stripping followed by HPLC analysis. The results showed that the new formula can deliver significantly more anti-aging actives at a faster rate. The increase of niacinamide resulted from the change of product formula and an increase of the niacinamide level in the new formula (Zhu et al., 2013).

Paper-2

Title of the paper: Nicotinic acid/niacinamide and the skin.

Authors name: Gehring W.

Journal title: Journal of Cosmetic Dermatology.

Date of publication: April 2004

Nicotinic acid and niacinamide are similarly effective as a vitamin because they can be converted into each other within the organism and the blanket term vitamin B_3 is used for both. Niacinamide is a component of important coenzymes involved in hydrogen transfer. Topical application of niacinamide has a stabilizing effect on epidermal barrier function, seen as a reduction in transepidermal water loss and an improvement in the moisture content of the horny layer, leads to an increase in protein synthesis, has a stimulating effect on ceramide synthesis, speeds up the differentiation of keratinocytes, and raises intracellular NADP levels. In ageing skin, topical application of niacinamide improves the surface structure, smoothes out wrinkles and inhibits photocarcinogenesis. It also provides anti-inflammatory effects in acne, rosacea and nitrogen mustard-induced irritation (Gehring, 2004).

Paper-3

Title of the paper: In vitro-in vivo correlation in skin permeation.

Authors name: Mohammed D et al

Journal title: Pharmaceutical Research

Date of publication: August 2013

In vitro skin permeation study was conducted in order to determine and optimize in vivo delivery of active ingredient. The study included niacinamide permeation from a range of vehicles. Single, binary or ternary systems were examined to determine the penetration of niacinamide. The same vehicles were subsequently examined to investigate niacinamide delivery in vivo. Analysis of in vitro samples was conducted using HPLC and in vivo uptake of niacinamide was evaluated using Confocal Raman spectroscopy (CRS).The amount of niacinamide permeated through skin in vitro was linearly proportional to the intensity of the niacinamide signal determined in the stratum corneum in vivo. A good correlation was observed between the signal intensities of selected vehicles and niacinamide signal intensity (Mohammed et al., 2014).

CHAPTER 2

MATERIALS and METHOD

2. Materials and Method

2.1 Materials

Chemicals:

Niacinamide	Gift sample from Eskayef Bangladesh Ltd., Fine Powder, Rmu05-16-0888, B/N: 10160503, S.D: 22.03.17
Day cream of international brand	manufactured by Procter & Gamble Manufacturing (Thailand) Ltd.
Day cream of local brand	manufactured by Unilever Bangladesh Limited
Solvit	vitamin B complex tablet manufactured by Eskayef Bangladesh Ltd
Methanol HPLC grade	Active Fine Chemicals, Dhaka, Bangladesh
Distilled water	Active Fine Chemicals, Dhaka, Bangladesh

Instruments:

Precision balance	Shimadzu, S/N:D307001084, model:ATX224
Sonicator	Power sonic 520
UV spectrophotometer	Shimadzu Corp. Serial No:A11454805768

2.2 Method

2.2.1 UV spectroscopic method development and validation for quantitative estimation of niacinmide (NIA)

2.2.1.1 UV spectrometric method development of NIA

Preparation of standard solution of NIA in water

NIA is a water soluble compound and it has 500 mg/ml water solubility, at 25 ℃ ("Niacinamide USP FCC Material Safety Data Sheet," 2001.). Therefore, standard solution of NIA was prepared in water. A stock solution of 100 µg/mL of NIA in water was prepared by accurately weighing 5 mg of NIA powder and dissolving it with distilled water in a 50 mL volumetric flask. Required amount of water was added to make it volume. The concentration of the solution was then 100 µg/mL. The stock solution was diluted further with water to prepare the following concentrations: 5, 10, 15, 20, 25 and 30 microgram/ml [no. of replicates (n) were 4].

Scanning NIA solution for maximum wavelength of absorbance (λ_{max})

A standard NIA solution of 10 µg/mL was used to perform UV scanning for $\lambda_{max}$from 200 to 400 nm. After completion of scanning the results were observed. As maximum absorption of light was found at 262 nm, it was taken as the λ_{max} of NIA. Several studies also confirmed the λ_{max} of NIA as 262 nm (Haque et al., 2017).

Construction of calibration curve

UV absorption of all standard solutions was observed at 262 nm wavelength. Data was collected and recorded. A calibration curve was constructed based on the average absorbance value from the four set of standards prepared. The calibration curve showed that the range of concentration from 5 to 30 µg/mL perfectly followed Beer-Lambert's law (R^2>0.999).

2.2.1.2 UV spectrometric method validation of NIA

Range

Range of analyte values that are clinically significant is known as maximum dilution/concentration range (Molinaro, 2013). Range of an analyte can be directly measured without modification is the dynamic range or analytical measurement range. The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity (ICH Guideline, 2005). Range is the lowest and highest concentration of an analyte between which the calibration curve will perfectly follow the Beer-Lambert's law.

Linearity

Linearity is another important characteristic of method validation. Linearity assessment is done by the value of correlation coefficient (R^2). Linear regression analysis may not be valid if the correlation coefficient is low. Correlation coefficient indicates the extent of linearity between the methods. Evaluation of slope and intercept values from the four methods help evaluating the linearity (Molinaro, 2013). Also correlation coefficient value must be greater than 0.975 to achieve linearity.

