PREPARATION AND EVALUATION OF VITAMIN D3 SUPPLEMENTATION AS TRANSDERMAL FILM FORMING SOLUTION

تحضير وفحص مستحضر فيتامين د على شكل محلول قابل لتشكيل غشاء لنقله عبر الجلد

This Thesis was submitted in partial fulfillment of the requirements for the Master's Degree in Industry Pharmaceutical Technology from the Faculty of Graduate Studies at Birzeit University, Palestine

MASTER OF SCIENCE

In

Industrial Pharmaceutical Technology

By

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26 FEB 2022

Thesis Title: Preparation and Evaluation of Vitamin D3 Supplementation as Transdermal Film Forming Solution.

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ACKNOWLEDGMENTS

All praises to **Allah** and His blessing for the completion of this thesis. First I would like to sincerely thank my supervisors **Dr. Mammal Qurt** and **Dr. Numan Malkieh** for their dedicated support and guidance. They provided encouragement and they were always ready and enthusiastic to assist in any way they could throughout the research project.

I would also like to thank **Dr. Hani Shtaya** and **Dr. Feras Qanaze** for their valuable help and support.

I would also like to thank **Eng. Ramzi Moqadi** for his assistance and providing advices regarding analysis, and to thank **Dr. Mohammad Enaya** for his assistance during the experimental work.

I would like to acknowledge **Jerusalem Pharmaceuticals Co. Ltd.** for their generous help and for their cooperating staff at Samih Darwazah Institute.

Finally, I would like to express my gratitude to my friends and my dear family, without their support and continued encouragement, I would not have been able to achieve this dissertation. Thank you.

FEB. 2022

Majd J. Kittaneh

DECLARATION

I declare that this dissertation submitted for my master's degree and titled "Preparation and Evaluation of Vitamin D3 Supplementation as Transdermal Film Forming Solution" was conducted by me in the Department of Pharmacy at Birzeit University. Any information taken from the literature was acknowledged throughout the text and cited in the references list. This thesis or any part of it has not been submitted to a diploma or other degree in at any institution before.

Signed: ……

Majd J. Kittaneh

Date: / /2022

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List of Abbreviations and acronyms

Abbreviation	Definition
A	Cross sectional area
Av. Area	Average area
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
BP	British Pharmacopeia
C	Concentration
C_1	High concentration in the membrane at the donner compartment
C_2	Low concentration in the membrane at the receptor compartment
CAS	Chemical Abstracts Service
C_{col}	Concentration within the sample
C_d	Concentration within the donor compartment
cm	Centimeter
C_n	Concentration within the receptor compartment
COA	Certificate of analysis
Conc.	Concentration

cp	Centipoise
^{0}C	Celsius
D	Diffusivity/ Diffusion coefficient
E	Young's modulus
EA	Ethyl acetate
e.g.	Example
EM	Eutectic mixture
EMA	European Medical Agency
Eq.	Equation
Ɛ	Strain
F	Force
FFP	Film forming polymer
FFS	Film forming solution
g	Gram
GRAS	Generally Regard as Safe
h	Membrane thickness
HPLC	High performance liquid chromatography
HPMC	Hydroxypropylmethylcellulose

hr.	Hour
Inj.	Injection
IPA	Isopropyl alcohol
IPM	Isopropyl myristate
IU	International unit
J	Flux
K	Partition Coefficient
kPs	Kilopascal
$L\circ$	The original length
$\triangle L$	The change in the material length
LOD	Limit of detection
LOQ	Limit of quantification
M	Amount
m^2	Meter square
mg	Milligram
Min.	Minute
ml	Milliliter
mm	Millimeter
MPs	Megapasscal

µg	Microgram
µl	microliter
N	Newton
ng	Nanogram
nm	Nanometer
No.	Number
OA	Oleic acid
P	Permeability coefficient
PBS	Phosphate buffer saline
PE	Penetration enhancer
PEG	Polyethylene glycol
PES	Polyether sulfone
PG	Propylene glycol
PP	Polypropylene
PTFs	Primary trial formulations
PVP	Polyvinylpyrrolidone
Q	Cumulative amount
QC	Quality control
RDA	Recommended daily allowance

RH	Relative humidity
Rpm	Round per minute
RSD%	Relative standard deviation%
S	Effective surface area
SA	Sample volume
SC	Stratum corneum
SD	Standard deviation
SE	Standard error
Sec.	Second
SLS	Sodium lauryl sulphate
STD	Standard
STFs	Successful trial formulations
t	Time
T_L	Lag time
US	United state
USP	United States Pharmacopeia
UV	Ultraviolet
V_i	Sample volume
V_R	The receptor compartment volume

w/v	Weight/ volume
w/w	Weight/weight
η	Viscosity
λ	Wavelength
ρ	Density
σ	Stress
#	Number

Note: Where (‘) is mentioned on a letter this means that the formulation is containing vitamin D3. e.g.:A1’=A1+ vitamin D3.

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Abstract

Vitamin D3 supplementation has become very important to prevent and treat many conditions related to vitamin D3 deficiency. Vitamin D3 is available as oral and injectable dosage forms. However, these routes suffer from several limitations. Other alternative routes of administration such as transdermal route may be beneficial.

The aim of this study was to prepare and evaluate vitamin D3 supplementation as transdermal film forming solution (FFS). Vitamin D3 has some properties that make it a good candidate for transdermal delivery. The FFS consists of the drug and other ingredients dissolved in highly volatile and non-volatile solvents. After being applied to the skin, the volatile solvents evaporate and leave thin, transparent, convenient and easily removed film.

In this study, number of FFSs capable to meet the proposed acceptance criteria for the film: having a drying time less than 5 minutes and being not sticky, adhesive, flexible, clear, transparent and smooth film. In vitro permeation studies through Strat M® membrane revealed that the cumulative amount of vitamin D3 permeated after 24 hours of selected FFSs was significant (around 800 IU). The main driving force of permeation was the supersaturation produced after the evaporation of the volatile solvents. The use of limonene and polyvinylpyrrolidone (PVP) had no significant effect on permeation. While the use of oleic acid with a eutectic mixture reduced the permeation by about half.

Chapter I: Introduction

1. Introduction

Vitamin D is considered a hormonal steroid. Naturally, vitamin D3 is synthesized upon skin exposure to ultraviolet (UV) B radiation, the 7-hydrocholesterol (vitamin D3 precursor of skin) converts into cholecalciferol (vitamin D3)[1]. Due to the current lifestyle, nutritional supplementation has become the main source of vitamin D3. Vitamin D3 is available as parenteral and oral dosage forms. However, due to compliance and absorption limitations, a new route of administration such as a transdermal route can be a good alternative [2].

Vitamin D3 is an extremely vital nutrient, the recommended daily allowance for normal children and adults is 400 IU/day (or 10μg/day). Measuring the serum level of 25-hydroxyvitamin D3 (25(OH) D3) is the best approach to evaluate vitamin D3 status. Vitamin D3 has an essential role in bone mineralization and skeleton growth[2]. Moreover, it has diverse roles in the treatment and prevention of many diseases. Vitamin D3 deficiency is associated with type 1 diabetes mellitus, rheumatoid arthritis, and Crohn's disease, it can induce immune cells differentiation and counteract inflammation of autoimmune diseases such as multiple sclerosis[3]. Vitamin D3 prevents malignancies in colon, prostate, breast, and ovarian cancer by local conversion of vitamin D3 into its metabolite 1,25-dihydroxycholecalciferol (1,25(OH)2D) in the healthy cell, it can inhibit angiogenesis, induce cellular maturation and cause apoptosis to prevent malignancy[4]. A low level of 25(OH) D3 is associated with congestive heart

failure, myocardial infarction, and calcific aortic stenosis. Also, a low level of calcitriol was found to be related to vascular calcification and increases mortality rate in end-stage heart failure and end-stage renal failure[5]. Vitamin D3 deficiency is also related to several lung diseases such as viral infection, tuberculosis, asthma, and chronic obstructive pulmonary disease [6]. Although many treatments are available for managing these conditions, vitamin D supplementation has a significant therapeutic efficiency; used as critical, adjuvant, and prophylactic treatment [7].

1.1. Transdermal drug delivery

The first knowledge that supports transdermal drug delivery was first documented by Ibn Sina, who proposed in his book; The Canon of Medicine that, topical drugs have the soft state which can penetrate the skin and the hard state that can't. Early observations of the systemic effect after skin application of belladonna plaster and nitroglycerine induced headache following exposure of the skin of the workers in an explosive factory. These observations provided that the skin is permeable only for lipophilic substances but not for hydrophilic. However, in the following years, other researchers succeeded in delivering more hydrophilic drugs. In the 1970s, the first US patents were given for scopolamine, nitroglycerin, and nicotine transdermal drug delivery systems. At present, the number of the approved transdermal drugs is continuously increasing in addition

to many drugs under research. Various conditions are now treated with already marketed transdermal drugs, such as central nervous system disorders (e.g. Selegiline), pain killer (e.g. Fentanyl), hormonal therapy (e.g. Testosterone), anti-emetic (Granisetron), cardiovascular diseases (e.g. Clonidine), enuresis (Oxybutinene) and smoking cessation (Nicotine)[8].

Transdermal delivery offers many advantages compared with the oral route, it can avoid first-pass metabolism, overcome Low oral bioavailability, more predictable drug delivery, reduce drug spike concentration, provide Long-term controlled drug release, can avoid gastrointestinal side effects such as nausea and vomiting, and overcome dysphagia problem for some patients. Compared with the parenteral route, it is considered more convenient since it is self-administered, non-invasive, dose termination is easy and simple by removing the transdermal system and has a lower risk of disease transmission especially in developing countries when a needle could be used more than once[8][9][10].

1.1.1. Recently conducted studies on transdermal delivery of vitamin D3

Several recent studies were conducted to investigate the transdermal delivery of vitamin D3. They tried to enhance vitamin D3 penetration through various possibilities. G. Costa et al used several penetration enhancers (PEs) (propylene glycol, ethoxydiglycol, isopropyl palmitate, cereal alcohol, and soybean lecithin) in cream and gel formulation. Even with the usage of a combined PEs, vitamin D3 in cream-based formulation remained at the surface. On the other hand, the

retention of vitamin D3 in the skin layers was significant in the gel-based formulation. They conclude that vitamin D3 retention was due to the high lipophilic properties, which may be helpful in psoriasis condition and more potent PEs or more hydrophilic analogs should be investigated to enhance transdermal penetration[11]. In another study, Ahmed Alsaqr and co-workers studied the penetration of vitamin D3 from ointment preparation contained oleic acid (OA) or dodecylamine as PEs. OA showed no significant improvement in penetration compared with control. However, the usage of dodecylamine improved the transdermal penetration of vitamin D3, and especially after pretreatment of the skin with 50% ethanol. The synergistic effect of dodecylamine and ethanol resulted in penetration of 760 ng of vitamin D3 as an accumulative amount. The recommended daily allowance (RDA) of vitamin D3 (400 IU or 10μg) could be achieved when covering the skin with 3.6cm^2 of the formulation[2]. In another study vitamin D3 was prepared as reservoir-type transdermal adhesive patch of 40 cm^2 size, it contained transcutol (diethylene glycol monoethyl ether) as a PE. The patch delivered more than 2000 μg within 5 hours, and more than 20,000 μg within 24 hours through unbroken intact living skin[12].

Other researchers used the polymeric nanoparticle (TyroSphere) and coated microneedles for transdermal penetration of vitamin D3. Other studies used more

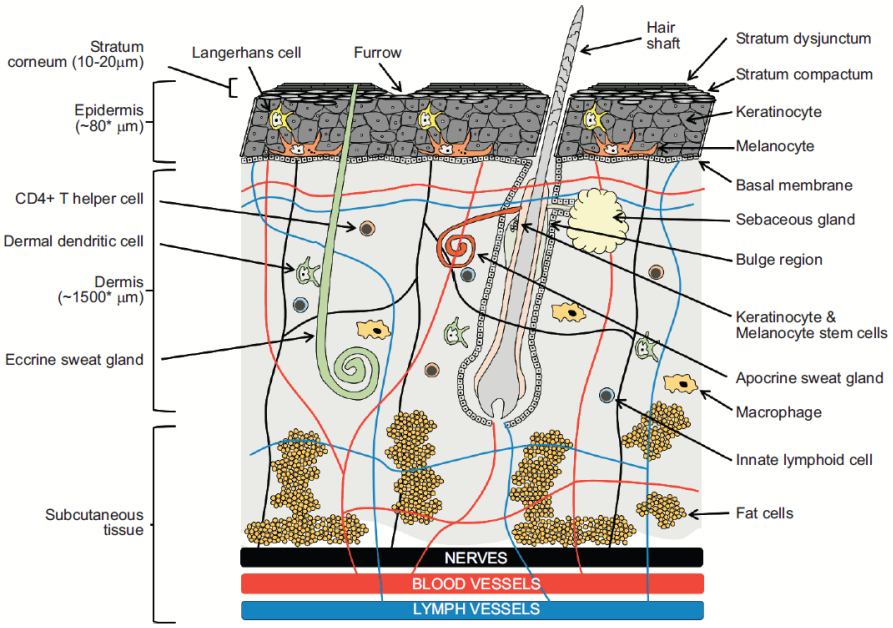

Figure #1. Skin structure[8].

hydrophilic analogs of vitamin D3 (calcitriol, 25(OH)D3, and oxacalcitriol), which were also had promising results[7].

1.2. Skin Barrier

The skin is the largest organ in the body with an area of about 2 m^2, it is 0.5 mm thick and accounts for 15% of the body weight. The skin has a physical barrier to the external environment. The skin also prevents the passage of the xenobiotics, electrolytes, and water. It has a temperature regulation function, and it protects the body from harmful UV radiation or possible invasive pathogens[13][14].

1.2.1. Skin structure

The skin has a unique structure. It consists from three main layers, starting from the surface are: the epidermis, the dermis, and the hypodermis (Figure #1). The

Skin appendages include hair follicles, nails, sweat, apocrine and sebaceous glands. The dermal layer differentiates and moves toward the surface of the skin to create epidermal cells and compensate for the continuous turnover loss[13]. The outer layer is called the stratum corneum (SC) layer. It is considered the main responsible for the barrier function of the skin. The SC thickness is nearly 20µm, contains several layers (18-21 layers) of rough, flattened, and keratin-rich dead cells (corneocytes) surrounded by crystalline lipid lamellar matrix arranged in the manner of "bricks and mortar" (Figure #2)[14][15]. The lipid portion of the SC is composed mainly of three lipids: ceramides, free fatty acid, and cholesterol. The highly dense back arrangements of lipid lamellas are responsible for the barrier function of the SC[16].

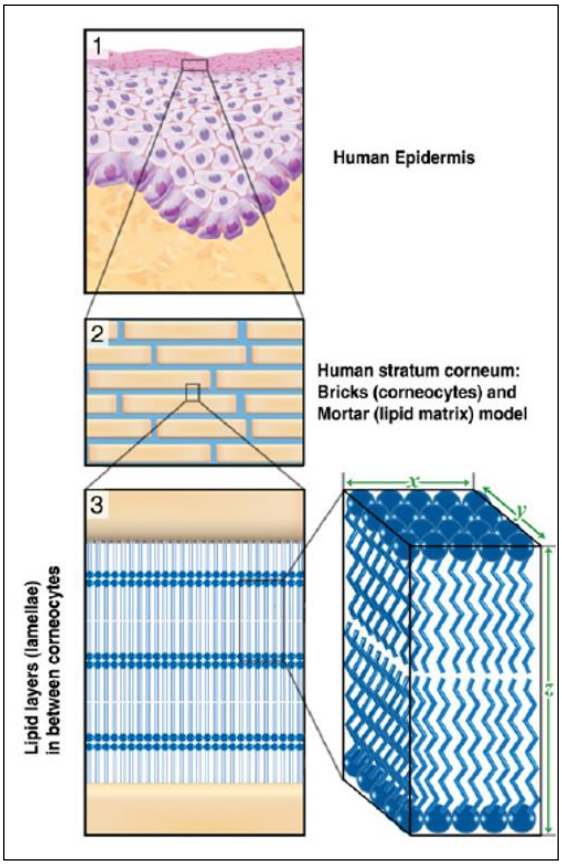

Figure #2. SC Layer structure**.** (1) The human epidermis. (2) Component of the SC: the corneocytes (bricks) and lipid envelope (mortar). (3) Lamellar organization of the intercellular lipids [15].

1.2.2. Percutaneous Absorption

The process of percutaneous or transdermal absorption involves a layer by layer of mass transfer of topically applied substances until they are uptake by the systemic circulation. Permeability of the viable layers and capillaries is high for a solute. The diffusion through the metabolically inactive dead SC layer is the rate-limiting step. The intercellular lipid in the SC is the essential pathway for percutaneous absorption. The skin allows a passive diffusion for relatively lipophilic substances. In Addition to the intercellular route, there are transcellular across the corneocytes and trasappendageal routes (Figure #3). The later one (the shunt route) accounts only for (0.1%) of the permeation area. However, it is essential for the permeation of polar or macromolecular substances [13][14].

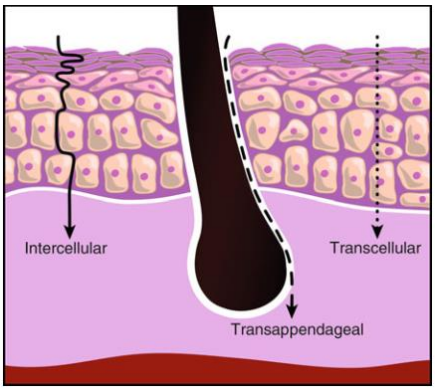

Figure #3. Permeation routes: intercellular, transcellular, and transappendageal[13].

Not all drugs are suitable for passive delivery through the skin to reach the blood capillaries or being absorbed by the deeper layers. Due to the unique skin structure and low water content in the SC, only moderately lipophilic drugs, low melting points, good solubility, and low molecular weight can cross the skin barrier (Table #1).

Table #1. Ideal properties for passive transdermal drug delivery [16][17].

Parameter	Ideal Properties
Partition coefficient (Log P)	1-3
Melting point	Less than 250 C^0
Molecular weight	less than 500 Dalton
Dose	less than 10 mg/day
Oral bioavailability	Low
Skin irritation	Non irritating and non-sensitizing
Water solubility	≈0.05 to 1 mg/mL

To overcome these limitations and increase the candidate drugs for cutaneous drug absorption, several passive and active approaches (Table #2) have been used to enable the permeation of more drugs of especial physicochemical properties (e.g. highly lipophilic drugs, large molecular weight or ionic, etc.)[13]

1.3. Vitamin D3

Vitamin D3 is a natural steroid fat-soluble hormone, the structure is shown in (Figure #4)[17]. In the skin, the UV-B converts 7-dehydrocholesterol to cholecalciferol (vitamin D3). Another natural source is food, such as oily fish (such as salmon). Vitamin D3 is considered inert, and it needs to be exposed to two steps of hydroxylation metabolism (Figure #5); the first one is in the liver, and the second one is in the kidneys. This metabolism convert it into the biologically active form, , 25(OH)2D [18][7].

Table #2. Passive and active approaches to enhance cutaneous drug absorption.

Passive approaches	**Active/ Physical enhancement approaches**
Supersaturation	*Indirect method:*
Eutectic system	Iontophoresis (Ionsys™)[8]
Prodrug	Electroporation (NanoKnife®)[19]
Ion pair formation	Sonophoresis (Sonoprep®)[8]
Complexation	Lazer- assisted delivery (under development) [8]
Liposomes	Magnetophoresis (under development) [8]
Microemulsions	
Organogels	*Direct method*:
Solid lipid nanoparticles	Thermal Ablation (Passport®)[8]
Liquid crystalline system	Needleless jet injectors (Glide SDI™)[10]
Chemical permeation enhancers [14]	Microneedles (Soluvia®)[8]
	Microdermabrasion (under development) [8]
	Elongated microparticles (under development) [20]

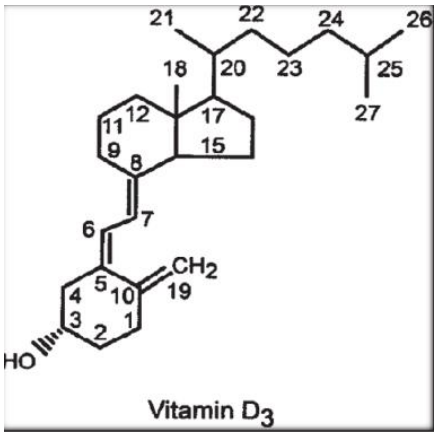

Figure #4. Vitamin D3 Structure [22].

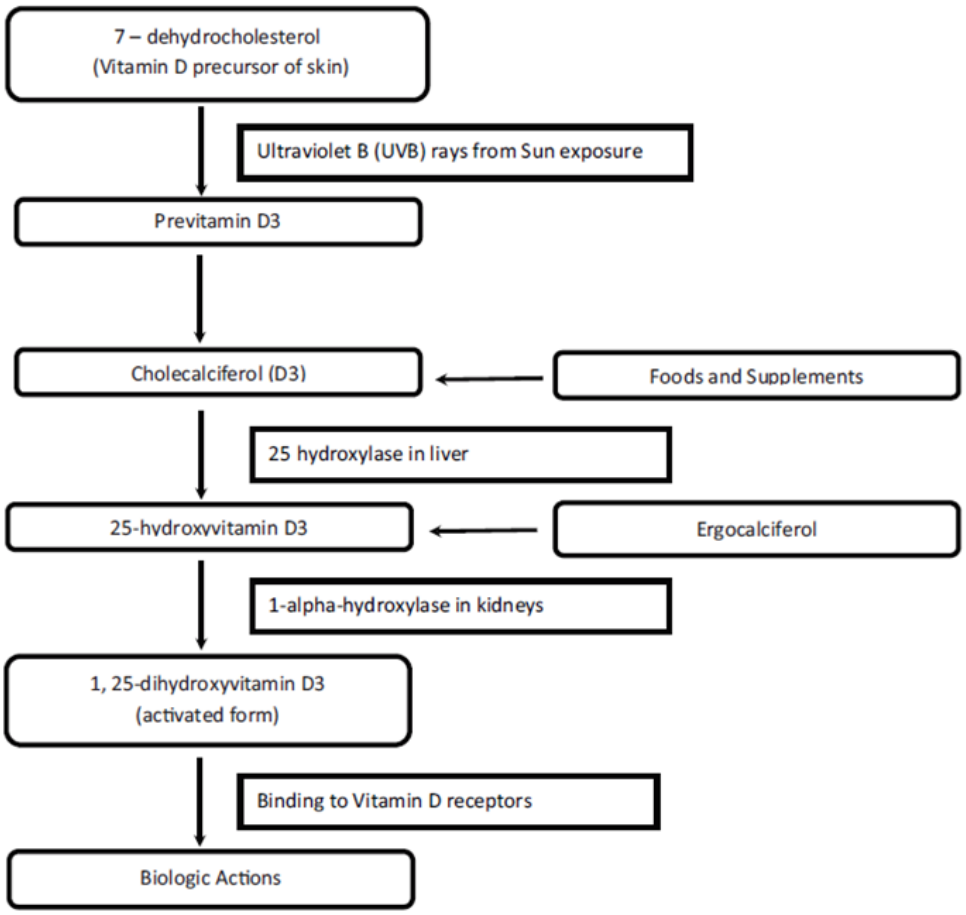

Figure #5. Vitamin D3 synthesis [23].