Accuracy

Accuracy is the next step in method validation. It is the closeness of measured value to the "true" value (Molinaro, 2013). To perform the accuracy or assay test Solvit (vitamin B complex tablet) of Eskyef Bangladesh Ltd. was used as the known standard. Each of this Solvit tablet contains 20 mg of niacinamide. 5 tablets were weighed in an electronic balance and the average weight was found 0.3204 grams. So, 20 mg of niacinamide is there in 0.3204g tablet. Therefore, 0.2mg of niacinamide is there in 0.003204g tablet or 3.2mg tablet. Five tablets were crushed in a mortar pestle and from there 3.2mg of powder was transferred into a 10ml volumetric flask. Distilled water was added to it and by vigorous shaking for a sufficient period of time was done to dissolve the whole content. Water was added up to mark to get a solution of 20µg/ml concentrated niacinamide. This solution was then filtered and the process was repeated to make 10µg/ml and 29 µg/ml solutions. The 3 sets of solutions were then analyzed in UV spectrophotometer at 262nm wavelength. Data was recorded and cross matched with the corresponding values of the standard curve to assess the accuracy.

In addition accuracy testing was also performed using NIA reference standard. A standard solution was prepared dissolving a known amount of NIA in distilled water. After proper dilution, the solution was analyzed for its absorbance values. Finally accuracy was calculated using Equation 1.

$$\text{Accuracy (\%)} = \frac{Observed\ concentration \times 100}{Original\ concentration} \quad \text{.....Equation 1}$$

Recovery testing

From the four sets of standard solutions, absorbance values were obtained. The average absorbance values were calculated and finally a calibration equation was established plotting concentration vs. average absorbance values. Using the equation, concentration was again calculated from the average absorbance values. Finally the observed or calculated concentration was checked (in terms of % recovery) against the original concentration using Equation 1. Recovery results indicate how accurate the observed absorbance values were.

Intra-day and inter-day precision

Precision is the dispersion of repeated measurements about the mean, is the indication of reproducibility (Molinaro, 2013). In this step of method validation the differences in UV absorbance of niacinamide solutions at different time in a particular day which is *intra-assay (within-run) precision* and at different day's interval that is *inter-assay (between-run) precision* were examined. To do so 3 sets of 5, 15 and 30 microgram/ml solutions were prepared from 100microgram/ml stock and the solutions were analyzed in the morning and afternoon and then after 2 days and after 4 days respectively. From the table mean, SD and RSD (relative standard deviation) values for extent of reproducibility were attained.

Sensitivity

Limit of quantitation (LOQ) and limit of detection (LOD)

LOD is the lowest analyte concentration likely to be reliably distinguished from the noise and at which detection is feasible (Armbruster & Pry, 2008). LOD also depends on the analyzing instrument. To simply say LOD is the lowest detectable concentration of a sample. In this study using UV spectrophotometer the LOD for NIA was found from the data recorded and analyzed.

LOQ is the concentration at which quantitation as defined by bias and precision goals is feasible, and the concentration at which the analyte can be quantitated with a linear response (Armbruster & Pry, 2008). The minimum working concentration of a sample is referred as LOQ. For NIA utilizing the UV spectrophotometer the lowest concentration of NIA that follows the Beer-Lambert law and maintains linearity was found from the recorded data.

2.2.2 Protocol development for tape stripping.

Skin penetration analysis of NIA requires adhesive tape stripping from skin where the formulation is applied. Tape stripping removes skin and allows to determine the depth of skin where the desired substance has reached. Therefore tape stripping protocol development involves the following steps.

2.2.2.1 Initial paper work

Written consent: participants who were to take part in this research work was fully informed about the purpose of the study, detailed procedure of conducting the work, advantages and any possible disadvantages and all necessary terms and conditions of the research through an ethical approval paper A consent form for the volunteer was prepared. After being fully aware of the facts mentioned earlier, if the participants agrees with the terms he/she has to give written consent of his/her voluntary participation in the research work. The consent form also contained some personal information, such as name, date of birth, age, gender, overall health and skin condition. The personal information along with the name were not used published separately.

2.2.2.2 Tape stripping protocol

A paper sheet was prepared with two sites of 4x4 cm^2 open areas in it. This paper was laminated afterwards and used to mark the area of skin where formulations were applied. A scotch tape with a dispenser, a large masking tape, a scissor, tweezers and a weight (approximately 8g) were arranged at first. Comfortable seats for both the researcher and volunteer were arranged next to the precision balance. The volunteers were asked previously not to use any cosmetic cream/lotion on their arms for at least 24 hour before the experiments. A pair of gloves was worn before starting the experiment to avoid contact with the tapes. The volar forearm of the volunteer was marked with a marker pen according to the defined area of paper sheet. Excess hair was cut from the specific area (where needed). A big spatula was taken and weighed. A large excess of formulation (approximately 0.20g) was taken on the spatula and weighed. The formulation was evenly spread on the marked area of the skin. The specific amount of formulation applied on the skin was calculated and recorded. The skin was then covered with a gauze followed by large masking tape and it was kept in this condition for 30 min. After 30 min, the formulation was removed from the skin and excess formulation was removed gently with the help of a soft facial tissue but not by rubbing. With a marker pen, a small area inside the marked area of the skin was

specified. A small portion of scotch tape was torn with the help of scotch tape dispenser and it was weighed by placing on the precision balance with the help of tweezers. The scotch tape was placed inside the small area facing the glued region towards the skin. Then the weight (8g) was applied on the scotch tape for 5 seconds (checking on the stop watch). Then the tape was removed in one go with the help of tweezers. The tape was weighed again with the initial set up and it was recorded. The difference between initial and final weight of the tape indicated the amount of skin (stratum corneum) removed. The tape was then placed in a previously marked centrifuge tube and the tube was kept in a holder. The same process was repeated at the same site on the arm for 15 times. Three tapes were taken in each tube for the first run and for the second run 5 tapes were taken in each tube. 5 mL methanol: water (20:80) mixture was added in the centrifuge tubes containing the tapes and it was shaken for at least for 1 min followed by sonication for 5 minutes at room temperature. Using Whatman filter paper the solutions were filtered and analyzed in the UV spectrophotometer at 262nm. All necessary data of the two run during this protocol development were recorded.