1.3.1. Vitamin D3 specifications

Vitamin D3 possesses some properties that make it a good candidate for transdermal delivery. It has a low melting point ≈ 83 C^0-86 C^0[21][22], it is considered very potent since the recommended daily allowance is small; between 400 IU – 800 IU (10-20 µg) , its tolerable upper intake level is up to 4,000 IU (100 µg)[23] and it has a relatively small molecular weight of 384.64 g/mol. Although it is insoluble in water it is very soluble in several solvents [2]. Vitamin D3 also

not produced any form of inflammation [24]. However, it is a very lipophilic substance (log P 10.2) [2], this makes a challenge that could be overcome with permeation enhancers with/or any other approaches (Table #2)[13].

1.3.2. Vitamin D3 stability

Vitamin D3 is a sensitive substance, it is labile to oxidative, photolytic, hydrolytic, and thermal conditions after forced degradation study[25]. All conditions during preparation, analysis, storage, and use should be controlled[26]. To protect vitamin D3 from photodegradation amber glass vials, flasks and bottles can be used and the light should be avoided even the indirect sunlight[11]. Oxidation can be prevented by using antioxidant excipients (such as ascorbic acid, butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA)[12].

1.3.3. Solubility of vitamin D3 in the literature

Several solvents are used in the preparation of FFS. Solubility studies concluded that vitamin D3 is very soluble in ethanol, (Isopropyl alcohol) IPA, and ethyl acetate (EA). It is also soluble in acetone, sparingly soluble in propylene glycol (PG) and polyethylene glycol-400 (PEG 400), and insoluble in water[2]. In another study the solubility of vitamin D3 was in the following order; propan-1-ol > ethanol> EA> Acetone> methanol > acetonitrile. The previous results showed that the solubility was decreased by increasing the polarity of the solvent[27].

1.4. Transdermal Film-Forming Solution (FFS):

In FFS the drug and other ingredients are dissolved in a highly volatile solvent, after application on the skin the solvent evaporates leaving thin residual transparent film containing the drug and other ingredients (such as; film-forming polymers (FFP), plasticizers, non-volatile solvents, and permeation enhancers) as shown in Figure #6. The presence of non-volatile solvents prevents drug precipitation after evaporation of the volatile solvents, and also enhances penetration through rapid partitioning into the SC. As the concentration of the solute increases, the permeation rate increases. This type of drug delivery provides an invisible depot for drugs that expected to permit sustained-release effect for a long time. The FFS is an attractive transdermal dosage form to deliver drugs through the skin which decreases the risk of drug transfer to clothes or other people as in the case of other transdermal preparations such as creams and ointments. After solvent evaporation, a thin, almost transparent, excellent adherent, non-tacky, flexible and wipe-off resistance film is formed[28][29].

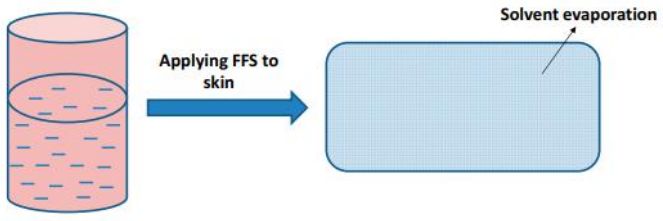

Figure #6. Simple illustration of film-forming solution after solvent evaporation[28].

1.4.1. Candidate ingredients for Film Forming System:

The selection of ingredients is based on their properties in order to obtain an acceptable film. Also we select ingredients that could increase vitamin D3 permeation.

1.4.1.1. Polymer

The solid backbone of the FFS is used mainly from polymer. Single or a combination of polymers can be used. It is important to choose polymer that possesses acceptable cosmetic appearance that able to form transparent, clear, flexible and adhesive film. Hydrophobic polymers usually need the addition of plasticizes and/or hydrophilic polymers to enhance film flexibility and uniformity. Several polymers were used before in the preparations of FFS include, Eudragit, Polyvinylpyrrolidone (PVP), polyvinyl alcohol, poloxamer, hydroxypropylmethylcellulose (HPMC), ethyl cellulose, methyl cellulose, and others [30].

Eudragit:

Eudragit is the commercial name for polymethacrylate. Polymethacrylates are used in pharmaceutical formulation as film forming agent, film coating agent, tablet binder, and tablet diluent. Several grades of polymrthacrylates are available. They consist of different ratio of synthetic anion and cation polymers of methacrylic acid ester, methacrylic acid, and dimethylaminoethyl methacrylates.

They differ in the supply form, solubility at different pH, and solubility in water[31].

Polymethacrylates are generally nonirritant and nontoxic material. Eudragit NE, RL-100, RS-100, and L30D-55 were used before in FFS. Other Hydrophobic polymers such as Eudragit L100-55, L100, and S100 can be dispersed in high volatile solvent such as acetone and alcohols, thus they are good candidate for FFS preparations[30][31].

Polyvinylpyrrolidone (PVP):

PVP is also known as Povidone in USP and BP. PVP is essentially composed from linear 1-vinyl-2-pyrrolidone group. The degree of polymerization is linked to the molecular weight. Variety of PVP polymers are available. The viscosity of these polymers vary in relative to water, and expressed by the K-value which is ranging from 10-120[31].

PVP is widely used in pharmaceutical preparations include oral and topical dosage forms. It is considered non-toxic in oral preparations and non-irritant for skin. It is freely soluble in water, ethanol and ketone. It is considered a hydrophilic polymer which can enhance drug penetration through the SC to form a drug reservoir [30].

1.4.1.2. Penetration enhancer

PEs or accelerants are substances that work to decrease SC barrier function resistance for drug permeation and so increase its diffusivity. Ideally, a PE should be compatible with other ingredients in the preparation, has reversible effects on the SC barrier function. It only allows substances to penetrate the skin but it doesn't allow internal substances to come out. It also predictable, reproducible, works rabidly, non-irritant, non-toxic, non-allergic, and pharmacologically inert. Although not all PEs have all of these properties, some possess most of them[32].

PEs include water, sulfoxides (e.g. Dimethylsulfoxide), pyrrolidones (e.g. 2-pyrrolidone), Azone (laurocapram), fatty acids (e.g. OA), alcohols (e.g. ethanol), fatty alcohol (1-nonanol), glycol (e.g. PG), surfactants (e.g. Tweens), urea, and terpenes (e.g. limonene). A synergistic effect is observed when using PE in a combination such as using Azone, OA, or terpenes in the presence of PG. The mechanisms of action of the PEs are complex. Most of the PEs promote permeation at an acceptable pharmacological concentration by interacting with the intercellular phospholipid bilayer within the SC (Figure #7) [32][33].

Isopropyl myristate:

Isopropyl myristate (IPM) consists of myristic acid, saturated high molecular weight fatty acids and esters of propan-2-ol. It is colorless, odorless, clear and low viscus liquid [31]. IPM is widely used in topical and transdermal preparation such

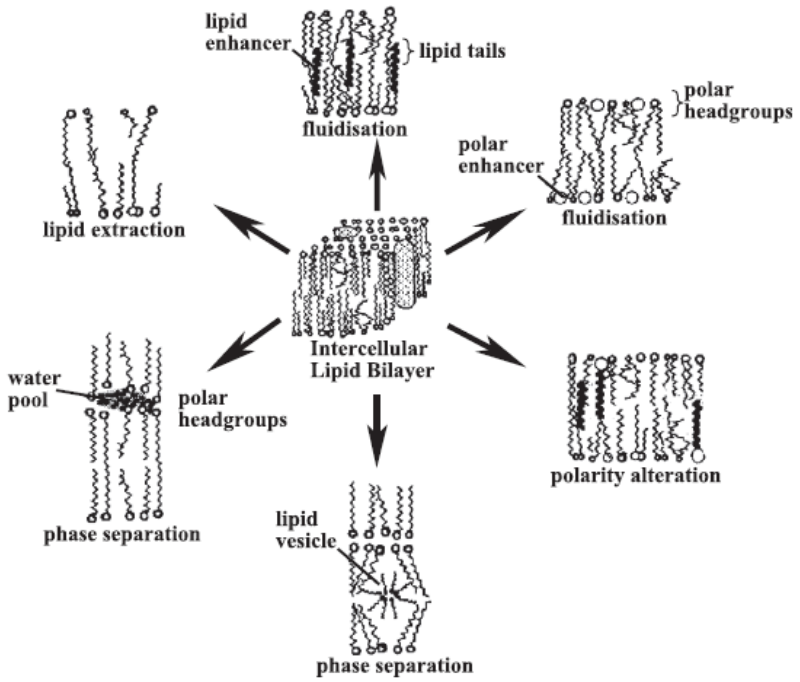

Figure #7. General actions of PEs within the phospholipid lamella [33].

as in FFS as PE [29][34][35]. It incorporates into the lipid matrix of SC layer, disrupt multilamellar lipid assembly, and form a separate phase through extraction of certain lipids in the SC layer [36].

Sodium lauryl sulfate:

Sodium lauryl sulfate (SLS) is an alkaline organic anionic surfactant. SLS is Generally Regarded as Safe (GRAS) excipient. In pharmaceutical preparations, it is used as PE, modified release agent, solubilizing agent, emulsifying agent, capsule and tablet lubricant. The usage of SLS in topical pharmaceutical preparations intended for long time may result in skin irritation. Thus in such

preparations, concentration should not exceeded 1%[37]. The mechanism of permeation involves SLS increases fluidization of the epidermal lipids below the applied site. It disrupts the skin structure by interacting with both the lipids and keratin. The alkyl chain interact hydroponically with skin structure and results in uncoiling the filament, separation of the protein matrix, and increase skin hydration[38].

Oleic acid (OA)

OA is an oily liquid. It color ranges from yellow to pale brown. It consists of different amount of saturated and unsaturated acids together with (Z)-9-octadecenoic acid[31]. It promotes the penetration by creation of more permeable and distinct fluid phase within the SC lipid[39].

Terpenes

Terpenes are organic compound found in essential oil. D-Limonene and eucalyptol (1,8-cineole) are examples on terpenes. The mechanism of action for D-limonene is by formation of phase separation in the SC [40]. D-limonene enhances the penetration of the lipophilic compound more than the hydrophilic compound[41]. On the other hand, eucalyptol increase SC lipid fluidity and disrupt its integrity[40]. it is considered a good penetration enhance for hydrophilic substances but has moderate penetration enhancement for lipophilic [14].

1.4.1.3. Volatile solvent:

Volatile solvents belong to class 3 residual solvents are favorable. Due to its low toxicity and low risk on human health. Ethanol, acetone, EA and IPA are solvents belong to this class, and their ability to solubilize vitamin D3 is very good, [2][31]. Refer to section (1.3.2).

Ethanol

Ethanol may be considered the first choice due to its additional function as PE. It can enhance drug penetration through different mechanisms, thus the increase in the contact time is in favor of penetration [20]. It has synergistic effect when combined with limonene [28]. Ethanol can increase drug solubility in the vehicle of the donor phase and increase drug flux especially for poorly soluble drugs, it can increase tissue solubility and drug partitioning through skin layers, and it can permeate through the skin carrying the solute with it or it can do so through rapid evaporation and increase the thermodynamic activity. Moreover, at high concentrations, ethanol can extract the intercellular lipid. However, at high concentrations, the penetration decreases due to skin dehydration [14].

Ethanol and acetone mixture

To speed up film formation and decrease drying time, ethanol can be mixed with acetone. The boiling point of ethanol and acetone are 78.15 ºC and 56.2 ºC

respectively. A mixture of ethanol and acetone (80:20) was used in previous studies and showed an acceptable drying time for FFSs [42][43][44].

1.4.1.4. Plasticizer

Polyethylene glycol 400 (PEG 400):

PEGs are widely used in pharmaceutical preparations such as topical, oral, parenteral, and others. According to molecular weight, different grades of PEGs are available. At ambient temperature the PEG of molecular weight (200-600) are liquids, while PEG of 1000 molecular weight and above are solids. PEG of molecular weight (200-600) is viscous, clear and it is colorless to slightly yellow color liquid [31].

PEG 400 is used as plasticizer in FFS, it also works as PE too[42][43].PEG 400 is suitable to be used in FFS due to Its ability to be dissolved in highly evaporated solvent such as acetone, it is nonirritant to the skin, and due to its hydrophilic characteristic it can be easily washed out by water[31].

Propylene glycol (PG)

PG is viscous, colorless, and odorless liquid. It is widely used in pharmaceutical and cosmetic preparations. It is considered non-toxic and minimally-irritant to the skin. PG is primarily used as plasticizer for the film[31]in the FFS. PG can also enhance drug penetration. It may be considered as non-volatile solvent for the

active ingredient after evaporation of the volatile solvent[43]. PG can also make a synergistic effect with other PEs, such as terpenes, Fatty acids, and others[40]

1.4.1.5. Eutectic mixture

The eutectic mixture (EM) is composed of two components that interact physically with each other without any change in the chemical structure. The melting point of the EM is lower than each of the components alone. It is formed when the two immiscible solid components become miscible in the liquid state. The EM can be used to enhance drug solubility by decreasing its melting point below the skin temperature and consequently enhance permeability [14].

Camphor and menthol mixture is an example on an EM that has a low viscosity property. The phase diagram for this EM (Figure #8) shows different phases based on the component proportion at different temperature. The Lower green zone is the solid state of the eutectic system, in this zone both component are solid. In yellow and blue zones, the eutectic system is in liquid state and excess solid of camphor and menthol respectively are exist. In the upper light green zoon, the EM is in liquid state, both component are liquid. The Yellow line refers to the melting point of excess camphor. The blue line refers to the melting point of the excess menthol. While the green line is the melting point of the eutectic system. At room temperature, camphor and menthol can form EM at 1:1, 6:4, 7:3, and 8:2 proportions. The melting point of menthol and camphor are 47.82ºC and 177.33ºC, respectively. The melting point of the EM is continue to decrease as the

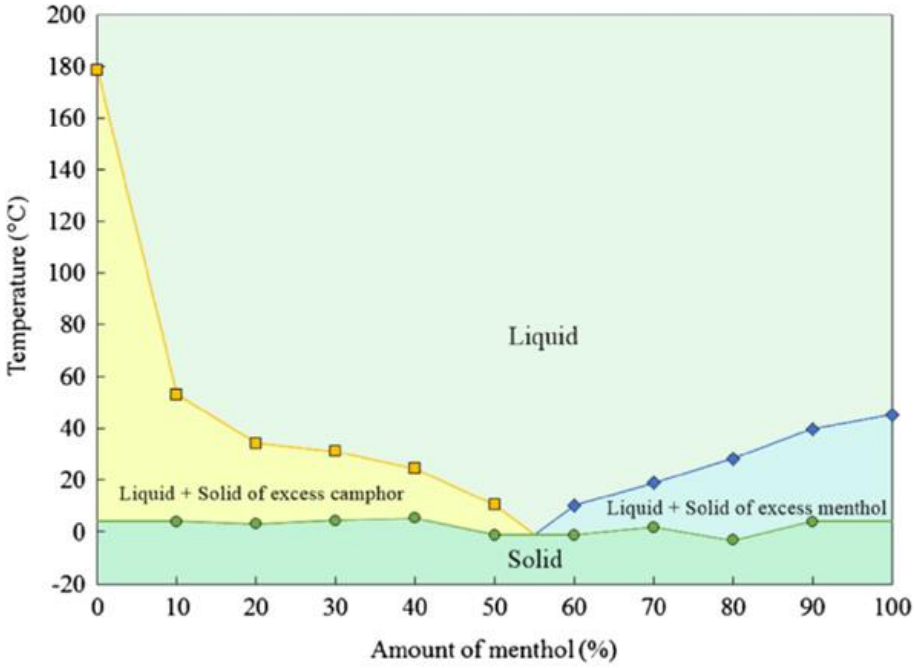

Figure #8. Phase diagram of menthol and camphor EM. [45].

mixture ratio is nearly close to 1:1[45]. The EM has been used \before in FFS. In which they used equal proportion (1:1) of camphor and menthol [42][43][44].

1.4.2. Supersaturation

After solvent evaporation, the concentration of the drug increases gradually, then it reaches saturation or even supersaturation. The supersaturation state increases the thermodynamic activity and consequently enhances drug permeation through the skin and overcome instability problem without skin barrier disruption. It is expected to have a burst effect after solvent evaporation and drug deposition into the upper layer of SC, followed by a gradual decrease in the flux [32].

To prevent drug precipitation, certain polymers are used as anti-nucleating agents. Supersaturation can be achieved by water uptake by the skin, by the evaporation of the volatile solvent, mixing of two solvents where the drug is more soluble in one of them due to the nature of the solvents or pH depending solubility[14] [32].

1.4.3. Impact of occlusion

The occlusion resulted from film formation leads to skin hydration. The increased water content of the SC works as a PE and contributes to a synergistic effect in the presence of other PEs in the preparation. The water content in the normal SC ranges between 5-15%, when the water content increases it can increase dissolution of the deposited drug in the skin and on the surface that can replenish the absorbed drug and maintain continuous flux. Also, water can increase intercellular lipid fluidity and polarity. These mechanisms have shown how the water can enhance the permeation of hydrophilic drugs. while, they fail to explain how water increases the penetration of lipophilic drugs[32].

1.5. Modulus of elasticity

Modulus of elasticity which is also known as Young's Modulus (E) is related to the material stiffness. It is a constant that can be determined from the linear part of the elastic region of the material in stress-strain curve and more specific during the first part of the curve until reaching the limit of proportionality (A) as seen in

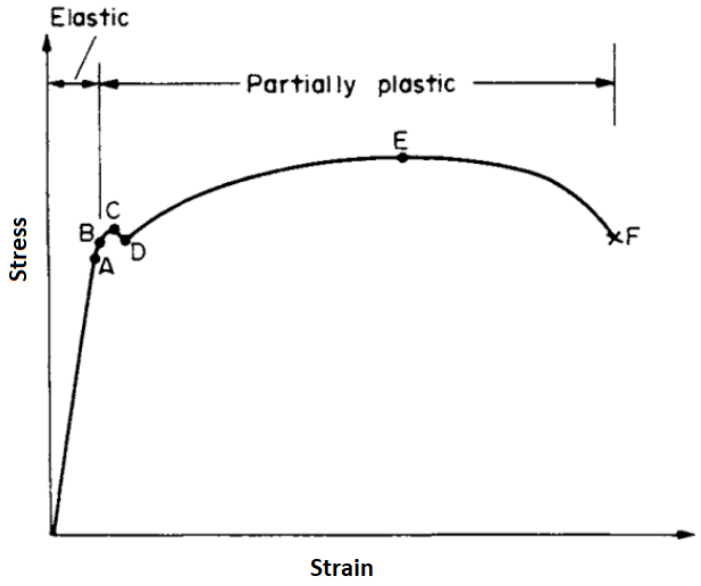

Figure #9. General stress-strain curve; (A) limit of proportionately, (B) Limit of elasticity, (C) upper yield point, (D) Lower yield point, (DE) elastic –plastic state, and (F) fracture [46].

Figure #9. This linear part obeys the simplest form of Hooke's Law, from which the Young's Modulus (E) is obtained (Eq. #1). For a short period after point A, the material is still elastic until reaching the elastic limit (B). During the elastic region the material can return to its original length after the removal of the stress (i.e. the strain is zero). If the stress is continued after point B, permanent or plastic deformation occurs and the strain is not totally recovered. C is the upper yield point and D is the lower yield point, these points maybe not exist for some materials, and for others, the difference between them is impossible to be detected. During the DE region which is also known as elastic-plastic state, only small increase in the stress results in relatively huge increase in the strain until

reaching point E. During this state, the material is not totally plastic and some section of the material can still be elastic. Beyond point E, and while reducing the stress, the cross sectional area reduced rabidly (necking) and the strain is increasing until fracture at point F [46].

Young's modulus $(E) = \frac{\text{Stress } (\sigma)}{\text{Strain } (\varepsilon)}$ = Slope of the straight line……..Eq. #1

The stress (σ) can be measured by dividing the uniform force (F) applied to the cross section area (A) of a material over (A). Thus the stress unit is newton (N) per square meter (m^2) (Eq. #2). The stress changes the material length and results in strain. The strain (ε) can be measured by dividing the change in the material length ($\triangle L$) over the original length ($L\circ$), so it has no unit. (Eq. #3).

Stress $(\sigma) = \frac{\text{Force}}{\text{Area}} = \frac{F}{A}$ ……Eq. #2

Strain $(\varepsilon) = \frac{Change\ in\ the\ lenght}{Original\ length} = \frac{\triangle L}{L\circ}$ .....Eq. #3

1.6. Viscosity

Viscosity is a measure that describes a fluid's resistance to flow[47]. For FFSs, low viscosity is favorable to enhance formulation spreadability on the skin, dispensing accurate dose, and enhance patient's compliance[43].

To measure the viscosity of Newtonian liquid, an apparatus called *Ostwald-Cannon-Fenske viscometer* can be used. The liquid formula of unknown viscosity (η_1) and known density (ρ_1) is let to flow by gravity. The time needed for the liquid to flow between a two timing marks (t_1) is compared with the time for another liquid (t_2) (usually water) of known viscosity (η_2) and known density (ρ_2). The unit of η_1 is in (dyne sec/cm^2), (g/cm sec) or more convenient in centipoise (cp). It can is calculated by using Eq. #4 [47].

$$\frac{\eta_1}{\eta_2} = \frac{\rho_1 t_1}{\rho_2 t_2} \quad \text{Eq. \#4}$$

1.7. Diffusion cells

For in vitro diffusion studies, Franz-type diffusion cells is used to detect the amount of vitamin D3 released or permeates through membrane. Franz cell

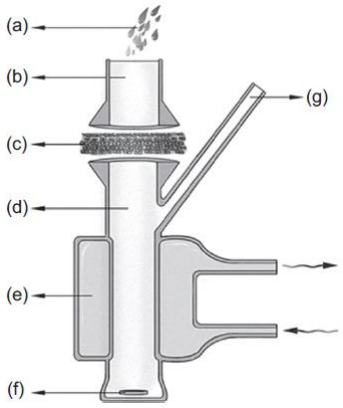

Figure #10. Franz diffusion; (a) tested formula, (b) donor compartment, (c) membrane, (d) receiver compartment, (e) heating jacket, and (g) sampling port [48].

(Figure #10) consists of donner and receiver compartments that are separated by a pretreated membrane. The heated jacket around each cell enable to maintain a constant temperature inside the receiver compartment (usually around 32 ºC - mimicking the temperature of the skin). The magnetic stirrer provides continuous stirring inside the receiver compartment to prevent the accumulation of permeates beneath the membrane. The buildup of local concentration can encounter the diffusion process and consequently affect the sink condition. The sampling port enable taking samples periodically from the receiver fluid, then samples are analyzed and the (*Q*) per unit area per unit of time is calculated [48].

1.7.1. Receiver compartment volume and effective diffusion area

Before using the Franz cell, the receptor chamber should be clean and dry. The volume of the receptor compartment can be determined by placing the magnetic stirrer at the bottom of the empty chamber, then the chamber is filled carefully to the top, and the volume is recorded. The mean of three trials is taken[49]. The inner diameter of the three-receiver compartments is measured by the caliper. The effective diffusion area (S) for each is calculated by using the formula πr^2, where r is the inner radius of the receiver compartment, then the mean is recorded[49][50].