Following the same procedure tape stripping was done without applying any formulation. This data was recorded as blank tape stripping to observe the UV absorbance of skin removed. In this case number of replicates was 3. As blank tapes showed some absorbance values at 262nm. Therefore, actual NIA content was analyzed by deducting the blank tape absorbance value from the NIA containing tape absorbance value.

2.3 Tape stripping from volunteers

According to the protocol developed, tape stripping was done on 10 volunteers consisting of 6 female and 4 male participants. All the volunteers aged between 20-25 years. Two commercial skin care product (cream) were used. One was a day cream from an international renowned brand and another was a vanishing cream from local renowned brand. The cosmetic creams were applied on the volar forearm area of left hand and right hand respectively. Approximately 0.20g of formulation was applied each time. The weight of tapes before and after taking the formulation, amount of formulation and amount of SC removed were recorded. Three tape strips were taken in a centrifuge tube and 5 mL water methanol (90:10) mixture was added. Then similar procedure was adapted as mentioned in section 2.2.2.2. Finally the UV absorbance values

were noted. Ultimately the absorbance values of blank tape measurement were deducted from the absorbance values to calculate the amount of NIA penetrating the skin.

2.4 Statistical analysis

All the data were analyzed using MS Excel 2013 and most of the cases the data were represented as mean±standard deviation (SD). In some cases relative standard deviation (RSD) and standard error of mean (SEM) were calculated using Equation 2 and 3, respectively.

RSD = $\frac{SD \times 100}{Mean}$ ……Equation 2

SEM = $\frac{SD}{\sqrt{n}}$ ……Equation 3

Finally comparison in penetration properties of NIA from international and local cream formulations in human volunteers were analyzed using Student's t-test. A p value of <0.05 indicates significant difference between the two groups.

CHAPTER 3
RESULTS and DISCUSSION

3. Results and Discussion

3.1 Quantitative estimation of NIA using UV spectroscopic method

The very first step is to determine the wavelength at which maximum absorption occurs by NIA (λ_{max}). A concentration of NIA in distilled water of 9.92µg/ml was scanned from 200-400 nm in the UV spectrophotometer and following data was observed-

Table 3.1: Absorbance values of NIA at different wavelengths

Wavelength	Absorbance
200	0.00
214	0.71
244	0.16
262	0.24
400	0.00

From this table although it is seen that the highest absorbance is at 214 nm but it cannot be used as λ_{max} because many solvent such as methanol and acetonitrile absorb UV light in the same region. That is why 262nm was considered as the $\lambda_{max}$for NIA (showing second highest absorbance value) which is also supported by other research works (Haque et al., 2017).

The next step was construction of a calibration curve. From 100µg/ml stock solution four sets of standard dilutions containing 5, 10, 15, 20, 25 and 30µg/ml solutions were prepared and analyzed. The mean from the four sets were calculated and a calibration curve was constructed using the concentration vs. mean absorbance values (Figure 3.1).

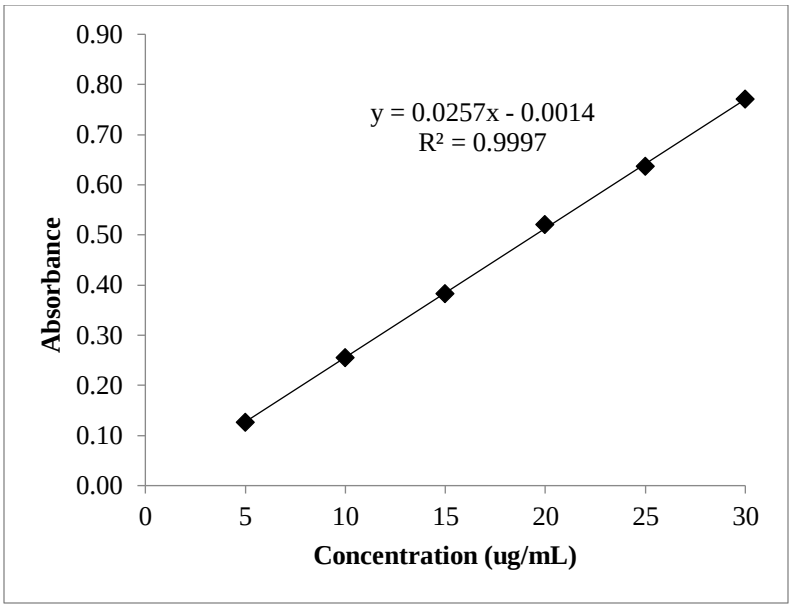


Figure no: 3.1: Calibration curve of NIA at 262nm

It is found that the calibration curve is linear (R^2>0.999) and follows Beer-Lambert's law from 5 µg/mL to 30 µg/mL concentration. The table also shows the slope and intercept values which help evaluating linearity. The values of slope and intercept shows linearity according to that. Also the range for NIA in this analytical procedure is between 5µg/ml to 30 µg/mL.

Necessary mean, standard deviation (SD) and relative standard deviation (RSD) values were calculated. RSD values for correlation coefficient and slope indicate the calibration curve is accurate (Table 3.2).

Table 3.2: Evaluation of linearity of the UV method of NIA

	Set-1	Set-2	Set-3	Set-4	Average	SD	RSD
R^2	1.00	1.00	1.00	1.00	1.00	0.00	0.07
Slope (m)	0.03	0.03	0.03	0.03	0.03	0.00	2.06
Intercept	0.00	-0.01	-0.01	0.01	0.00	0.01	-629.95

Accuracy testing was performed according to the method stated in chapter 2 section 2.2.1.2 using 9 replicates. The recovery value was found to be 95.85±7.82% with a RSD value of 8.15 (Table 3.3).

Table 3.3: Evaluation of accuracy of UV method of NIA

Absorbance	Observed conc. (µg/mL)	Original conc. (µg/mL)	% Recovery	Mean	SD	RSD
0.24	9.64	10	96.40	95.85	7.82	8.15
0.29	11.64	10	116.40			
0.233	9.36	10	93.60			
0.462	18.52	20	92.60			
0.464	18.60	20	93.00			
0.46	18.44	20	92.20			
0.665	26.64	29	91.86			
0.673	26.96	29	92.97			
0.678	27.16	29	93.66			

The recovery value was tested using the method stated in chapter 2 section 2.2.1.2. The recovery value is given in Table 3.4. From the table it can be seen that 100.06±0.83% NIA was recovered using the UV method with a RSD value of 0.83%.

Table 3.4: Evaluation of recovery value of UV method of NIA

Conc. (µg/mL)	Standard 1	Standard 2	Standard 3	Standard 4	Observed conc (ug/mL)	%Recovery	Mean (% Recovery)	SD (% Recovery)	RSD
5	0.125	0.125	0.125	0.132	4.99	99.81	100.06	0.83	0.83
10	0.255	0.249	0.253	0.262	9.97	99.71			
15	0.384	0.381	0.385	0.382	14.96	99.74			
20	0.521	0.521	0.521	0.521	20.33	101.65			
25	0.64	0.638	0.62	0.648	24.82	99.30			
30	0.764	0.777	0.793	0.748	30.04	100.13			

Precision of an analytical method refers to the intra- day repeatability and inter-day repeatability. In both cases NIA solutions of 5, 15 and 30 µg/ml were used where the number of replicates was three. In the within day or intra-day repeatability test the absorbance of the solutions was measured in the morning and in the afternoon within the same day. The data were presented in Table 3.5. The table shows that means RSD of intra-day precision is 1.13% which is below 2%.

Table 3.5: Evaluation of intra-day precision of UV method of NIA

Concentration (µg/mL)	Absorbance (obtained in the morning)	Absorbance (obtained in the evening)	Conc. (obtained in the morning)	Conc. (obtained in the evening)	Average conc. in the morning	Average conc. in the evening	Mean conc. (between morning & evening)	SD of conc. (between morning & evening)	RSD	Avg. RSD
30	0.826	0.822	32.20	32.04	32.60	32.54	32.57	0.05	0.14	1.13
	0.846	0.849	32.98	33.09						
	0.837	0.833	32.63	32.47						
15	0.424	0.424	16.56	16.56	16.35	16.56	6.45	0.15	0.89	
	0.414	0.43	16.17	16.79						
	0.418	0.418	16.32	16.32						
5	0.137	0.146	5.39	5.74	5.36	5.54	5.45	0.13	2.35	
	0.136	0.14	5.35	5.51						
	0.136	0.137	5.35	5.39						
4(precision of LOQ)	0.106	0.109	4.18	4.30	4.46	4.11	4.28	0.25	5.79	
	0.116	0.106	4.57	4.18						
	0.117	0.097	4.61	3.83						

Repeatability or precision of the method was also determined between days which is termed as inter-day precision. At first the solutions analyzed at day 0. The solutions were again analyzed in the same procedure after 2 days and 4 days. The data were presented in Table 3.6 and 3.7. The tables show that inter-day precision at 2 days and 4 days intervals were 1.42 and 4.67%, respectively. After 4 days interval, the UV precision value was more than 2%. Therefore, it is suggested to analyze the sample within 2 days intervals where precision value was found to be within acceptable limit.

Table 3.6: Evaluation of inter-day precision (2 days) of UV method of NIA

Concentration (µg/mL)	Absorbance (Day 0)	Absorbance (Day 2)	Conc. (Day 0)	Conc. (Day 2)	Avg. conc in Day 0	Avg. conc in Day 2	Mean conc (between Day 0 & Day 2)	SD of conc (between Day 0 & Day 2)	RSD	Average RSD
30	0.808	0.797	31.50	31.07	31.65	31.30	31.48	0.25	0.79	1.42
	0.811	0.809	31.61	31.54						
	0.817	0.803	31.85	31.30						
15	0.391	0.398	15.27	15.54	15.79	15.29	15.54	0.36	2.30	
	0.408	0.379	15.93	14.81						
	0.414	0.397	16.17	15.51						
5	0.135	0.133	5.31	5.23	5.51	5.42	5.46	0.06	1.18	
	0.143	0.144	5.62	5.66						
	0.142	0.136	5.58	5.35						
4(precision of LOQ)	0.106	0.109	4.18	4.30	4.46	4.14	4.30	0.22	5.12	
	0.116	0.103	4.57	4.07						
	0.117	0.103	4.61	4.07						

Table 3.7: Evaluation of inter-day precision (4 days) of UV method of NIA

Concentration (µg/mL)	Absorbance (Day 0)	Absorbance (Day 4)	Conc. (Day 0)	Conc. (Day 4)	Avg. conc in Day 0	Avg. conc in Day 4	Mean conc (between Day 0 & Day 4)	SD of conc (between Day 0 & Day 4)	RSD	Average RSD
30	0.808	0.786	31.50	30.64	31.65	30.63	31.14	0.72	2.33	4.67
	0.811	0.775	31.61	30.21						
	0.817	0.796	31.85	31.03						
15	0.391	0.392	15.27	15.31	15.79	14.86	15.32	0.66	4.31	
	0.408	0.366	15.93	14.30						
	0.414	0.383	16.17	14.96						
5	0.135	0.123	5.31	4.84	5.51	4.96	5.23	0.39	7.36	
	0.143	0.13	5.62	5.12						
	0.142	0.125	5.58	4.92						
4(precision of LOQ)	0.106	0.114	4.18	4.49	4.46	4.29	4.37	0.12	2.73	
	0.116	0.107	4.57	4.22						
	0.117	0.105	4.61	4.14						

Sensitivity of this analytical method is expressed in terms of LOD and LOQ. NIA solutions at different low concentrations were prepared and analyzed in UV spectrophotometer at 262nm. The values were given in Table 3.8

Table 3.8: Evaluation of sensitivity test and determining LOQ and LOD.