1.7.2. Solubility of vitamin D3 in receptor fluid

According to the European Medical Agency (EMA), sink condition should be verified to guarantee that the permeation is not limited by the receptor medium. The maximum concentration of the active ingredient should not exceed 10-30% of the maximum solubility in the receptor compartment[51]. According to the literature, sink condition was maintained in a mixture of phosphate buffer saline (PBS) (pH 7.4) and ethanol (50:50) after 24 hours under stirring (100 rpm), at 35 ^{0}C and protection from daylight[11]. Under these conditions the maximum solubility of vitamin D3 was 110.22 ± 3.02 µg/ml while maintaining skin integrity [11]. Phosphate buffer saline is prepared according to European pharmacopeia[52].

1.7.3. Strat-M Membrane

The synthetic artificial membrane Strat-M® is manufactured to mimic human skin. It is considered a substitute to human and animal skin in permeation studies[53][54][55][56]. Strat-M® consists of several layers of polyester sulfone that differ in diffusivity (Figure #11). The outermost layer is tightly packed surface layer which resemble the function of the SC, underneath this layer, there are two layers of polyethersulfone that resemble the dermis, and at the bottom there is a more diffusive polyolefin layer that resample the subcutaneous fat layer.

The porous structure of this membrane provides a permeability gradients to mimic the permeability of human skin[53][57].

In contrast to biological membranes, the use of artificial membrane like Strat-M is easy, simple (no need for special storage conditions), and provides reproducible results without any ethical concern. However, it is very difficult to separate the layers of synthetic membranes to study the entrapped permeates[53].

Strat-M® has been used in several publications. It was used to predict the permeation of both lipophilic and hydrophilic drugs. But the permeability of hydrophilic compound was higher. These studies, showed that the permeation of Strat-M® and human skin were comparable. This makes Strat-M® a reasonable alternative to predict drug permeation through real human skin[53].

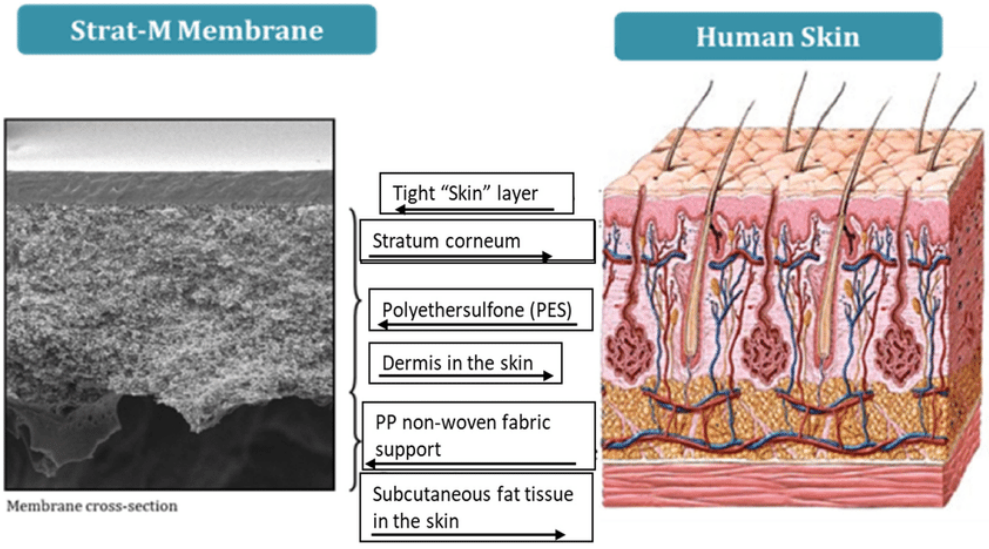

Figure #11. The cross section of Strat-M® membrane and the main three layers that mimic the human skin[57].

1.7.4. The Principle of diffusion through membranes

Diffusion is defined as mass transfer of molecules in response to concentration gradient from high to low concentration to reach thermodynamic equilibrium state. The flux (J) is the material amount transferred (M) per unit area of barrier (S) in unit time (t) (Eq. #5)[47].

$$J = \frac{dM}{S.dt} \quad \text{.....Eq. \#5}$$

When the concentration gradient in no longer exists, the diffusion will stop. According to Fick's first law (Eq. #6), the flux (J) is proportional with the concentration gradient (dC/dx) [47]:

$$J = -D\frac{dC}{dx} \quad \text{.....Eq. \#6}$$

Where (D) is the diffusivity or diffusion coefficient in cm^2/sec. The concentration (C) is in g/cm^3, the distance (x) is in cm, and (J) in g/cm^2 sec. (J) it is a positive quantity. The negative sign indicates for the opposite direction of the diffusion relative to the increase of concentration gradient. (D) is not constant, and it can be changed with concentration, solvent properties, pressure and temperature[47].

The molecules continue to flow from the donner compartment where the concentration of molecules is high through the membrane toward the receptor fluid where the concentration is very low (sink condition). After enough time, the system reaches steady state, which is described by Fick's first law. However, at

Nonsteady state the Fick's second law (Eq. #7) is generally used to describe the change in permeant concentration with time at any distance, and it can also be use to describe the steady state condition[47].

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} \quad \text{Eq. \#7}$$

At steady state the rate of change in the concentration will become zero

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} = 0 \quad \text{Eq. \#8}$$

If the later equation integrated twice, the Eq. #9 is obtained

$$J = \frac{D}{h} (C_1 - C_2) \quad \text{Eq. \#9}$$

Where (C_1) and (C_2) are the high concentration in the membrane at the donner compartment and the low concentration in the membrane at the receptor compartment, respectively. (h) is the membrane thickness and (D/h) is the diffusional resistance. If (Eq. #9) is substituted in Fick's first law (Eq. #5), it becomes:

$$J = \frac{dM}{S\,dt} = D \left(\frac{C_1 - C_2}{h}\right) \quad \text{Eq. \#10}$$

The concentration in the membrane (C_1) and (C_2) are generally difficult to be measure. Thus, they are replaced by the partition coefficient (K), the concentration within the donor (C_d) and the concentration within the receptor compartment (C_n)[47], where

$$K=\frac{C_1}{C_d}=\frac{C_2}{C_n}$$ …… Eq. #11

$$\frac{dM}{dt}=\frac{DSK(C_d-C_r)}{h}$$ ……Eq. #12

In sink condition, $C_d >> C_r$.

$$\frac{dM}{dt}=\frac{DSKC_d}{h}$$ …… Eq. #13

Where,

$$P=\frac{DK}{h}$$ …… Eq. #14

Where, (P) is the permeability coefficient.

Hence,

$$\frac{dM/S}{dt}=PC_d$$ …… Eq. #15

In Diffusion study, the cumulative amount per unit area (dM/S) or (Q) can be measured based on (Eq. #16) the diffused amount of drug in the receptor compartment through the effective surface area (S), in addition to the previous amount removed from the receptor compartment during sampling. (C_n) and (C_{col}) are the concentration of drug in the receptor compartment and the concentration of drug in the sample, respectively. (V_R) and (V_i) are the receptor compartment volume and the sample volume, respectively[58].

$$Q=\frac{C_nV_R+\sum_{i=1}^{i=n-1}C_iV_{col}}{S}$$ …… Eq. #16

When (Q) is plotted against time (Figure #12), and concentration of drug in the donor compartment (C_d) is known, (P) in cm/sec can be calculated from the slope (Eq. #17) [49].

$$\text{Slope} = J = \frac{dM/S}{dt} = \frac{Q}{t} = PC_d \quad \text{.....Eq. \#17}$$

From extrapolation of the trend line with the x axes, lag time (T_L) can be obtained. The membrane thickness can be measured by the caliper. Then (D) can be calculated according to Eq. #18 [59].

$$D = \frac{h^2}{6T_L} \text{.....Eq. \#18}$$

From (P) and (D), the partition coefficient (K) can be calculated (Eq. #14).

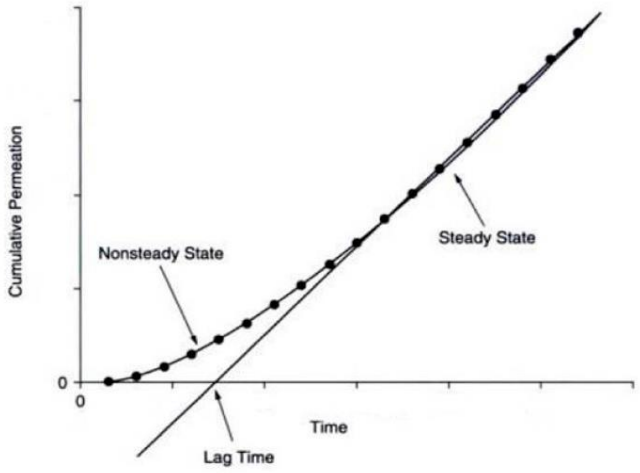

Figure #12. Typical plot for permeation study. The plot of cumulative amount against time results in curve start with Nonsteady State (nonlinear part) then reach the Steady State (linear part)[59].

Chapter II: Objective and significance

2. Objective and significance

2.1 Significance of the study

Vitamin D3 deficiency is now considered a worldwide problem with an incidence of 30-50% of children and adults. Vitamin D3 deficiency can lead to potential health consequences; it increases the risk of osteoporosis, osteomalacia, rickets, schizophrenia, depression, infections (urinary tract infection, tuberculosis), Asthma, high blood pressure, coronary heart disease, autoimmune disease (type 1 diabetes, multiple sclerosis, Crohn Disease, rheumatoid arthritis), muscle weakness and muscle aches[18][60].

The main source of vitamin D3 is moderate skin exposure to sunlight. The active form; 1,25(OH)2D enhances the absorption of calcium by 30-40% and phosphate by 80% compared to 10-15% and 60 % respectively without vitamin D3. The 1,25(OH)2D binds to the vitamin D receptors which distribute in many tissues in the body and consequently exerts several biological effects including macrophages production stimulation, renin inhibition, stimulation of insulin production, angiogenesis inhibition, cellular proliferation inhibition, inducing of terminal differentiation and regulation more than 200 genes that may be related to the benefits of vitamin D3[18].

Elderly people have only about 25% of 7-hydrocholesterol (vitamin D3 precursor), which decreases their ability to synthesis Vitamin D3 by 75% in their skin. Most of the human do not reach the desired level of 25(OH)D3 (> 30 ng/ml)

in their blood due to lack of appreciation of sun exposure, the usage of sunscreens, traditional clothes, high melanin in the dark skin, decrease UV-B photons in winter, living at high latitude, hepatic failure, renal failure, obesity and taking certain medications (e.g. glucocorticoids and anti-seizure medications). Other sources for vitamin D3 are few types of food (e.g. salmon) which are naturally containing vitamin D3, fortified products and multivitamins[1].

Now the multivitamin supplementations (oral and parenteral preparation) have become the main source of vitamin D3[2]. However, these routes of administration are associated with drawbacks and limitations. Absorption of vitamin D3 is highly variable in many populations including, patients who take bile acid medication (e.g. cholestyramine), patients who undergo gastric bypass and those with fat malabsorption (e.g. celiac disease, biliary obstruction, Crohn disease, and chronic pancreatitis), the absorption in these population is highly affected compared with normal subjects[61]. Patient compliance decreases when supplementation therapy is taken multiple times daily, which may be difficult for geriatric and Alzheimer's patients. On the other hand, parenteral route is considered an invasive route and needs continuous supervision which also affects patients' compliance. Thus another route of administration for vitamin D3 is important to overcome these limitations such as transdermal drug delivery system such as FFS[7].

2.2 Objectives

- Developing of high performance liquid chromatography (HPLC) method for accurate determination of vitamin D3 in solutions and FFSs.
- Preparation and characterization of different FFSs using various polymers.
- Loading of vitamin D3 in the different formulation trials and its characterization.
- Studying vitamin D3 release from formulation trials through polyamide membrane and permeation through the synthetic membrane (Strat M®) by using Franz diffusion cell.
- Optimizing the permeation of vitamin D3 using chemical PEs.
- Studying the stability of the selected formulation at long-term and accelerated storage conditions.

Chapter III: Research Methodology

3. Research methodology

3.1 Formulation materials, equipment and tools

3.1.1. Materials

All material used in this study are listed in Table #3. The cholecalciferol (vitamin D3) and camphor were gifted from Jerusalem Pharmaceuticals Co. Ltd.

Table #3. Name of materials needed for formulation trials and their functions.

No.	Name of Component	CAS No	Description/ Source	Function
1	Cholecalciferol (vitamin D3)	67-97-0	FERMENTA BIOTECH LIMITED	Active ingredient
2	Eudragit L100-55	25212-88-8	Evonik Industries	FFP
3	Eudragit L100	25806-15-1	Evonik Industries	FFP
4	Eudragit S100	25086-15-1	Evonik Industries	FFP
5	Polyvinylpyrrolidone K30	9003-39-8	Pharmaceutical grade	FFP
6	Poly ethylene glycol 400	25322-68-3	Acros Organics	PE, plasticizer
7	Propylene glycol	57-55-6	Sigma Aldrich	PE , plasticizer
8	Oleic acid	112-80-1	SIGMA-ALDRICH	PE , plasticizer
9	Isopropyl myristate	110-27-0	ARCOS ORGANICS	PE , plasticizer
10	Sodium Lauryl sulfate	151-21-3	Pharmaceutical grade	PE , solubilizer

11	Limonene	5989-27-5	ALFA Aesar	PE
12	Eucalyptol	470-82-6	SIGMA-ALDRICH	PE
13	Transcutol	111-90-0	SIGMA-ALDRICH	PE
14	Ethanol 99.9%	64-17-5	Fisher Scientific	Solvent, PE
15	Ethyl acetate	141-78-6	Fisher Scientific	Solvent
16	Isopropyl alcohol	67-63-0	DAEJUNG	Solvent
17	Acetone	67-64-1	CARLO ERBA REAGENTS	Solvent
18	Camphor	76-22-2	Pharmaceutical grade	EM
19	Menthol	2216-51-5	Pharmaceutical grade	EM
20	Disodium hydrogen phosphate	7558-79-4	SIGMA-ALDRICH	Buffering agent
21	potassium dihydrogen phosphate	7778-77-0	SIGMA-ALDRICH	Buffering agent
22	Sodium chloride (Pure)	7647-14-5	DAEJUNG	For buffer saline
23	Purified water	7732-18-5	RO-water treatment system at Samih Darwazah institute	Buffer preparation

3.1.2. Equipment and tools.

All Equipments and tools used in analyses, formulation, stability studies and compatibility studies are listed in Table #4.

Table #4. Equipment and tools needed for the experiment.

No.	Name of Equipment/tool	Used for	Brand/Source
1	HPLC equipped with UV detector	Analysis test	Agilent 1200 Series
2	Modified Franz Diffusion Cell	Diffusion studies	ORCHID Scientific™
3	Refrigerator	Stability and storage	beko®
4	PH/ORP meter	PH adjustment	HANNA instruments
5	Computer	Data collection and data analysis	HP Elite Desk 705 G2MT
6	Analytical balance	Weighting	METTLER TOLEDO balance (5 digits), OHAUS®
7	Bath sonicator	Degassing and solubilization	ELMA S300H Elmasonic
8	Stop watch	Viscosity test	Samsung
9	Artificial membrane (Strat M)	Permeation studies	Merck Millipore
10	Polyamide membrane 0.45 µm	Release studies	SUPELCO®
11	Amber glass vials	HPLC analysis test	ROMICAL®
12	Micropipette	Formulation	KIRGEN®
13	Parafilm	Formulation and permeation test	Bemis
14	Ostwald Viscometer	Viscosity test	From Jerusalem pharmaceuticals company

15	Amber glass injection vials	Stability studies	
16	Incubators	Stability studies	
17	Weights	Elasticity test	From Samih Darwazah institute
18	Magnetic stirring bar	Mixing	
19	Amber glass volumetric flasks	Formulation, class A	
20	Volumetric and graduated pipettes	Formulation, class (A/AS)	
21	Beakers (different sizes)	Formulation	
22	Plastic droppers	Formulation	
23	Plastic dishes	Formulation	
24	Flexible needles and syringes	Diffusion studies	
25	Caliper	Effective surface area determination, Elasticity test	
26	Thermometer	Temperature measurement	
27	Tweezer	Diffusion studies	
28	Cotton	Sickness test	
29	Glass slides	Sickness test	
30	Paper clips	Elasticity test	
31	Non elastic Nylon string	Elasticity test, Sickness test	
32	Metal ball	Elasticity test	
33	Glass mortar and pestle	Eutectic Mixture formulation	

3.1.3. Analytical material

Analytical materials used in HPLC analysis are listed in (Table #5).

Table #5. Analytical material needed for vitamin D3 analysis.

No.	Name	CAS No	Description/ Source	Function
1	Acetonitrile	75-05-8	Carlo Erba Reagents	HPLC mobile phase
2	Ethanol 99.9%	64-17-5	Fisher Scientific	Solvent for stock solution
3	Methanol	67-56-1	Fisher Scientific	Diluent for vitamin D3 standards

3.2 Methods

3.2.1. HPLC analysis

Two calibration curves were constructed. Methanol is used as diluent in the first one. Its concentration range is used to cover vitamin D3 assay for stability and compatibility studies. While in the second calibration curve, fluid (ethanol: PBS (50:50)) as diluent. The latter one was used to detect and quantified the low vitamin D3 concentration during diffusion studies.

A. Stock solution preparation:

Stock solution was prepared in order to get several standards by serial dilutions. 0.2 gr of oil containing vitamin D3 (each 1 gr of oil contains 1,000,000 IU vitamin D3, potency was 106.2%) was diluted in 200 ml ethanol, and sonicated for 15 minutes to guarantee complete oil dissolving.

B. Standard solutions preparation:

Two sets of standard (STD) solutions were prepared by stock solution dilution. The first set the STDs were diluted in methanol for high concentration calibration curve. While the other set of STDs were diluted in ethanol:PBS (50:50) for low concentration calibration curve. STDs concentrations were calculated by multiplication with the proper dilution factor.

C. HPLC analysis method

The HPLC analytical method is based on a previously published validated reversed-phase HPLC method. [25]. Each STD solution was injected three times and analyzed by HPLC (Agilent 1200) under the following conditions in Table #6.

D. Linearity, ranges, LOD and LOQ

To construct the calibration curves, we took the average peak area of the three injections for each STD obtained from chromatograms, and then we plotted them

Table #6. HPLC analytical method conditions used for vitamin D3 analysis

No.	Analytical method Conditions	
1	Column	C18: 150 × 4.6 mm, 5 µm.
2	Injection volume	100 µl
3	Temperature	25^0C
4	Stop time	15 minutes
5	Retention time	Around 9 minutes
6	Mobile phase	Acetonitrile 100%.
7	Wavelength detection (λ)	265 nm
8	Flow rate	1.5 ml/min

against the corresponding concentrations. From the R^2 of the regression line of the calibration curve we checked the linearity that covers the studied concentration range.

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated based on the standard deviation of the intercept and the slope of the calibration curve for low vitamin D3 concentration (Table #7).

Table #7. LOD and LOQ determination

	Determination
SE[1]	Regression statistics
SD[2]	SE/√n , (n=7)
LOD	3.3 * SD/ Slope
LOQ	10 * SD/ Slope

SE : Standard error of the intercept

SD: Standard deviation of the intercept

3.2.2. Compatibility studies

The compatibility of vitamin D3 in four candidate solvent was tested at room temperature. These solvents were ethanol, IPA, EA, and acetone. 0.1 g of the oil containing vitamin D3 was weighed directly into 100 ml amber volumetric flask, and then the tested solvent was added up volume. Three samples (n=3) were prepared for each. At 0 time, 24 hours, and 4 month, we took 2 ml from each sample and diluted it in 25 ml methanol before injection into HPLC three times. We calculated the average area (Av. Area) under the curve appeared in HPLC chromatogram for the three injection (Area of sample), then we substituted it in

the (Eq. #19) to calculate the assay % for each sample. Finally, we took the average of assay%.

$$\text{Assay \%} = \frac{Area\ of\ sample}{Area\ of\ STD} \times \frac{Concentration\ of\ STD}{Concentration\ of\ sample} \times \text{Potency of STD} \quad \text{.....Eq. \#19}$$

Where the concentration of sample is the actual concentration after the dilution in 25 ml methanol. And the potency of standard % according to certificate of analysis (COA) is 106.2%. (Please see the Appendix)

3.2.3. Formulation development

The process of the formulation development involved the preparation of several primary trial formulations (PTFs). These PTFs were characterized and tested to meet the acceptance criteria. The passed trial formulation or the successful trial formulations (STFs) were characterized by pH, viscosity and elasticity tests. Trial formulations were chosen from the STFs, then vitamin D3 was added to them to prepare the primary formulations. These formulations were retested again to grantee the reservation of the acceptable film properties. And then, they were subjected to diffusion studies to check and calculate vitamin D3 release and permeation.

3.2.3.1. General method of preparation

For all trial formulations, all components were weighed into a volumetric flask and dissolved in 50% -90% of evaporating solvent. Shaking and sonication were

used to ensure complete polymer dissolution, then cooled down filled with solvent up to volume. At the end, it is sonicated again for a few seconds to ensure complete homogenization. In case of PVP polymer, it was dissolved first by sonication in the evaporating solvent then the other ingredients were added and dissolved, the use of magnetic stirrer can facilitate Eudragit L100-55 dissolution in PVP containing solutions.

To prepare formulations loaded with vitamin D3, start by directly weighing the exact amount of vitamin D3 in an amber volumetric flask then the rest of components are dissolved as mentioned in trial formulations.

The Eutectic Mixture (EM) is freshly prepared by weighing equal amount of camphor and menthol. Grounded the binary mixture in circular motion in a glass mortar and pestle for 5 minutes until all solid particles melt into one liquid phase.

3.2.3.2. The primary trial formulations (PTFs)

Generally, the trial formulations are composed of two or more components among the following:

- Polymer or combination of polymers
- Solvent or mixture of solvents
- Plasticizer / PE or combination of plasticizers /PEs

A. **The polymeric trial formulations**: Simple polymeric solutions were prepared by dissolving the amount of one polymer (w/v) in ethanol as a volatile solvent (Table #8).

Table #8: Ingredients of the polymeric trial formulations.

No.	Eudragit			PVP	Ethanol
	L100-55	L100	S100		
X1	5%	-	-	-	Up to volume
X2	-	5%	-	-	
X3	-	-	5%	-	
X4	-	-	-	5%	

B. **The initial trial formulations**: to enhance polymeric films flexibility and/or adhesiveness, other components were added to the polymeric solution; these components include (PEG, OA, or PG) (Table #9).

Table #9. Ingredients of the initial trial formulations.

No.	Eudragit			PEG	PG	O.A	Ethanol
	L100-55	L100	S100				
XP1	5%	-	-	2%	-	-	Up to volume
XP2	5%	-	-	-	2%	-	
XP3	5%	-	-	-	-	2%	
XP4	-	5%	-	2%	-	-	
XP5	-	5%	-	-	2%	-	
XP6	-	5%	-	-	-	2%	
XP7	-	-	5%	2%	-	-	
XP8	-	-	5%	-	2%	-	
XP9	-	-	5%	-	-	2%	

C. The modified trial formulations*:* ethanol is replaced by binary evaporating solvent; ethanol: acetone (80:20 w/w) in an attempt to decrease the drying time. (Table #10).

Table #8. Ingredients of the modified trial formulations.

No.	Eudragit			PEG	PG	O.A	Ethanol: acetone (80:20)
	L100-55	L100	S100				
P1	5%	_	_	2%	_	_	Up to volume
P2	5%	_	_	_	2%	_	
P3	5%	_	_	_	_	2%	
P4	_	5%	_	2%	_	_	
P5	_	5%	_	_	2%	_	
P6	_	5%	_	_	_	2%	
P7	_	_	5%	2%	_	_	
P8	_	_	5%	_	2%	_	
P9	_	_	5%	_	_	2%	

D. The complex trial formulations: more complex trial formulations (Table #11) were prepared containing a mixture of different polymers, PEs, and/or EM. The aim of this stage was to get trial formulations that contain variety of ingredients and at the same time are able to form good films. The preparation of A1- A7 trail formulations was by using one polymer and a combination of two other excipients that may enhance film flexibility and adhesiveness without increase sickness or affect cosmetic appearance. In B1- B4 trial formulations two polymers and two excipients were used. Different PEs were

used to prepare C1-C4 trial formulations. And finally, D1-D5 trial formulations were prepared by adding another excipient; the EM camphor: menthol (1:1).

Table #9. Ingredients of the complex trial formulations.

No.	Eudragit			PVP	PEG	PG	O.A	IPM	SLS	limonene	Eucalyptol	Transcutol	EM	Ethanol: acetone (80:20)
	L100-55	L100	S100											
A1	8%	_	_	_	_	2%	1%	_	_	_	_	_	_	Up to volume
A2	8%	_	_	_	2%	_	1%	_	_	_	_	_	_	
A3	8%	_	_	_	2%	2%	_	_	_	_	_	_	_	
A4	8%	_	_	_	_	2%	_	2%	_	_	_	_	_	
A5	8%	_	_	_	2%	_	_	2%	_	_	_	_	_	
A6	_	8%	_	_	2%	_	_	2%	_	_	_	_	_	
A7	_	5%	_	_	2%	_	1%	_	_	_	_	_	_	
B1	6%	-	-	1%	-	2%	-	-	-	-	-	-	-	
B2	4%	4%	-	-	2%	2%	-	-	-	-	-	-	-	
B3	6%	2%	-	-	2%	2%	-	-	-	-	-	-	-	
B4	7%	1%	-	-	2%	2%	-	-	-	-	-	-	-	
C1	5%	-	-	-	-	2%	-	-	1%	-	-	-	-	
C2	5%	-	-	-	-	2%	-	-	-	1%	-	-	-	
C3	5%	-	-	-	-	2%	-	-	-	-	1%	-	-	
C4	5%	-	-	-	-	2%	-	-	-	-	-	1%	-	
D1	8%	-	-	-	2%	-	1%	-	-	-	-	-	2%	
D2	8%	-	-	-	2%	-	1%	-	-	-	-	-	5%	
D3	5%	-	-	-	-	2%	-	-	-	1%	-	-	5%	
D4	8%	-	-	-	-	2%	1%	-	-	-	-	-	5%	
D5	6%	-	-	1%	-	2%	-	-	-	-	-	-	5%	

E. Characterization of the PTFs

The PTF were evaluated to meet the following film acceptance criteria (Table #12):

Table #10. Acceptance criteria for the selection of PTF after solvent evaporation.

Step	**Acceptance criteria**	
Step 1 (Thin film tests)	Drying time [43]	Less than 5 minutes
	Stickiness [43]	Not sticky
	Cosmetic appearance [43]	Clear ,transparent, and smooth
Step 2 (Thick film tests)	Adhesiveness	Good or high
	Flexibility	Flexible/ Bendable
	Cosmetic appearance	Clear ,transparent, and smooth

A. **<u>Step 1 (Thin film tests):</u>** By using a micropipette, spread an exact volume of liquid preparation (100 µl) on a slide of glass to cover the predefined area (5×2 cm^2), and measure / describe the drying time, stickiness, and cosmetic appearance properties as follows:

A.1. Drying time:

- **Visual method:** leave the solution to dry at room temperature. Start the timer immediately after the application of the liquid preparation on a slide of glass. While the solvent evaporated, a film was formed gradually. Put another clear and clean slide of glass on the formed film gently without pressing, then took the later slide off and look for any remaining traces. If

the slide was completely dry and clean, the film was considered dry and the time was recorded[43].

- **The scale method:** immediately after the application of the liquid preparation, the timer was started. Then the decrease in weight was monitored on an analytical scale. When the loss in the weight was ≤ 10^{-4} mg /15 seconds the drying time was recorded. If the drying time was less than 5 minutes, it was considered acceptable [43].

A.2. Stickiness property: After the film dried, the stickiness property was evaluated by pulling a metallic ball covered with cotton along the film three consecutive times. The film should be non-sticky after 5 minutes from the application. The amount of fiber is proportional to the stickiness property. However, the film was considered non sticky only if no fiber was left on the film [43].

A.3. Cosmetic appearance: The film formed on the slide of glass was considered acceptable if it was clear, transparent/ semi-transparent [43].

B. **Step 2 (thick film):** Prepared thick films for the trial formulations by pouring each one of them carefully into a silicon mold. Keep the mold on a straight surface at room temperature without moving until the film is completely dry. Test the thick film as follow:

B.1. Adhesiveness: After the thick film was completely dried in the mold, a trial to take the film out is made. According to the ease of film removal, the adhesiveness of the film was evaluated into low, good, and high

B.2. Flexibility: After removing the film from the mold. The film was bent, rolled up, and twisted to evaluate its flexibility if the film was easily bent and remained intact without breaking or cracking, the film was considered flexible. The film flexibility was classified into not flexible, Low, good, and high.

B.3. Cosmetic appearance*:* the thick film was considered acceptable if it was clear, transparent/ semi-transparent

3.2.3.3. The successful trial formulations (STFs)

Based on the tests in step 1 and step 2, the STF were selected. These formulation were subjected to pH, viscosity, and elasticity tests.

A. pH test: pH was measured as quality control (QC) test. 1 ml of STF was diluted in 100 ml purified water. The pH of the supernatant was measured by pH meter at room temperature.

B. Viscosity test: calculate the density (ρ_1) of each STF by dividing its weight by its volume. Before starting the test, check–that the *Ostwald -Cannon-Fenske* viscometer (Figure #13) is clean, dry and free from particles. The viscometer and the flask containing the STF were immersed in a water bath (25 ^{0}C ± 1) and left for 5 minutes to reach equilibrium. The viscometer was hung vertically on a retort stand and the reservoir was filled with the STF

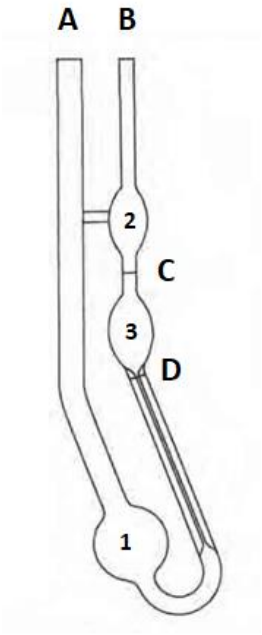

Figure #13. *Ostwald-Cannon-Fenske* viscometer. (A) Venting tube, (B) The tube with capillary, (C) Upper timing mark, (D) Lower timing mark, (1) Reservoir, (2) Pre-run sphere, and (3) Measuring sphere[47].

from the venting tube (A). From the tube with capillary (B), suck the liquid to reach the pre-run sphere above the upper timing mark (C). Then the suction is stopped to let the liquid to flow freely. The time needed for the meniscus to pass between (C) and (D) is recorded by using a precise stop watch. The experiment was performed three times (n=3) for each STF, and the average time was recorded (t_1). Finally, the viscometer was cleaned twice with acetone and then was dried completely to reuse it again. The test was also performed in triplicate for water to get (t_2) .The viscosity of purified water at 25^0C (η_2) is 0.8904 cp [47] and its density ($\rho 2$) at 25 ^{0}C is

0.997 g/ml [62]. η_2 and $\rho 2$ were used in Eq. #4 to calculate the viscosity of the STF (η_1).

C. **Elasticity test**: to determine Young's modulus, prepare a thick film in a mold, then cut a rectangular piece from it. From the thickness and the width of the rectangle tape the cross sectional area (A) was calculated in (m^2). We clamped a paperclip to both ends of the rectangle tape (Figure #14). The initial length (L○) between the two clamps was measured. Then, we hung the tape vertically on a retort stand. Weights were attached to the lower

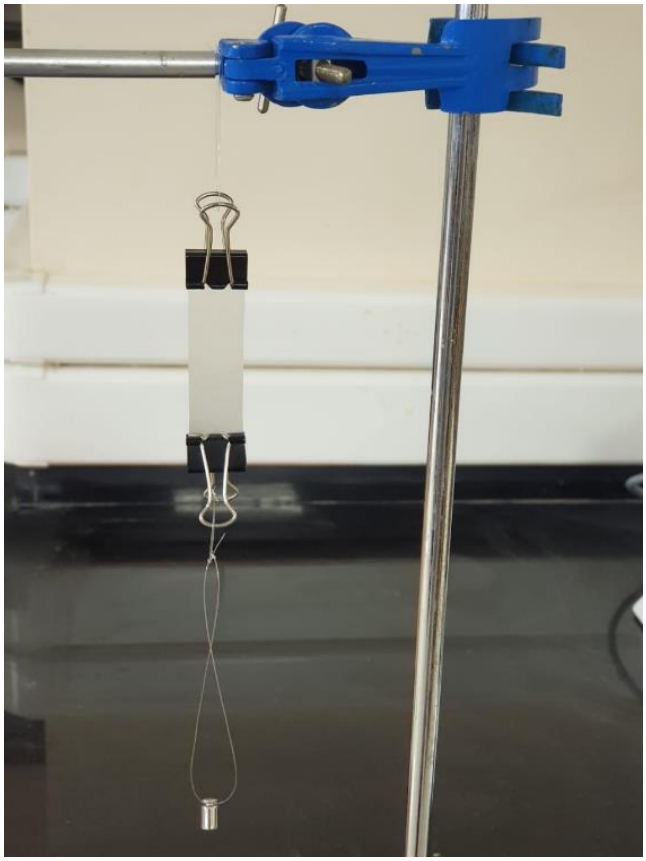

Figure #14. A rectangular tape is hanging freely from one end on retort stand for elasticity test. From the other end a weight is attached.

clamp, the increase in length (ΔL) was calculated by subtraction the initial length (L○) from the new length (L1). The Force in Newton (N) was calculated by multiplying the cumulative weights in kilogram by the acceleration due to gravity 9.8 m/s^2[63]. At some point, after two or three reading, the weights were removed and the length was re-measured again to check whether the film had returned to its initial length or not, and ensure that the film was remaining in the elastic region with no deformation. We increased the hanging weights gradually and recording the increase in the length until the increase in the length is significantly greater for the hanging weights.

For the transdermal thin films, no limit available yet in the literature for the Young's modulus. In FFS studies they relayed on the clinical test on healthy volunteers to evaluate the elasticity of the film. To make a prediction for the acceptable limit, a comparison was made with the Young's modulus for transdermal patches available in the market which range between 4-501 N/mm^2 [64].

3.2.3.4. The primary formulations

Number of trial formulations (of different ingredients) were chosen from the STFs to prepare the primary formulations loaded with vitamin D3 (6000 IU/ml). These formulations were subjected to step 1 and step 2 tests to check whether the films will still meet the acceptance criteria or not.

On these primary formulations, diffusion studies done include in vitro release studies and in vitro permeation studies. The polymeric formulation #1' loaded with vitamin D3 was tested also in release study.

A. Diffusion studies:

Franz diffusion cell used for in vitro release and permeability studies:

A.1. In vitro release study using polyamide membrane

To perform the release studies, prepare the receptor fluid PBS: ethanol (50:50). The PBS (7.4 pH) was prepared according to European Pharmacopeia [52] (Table #13). The solid ingredients were dissolved in exact volume of purified water. Put the volumetric flask -containing the PBS- in the sonicator for a few minutes to ensure complete dissolving for all ingredients. Then, the pH (7.4) was adjusted if required at 25ºC ± 1^0C. After that, mix equal volumes from PBS (7.4) and ethanol (50:50) v/v to get the receptor fluid. Polyamide membranes (0.45 µm) were cut to cover the inner orifice of the receiver compartment but smaller than the outer edge of the orifice. Then, soak them for half an hour in nearly 5 ml receptor fluid.

Table #11. Phosphate buffer solution (7.4) ingredients [52].

No.	Ingredient	Weight (g)
1	Disodium hydrogen phosphate	2.38
2	Potassium dihydrogen phosphate	0.19
3	Sodium chloride	8
4	Purified water	Up to 1000 ml

While the heat jacket of the Franz diffusion cell is up to 32 ± 1^0C, the receptor fluid was degassed in the sonicator and heated to reach the same temperature. Then fill the receptor compartment with the receptor fluid to the top of the lid in the presence of a magnetic stirrer. The rotation of the magnetic stirrer was adjusted to 600 rpm to provide well mixing and keep sink condition. By using a tweezer, mount the polyamide nylon membranes on the receptor compartment with caution to prevent trapping bubbles. Then, put a ring of rubber on the edge of the membrane before assembling the donner compartment. Close the rim where the donner and the receptor compartment met, as well as the open of the sampling port with a parafilm. A clamp of metal was used to tighten and keep the donner compartment in place.

Release studies were performed for the liquid formulations and the dry films. Load the liquid formulations (2 ml) in the center of the membrane by using a volumetric pipette. In the case of the dry film, prepare the film first by letting (1 g) of formulations to dry in the donner compartment inside a mold before assembling it with the film as one unit. An accurate volume of 1 ml was taken from the sampling port at each timing point during the 6 hours of the experiment. We analyzed samples via HPLC to determine the (Q) released per unit area. Through the sampling port, the taken samples were replaced with fresh receptor fluid of same temperature (32ºC± 1^0C) to maintain sink condition. The diffusion cells were protected from light throughout the experiment.

A.2. In vitro permeation study using Strat M® membrane

For in vitro permeation study, the same method and conditions used in release studies were used in permeation studies. However, the artificial membrane (Strat M®) is used instead of polyamide membrane. Permeation studies were conducted on the liquid primary formulations. 0.5 ml containing 3000 IU was dispensed in the donner compartment of each cell. For each primary formulation the test was performed in triplicate (n=3). Samples were taken at 0.5, 1, 2, 3, 4, 6, 7, 8, and 24 hours. The (Q) per unit area (IU/cm^2) was plotted against time (hr.)[43].

3.2.4. Stability studies

The stability of the successful formulations were studied under four storage conditions at zero time and after incubation for 2 and 3 weeks (Table #14). Fill each formulation into individual injection vials (type A) protected from light for each time period for each storage condition. To calculate the assay, take 2 ml from the each vial then dilute it in 50 ml methanol prior the injection in HPLC three times, then substituted the average area in Eq. #19. A STD of the same concentration (6000 IU/ml) was prepared in ethanol. Take 2 ml from STD and diluted it in 50 ml methanol. Inject the diluted STD 6 times in HPLC prior samples analysis and twice at the end of the analysis sequence.

For STD preparation verification, prepare another STD of same concentration and inject it in HPLC twice before sample analyses. Calculate the %Cross Check according to Eq. #20.

% Cross Check= $\frac{Area\ of\ STD1}{Area\ of\ STD2} \times \frac{Concentration\ of\ STD2}{Concentration\ of\ STD1} \times 100\%$..... Eq. #20

Table #12. Incubation period of stability tests under specific storage conditions.

Study	**Storage condition**	**Incubation period (weeks)**
Long term	25°C ± 2°C/60% RH ± 5%	2, 3
Intermediate	30°C ± 2°C/65% RH ± 5% RH	2, 3
Accelerated	40°C ± 2°C/75% RH ± 5% RH	2, 3
Stress	5°C ± 3°C	2, 3

Chapter IV: Results and discussion

4. Results and discussion

4.1. HPLC Analysis

4.1.1. Linearity, ranges LOD and LOQ

In the two linearity studies for high vitamin D3 concentrations and low vitamin D3 concentrations, the peak areas of the three injections (RSD<2%) for the seven STDs and for the six STDs are shown Table #15 and Table #16, respectively. By plotting the concentrations against the corresponding average peak areas (Figure #15), we can see the regression line equation for low vitamin D3 concentrations ($y = 4.3262x - 0.463$) and the correlation coefficient R^2 which equals 0.999.

Table #13. Concentration of seven STDs (low concentrations) and the related peak area in HPLC chromatogram using ethanol: PBS (50:50) as diluent.

STD #	Conc.[1]	Peak area			Average Peak area	SD	RSD %
		Inj[2]. 1	Inj. 2	Inj. 3			
STD 1	0.49	1.84	1.87	1.80	1.84	0.03	1.85
STD 2	0.98	3.90	3.89	3.95	3.91	0.03	0.87
STD 3	2.00	8.22	8.39	8.32	8.31	0.09	1.04
STD 4	3.20	13.65	13.39	13.38	13.47	0.15	1.13
STD 5	3.99	17.42	16.85	17.33	17.20	0.31	1.80
STD 6	4.82	19.05	19.12	19.33	19.16	0.15	0.76
STD 7	12.05	52.19	52.12	51.63	51.98	0.30	0.58

1: Concentration in IU/ml.

2: Injection

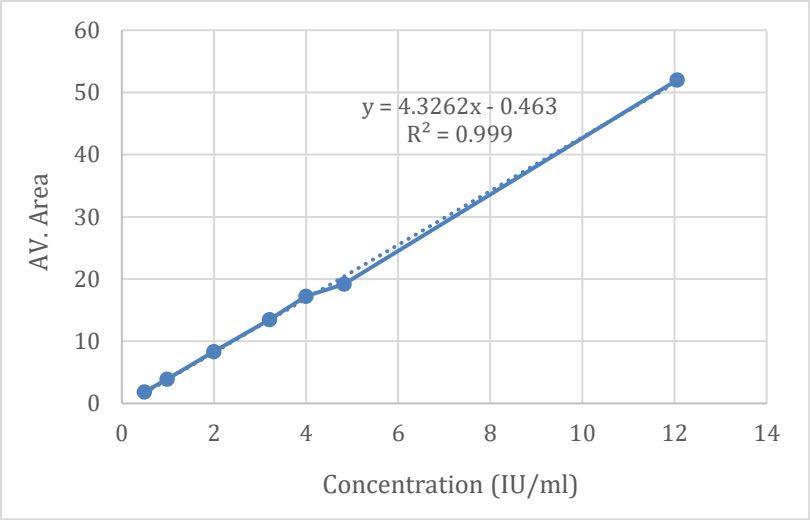

Figure #15. Calibration curve for low vitamin D3 concentrations.

Table #14. Concentration of six STDs (High concentrations).and the related peak area in HPLC chromatogram using methanol as diluent

STD #	Conc.[1]	Peak area			Average Peak area	SD	RSD %
		Inj[2]. 1	Inj. 2	Inj. 3			
STD 1	12.3	52.2	51.8	52.1	52.0	0.21	0.40
STD 2	24.1	115	114.2	113.8	114.3	0.61	0.53
STD 3	51.2	227.3	228	224.8	226.7	1.68	0.74
STD 4	102.4	457.2	457	457.1	457.1	0.10	0.02
STD 5	204.7	939.8	940.3	935.8	938.6	2.47	0.26
STD 6	409.4	1852.4	1850.7	1864.5	1855.9	7.52	0.41

1: Concentration in IU/ml.

2: Injection

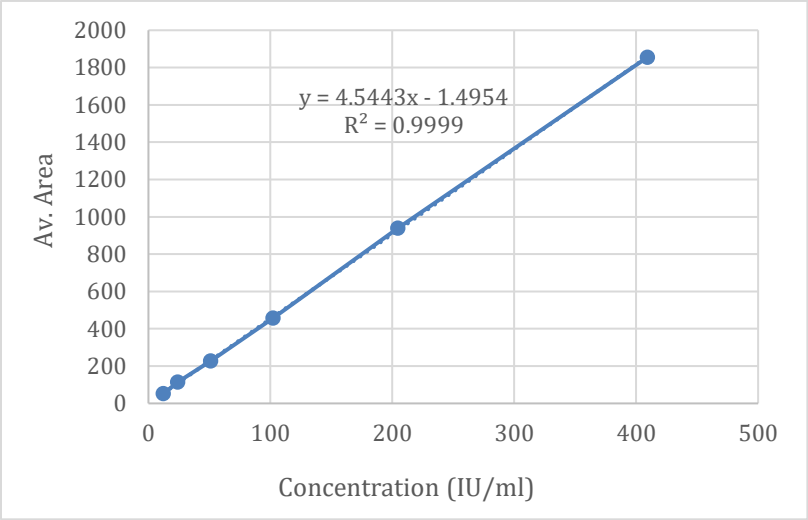

Figure #16. Calibration curve for high vitamin D3 concentrations.

In Figure #16 for high vitamin D3 concentrations, we can see the regression line equation (y = 4.5443x – 1.4954) and the value of R^2 equals 0.9999. The. The R^2 values indicates for a linear relationship between the concentrations and peak areas over the ranges (12.3- 409.4 IU/ml) and (0.49- 12.05 IU/ml) respectively. (Please see the Appendix for examples on Chromatogram)

LOD and LOQ

The LOD and LOQ were calculated depending on the calibration curve for low vitamin D3 concentrations (Figure #16). From the LOD and LOQ shown in Table #17, we knew the lowest detectable vitamin D3 concentration (0.098 IU/ml) and the lowest quantifiable concentration (0.296 IU/ml). These values are low enough to be relied upon on during the release and permeation studies of vitamin D3 preparations containing a concentration of 6000 IU/ml.

Table #15. Results of LOD and LOQ.

	Determination	Value
SE	Regression statistics	0.339
SD	SE/√n , (n=7)	0.128
LOD	3.3 * SD/ Slope [65]	0.098 IU/ml
LOQ	10 * SD/ Slope [65]	0.296 IU/ml

SE : Standard error of the intercept

SD: Standard deviation of the intercept

4.1.2. Compatibility studies

The results of compatibility studies of vitamin D3 in four solvents are shown in Table #18. All samples were tested at room temperature directly after preparation at 0 time, after 24 hr., and finally after 4 month. Assuming the Assay at 0 time is 100%.

The assay of vitamin D3 in ethanol, IPA, and EA were calculated according to Eq. #19 and they were acceptable (±2 %, <2 RSD %). However, the assay dropped to (74.6 %) in acetone after 4 month. In acetone chromatogram, we observed the appearance of other peaks in addition to the peak of vitamin D3 (please see the Appendix). This prompted us to identify and quantify previtamin D3 which is part of vitamin D3[66]. With the help of Jerusalem Pharmaceutical Company we analyzed vitamin D3 again after 4 month in their laboratories using their own method. They provided us the chromatogram that identifies the previtamin D3 (please see the Appendix). However, after addition the area of the previtamin D3 to the assay, it was still unacceptable (84.6 %). This can be explained by the ability

Table #16. Results of vitamin D3 assay in compatibility studies.

No.	Solvent	Time	Assay %	SD	RSD %
1	**Ethanol**	0	100.0	0.45	0.45
		24 hr.	98.2	1.01	1.03
		4 month	101.9	1.82	1.79
2	**IPA**	0	100.0	0.37	0.37
		24 hr.	99.3	0.08	0.08
		4 month	100.3	1.90	1.95
3	**EA**	0	100.0	1.12	1.07
		24 hr.	98.5	1.94	1.90
		4 month	103.8	1.17	1.09
4	**Acetone**	0	100.0	0.51	0.52
		24 hr.	97.9	0.79	0.84
		4 month	74.6	1.32	1.83

of acetone to produce intermediate oxygenates and free radical, which can result in vitamin D3 oxidation [67]. Adding antioxidant like ascorbic acid, BHT, or BHA can solve the issue.