Conc (ug/mL)	Absorbance	LOQ/LOD
0.5	0.015	**LOD**
1	0.042	
1.5	0.062	
2	0.065	
2.5	0.087	
3	0.079	
4	0.113	**LOQ**

The table shows 0.5 µg/ml is the lowest concentration that is detectable in the UV spectrophotometer that is why it was considered as the LOD of this UV method. While 4 µg/ml is the lowest concentration that maintains linearity, good recovery values and precision (Table 3.5, 3.6 and 3.7) value as well. Therefore, 4µg/ml was considered as the LOQ value of this method.

The results of method validation of NIA using UV spectrophotometer were summarized in Table 3.9

Table 3.9: Summary of UV method validation parameter of NIA

Parameter	Unit		
	µg/mL	**mean±SD**	**RSD (%)**
Range	5 to 30		
Linearity (n=3)			
R^2		0.9993±0.001	0.07
Slope		0.03±0.001	2.06
Intercept		(-)0.0014±0.009	
% Recovery		100.06±0.83	0.83
Accuracy (n=9)		95.85±7.82	
Precision (n=9)			
Intraday			1.13
Interday (2 days variation)			1.42
Interday (4 days variation)			4.67
Sensitivity			
LOD	0.5		
LOQ	4		

3.2 **Protocol development for tape stripping**

Protocol development included the number of tape strips should be taken, how many tape strips should be extracted at a time, how much extracted solvent will fill up the UV quartz cell, blank tape analysis, etc. After taking written consent from the participants who wished to take part in the research work, one of them was taken for the protocol development study. The procedure for developing protocol was given in Chapter 2 section 2.2.2.2. A total of 15 tapes were taken from the formulation applied skin from the volunteer. 5 of the formulation containing tapes were extracted together and analyzed in the UV method. The results are given in Figure 3.2 and 3.3. It can be seen from Figure 3.2 that while tape stripping deeper higher cumulative amount of SC was removed. However, Figure 3.3, it shows that with the increased number of tape strips, NIA content decreased. As higher amount of NIA removed from a total of 5 tape strips, the number was reduced to 3 tape strips while tape stripping the volunteers. At the same time as NIA was still quantified up to 15 tape strips, the number to total tape strips was increased to 21 in the volunteers in order to track NIA deeper into the skin.

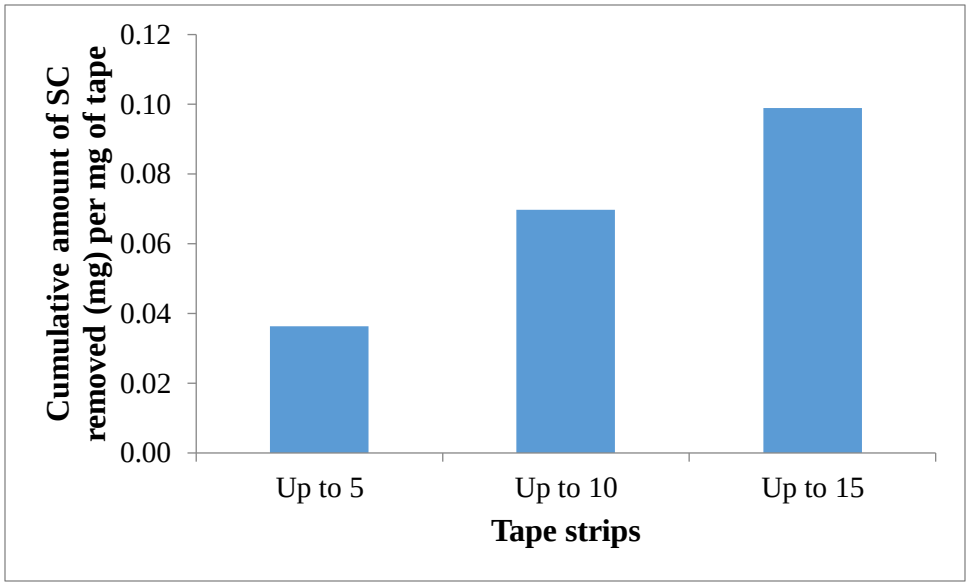


Figure 3.2: Cumulative amount of SC (mg) removed per mg of tape in protocol development procedure (n=1)

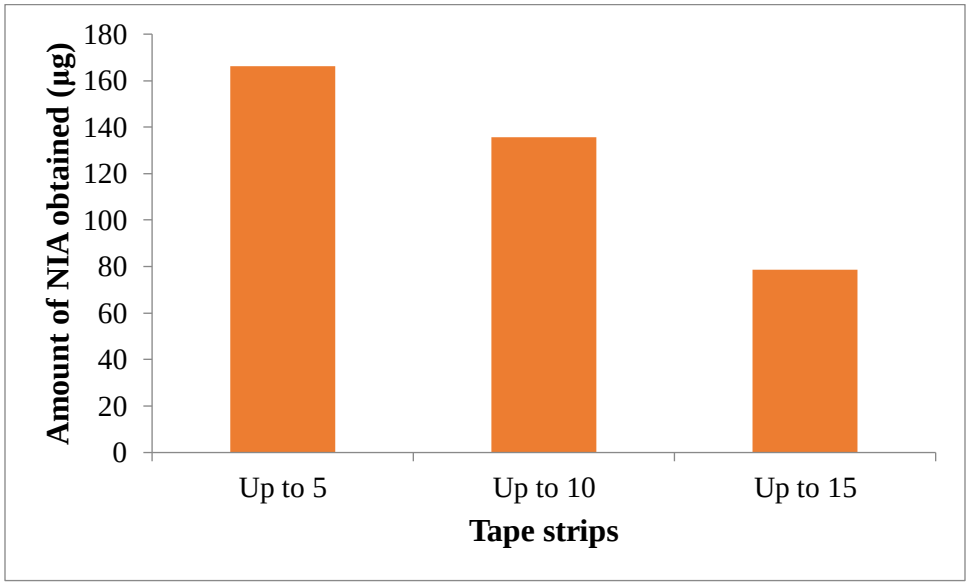


Figure 3.3: Amount of NIA obtained during the protocol development process (n=1)