4.2. Formulation development

4.2.1. The primary trial formulations

4.2.1.1. The polymeric trial formulations

The formulation development started by the preparation and evaluation of the polymeric trial formulations. The aim of the step 1 and step 2 tests (Table #19) was to evaluate polymer behavior alone without other excipients.

X1 and X4 formed clear and transparent films. The invisible cosmetic appearance is preferred for patient acceptance and compliance[43]. On the other hand, X2 and X3 formed white films. The drying time using scale method was stricter and more precise than the visual method. X4 formed very adhesive film which looked like a glue, and we couldn't remove it from the mold to check its flexibility. All these trial formulations failed to achieve the acceptance criteria. However, all these properties could be modified and enhanced after the addition of other excipients.

Table #17. Results of step 1 and step 2 testes of the polymeric trial formulations using ethanol as a solvent.

No.	Ingredients	Step 1				Step 2			Pass/Fail
		Drying time		Stickiness	Cosmetic appearance	Adhesiveness	Flexibility	Cosmetic appearance	
		Visual	Scale						
X1	Eudragit L100-55 (5%)	3 min	5 min 15 sec	Non-sticky	Transparent, clear	Low	Not flexible	Transparent, clear,	Fail
X2	Eudragit L100 (5%)	2.5 min	4 min 30 sec	Not sticky	White	Low	Not flexible	White	Fail
X3	Eudragit S100 (5%)	2.5 min	6 min 45 sec	Not sticky	White	Low	Not flexible	White	Fail
X4	PVP (5%)	3 min	4 min	Not sticky	Transparent, clear	High	N.A.	Transparent, clear	Fail

4.2.1.2. The initial trial formulations

The results of the Initial trial formulations tests, which contained one polymer and one excipient (Table #20) showed significant improvement in film adhesiveness and flexibility in number of films without increasing film stickiness. PG and PEG enhanced film flexibility and adhesiveness especially in XP1 and XP3 trial formulations. In the opposite, OA had no effect on film flexibility and low effect on film adhesiveness. The white color of the polymeric films Eudragit L100 and Eudragit S100 changed to transparent or opaque. After ethanol evaporation the polymers were still dissolved in these excipients (PG, PEG, or OA), this can explain the reason for color changing. Although film properties were improved, the drying times for all of them were still more than the acceptable limit.

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Table #18. Results of step 1 and step 2 testes for the initial trial formulations using ethanol as an evaporating solvent.

No.	Ingredients	Step 1				Step 2			Pass/ Fail
		Drying time		Stickiness	Cosmetic appearance	Adhesiveness	Flexibility	Cosmetic appearance	
		Visual	Scale						
XP1	Eudragit L100-55 5% + PEG 2%	3 min 17 sec	5 min 45 sec	Non-sticky	Transparent	High	High	Transparent, clear	Fail
XP2	Eudragit L100-55 5% + OA 2%	3 min 15 sec	5 min 45 sec	Non-sticky	Transparent	Low	Not flexible	Semi-transparent, clear	Fail
XP3	Eudragit L100-55 5% + PG 2%	3 min 40 sec	6 min	Non-sticky	Transparent	Good	Good	Transparent, clear	Fail
XP4	Eudragit S100 5% + PEG 2%	3 min 35 sec	5 min 45 sec	Non-sticky	Transparent	Low	Not flexible	Opaque	Fail
XP5	Eudragit S100 5% + OA 2%	3 min 9 sec	5 min 45 sec	Non-sticky	Transparent	Low	Not flexible	Opaque	Fail
XP6	Eudragit S100 5% + PG 2%	3 min 20 sec	5 min 30 sec	Non-sticky	Transparent	Low	Not flexible	Opaque	Fail
XP7	Eudragit L100 5% + PEG 2%	3 min 5 sec	5 min	Non-sticky	Transparent	Low	Low	Transparent, Clear	Fail
XP8	Eudragit L100 5% + OA 2%	3 min	5 min15 sec	Non-sticky	Transparent	Low	Not flexible	Opaque, not smooth.	Fail
XP9	Eudragit L100 5% + PG 2%	2 min 46 sec	5 min	Non-sticky	Transparent	Low	Low	Transparent, clear	Fail

4.2.1.3. The modified trial formulations

We tested the drying time for the modified trial formulations using scale method, then we compared it with the drying time for the polymeric and the initial trial formulations (Table #21). We noticed satisfying decrease in the drying time for all modified formulations (less than 5 min)[43]. This was expected because of the boiling point of acetone, which is less than that for ethanol (56.2 ^{0}C vs 78.15^{0}C, respectively)[31] . Since the drying time decreased, the problem in XP1 and XP3 was solved, and so, P1 and P3 in the modified trial formulation were successful

Table #19. Drying time comparison between trial formulations dissolved in ethanol vs ethanol: acetone (80:20) as evaporating solvents.

No.	Drying time for ethanol formulations	No.	Drying time for ethanol: acetone (80:20) formulations
X1	5 min 15 sec	1	3 min and 45 sec
X2	4 min 30 sec	2	3 min
X3	6 min 45 sec	3	2 min and 45 sec
X4	4 min	4	2 min and 15 sec
XP1	5 min 45 sec	P1	4 min and 30 sec
XP2	5 min 45 sec	P2	4 min and 30 sec
XP3	6 min	P3	4 min and 30 sec
XP4	5 min 45 sec	P4	3 min and 45 sec
XP5	5 min 45 sec	P5	3 min and 45 sec
XP6	5 min 30 sec	P6	3 min and 30 sec
XP7	5 min	P7	3 min
XP8	5 min15 sec	P8	3 min
XP9	5 min	P9	3 min

4.2.1.4. The complex trial formulations

The results of step1 and step 2 tests for complex trial formulations illustrated in Tables #22-25. In A1-A6 trial formulations we increased the percentage of polymer to 8% in the presence of two excipients to make some balance between stickiness in one hand and flexibility and adhesiveness in the other hand. The combination between PEG and PG increased the chance of stickiness. However, all trial formulations except A2 failed to meet the acceptance criteria (Table #22).

In B trial formulations (Table #23) we used a combination of two polymer and two excipients. In B1 trial formulation, we use the hydrophilic polymer (PVP) that may promote vitamin D3 permeation by decrease its affinity in polymer structure. While in B2-B4 trial formulations, the combination of polymers was to overcome the stickiness effect resulted from the combination of PG and PEG together. All B trial formulations except B2 –due to lack of smoothness- met the acceptance criteria.

We modified the P3 trial formulation, and prepared C trial formulations by using several PEs (Table #24). The addition of limonene, eucalyptol, and transcutol in C2, C3, and C4 respectively, gave successful trial formulations. While the SLS (1%) in C1 trail formulation gave sticky film.

In D trial formulations (Table #25) we increased the complexity of the film by the adding the EM to the number of trial formulations that were expected to handle

it. A1 formed non-sticky and not flexible film. After adding the EM in D1, it was still non-sticky but it became flexible. C2 was non-sticky film, but after adding the EM in D4, it became very sticky. In general the EM can increase film flexibility and stickiness. Only D1 and D4 were successful trial formulations.

Table #20. Results of step 1 and step 2 testes for the complex trial formulations (A1-A7) using ethanol: acetone (80:20) as an evaporating solvent.

No.	Ingredients	Step 1			Step 2			Pass/fail
		Drying time	Stickiness	Cosmetic appearance	Adhesiveness	Flexibility	Cosmetic appearance	
A1	Eudragit L100-55 8% + PG 2%+ OA 1%	5 min 30 sec	Non-sticky	Transparent	Good	Not flexible	Semi-transparent	Fail
A2	Eudragit L100-55 8% + PEG 2%+ OA 1%	3 min 30 sec	Non-sticky	Transparent	Good	High	Semi-transparent	Pass
A3	Eudragit L100-55 8% + PG 2%+ PEG 2%	5 min	Sticky	Transparent	High.	High	Transparent, clear	Fail
A4	Eudragit L100-55 8% + PG 2% + IPM 2%	4 min 14 sec	Non-sticky	Transparent	Good	Low	Opaque	Fail
A5	Eudragit L100-55 8% + PEG 2% + IPM 2%	4 min	Non-sticky	Transparent	Good	Good	Opaque	Fail
A6	Eudragit L100 8% + PEG 2%+ PG 2%	2 min 45 sec	Not sticky	Transparent	Low	Good	Transparent, clear	Fail
A7	Eudragit L100 5%+ PEG 2%+ OA 1%	3 min 30 sec	Not sticky	Transparent	Low	Low	Opaque, smooth	Fail

Table #21. Results of step 1 and step 2 testes for the complex trial formulations (B1-B4) using ethanol: acetone (80:20) as an evaporating solvent.

No.	Ingredients	Step 1			Step 2			Pass/ Fail
		Drying time	Stickiness	Cosmetic appearance	Adhesiveness	Flexibility	Cosmetic appearance	
B1	Eudragit L100-55 6% + PVP 1%+ PG 2%	3 min 15 sec	Not sticky	Transparent,	Good	Good	Transparent, clear	Pass
B2	Eudragit L100 4% + Eudragit L100-55 4% + PG 2%+ PEG 2%	4 min 15 sec	Not sticky	Transparent	Good	High	Transparent, not smooth	Fail
B3	Eudragit L100 2% + Eudragit L100-55 6% + PG 2% + PEG 2%	3 min 15 sec	Not sticky	Transparent	Good	Good	Semi-transparent, clear	Pass
B4	Eudragit L100 1% + Eudragit L100-55 7% + PG 2% + PEG 2%	3 min	Not sticky	Transparent	High	High	Semi-transparent clear,	Pass

Table #22. Results of step 1 and step 2 testes for the complex trial formulations (C1-C4) using ethanol: acetone (80:20) as an evaporating solvent.

No.	Ingredients	Step 1			Step 2			Pass/Fail
		Drying time	**Stickiness**	**Cosmetic appearance**	**Adhesiveness**	**Flexibility**	**Cosmetic appearance**	
C1	Eudragit L100-55 5% + PG 2% + SLS 1% (P3 + SLS 1%)	3 min 15 sec	Sticky	Semi-Transparent	Good	Good	White clusters, not transparent.	Fail
C2	Eudragit L100-55 5% + PG 2% + limonene 1% (P3 + limonene 1%)	3 min 30 sec	Not sticky	Transparent	Good	Good	Transparent, clear	Pass
C3	Eudragit L100-55 5% + PG 2% + Eucalyptol 1% (P3 + Eucalyptol 1%)	3 min 30 sec	Not sticky	Transparent	Good	Good	Transparent, clear	Pass
C4	Eudragit L100-55 5% + PG 2% + transcutol 1% (P3 + transcutol 1%)	4 min	Not sticky	Transparent	Good	Good	Transparent, clear	Pass

Table #23. Results of step 1 and step 2 testes for the complex trial formulations (D1-D5) using ethanol: acetone (80:20) as an evaporating solvent.

No.	Ingredients	Step 1			Step 2			Pass/Fail
		Drying time	Stickiness	Cosmetic appearance	Adhesiveness	Flexibility	Cosmetic appearance	
D1	Eudragit L100-55 8% + PEG 2% + OA 1% + EM 2% (A2+ eutectic mixture 2%)	3 min 30 sec	Non-sticky	Transparent	Good	Good	Semi-transparent, smooth	Pass
D2	Eudragit L100-55 8% + PEG 2% + OA 1% + EM 5% (A2+ EM 5%)	4 min 30 sec	Slightly sticky	Transparent	Good	Good	Semi-transparent, smooth	Fail
D3	Eudragit L100-55 5%+ PG 2%+ Limonene 1% +EM 5% (C2 + EM 5%)	3 min 45 sec	Very sticky	Transparent	Good	Good	Transparent, clear	Fail
D4	Eudragit L100-55 8% + PG 2%+ OA 1% + EM 5% (A1 + EM 5%)	3 min 45 sec	Non-sticky	Transparent	Good	Good	Semi-transparent	Pass
D5	Eudragit L100-55 6% + PVP 1%+ PG 2% + EM 5% (B1 + EM 5%)	4 min 15 sec	Slightly sticky	Transparent	Good	Good	Transparent, clear	Fail

Figures (#17-20) show the ways we used to evaluate stickiness, Cosmetic appearance, adhesiveness, and flexibility. In Figure #21 we applied D4 trial formulation on rubber surface then we try to remove it, we can see how the elastic film was easily stretched and removed.

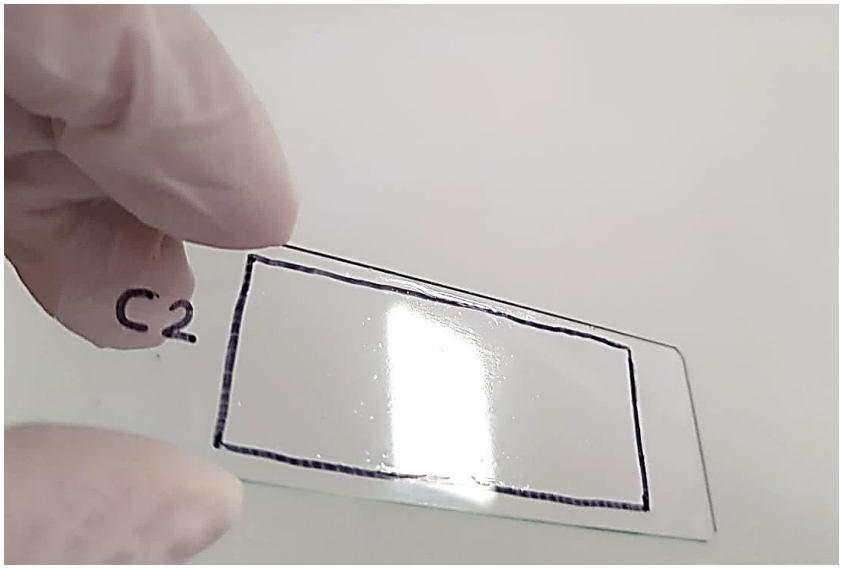


Figure #17. Preparation of dry film (C2) covering 5×2 cm^2 on a slide of glass to check its cosmetic appearance.

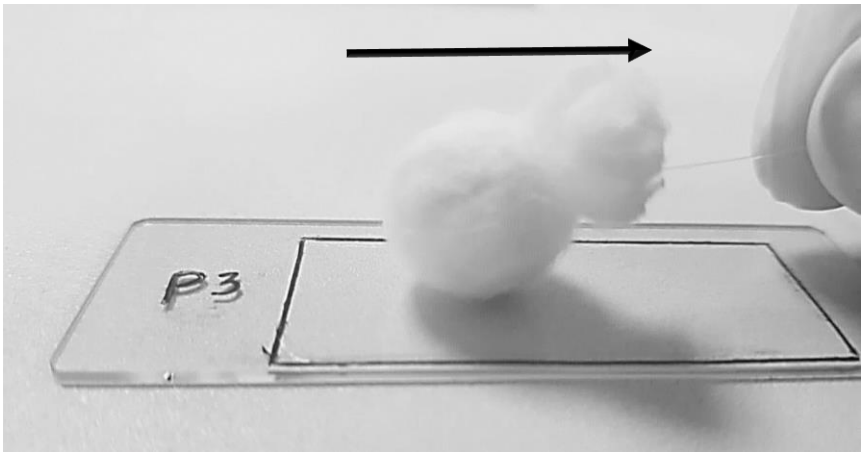


Figure #18. Stickness evaluation test. The arrow showes the direction of pulling the cotton ball.

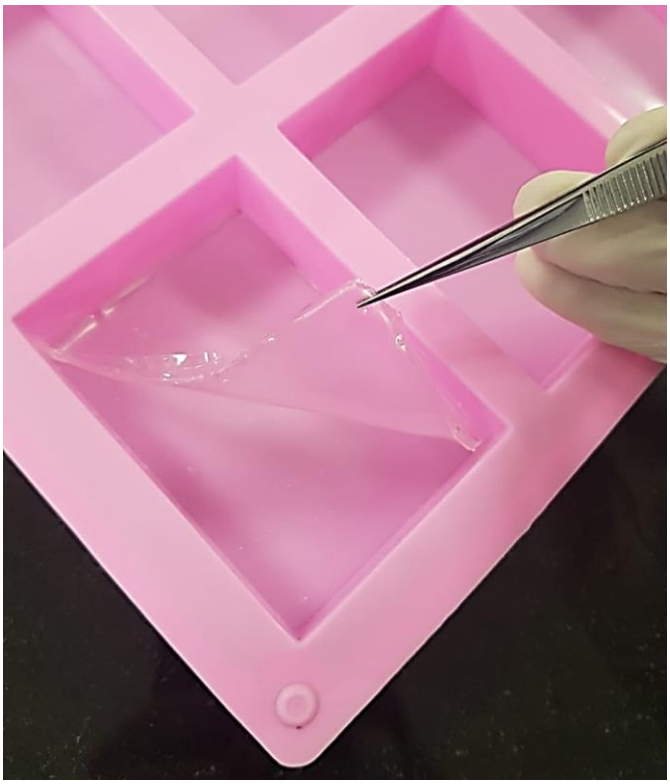


Figure #19. Taking out a thick film (D4) from a mold to evaluate adhesiveness.

Figure #20. Thick film (D4) was rolled up to assess its flexibility.

Figure #21. Removing thin film applied on rubber surface after washing with water.

4.2.2. The successful trial formulations

We test the pH, viscosity, and elasticity of the STFs.

A. pH

The results of pH are shown in Table #26. This test was performed at room temperature (25 ºC ±1) as QC test.

Table #24. the pH of the STFs.

No.	pH
P1	3.5
P3	3.9
A2	3.6
B1	3.5
B3	3.4
B4	3.4
C2	4.1
C3	5.0
C4	4.2
D1	4.7
D4	5.1

B. Viscosity:

Table #27 shows the calculated density for STFs and water, and the time needed for them to flow between the two timing marks in *Ostwald-Cannon-Fenske viscometer*. The viscosity of STFs were calculated by using Eq. #4.

The viscosity for all STFs in Table #27 ranges between 5.1957 and 14.3061 cp. These values are relatively small and acceptable. They were water-like if compared with the viscosity of water (0.8904 cp).

Table #25. The results of viscosity testes for STFs using Ostwald-Cannon-Fenske viscometer.

Name	Density (mg/ml)	Time[1] ± SD (second)	Viscosity (cp)
P1	0.818	42.0 ± 0.1	5.1957
P3	0.814	45.3 ± 0.1	5.5859
A2	0.828	65.6 ±1.3	8.2140
B1	0.823	70.5 ±0.7	8.7714
B3	0.841	95.4 ± 0.8	12.1330
B4	0.834	150.2 ± 1.0	18.9423
C2	0.816	44.5 ± 0.2	5.5018
C3	0.816	45.8 ± 0.1	5.6492
C4	0.817	47.4 ± 0.1	5.8561
D1	0.830	114.0 ± 0.9	14.3061
D4	0.842	87.8 ±1.4	11.1796
water	0.997 [62]	5.9 ± 0.0	0.8904[47]

1: Average flow time between the two timing marks ± standard deviation

C. Module of elasticity:

We performed simple elasticity tests on all STFs to obtain the Young's modulus (E). We used the Eq. #2 and Eq. #3 to calculate stress (σ) and strain ($\mathcal{E}$). The results of the elasticity testes are shown in Tables (#28-36). By using

Eq. #1 we obtained (*E*) for STFs from the slope of the linear segment during the elastic region in stress-strain curves (Figure #22-30).

- **P3 trial formulation:**

Table #26. Results of elasticity tests for P3 trial formulation.

Length L (mm)	(L-L○) ΔL	Strain Ɛ ΔL/L	Weight (g)	Force (N)	Stress σ (N/A*) kPs
24.00	0.00	0.00	0.00	0.00	0.00
24.70	0.70	0.03	20.01	0.20	25.63
24.90	0.90	0.04	27.14	0.27	34.77
25.10	1.10	0.05	34.31	0.34	43z.95
25.25	1.25	0.05	41.44	0.41	53.09
25.45	1.45	0.06	48.61	0.48	62.27
25.65	1.65	0.07	55.75	0.55	71.42
25.80	1.80	0.08	62.91	0.62	80.58
25.95	1.95	0.08	70.07	0.69	89.77
26.05	2.05	0.09	77.24	0.76	98.95
26.10	2.10	0.09	84.39	0.83	108.11
26.15	2.15	0.09	91.54	0.90	117.27
26.20	2.20	0.09	98.67	0.97	126.40
26.25	2.25	0.09	105.83	1.04	135.57
26.30	2.30	0.10	112.92	1.11	144.66
26.35	2.35	0.10	120.08	1.18	153.83
26.60	2.60	0.11	127.24	1.25	163.01
27.00	3.00	0.13	134.42	1.32	172.19
27.70	3.70	0.15	141.58	1.39	181.37
28.30	4.30	0.18	148.74	1.46	190.55
28.90	4.90	0.20	155.90	1.53	199.71
29.20	5.20	0.22	163.41	1.60	209.33
30.20	6.20	0.26	170.56	1.67	218.49
40.90	16.90	0.70	177.71	1.74	227.66

*A = 7.65×10^{-6} m^2

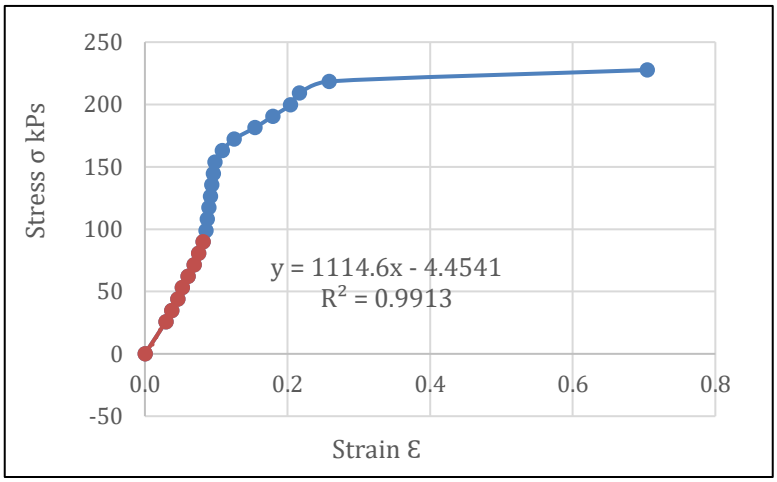


Figure #22. Stress- Strain curve for P3 trial formulation.

- **A2 trial formulation**

Table #27. Results of elasticity tests for A2 trial formulation.