At the same time, without applying formulation, tape strips were taken out from the same volunteer as well (a total of 21 tapes) and 3 of the skin containing tapes were analyzed together.

A blank tape striping was conducted on the volunteers without applying any formulation on the skin (Chapter 2, section 2.2.2.2). Surprisingly the blank tape strips showed different absorbance values, which may be due to different skin components which absorb UV light in UV

Spectrophotometer at 262 nm. The SC (mg) removed per mg of tape along with the absorbance values are given in Table 3.10.

Table 3.10: Table showing cumulative SC removed (mg) per mg of tape, mean absorbance value (from 3 sites of a volunteer) and absorbance values per mg of tape

No. of tapes	Cumulative amount of SC removed (mg) per mg of tape	Mean absorbance value	Mean absorbance value per mg of tape
1 to 3	0.03	0.21	0.01
4 to 6	0.05	0.26	0.01
7 to 9	0.06	0.23	0.01
10 to 12	0.09	0.27	0.01
13 to 15	0.10	0.25	0.01
16 to 18	0.12	0.27	0.01
19to 21	0.13	0.26	0.01

The mean absorbance value per mg of tape was deducted from the NIA containing tape strips obtained from volunteers in order to avoid interference the SC components.

3.3 Tape stripping from volunteer's volar forearm and quantitative analysis of NIA

The study was conducted on 10 participants consisting of 6 female and 4 male volunteer. To determine the skin penetration of NIA in both the international brand and local brand creams the formulations were applied according to the protocol and absorbance values from the tapes stripped were utilized to calculate the amount of NIA. Four sites were selected in the lower and upper portion of left and right volar forearm and assigned as L-1, L-2, R-1, and R-2 respectively. About 200mg of formulation was applied in the site each time. According to the method stated in chapter 2 section 2.2.2.2 tape stripping was done from each volunteer for both the international and local branded day cream. The cumulative stratum corneum removal in mg per mg of tape weight was plotted corresponding to the number of tapes stripped in Figure 3.4. t-test gives rise to p value >0.05 in case of SC removal which indicates that there is no statistically significant difference in SC removal in tape stripping for international and local branded face cream.

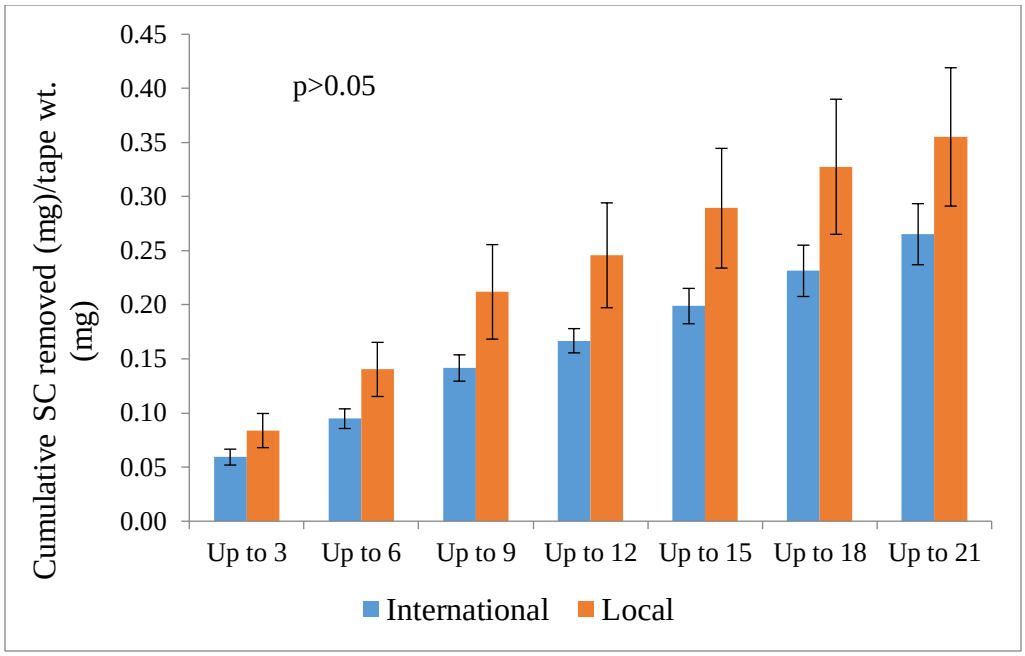


Figure 3.4: Cumulative thickness of SC removed with tape strips for international and local NIA face cream formulations (mean±SEM, n=10).

Figure 3.4 shows that cumulative amount of SC removed increased with increased number of tape strips. Up to 21 tape strips, the site where cream of internal brand was applied removed an amount of 0.27±0.03 mg of SC whereas from the site of application of local brand 0.36±0.06 mg of SC was removed. However, there was no statistically significant difference between the SC removal values between these two sites ($p>0.05$). Figure 3.5 sums up the amount of NIA obtained in tape strips from volunteers after applying face cream from both international and local brand. The p value from t-test is <0.05 up to 15tape stripped. This indicates that there is statistically significant difference in the amount of NIA obtained from tape stripping from international and local branded formulations. At 15 tape strips, 38.24±5.53 µg and 19.76±5.22 µg of NIA were recovered, respectively. Amount of NIA penetrated into the skin up to 15 tape strips from international brand was significantly higher than that from the local formulation. Although the difference in amount of NIA is insignificant obtained in the last 6 tape strips. There is reduction in the amount of NIA as the tape strips increased in number in the deeper layer of SC for both formulations.

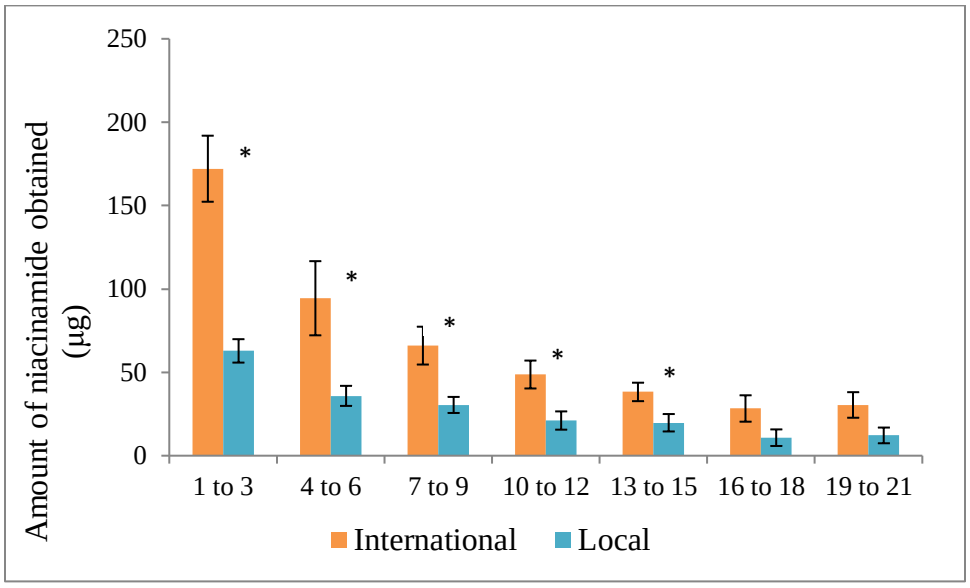


Figure 3.5: Amount of NIA obtained in the tape strips for international and local NIA face cream formulations (mean±SEM, n=10). Asterisk (*) indicates $p<0.05$.

Among the 10 volunteers 6 were female and 4 were male. In this study the differences in cumulative SC removal and amount of NIA obtained from these two gender groups were also analyzed. Figure 3.6 shows that there was no significant difference in cumulative SC removal from international branded formulation (A) between 6 female and 4 male volunteers as well as for the local brand formulation (B) ($p>0.05$). Also cumulative thickness of SC removal increased gradually as the number of tape strip increased in case of both the formulations.