Length L (mm)	(L-L○) ΔL	Strain Ɛ ΔL/L	Weight (g)	Force (N)	Stress σ (N/A*) kPs
23.50	0.00	0.00	0.00	0.00	0.00
23.90	0.40	0.02	3.03	0.03	1.66
24.35	0.85	0.04	5.66	0.06	3.11
24.70	1.20	0.05	8.01	0.08	4.40
25.00	1.50	0.06	10.41	0.10	5.72
26.00	2.50	0.11	12.82	0.13	7.04
26.20	2.70	0.11	15.18	0.15	8.34
26.95	3.45	0.15	17.90	0.18	9.83
27.40	3.90	0.17	20.60	0.20	11.31
90.75	67.25	2.86	22.77	0.22	12.50

*A= 1.785×10^{-5} m^2

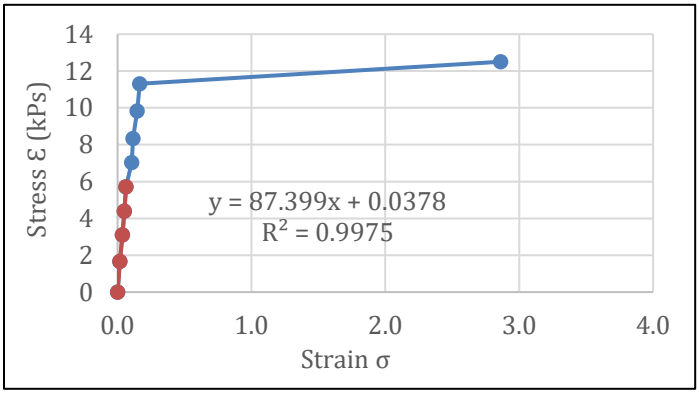


Figure #23. Stress- Strain curve for A2 trial formulation.

- **B1 trial formulation**

Table #28. Results of elasticity tests for B1 trial formulation.

Length L (mm)	(L-L○) ΔL	Strain ε ΔL/L	Weight (g)	Force (N)	Stress σ (N/A*) kPs
37.35	0.00	0.00	0.00	0.00	0.00
38.45	1.10	0.03	48.59	0.48	54.89
38.90	1.55	0.04	63.27	0.62	71.48
39.35	2.00	0.05	77.56	0.76	87.61
39.80	2.45	0.07	91.89	0.90	103.81
40.25	2.90	0.08	106.20	1.04	119.98
40.70	3.35	0.09	120.52	1.18	136.15
41.15	3.80	0.10	134.81	1.32	152.29
42.15	4.80	0.13	149.04	1.46	168.36
43.35	6.00	0.16	163.36	1.60	184.54
100.35	63.00	1.69	220.61	2.16	249.22
*A= 7.1775×10^{-6} m^2					

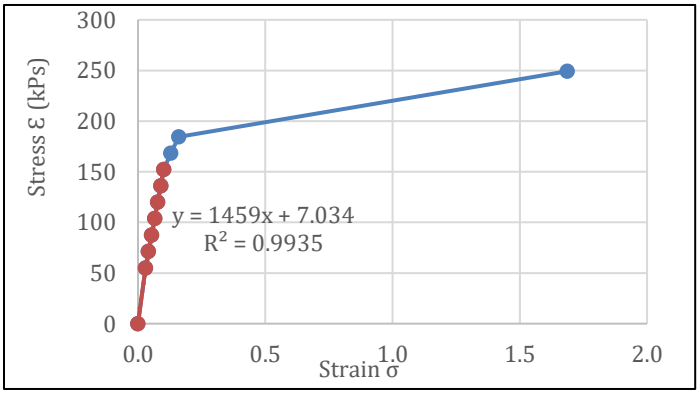


Figure #24. Stress- Strain curve for B1 trial formulation.

- **B4 Trial formulation**

Table #29. Results of elasticity tests for B4 trial formulation.

Length L (mm)	(L-L○) ΔL	Strain ε ΔL/L	Weight (g)	Force (N)	Stress σ (N/A*) kPs
38.58	0.00	0.00	0.00	0.00	0.00
39.85	1.27	0.03	2.95	0.03	2.11
40.45	1.87	0.05	4.58	0.04	3.28
40.90	2.32	0.06	5.84	0.06	4.18
41.35	2.77	0.07	7.05	0.07	5.05
41.80	3.22	0.08	8.32	0.08	5.96
42.25	3.67	0.09	9.57	0.09	6.86
42.70	4.12	0.10	10.83	0.11	7.76
43.15	4.57	0.11	12.09	0.12	8.66
44.45	5.87	0.13	13.35	0.13	9.56
45.10	6.52	0.14	14.61	0.14	10.47
45.95	7.37	0.16	15.88	0.16	11.38
46.85	8.27	0.18	17.14	0.17	12.28
47.95	9.37	0.20	18.41	0.18	13.19
48.90	10.32	0.21	19.67	0.19	14.09
49.70	11.12	0.22	20.93	0.21	15.00
489.50	450.92	0.92	22.20	0.22	15.90

*A= 1.368×10^{-5} m^2

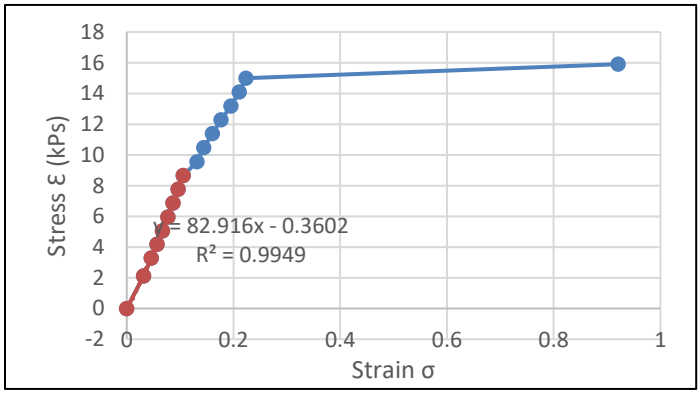


Figure #25. Stress- Strain curve for B4 trial formulation.

- **C2 trial formulation**

Table #30. Results of elasticity tests for C2 trial formulation.

Length L (mm)	(L-L○) ΔL	Strain ε ΔL/L	Weight (g)	Force (N)	Stress σ (N/A*) kPs
31.90	0.00	0.00	0.00	0.00	0.00
32.40	0.50	0.02	20.01	0.20	27.32
32.90	1.00	0.03	34.33	0.34	46.87
33.50	1.60	0.05	48.66	0.48	66.44
34.10	2.20	0.06	62.98	0.62	85.99
34.70	2.80	0.08	77.26	0.76	105.48
35.30	3.40	0.10	91.93	0.90	125.52
37.25	5.35	0.14	106.24	1.04	145.06
38.15	6.25	0.16	120.54	1.18	164.59
39.50	7.60	0.19	134.86	1.32	184.13
40.65	8.75	0.22	149.12	1.46	203.61
42.40	10.50	0.25	163.45	1.60	223.18
111.70	79.80	0.71	177.75	1.74	242.70

*A= 7.1775 × 10^{-6} m^2

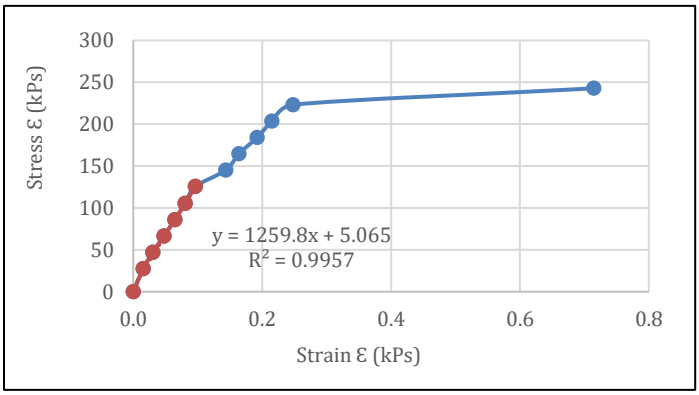


Figure #26. Stress- Strain curve for C2 trial formulation.

- **C3 trial formulation**

Table #31. Results of elasticity tests for C3 trial formulation.

Length L (mm)	(L-L○) ΔL	Strain ε ΔL/L	Weight (g)	Force (N)	Stress σ (N/A*) kPs
35.60	0.00	0.00	0.00	0.00	0.00
36.00	0.40	0.01	34.32	0.34	45.30
36.20	0.60	0.02	48.58	0.48	64.12
36.40	0.80	0.02	62.92	0.62	83.05
36.75	1.15	0.03	77.24	0.76	101.95
37.65	2.05	0.05	91.59	0.90	120.88
38.60	3.00	0.08	105.93	1.04	139.82
39.55	3.95	0.10	120.19	1.18	158.63
42.75	7.15	0.17	134.46	1.32	177.47
45.45	9.85	0.22	148.78	1.46	196.37
47.65	12.05	0.25	163.10	1.60	215.26
50.10	14.50	0.29	177.41	1.74	234.15
53.75	18.15	0.34	192.08	1.88	253.52
57.10	21.50	0.38	206.35	2.02	272.36
79.45	43.85	0.55	220.64	2.16	291.21

*A=7.425 × 10^{-6} m^2

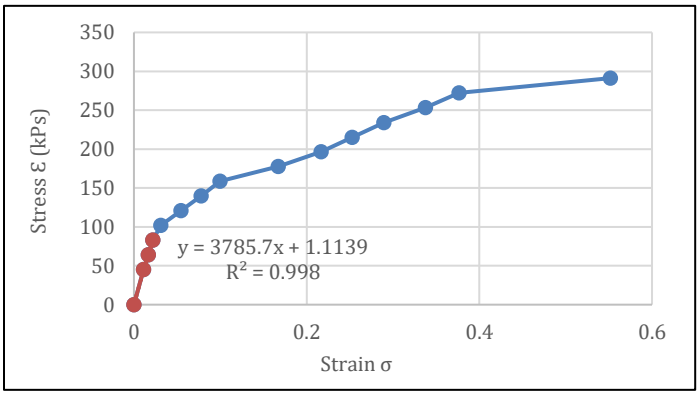


Figure #27. Stress- Strain curve for C3 trial formulation.

- **C4 trial formulation**

Table #32. Results of elasticity tests for C4 trial formulation.

Length L (mm)	(L-L○) ΔL	Strain ε ΔL/L	Weight (g)	Force (N)	Stress σ (N/A*) kPs
34.40	0.00	0.00	0.00	0.00	0.00
34.70	0.30	0.01	4.58	0.04	6.04
35.00	0.60	0.02	9.15	0.09	12.08
35.30	0.90	0.03	13.66	0.13	18.03
35.60	1.20	0.03	17.97	0.18	23.72
36.20	1.80	0.05	22.45	0.22	29.62
36.95	2.55	0.07	26.64	0.26	35.16
37.60	3.20	0.09	31.20	0.31	41.18
38.30	3.90	0.10	35.51	0.35	46.87
39.40	5.00	0.13	39.83	0.39	52.57
40.05	5.65	0.14	44.14	0.43	58.26
40.85	6.45	0.16	48.65	0.48	64.22
41.50	7.10	0.17	53.17	0.52	70.18
112.35	77.95	0.69	57.61	0.56	76.04

*A=7.425 × 10^{-6} m^2

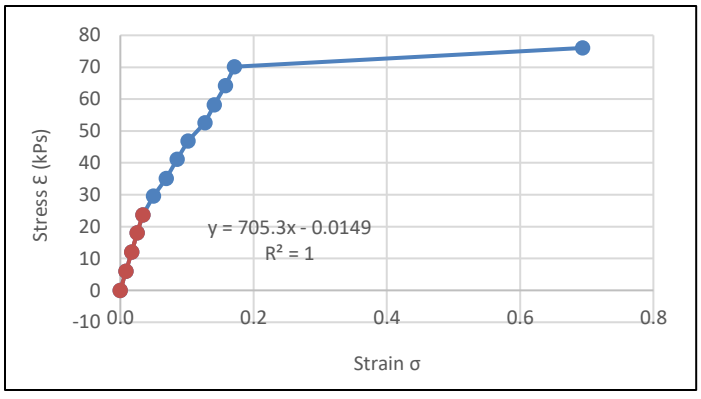


Figure #28. Stress- Strain curve for C4 trial formulation.

- **D1 trial formulation**

Table #33. Results of elasticity tests for D1 trial formulation.

Length L (mm)	(L-L○) ΔL	Strain ε ΔL/L	Weight (g)	Force (N)	Stress σ (N/A*) kPs
22.00	0.00	0.00	0.00	0.00	0.00
22.25	0.25	0.01	4.58	0.04	2.76
22.30	0.30	0.01	5.84	0.06	3.52
22.35	0.35	0.02	6.95	0.07	4.19
22.80	0.80	0.04	8.28	0.08	4.99
23.45	1.45	0.06	9.43	0.09	5.69
23.70	1.70	0.07	10.59	0.10	6.39
24.15	2.15	0.09	11.90	0.12	7.18
30.10	8.10	0.27	13.19	0.13	7.96
31.00	9.00	0.29	14.40	0.14	8.69
32.05	10.05	0.31	15.60	0.15	9.41
33.15	11.15	0.34	16.73	0.16	10.09
34.00	12.00	0.35	17.87	0.18	10.77
35.00	13.00	0.37	19.00	0.19	11.46
36.45	14.45	0.40	20.26	0.20	12.22
37.95	15.95	0.42	21.51	0.21	12.97
190.20	168.20	0.88	22.78	0.22	13.74

*A= 1.625 × 10^{-5} m^2

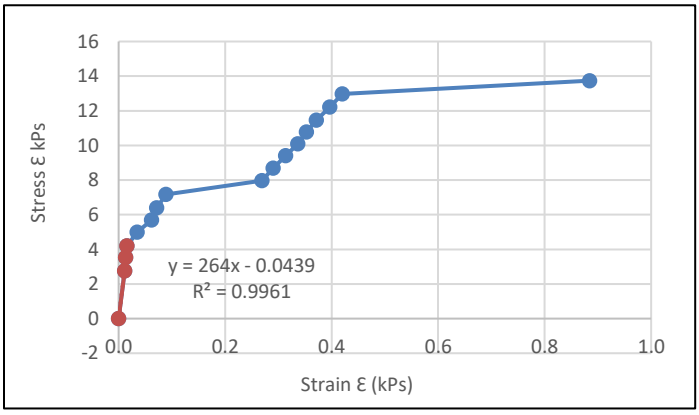


Figure #29. Stress- Strain curve for C4 trial formulation.

- **D4 trial formulation**

Table #34. Results of elasticity tests for D4 trial formulation.

Length L (mm)	(L-L○) ΔL	Strain ε ΔL/L	Weight (g)	Force (N)	Stress σ (N/A*) kPs
31.50	0.00	0.00	0.00	0.00	0.00
33.35	1.85	0.06	5.08	0.05	3.09
33.85	2.35	0.07	6.24	0.06	3.80
34.30	2.80	0.08	7.38	0.07	4.49
34.75	3.25	0.09	8.51	0.08	5.18
35.20	3.70	0.11	9.63	0.09	5.86
36.60	5.10	0.14	10.77	0.11	6.56
37.65	6.15	0.16	11.97	0.12	7.29
38.35	6.85	0.18	13.19	0.13	8.03
39.20	7.70	0.20	14.39	0.14	8.76
99.20	67.70	0.68	15.61	0.15	9.50

*A= 1.61 × 10^{-5} m^2

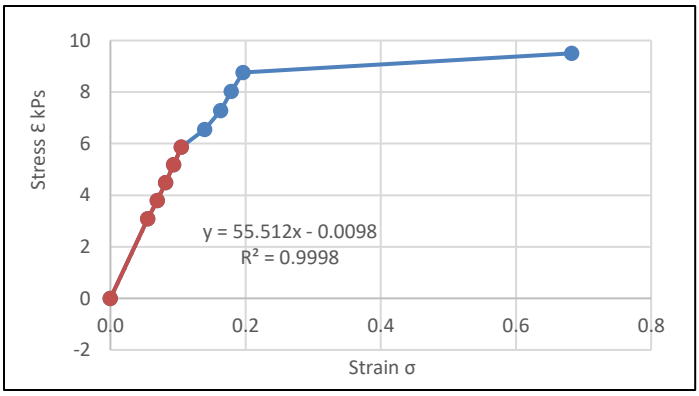


Figure #30. Stress- Strain curve for D4 trial formulation.

Table #35. Summary of The Young's module (E) of the STFs obtained from stress-strain curves.

No.	The Young's module (E) kPs
P1	N.A.
P3	1114.6
A2	87.399
B1	1459
B3	N.A.
B4	82.916
C2	12598
C3	3785.7
C4	705.3
D1	264
D4	55.512

(*E)* can only be determined at a very small strain, and it reflects material rigidity. Rigid material had high (*E*) value (Table #37). A2, B4, and D4 films were the least rigid films, stretched easily under a small load (low stress) and had Low (*E*) values. Both C4 and D1 showed greater resistance to stretching. Higher load was needed to reach the same stain and they had higher (*E*) values. P3, B1, C2 and C3 films were stiffer, they showed much more resistance to stretching and their (*E*) values were much higher especially the C2 film. P1 and B3 were very soft, even very small load (low stress) resulted in very large elongation (high strain) that exceeded the elastic region. Although we couldn't determine (*E*) for them by this method, we would expect them to have low (*E*) values.

Elasticity tests were performed on STFs as QC tests. We compared the (E) values for STFs with the (E) of marketed patches (4-501 MPs) or (4000-501,000 kPs). STFs were softer and more easily stretched than most of patches, which is more comfortable and convenient for patients. We assumed that (E) value less than 501 MPs is acceptable.

4.2.3. The primary formulations

The primary formulations are the trial formulations selected from the STFs loaded with vitamin D3 (P3', B1', C2' and D4'). We chose different films of different ingredients that were expected to have positive effect on vitamin D3 permeation. The primary formulation were characterized by step 1, step2. According to the results in Table #38, we can see that film properties didn't changed too much before and after the addition of vitamin D3. The PVP polymer in B1' formulation was very difficult to be dissolved in the presence of vitamin D3, so we decreased its concentration from 1% to 0.5% in B1' (new) formulation.

Table #36. Characterization of primary formulations containing vitamin D3 6000 IU/ml, using ethanol: acetone (80:20).

No.	Ingredients	Step 1			Step 2		
		Drying time	Stickiness	Cosmetic appearance	Adhesiveness	Flexibility	Cosmetic appearance
P3'	Eudragit L100-55 5% + PG 2% + vitamin D3 6000 IU/ml	3 min. 30 sec.	Non-sticky	Transparent	Good	Good	Transparent, clear
B1' (new)	Eudragit L100-55 6% + PVP 0.5%+ PG 2% + vitamin D3 6000 IU/ml	3 min. 45 sec.	Non-sticky	Transparent	Good	Good	Transparent, clear
C2'	Eudragit L100-55 5% + PG 2% + limonene 1%+ vitamin D3 6000 IU/ml	3 min. 30 sec	Non-sticky	Transparent	Good	Good	Transparent, clear
D4'	Eudragit L100-55 8% + PG 2%+ OA 1% + EM 5% + vitamin D3 6000 IU/ml	3 min. 45 sec.	Non-sticky	Transparent	Good	Good	Semi-transparent

The Primary formulations P3', B1' (new), C2' and D4' in addition to the polymeric formulation #1' loaded with vitamin D3 were subjected to diffusion studies:

4.2.3.1. Diffusion studies

A. In vitro release study using polyamide membrane:

The FFS was applied as solution and gradually the solvents evaporated leaving vitamin D3 dissolved in other excipients. We performed a release study for each liquid formulation (n=3) and also for dry film (n=3) to get complete understanding of vitamin D3 release from both state. The results of vitamin D3 (6000 IU/ml) release from liquid formulations and dry films are shown in Tables (#39-46) and Tables (#47-56) respectively. Each sample taken over time was injected three times in HPLC. Q was measured based on the Eq. #16. We get C_n and C_i from the substitution of average area of the three injection in the linearity equation of the calibration curve, S was (3.14 cm^2), V_R was (20 ml) and V_{col} was (1ml).

From the results of release studies we proof that all formulation were able to release vitamin D3. The release from liquid formulations was significantly more than dry films. Which can be explained by the presence of saturation or supersaturation driving force appeared with the continuous solvent evaporation. The release from dry films was a good indication for the continuous ability of the film to let vitamin D3 get free from the formulation and become available to penetrate the skin if enough driving force were present together with PEs.

A.1. Release studies for liquid formulation

1. Release study of P3' liquid formulation

Table #37. Results of release study for P3' liquid formulation.

#	Time (hr.)	Av. area	C_n (IU/ml)	$Cn*V_R$ (IU)	Ci (IU/ml)	$Ci*V_{col}$ (IU)
SA1	0.25	28.3	6.7	133.1	6.7	6.7
	0.50	57.5	13.0	259.8	13.0	13.0
	1.00	107.3	23.9	478.7	23.9	23.9
	2.00	186.1	41.3	825.5	41.3	41.3
	4.00	320.7	70.9	1417.9	70.9	70.9
	6.00	390.1	86.2	1723.6	86.2	86.2
SA2	0.25	30.9	7.3	145.3	7.3	7.3
	0.50	60.2	13.6	271.5	13.6	13.6
	1.00	100.3	22.4	448.2	22.4	22.4
	2.00	159.0	35.3	706.4	35.3	35.3
	4.00	280.4	62.0	1240.5	62.0	62.0
	6.00	388.3	85.8	1715.4	85.8	85.8
SA3	0.25	26.6	6.3	125.1	6.3	6.3
	0.50	56.1	12.7	253.6	12.7	12.7
	1.00	108.9	24.3	485.7	24.3	24.3
	2.00	190.9	42.3	846.9	42.3	42.3
	4.00	338.9	74.9	1498.1	74.9	74.9
	6.00	394.1	87.1	1741.1	87.1	87.1

Table #38. Results of the cumulative amount released per unit area from P3' liquid formulation.

Time (hr.)	Q (IU/cm^2)			Av. Q (IU/cm^2)	SD	RSD%
	SA1	SA2	SA3			
0.25	42.4	46.3	39.8	42.8	3.2	7.6
0.50	84.9	88.8	82.8	85.5	3.1	3.6
1.00	158.7	149.4	160.7	156.3	6.1	3.9
2.00	276.8	238.7	283.5	266.3	24.1	9.1
4.00	478.6	420.1	504.4	467.7	43.2	9.2
6.00	598.5	591.1	605.6	598.4	7.3	1.2

2. Release study of B1' (new) liquid formulation

Table #39. Results of release study for B1' (new) liquid formulation**.**

#	Time (hr.)	Av. area	C_n (IU/ml)	$Cn*V_R$ (IU)	Ci (IU/ml)	$Ci*V_{col}$ (IU)
SA1	1	37.2	8.7	174.4	8.7	8.7
	2	117.4	26.2	523.4	26.2	26.2
	3	191.9	42.6	851.2	42.6	42.6
	4	243.4	53.9	1077.8	53.9	53.9
	5	272.6	60.3	1206.2	60.3	60.3
	6	337.4	74.6	1491.4	74.6	74.6
SA2	1	61.7	13.9	278.1	13.9	13.9
	2	150.1	33.4	667.3	33.4	33.4
	3	218.8	48.5	969.7	48.5	48.5
	4	272.8	60.4	1207.2	60.4	60.4
	5	319.2	70.6	1411.3	70.6	70.6
	6	390.8	86.3	1726.5	86.3	86.3
SA3	1	47.4	11.1	221.6	11.1	11.1
	2	135.0	30.0	600.7	30.0	30.0
	3	180.0	39.9	798.6	39.9	39.9
	4	228.1	50.5	1010.3	50.5	50.5
	5	282.2	62.4	1248.6	62.4	62.4
	6	371.4	82.1	1641.0	82.1	82.1

Table #40. Results of cumulative amount released per unit area from B1' (new) liquid formulation.