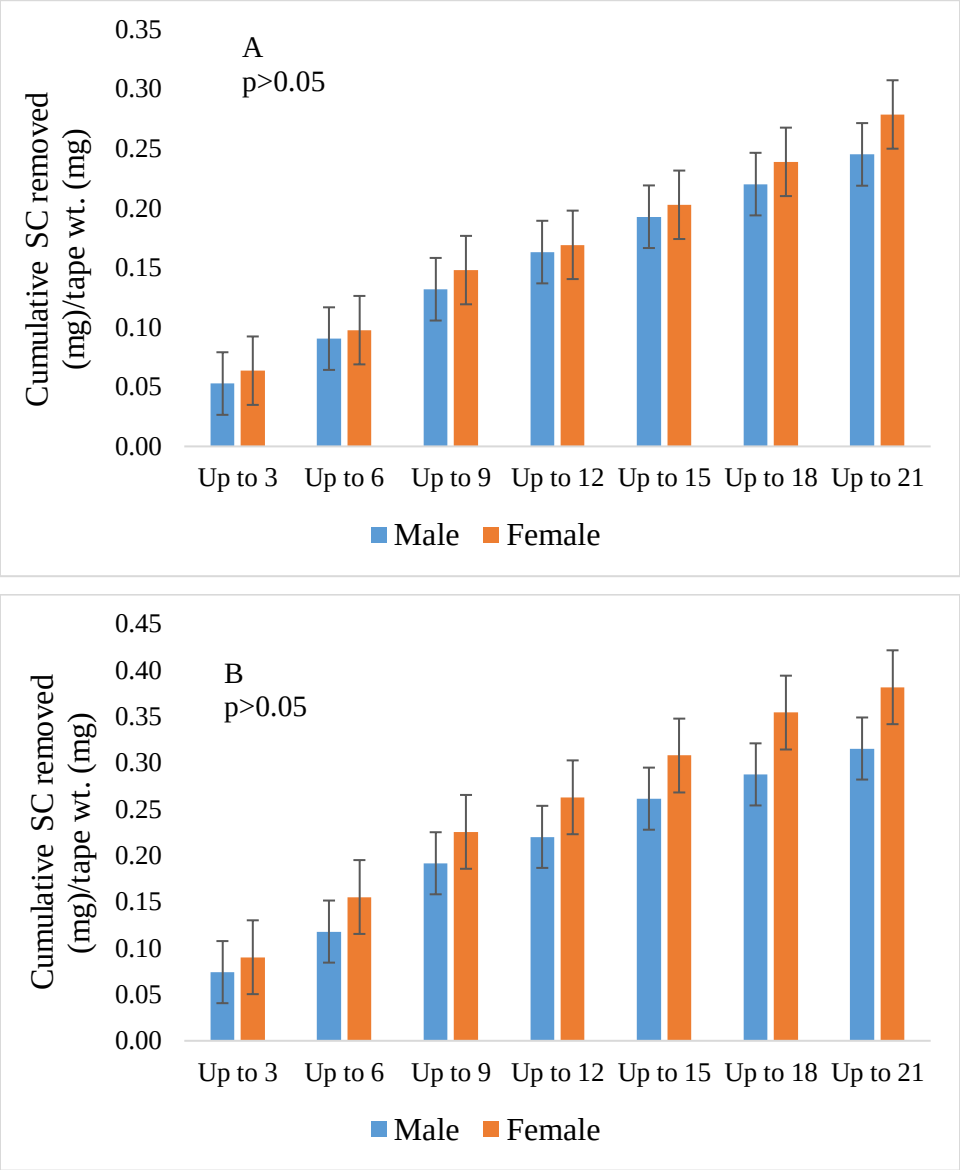


Figure 3.6: Cumulative thickness of SC removed with tape strips for male (mean±SEM, n=4) and female (mean±SEM, n=6) volunteers in (A) International and (B) Local brand of face cream

Variability in the gender of the volunteers did not affect the amount of NIA that penetrated the skin ($p>0.05$). Up to 21 tape strips, ~24 and ~34 µg of NIA were recovered after applying the international face cream from male and female volunteers, respectively. Whereas, after applying local face cream formulation, ~11 and ~13 µg NIA was observed at 21 tape strips respectively from male and female volunteers. Both cream formulations showed that NIA penetrated female skin in higher quantities than male skin. Figure 3.7 demonstrates these statements very clearly.

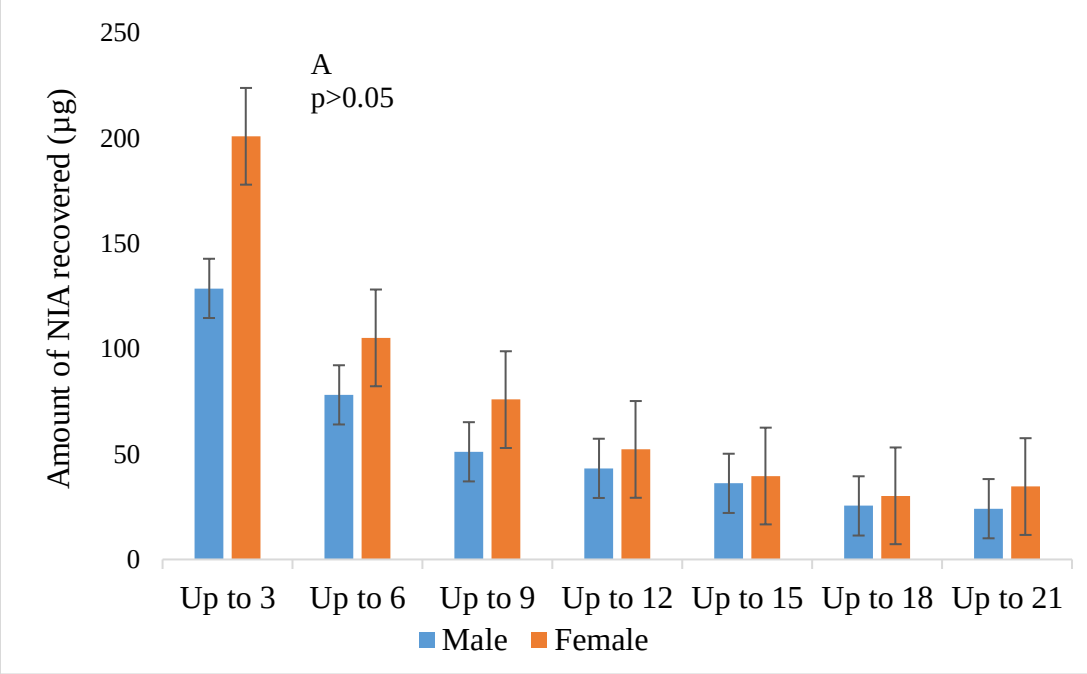


Figure 3.7: Amount of NIA obtained from the tape strips for male (mean±SEM, n=4) and female (mean±SEM, n=6) volunteers in (A) International and (B) Local brand of face cream

From Chapter 1 section 1.5 it is easy to understand why the two formulations differ in penetration of NIA. It is because the international cream has skin conditioning agents such as C13-14 isoparaffin, panthenol, sucrose polycottonseedate and glycerin which conditions the skin as well as presence of benzyl alcohol (among other chemical penetration enhancers such as panthenol and PEG-100 stearate) associates better penetration of NIA. Whereas the local cream formula contains only stearic acid, polyethylene glycol, dimethicone (skin conditioning agent), chelating agent(disodium EDTA), an emulsifierand preservatives (methyl paraben, propyl paraben) along with vitamin B_3 (NIA). The number of penetration enhancer ingredient in the formula of local cream is only one that is polyethylene glycol and not a very efficient one. This is responsible for lower penetration of NIA from local formulation through SC compared to the international formulation.

CHAPTER 4
CONCLUSIONS

4. Conclusions

According to the study it was observed that there was a remarkable difference in the skin penetration of niacinamide between the local and international brand cream formulations. It was found that the amount of formulation applied was within a controlled limit and removal of stratum corneum in course of tape stripping also differs insignificantly. Gender group of the volunteer (male and female) did not cause any notable variability as well. The only significant difference obtained in this study is the difference in the amount of NIA penetration from two different brands (international and local) through SC.

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APPENDIX

Patient Identification Number for this trial:

CONSENT FORM

Title of Project: Penetration of niacinamide (vitamin B_3) in human skin from commercially available creams

Date of birth: Age: Gender:

Your overall health: Skin condition:

Please initial box

☐ I confirm that I have read and understand the information sheet for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

☐ I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

☐ I agree to take part in the above study.

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Researcher	Date	Signature