Time (hr.)	Q (IU/cm^2)			Av. Q (IU/cm^2)	SD	RSD%
	SA1	SA2	SA3			
1	55.6	88.6	70.6	71.6	16.5	23.1
2	169.5	217.0	194.8	193.8	23.8	12.3
3	282.2	323.9	267.4	291.2	29.3	10.1
4	367.9	415.0	347.6	376.8	34.6	9.2
5	426.0	499.2	439.5	454.9	38.9	8.6
6	536.0	622.0	584.4	580.8	43.1	7.4

3. Release study of C2' liquid formulation

Table #41. Results of release study for C2' liquid formulation.

#	Time (hr.)	Av. area	C_n (IU/ml)	$Cn*V_R$ (IU)	Ci (IU/ml)	$Ci*V_{col}$ (IU)
SA1	1	51.5	12.0	240.5	12.0	12.0
	2	95.1	21.2	425.0	21.2	21.2
	3	138.4	30.8	615.7	30.8	30.8
	4	192.0	42.6	851.4	42.6	42.6
	5	224.5	49.7	994.6	49.7	49.7
	6	274.4	60.7	1214.4	60.7	60.7
SA2	1	66.5	15.0	299.3	15.0	15.0
	2	112.8	25.1	502.9	25.1	25.1
	3	153.4	34.1	681.7	34.1	34.1
	4	189.9	42.1	842.4	42.1	42.1
	5	213.2	47.2	944.7	47.2	47.2
	6	254.3	56.3	1125.9	56.3	56.3
SA3	1	63.6	14.3	286.6	14.3	14.3
	2	115.1	25.7	513.0	25.7	25.7
	3	156.8	34.8	696.8	34.8	34.8
	4	203.9	45.2	904.0	45.2	45.2
	5	224.5	49.7	994.5	49.7	49.7
	6	272.0	60.2	1203.8	60.2	60.2

Table #42. Results of the cumulative amount released per unit area from C2' liquid formulation.

Time (hr.)	Q (IU/cm^2)			Av. Q (IU/cm^2)	SD	RSD%
	SA1	SA2	SA3			
1	76.6	95.3	91.3	87.7	9.8	11.2
2	139.2	164.9	167.9	157.3	15.8	10.0
3	206.7	229.9	234.7	223.7	15.0	6.7
4	291.6	291.9	311.7	298.4	11.5	3.9
5	350.7	337.9	354.9	347.9	8.9	2.5
6	436.5	410.7	437.4	428.2	15.2	3.6

4. Release study of D4' liquid formulation

Table #43. Results of release study for D4' liquid formulation.

#	Time (hr.)	Av. area	C_n (IU/ml)	$Cn*V_R$ (IU)	Ci (IU/ml)	$Ci*V_{col}$ (IU)
SA1	1	36.2	8.5	169.9	8.5	8.5
	2	77.5	17.4	347.8	17.4	17.4
	3	114.1	25.4	508.9	25.4	25.4
	4	271.1	60.0	1199.9	60.0	60.0
	5	300.2	66.4	1327.7	66.4	66.4
	6	366.2	80.9	1618.3	80.9	80.9
SA2	1	80.1	24.8	495.6	24.8	24.8
	2	129.9	28.9	578.1	28.9	28.9
	3	168.7	37.5	749.2	37.5	37.5
	4	212.0	47.0	939.5	47.0	47.0
	5	232.3	51.5	1029.1	51.5	51.5
	6	263.1	58.2	1164.7	58.2	58.2
SA3	1	62.2	20.4	408.5	20.4	20.4
	2	114.3	25.5	509.8	25.5	25.5
	3	163.7	36.4	727.2	36.4	36.4
	4	188.2	41.7	834.9	41.7	41.7
	5	212.9	47.2	943.7	47.2	47.2
	6	341.9	75.6	1511.5	75.6	75.6

Table #44. Results of the cumulative amount released per unit area from D4' liquid formulation.

Time (hr.)	Q (IU/cm^2)			Av. Q (IU/cm^2)	SD	RSD%
	SA1	SA2	SA3			
1	54.1	157.8	130.1	114.0	53.7	47.1
2	113.5	192.0	168.9	158.1	40.4	25.5
3	170.3	255.7	246.2	224.1	46.8	20.9
4	398.5	328.2	292.1	339.6	54.1	15.9
5	458.3	371.7	340.0	390.0	61.2	15.7
6	572.0	431.3	535.9	513.0	73.1	14.2

A.2. Release studies of dry films

1. Release study from #1 dry film

Table #45. Results of release study for #1' dry film.

#	Time (hr.)	Av. area	C_n (IU/ml)	$Cn*V_R$ (IU)	Ci (IU/ml)	$Ci*V_{col}$ (IU)
SA1	0.5	Not detected	-	-	-	-
	1.0	2.9	0.8	16.1	0.8	0.8
	2.0	14.6	3.5	70.2	3.5	3.5
	3.0	33.7	7.9	158.3	7.9	7.9
	4.0	44.1	10.3	206.2	10.3	10.3
	6.0	66.0	15.4	307.7	15.4	15.4
SA2	0.5	Not detected	-	-	-	-
	1.0	6.3	1.6	31.5	1.6	1.6
	2.0	21.5	5.1	102.1	5.1	5.1
	3.0	45.8	10.7	214.3	10.7	10.7
	4.0	56.9	13.3	265.3	13.3	13.3
	6.0	91.8	21.3	426.9	21.3	21.3
SA3	0.5	3.1	0.8	16.7	0.8	0.8
	1.0	8.6	2.1	42.3	2.1	2.1
	2.0	17.4	4.1	83.0	4.1	4.1
	3.0	34.5	8.1	162.0	8.1	8.1
	4.0	41.5	9.7	194.2	9.7	9.7
	6.0	61.8	14.4	288.1	14.4	14.4

Table #46. Results of the cumulative amount released per unit area from #1' dry film.

Time (hr.)	Q (IU/cm^2)			Av. Q (IU/cm^2)	SD	RSD%
	SA1	SA2	SA3			
0.5	Not detected	Not detected	5.3	-	-	-
1.0	5.1	10.0	13.7	9.6	4.3	44.8
2.0	22.6	33.0	27.4	27.7	5.2	18.8
3.0	51.8	70.4	53.8	58.7	10.2	17.4
4.0	69.6	90.0	66.7	75.4	12.7	16.9
6.0	105.2	145.7	99.7	116.9	25.1	21.5

2. Release study from P3' dry film

Table #47. Results of release study for P3' dry film.

#	Time (hr.)	Av. area	C_n (IU/ml)	$Cn*V_R$ (IU)	Ci (IU/ml)	$Ci*V_{col}$ (IU)
SA1	0.5	2.2	0.6	12.9	0.6	0.6
	1.0	11.0	2.7	53.2	2.7	2.7
	2.0	31.1	7.3	146.3	7.3	7.3
	3.0	46.5	10.9	217.6	10.9	10.9
	4.0	60.3	14.0	280.9	14.0	14.0
	6.0	107.4	24.9	498.8	24.9	24.9
SA2	0.5	3.1	0.8	16.9	0.8	0.8
	1.0	11.4	2.8	55.2	2.8	2.8
	2.0	39.0	9.1	182.6	9.1	9.1
	3.0	54.2	12.7	253.1	12.7	12.7
	4.0	66.1	15.4	307.9	15.4	15.4
	6.0	111.2	25.8	516.2	25.8	25.8
SA3	0.5	3.3	0.9	17.8	0.9	0.9
	1.0	11.2	2.7	54.2	2.7	2.7
	2.0	31.4	7.4	147.8	7.4	7.4
	3.0	50.3	11.7	234.8	11.7	11.7
	4.0	64.9	15.1	302.4	15.1	15.1
	6.0	107.8	25.0	500.6	25.0	25.0

Table #48. Results of the cumulative amount released per unit area from P3' dry film.

Time (hr.)	Q (IU/cm^2)			Av. Q (IU/cm^2)	SD	RSD%
	SA1	SA2	SA3			
0.5	4.1	5.4	5.7	5.1	0.8	16.5
1.0	17.2	17.9	17.5	17.5	0.4	2.0
2.0	47.6	59.3	48.2	51.7	6.6	12.7
3.0	72.7	84.7	78.3	78.5	6.0	7.6
4.0	96.3	106.1	103.6	102.0	5.1	5.0
6.0	170.2	177.4	171.5	173.0	3.8	2.2

3. Release study from B1' (new) dry film

Table #49. Results of release study for B1' (new) dry film.

#	Time (hr.)	Av. area	C_n (IU/ml)	$Cn*V_R$ (IU)	Ci (IU/ml)	$Ci*V_{col}$ (IU)
SA1	0.5	2.1	0.6	12.2	0.6	0.6
	1.0	8.4	2.1	41.6	2.1	2.1
	2.0	21.9	5.2	103.8	5.2	5.2
	3.0	38.8	9.1	181.6	9.1	9.1
	4.0	42.7	10.0	199.7	10.0	10.0
	6.0	84.0	18.8	376.1	18.8	18.8
SA2	0.5	2.6	0.7	14.6	0.7	0.7
	1.0	10.9	2.6	52.8	2.6	2.6
	2.0	26.8	6.3	126.2	6.3	6.3
	3.0	43.2	10.1	202.2	10.1	10.1
	4.0	57.1	12.9	257.7	12.9	12.9
	6.0	96.7	21.6	432.3	21.6	21.6
SA3	0.5	1.8	0.5	10.9	0.5	0.5
	1.0	8.5	2.1	42.0	2.1	2.1
	2.0	23.6	5.6	111.8	5.6	5.6
	3.0	40.3	9.4	188.8	9.4	9.4
	4.0	56.9	12.9	257.2	12.9	12.9
	6.0	82.6	18.5	370.0	18.5	18.5

Table #50. Results of the cumulative amount released per unit area from B1' (new) dry.

Time (hr.)	Q (IU/cm^2)			Av. Q (IU/cm^2)	SD	RSD%
	SA1	SA2	SA3			
0.5	3.9	4.6	3.5	4.0	0.6	14.7
1.0	13.4	17.0	13.5	14.7	2.1	14.0
2.0	33.9	41.3	36.4	37.2	3.7	10.1
3.0	60.3	67.5	62.7	63.5	3.6	5.7
4.0	69.0	88.4	87.5	81.6	11.0	13.4
6.0	128.4	148.1	127.5	134.7	11.6	8.6

4. Release study from C2' dry film

Table #51. Results of release study for C2' dry film.

#	Time (hr.)	Av. area	C_n (IU/ml)	$Cn*V_R$ (IU)	Ci (IU/ml)	$Ci*V_{col}$ (IU)
SA1	0.5	1.3*	0.4	8.5	0.4	0.4
	1.0	5.7	1.4	29.0	1.4	1.4
	2.0	20.7	4.9	98.1	4.9	4.9
	3.0	38.1	8.9	178.4	8.9	8.9
	4.0	59.5	13.9	277.5	13.9	13.9
	6.0	88.6	20.6	411.9	20.6	20.6
SA2	0.5	1.9	0.6	11.1	0.6	0.6
	1.0	7.8	1.9	38.7	1.9	1.9
	2.0	23.2	5.5	109.6	5.5	5.5
	3.0	45.9	10.7	214.6	10.7	10.7
	4.0	69.8	16.2	324.8	16.2	16.2
	6.0	106.4	24.7	494.2	24.7	24.7
SA3	0.5	1.7	0.5	10.4	0.5	0.5
	1.0	5.9	1.5	29.7	1.5	1.5
	2.0	21.2	5.0	100.7	5.0	5.0
	3.0	42.1	9.8	196.9	9.8	9.8
	4.0	65.7	15.3	306.1	15.3	15.3
	6.0	96.5	22.4	448.4	22.4	22.4

* 1.3 and 1.7 are out of linearity range, but more than LOQ

Table #52. Results of the cumulative amount released per unit area from C2' dry film.

Time (hr.)	Q (IU/cm^2)			Av. Q (IU/cm^2)	SD	RSD%
	SA1	SA2	SA3			
0.5	2.7	3.5	3.3	3.2	0.4	13.3
1.0	9.4	12.5	9.6	10.5	1.7	16.6
2.0	31.8	35.7	32.7	33.4	2.0	6.1
3.0	59.0	70.9	65.0	64.9	6.0	9.2
4.0	93.4	109.4	102.9	101.9	8.1	7.9
6.0	140.6	168.5	153.1	154.1	14.0	9.1

5. Release study from D4' dry film

Table #53. Results of release study for D4' dry film.

#	Time (hr.)	Av. area	C_n (IU/ml)	$Cn*V_R$ (IU)	Ci (IU/ml)	$Ci*V_{col}$ (IU)
SA1	0.5	Not detected	-	-	-	-
	1.0	2.2	0.6	12.6	0.6	0.6
	2.0	9.3	2.3	45.5	2.3	2.3
	3.0	18.8	4.5	89.6	4.5	4.5
	4.0	25.6	6.0	120.6	6.0	6.0
	6.0	40.6	9.5	190.3	9.5	9.5
SA2	0.5	Not detected	-	-	-	-
	1.0	1.6	0.5	9.9	0.5	0.5
	2.0	9.7	2.4	47.2	2.4	2.4
	3.0	18.5	4.4	87.9	4.4	4.4
	4.0	25.6	6.0	120.8	6.0	6.0
	6.0	42.5	10.0	199.1	10.0	10.0
SA3	0.5	Not detected	-	-	-	-
	1.0	3.4	0.9	18.2	0.9	0.9
	2.0	12.9	3.1	62.3	3.1	3.1
	3.0	21.0	5.0	99.4	5.0	5.0
	4.0	27.5	6.5	129.6	6.5	6.5
	6.0	46.7	10.9	218.3	10.9	10.9

Table #54. Results of the cumulative amount released per unit area from D4' dry film.

Time (hr.)	Q (IU/cm^2)			Av. Q (IU/cm^2)	SD	RSD%
	SA1	SA2	SA3			
0.5	-	-	-	-	-	-
1.0	4.0	3.2	5.8	4.3	1.3	31.0
2.0	14.7	15.2	20.1	16.7	3.0	18.0
3.0	29.5	28.9	33.0	30.4	2.2	7.2
4.0	40.8	40.8	44.1	41.9	1.9	4.6
6.0	64.9	67.6	74.5	69.0	4.9	7.2

Immediately after loading the formulations into the donor compartment, ethanol and acetone begun to evaporate very quickly. This increased the concentration of vitamin D3 which gradually reached saturation or even supersaturation, and formed a driving force on vitamin D3 to leave the formulation and diffuse through the soaked membrane to reach the receptor fluid where the sink condition is.

In Figure #31 we can see a comparison between the fluxes (Q per unit time) for liquid formulations. We got the flux (*f*) from the slope of the linear part at steady state. For all batches, (*f*) reached steady state after about 2 hours. (*Q*) was relatively high at 1 hour. To more accurately check the release start time, we sampled earlier in P3' at 0.25 hr. and 0.5 hr., and the results showed that the release had already started. This indicates that the release of vitamin D3 from the liquid formulations started instantly or very close to zero time. The descending order for (*f*) values was: B1' (new)> P3' > D4'> C2'.

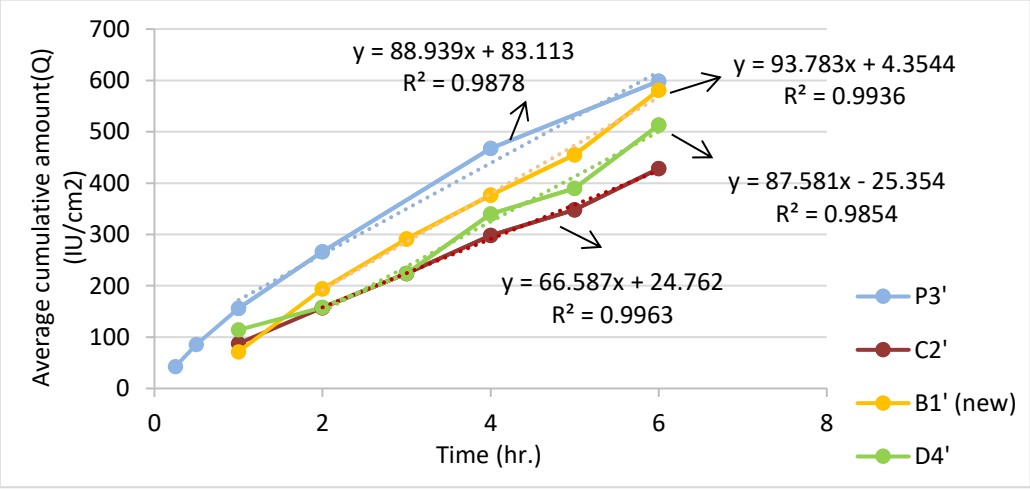


Figure #31. The average cumulative amount released per unit area versus time for liquid formulations (P3', C2', B1' (new) and D4').

All formulations contained the same percentage of PG. The release amount was the best in case of P3' liquid formulation which contained PG (2%) and Eudragit L100-55 (5%) dissolved in the ethanol: acetone (80:20). In addition to PG's solubilizing and plasticizing effects, it acts as penetration enhancer[43]. Vitamin D3 is sparingly soluble in PG[2], while it is considered soluble in the receptor fluid ethanol: PBS pH 7.4 (110.22 ± 3.02 µg/ml) [11], this promotes the release of vitamin D3 and its penetration through the polyamide membrane to reach the receptor fluid where it is more soluble under sink condition.

The addition of the hydrophilic polymer PVP (0.5%) with Eudragit L100-55 (6%) in B1' (new) decreased the enhancement in the release. This may be due to the increase in the percentage of Eudragit L100-55 that may counteract the driving force formed by saturation and that may mask the effect of the hydrophilic polymer PVP. The same thing was seen in D4' where Eudragit percentage was 8%. Also the presence of PEs those have the ability to dissolve vitamin D3 (non-evaporating solvents) such as EM, OA and Limonene can explain the negative effect on the release compared with P3'. The descending order for (Q) released after 6 hours. was P3'> B1' (new) > D4'> C2'.

The release from dry films are shown in Figure #32. We tested the release from the polymeric film #1' too, which contained Eudragit L100-55 (5%) and vitamin D3 without any other components. The addition of PG alone in P3' film

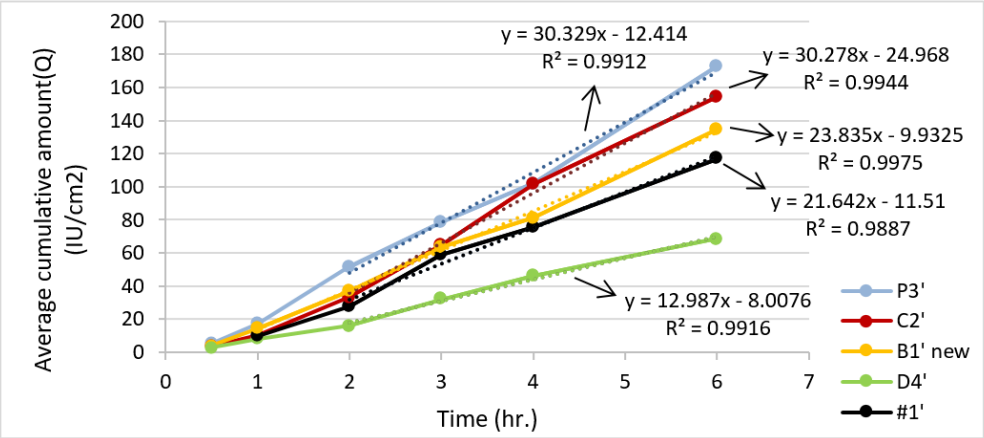


Figure #32. The average cumulative amount released per unit area per versus time for dry films (P3', C2', B1' (new), D4' and #1).

had the best positive effect on the release as seen previously in liquid state. The release form C2' and B1' (new) films were also better than that for #1' film. However, in comparison with P3', the addition of Limonene in C2' and the increase in the total polymer concentration in B1' (new) film reduced the enhancement in release. While the combination of the EM and OA with increasing polymer concentration in D4' film had a negative effect on release. The descending order of (Q) for dry films was different from that for the liquid formulations: P3'> C2'> B1' (new)> #1> D4'.

B. In vitro permeation study using Strat-M® membrane

The results of permeation studies conducted on P3', B1' (new), C2' and D4' are shown in Tables (#57-64). Each formulation was tested in triplicate (n=3). Samples were taken with time and injected three times in HPLC. From the average

area, the concentration was calculated by using the equation of the calibration curve. Then the cumulative permeated amount was calculated by using Eq. #16. We put small volume of sample (0.5 ml) to enable studying the permeation from both liquid and dry state. In this way we try to mimic the real application on skin over 24 hours.

1. Permeation study from P3' formulation

Table #55. Results of permeation study for P3' formulation

#	Time (hr.)	Av. area	C_n (IU/ml)	$Cn*V_R$ (IU)	Ci (IU/ml)	$Ci*V_{col}$ (IU)
SA1	0.5	24.2	5.7	114.3	5.7	5.7
	1.0	37.3	8.7	174.9	8.7	8.7
	2.0	53.7	12.1	242.8	12.1	12.1
	3.0	58.8	13.3	265.2	13.3	13.3
	4.0	65.3	14.7	294.0	14.7	14.7
	6.0	68.5	15.4	308.1	15.4	15.4
	7.0	73.7	16.5	330.9	16.5	16.5
	8.0	80.6	18.1	361.3	18.1	18.1
	24.0	157.5	35.0	699.8	35.0	35.0
SA2	0.5	21.3	5.1	101.1	5.1	5.1
	1.0	30.7	7.2	144.5	7.2	7.2
	2.0	53.0	12.0	239.7	12.0	12.0
	3.0	56.0	12.7	253.0	12.7	12.7
	4.0	60.9	13.7	274.6	13.7	13.7
	6.0	67.6	15.2	304.2	15.2	15.2
	7.0	71.4	16.0	320.8	16.0	16.0
	8.0	78.3	17.6	351.3	17.6	17.6
	24.0	158.1	35.1	702.4	35.1	35.1
SA3	0.5	19.9	4.7	94.3	4.7	4.7
	1.0	29.9	7.0	140.5	7.0	7.0
	2.0	53.3	12.1	241.3	12.1	12.1
	3.0	57.1	12.9	258.0	12.9	12.9
	4.0	62.0	14.0	279.3	14.0	14.0
	6.0	68.2	15.3	306.6	15.3	15.3
	7.0	71.8	16.1	322.7	16.1	16.1
	8.0	79.7	17.9	357.4	17.9	17.9
	24.0	162.7	36.1	722.8	36.1	36.1

Table #56. Results of the cumulative amount permeated per unit area from P3' formulation.

Time (hr.)	Q (IU/cm^2)			Av. Q (IU/cm^2)	SD	RSD%
	SA1	SA2	SA3			
0.5	36.4	32.2	30.0	32.9	3.2	9.9
1.0	57.5	47.6	46.3	50.5	6.1	12.2
2.0	81.9	80.2	80.6	80.9	0.9	1.1
3.0	92.9	88.3	89.8	90.3	2.4	2.6
4.0	106.3	99.2	100.6	102.1	3.8	3.7
6.0	119.1	116.6	117.5	117.7	1.3	1.1
7.0	128.5	124.4	125.3	126.1	2.1	1.7
8.0	139.6	135.4	137.6	137.5	2.1	1.5
24.0	248.9	248.8	255.6	251.1	3.9	1.6

2. Permeation study from B1' (new) formulation

Table #57. Results of permeation study for B1' (new) formulation

#	Time (hr.)	Av. area	C_n (IU/ml)	$Cn*V_R$ (IU)	Ci (IU/ml)	$Ci*V_{col}$ (IU)
SA1	0.5	20.3	4.8	96.2	4.8	4.8
	1.0	53.2	12.0	240.6	12.0	12.0
	2.0	66.3	14.9	298.5	14.9	14.9
	3.0	68.8	15.5	309.2	15.5	15.5
	4.0	71.2	16.0	319.9	16.0	16.0
	6.00	78.0	17.5	349.9	17.5	17.5
	7.00	80.4	18.0	360.3	18.0	18.0
	8.00	86.1	19.3	385.7	19.3	19.3
	24.00	159.3	35.4	707.8	35.4	35.4
SA2	0.5	19.9	4.7	94.3	4.7	4.7
	1.0	52.4	11.9	237.1	11.9	11.9
	2.0	56.9	12.9	257.0	12.9	12.9
	3.0	68.0	15.3	305.7	15.3	15.3
	4.0	70.3	15.8	316.0	15.8	15.8
	6.0	77.5	17.4	347.5	17.4	17.4
	7.0	79.2	17.8	355.3	17.8	17.8
	8.0	84.3	18.9	377.7	18.9	18.9
	24.0	157.9	35.1	701.4	35.1	35.1
SA3	0.5	24.9	5.9	117.4	5.9	5.9
	1.0	44.9	10.2	204.2	10.2	10.2
	2.0	67.8	15.2	305.0	15.2	15.2
	3.0	75.8	17.0	340.0	17.0	17.0
	4.0	78.4	17.6	351.8	17.6	17.6
	6.0	83.8	18.8	375.2	18.8	18.8
	7.0	85.4	19.1	382.3	19.1	19.1
	8.0	88.6	19.8	396.7	19.8	19.8
	24.0	168.4	37.4	747.6	37.4	37.4

Table #58. Results of the cumulative amount permeated per unit area from B1' (new) formulation.

Time (hr.)	Q (IU/cm^2)			Av. Q (IU/cm^2)	SD	RSD%
	SA1	SA2	SA3			
0.5	30.6	30.0	37.4	32.7	4.1	12.5
1.0	78.1	77.0	66.9	74.0	6.2	8.4
2.0	100.4	87.1	102.2	96.6	8.3	8.5
3.0	108.6	106.7	118.3	111.2	6.2	5.6
4.0	116.9	114.9	127.4	119.7	6.7	5.6
6.0	135.5	133.9	144.5	138.0	5.8	4.2
7.0	140.5	138.1	149.5	142.7	6.0	4.2
8.0	149.6	146.8	155.3	150.6	4.3	2.9
24.0	253.4	251.0	268.0	257.5	9.2	3.6

3. Permeation study from C2' formulation

Table #59. Results of permeation study for C2' formulation

#	Time (hr.)	Av. area	C_n (IU/ml)	$Cn*V_R$ (IU)	Ci (IU/ml)	$Ci*V_{col}$ (IU)
SA1	0.5	22.4	5.3	106.0	5.3	5.3
	1.0	40.2	9.4	188.1	9.4	9.4
	2.0	60.5	13.6	272.7	13.6	13.6
	3.0	63.3	14.3	285.3	14.3	14.3
	4.0	67.9	15.3	305.6	15.3	15.3
	6.00	74.1	16.6	332.7	16.6	16.6
	7.00	76.9	17.3	345.0	17.3	17.3
	8.00	88.2	19.7	394.6	19.7	19.7
	24.00	157.6	35.0	700.3	35.0	35.0
SA2	0.5	20.8	4.9	98.8	4.9	4.9
	1.0	39.4	9.2	184.6	9.2	9.2
	2.0	59.2	13.4	267.1	13.4	13.4
	3.0	61.4	13.8	277.0	13.8	13.8
	4.0	68.7	15.5	309.1	15.5	15.5
	6.0	73.7	16.5	330.8	16.5	16.5
	7.0	78.3	17.6	351.3	17.6	17.6
	8.0	90.4	20.2	404.4	20.2	20.2
	24.0	159.0	35.3	706.5	35.3	35.3
SA3	0.5	18.5	4.4	87.7	4.4	4.4
	1.0	37.7	8.8	176.4	8.8	8.8
	2.0	59.1	13.3	266.7	13.3	13.3
	3.0	62.8	14.1	282.8	14.1	14.1
	4.0	71.5	16.1	321.1	16.1	16.1
	6.0	74.2	16.7	333.1	16.7	16.7
	7.0	78.9	17.7	353.8	17.7	17.7
	8.0	92.5	20.7	413.5	20.7	20.7
	24.0	159.1	35.3	706.8	35.3	35.3

Table #60. Results of the cumulative amount permeated per unit area from C2' formulation.

Time (hr.)	Q (IU/cm^2)			Av. Q (IU/cm^²)	SD	RSD%
	SA1	SA2	SA3			
0.5	33.8	31.5	27.9	31.0	2.9	9.5
1.0	61.6	60.4	57.6	59.8	2.1	3.4
2.0	91.5	89.6	89.1	90.1	1.3	1.4
3.0	99.9	97.0	98.5	98.5	1.5	1.5
4.0	110.9	111.6	115.2	112.6	2.3	2.1
6.0	128.2	127.4	128.5	128.0	0.6	0.4
7.0	134.4	136.3	137.6	136.1	1.6	1.2
8.0	151.4	154.5	158.0	154.6	3.3	2.1
24.0	250.5	252.8	253.4	252.2	1.6	0.6

4. Permeation study from D4' formulation

Table #61. Results of permeation study for D4' formulation

#	Time (hr.)	Av. area	C_n (IU/ml)	$Cn*V_R$ (IU)	Ci (IU/ml)	$Ci*V_{col}$ (IU)
SA1	0.5	10.4	2.5	50.7	2.5	2.5
	1.0	20.7	4.9	98.2	4.9	4.9
	2.0	33.8	7.9	158.5	7.9	7.9
	3.0	37.1	8.7	173.9	8.7	8.7
	4.0	37.5	8.8	175.9	8.8	8.8
	6.00	38.6	9.1	181.0	9.1	9.1
	7.00	39.6	9.3	185.6	9.3	9.3
	8.00	41.2	9.6	192.7	9.6	9.6
	24.00	73.1	16.4	328.3	16.4	16.4
SA2	0.5	9.7	2.4	47.2	2.4	2.4
	1.0	22.5	5.3	106.3	5.3	5.3
	2.0	40.2	9.4	188.1	9.4	9.4
	3.0	42.5	9.9	198.7	9.9	9.9
	4.0	52.5	11.9	237.6	11.9	11.9
	6.0	52.0	11.8	235.4	11.8	11.8
	7.0	53.7	12.1	242.9	12.1	12.1
	8.0	53.4	12.1	241.5	12.1	12.1
	24.0	98.6	22.0	440.5	22.0	22.0
SA3	0.5	11.9	2.9	57.2	2.9	2.9
	1.0	23.7	5.6	111.5	5.6	5.6
	2.0	41.1	9.6	192.3	9.6	9.6
	3.0	43.2	10.1	202.0	10.1	10.1
	4.0	53.5	12.1	241.9	12.1	12.1
	6.0	12.1	241.0	12.1	12.1	92.5
	7.0	12.3	245.6	12.3	12.3	96.0
	8.0	12.4	247.3	12.4	12.4	97.4
	24.0	22.6	451.8	22.6	22.6	163.3

Table #62. Results of the cumulative amount permeated per unit area from D4' formulation.

Time (hr.)	Q (IU/cm^2)			Av. Q (IU/cm^²)	SD	RSD%
	SA1	SA2	SA3			
0.5	16.2	15.0	18.2	16.5	1.6	9.8
1.0	32.1	34.6	36.4	34.4	2.2	6.4
2.0	52.9	62.4	63.9	59.7	6.0	10.0
3.0	60.3	68.7	70.1	66.4	5.3	8.0
4.0	63.7	84.3	86.0	78.0	12.4	15.9
6.0	70.3	90.4	92.5	84.4	12.3	14.5
7.0	73.1	94.8	96.0	88.0	12.9	14.7
8.0	75.8	95.2	97.4	89.5	11.9	13.3
24.0	119.2	159.3	163.3	147.3	24.4	16.5

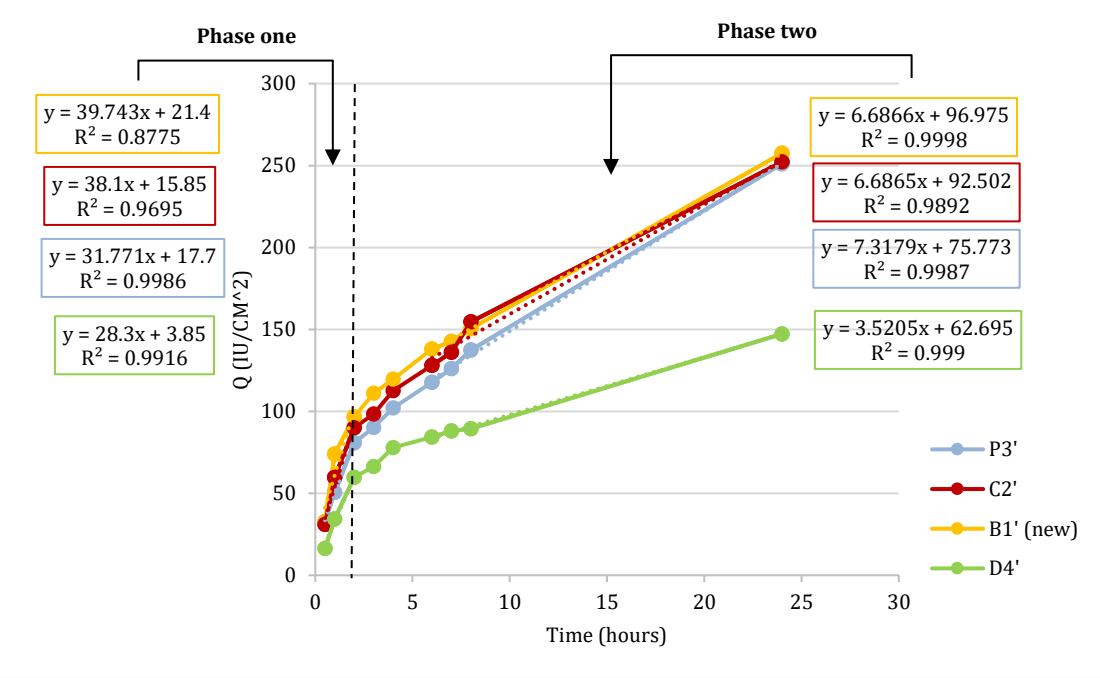


Figure #33. Average cumulative amount permeated per unit area per unit time from P3', C2', B1' (new) and D4' formulations through Strat-M® membrane.

We can see in Figure #33 that the penetration of vitamin D3 from FFS was biphasic. The flux in phase one was rapid (0.5-2 hours). It was represented by steep regression lines. This rapid phase could be the result of reaching supersaturation after evaporation of the highly volatile solvents (ethanol and acetone). Under this condition, the thermodynamic activity increased in the nonvolatile solvent and vitamin D3 was forced to leave the formulation to find a way out, which was the artificial skin (Strat-M® membrane). Ethanol before evaporation could also be involved in accelerating this phase. It acts as a penetration enhancer by carrying vitamin D3 across the membrane. After

evaporation and film formation, the release changed significantly in phase two. At the beginning of phase two, the flux gradually decreased until it reached a steady flux (5-6 hours). The flux was slower compared to phase one which was represented by less steep regression lines. The occlusive film continued with a constant depot flux until the end of the study (24 hours) for all formulations[68].

Comparing with P3', the addition of Limonene or PVP in C2' and B1' (new) respectively, had no significant effect on (*f*) obtained from the slop or on (*Q*) permeated. However, the addition of EM with OA at D4' had a negative effect on (*f*) and (*Q*). We can see in Table #65 that the (*Q*) permeated (after 24 hours through S= 3.14 cm^2) of P3', C2' and B1' (new) was significant amount (about 800 IU). Whereas in the case of D4' it has decreased nearly by a half. This can be explained by the ability of EM and OA to dissolve vitamin D3 in relatively significant amount that counteract the main driving force of penetration; the supersaturation.

Table #63. The total cumulative amount permeated through the effective diffusion area after 24 hours.

No.	The cumulative amount permeated (IU)
P3'	788.4 ± 12.2
B1' (new)	808.5 ± 28.8
C2'	791.9 ± 4.8
D4'	462.5 ± 76.5

4.3. Stability studies

Stability were tested for P3', C2', B1' (new) and D4' under four storage conditions. The results of the analysis at zero time and after incubation for 2, and 3 weeks are shown in Tables #66-69. All formulation were stable (< ±5% from initial) under the studied storage conditions except D4' which was unstable under 30°C ± 2°C/65% RH ± 5% RH and under 40°C ± 2°C/75% RH ± 5% RH after incubation for 2 and 3 weeks.

Table #64. Results of the stability study of the P3' formulation.

No.	Time (weeks)	Storage conditions	Av. Area	SD	RSD %	Assay %
P3'	0	Room temperature	1045.4	2.67	0.26	99.3
	2	25°C ± 2°C/60% RH ± 5%	1038.7	0.79	0.08	103.6
		30°C ± 2°C/65% RH ± 5% RH	997.9	0.67	0.07	99.5
		40°C ± 2°C/75% RH ± 5% RH	1003.5	0.80	0.08	100.1
		5°C ± 3°C	1013.0	1.5	0.15	101.0
	3	25°C ± 2°C/60% RH ± 5%	1050.5	0.45	0.04	103.8
		30°C ± 2°C/65% RH ± 5% RH	1041.1	0.64	0.06	102.8
		40°C ± 2°C/75% RH ± 5% RH	1006.4	3.92	0.39	99.4
		5°C ± 3°C	1045.6	0.84	0.08	103.3

Table #65. Results of the stability study of the B1' (new) formulation.

No.	Time (weeks)	Storage conditions	Av. Area	SD	RSD %	Assay %
B1' (new)	0	Room temperature	1080.2	0.15	0.01	103.2
	2	25°C ± 2°C/60% RH ± 5%	1058.7	0.95	0.09	106.3
		30°C ± 2°C/65% RH ± 5% RH	1028.4	1.98	0.19	103.2
		40°C ± 2°C/75% RH ± 5% RH	1011.4	1.30	0.13	101.5
		5°C ± 3°C	1043.7	5.12	0.49	104.8
	3	25°C ± 2°C/60% RH ± 5%	1068.0	0.60	0.06	106.1
		30°C ± 2°C/65% RH ± 5% RH	1069.6	1.10	0.10	106.3
		40°C ± 2°C/75% RH ± 5% RH	995.1	4.97	0.05	98.9
		5°C ± 3°C	1083.2	0.32	0.03	107.7

Table #66. Results of the stability study of the C2' formulation.

No.	Time (weeks)	Storage conditions	Av. Area	SD	RSD %	Assay %
C2'	0	Room temperature	1084.2	1.18	0.11	103.5
	2	25°C ± 2°C/60% RH ± 5%	1053.0	0.97	0.09	105.6
		30°C ± 2°C/65% RH ± 5% RH	1027.9	0.42	0.04	103.1
		40°C ± 2°C/75% RH ± 5% RH	1011.8	1.51	0.15	101.5
		5°C ± 3°C	1039.2	0.55	0.05	104.2
	3	25°C ± 2°C/60% RH ± 5%	1070.9	0.30	0.03	106.4
		30°C ± 2°C/65% RH ± 5% RH	1066.2	0.61	0.06	105.9
		40°C ± 2°C/75% RH ± 5% RH	996.0	0.70	0.07	98.9
		5°C ± 3°C	1072.1	0.75	0.07	106.5

Table #67. Results of the stability study of the D4' formulation.

No.	Time (weeks)	Storage conditions	Av. Area	SD	RSD %	Assay %
D4'	0	Room temperature	1062.5	0.82	0.08	101.5
	2	25°C ± 2°C/60% RH ± 5%	1017	0.75	0.07	102.1
		30°C ± 2°C/65% RH ± 5% RH	1028.4	1.97	0.19	89.1
		40°C ± 2°C/75% RH ± 5% RH	895.4	0.20	0.02	89.9
		5°C ± 3°C	1016.4	0.40	0.04	102.0
	3	25°C ± 2°C/60% RH ± 5%	1030	0.79	0.08	102.4
		30°C ± 2°C/65% RH ± 5% RH	885.5	0.84	0.09	88.0
		40°C ± 2°C/75% RH ± 5% RH	882.1	2.28	0.26	87.7
		5°C ± 3°C	1055.7	0.17	0.02	104.9

We used STD2 to calculate the assay at each time point. The results of %Cross Check (Eq. #20) between two STDs at each time point was acceptable (98.0-102.0%) (Table #69). This gives an indication that the STD2 is reliable in %assay calculation (Eq. #16). Also the %RSD was <2% for the eight injections of the STD2 (6 at the beginning and 2 at the end) showed that the HPLC still efficient throughout the analyses and gave reproducible results.

Table #68. %Cross Check of two standards prepared for assay calculation in stability studies

Time	Av. Area for STD1	Av. Area for STD2	SD	RSD%	% Cross Check
At zero	1045.4	1071.3	1.5	0.11	100.6
2 weeks	1040.6	1032.6	1.6	0.16	98.8
3 weeks	1047.7	1042.7	1.6	0.16	99.1

Chapter V: Conclusion

5. Conclusion

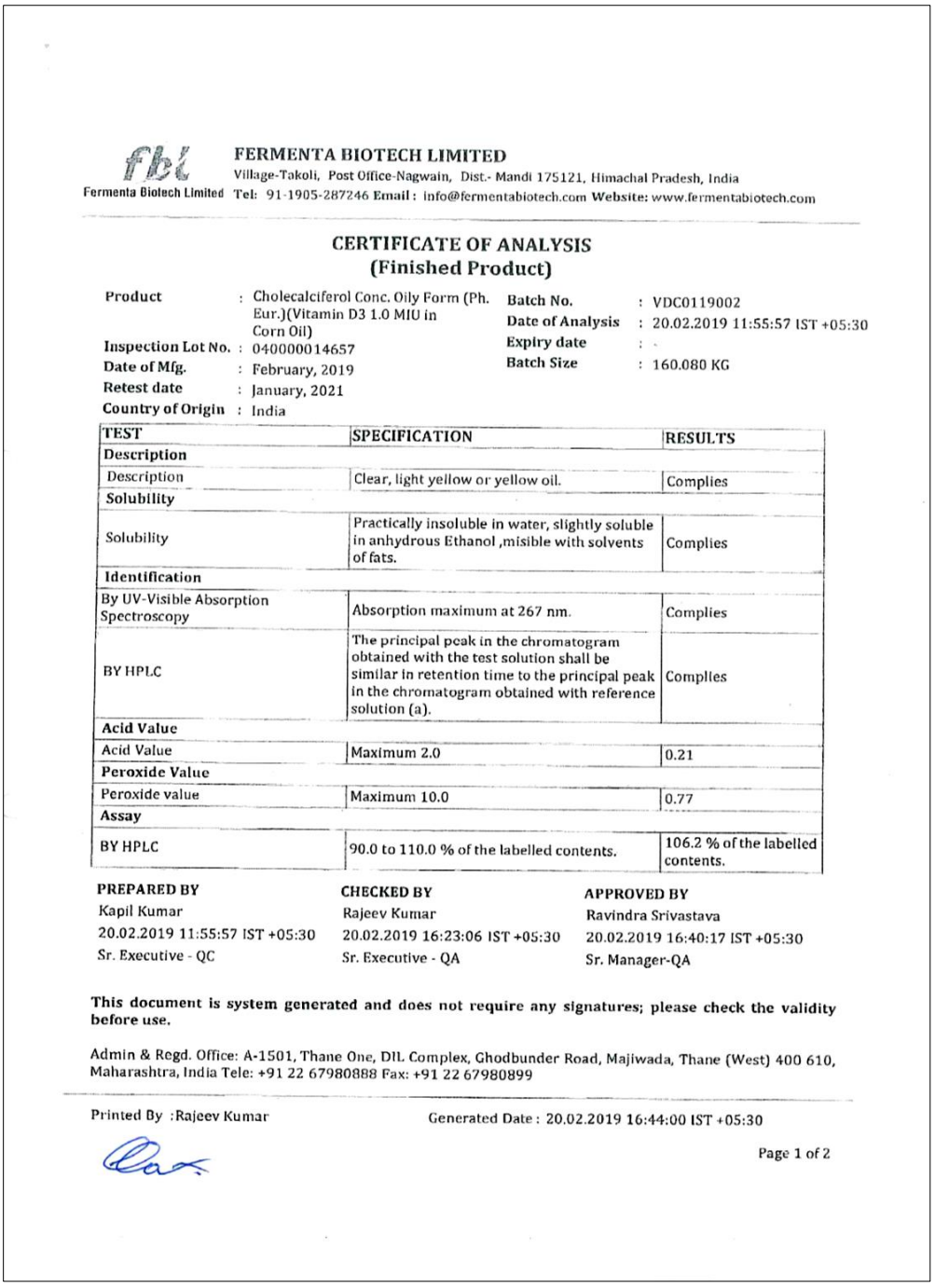
In this study we found that the FFS is an efficient system for vitamin D3 administration through transdermal route. And could be an alternative for oral and parenteral routes. The FFS evaporated in short period of time (< 5 min) after application, and it formed transparent, thin, elastic and adhesive layer. The highly lipophilic vitamin D3 penetrated the artificial skin (Strat-M®) in acceptable amount over 24 hours (about 800 IU).

After the application of the FFS containing the binary solvent ethanol: acetone, the concentration increased and reached supersaturation and the thermodynamic activity increased in the nonvolatile solvent (PG), this has a great impact and considered the main driving force for vitamin D3 o be released and become ready for penetration. Ethanol also improved the penetration in different mechanism prior evaporation. The addition of limonene and PVP in C2' and B1' (new) respectively didn't increase the penetration significantly in comparing with P3'. On the other hand, the use EM with OA had a negative effect on vitamin D3 penetration seen as the (Q) permeated after 24 hours decreased by a half.

All formulations were stable after 3 weeks except D4' which was stable in the refrigerator (5 ± 3 ºC) and in long-term storage condition, while it was unstable under intermediate and accelerated storage conditions.

For future, we recommend to do further studies to improve the penetration of vitamin D3 from FFS, by increasing the concentration of the PEs, testing more potent PEs and try other FFPs. To prevent the risk of vitamin D3 oxidation resulted from acetone, we recommend to add suitable antioxidant. We also recommend to evaluate the efficiency of several types of convenient dispenser such as sprays and roll on bottles. Finally, in vivo permeation studies on animal models is recommended for dose adjustment.

Appendix



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CERTIFICATE OF ANALYSIS
(Finished Product)

Product	: Cholecalciferol Conc. Oily Form (Ph. Eur.)(Vitamin D3 1.0 MIU in Corn Oil)	**Batch No.**	: VDC0119002
Inspection Lot No.	: 040000014657	**Date of Analysis**	: 20.02.2019 11:55:57 IST +05:30
Date of Mfg.	: February, 2019	**Expiry date**	: -
Retest date	: January, 2021	**Batch Size**	: 160.080 KG
Country of Origin	: India		

TEST	SPECIFICATION	RESULTS
Description		
Description	Clear, light yellow or yellow oil.	Complies
Solubility		
Solubility	Practically insoluble in water, slightly soluble in anhydrous Ethanol ,misible with solvents of fats.	Complies
Identification		
By UV-Visible Absorption Spectroscopy	Absorption maximum at 267 nm.	Complies
BY HPLC	The principal peak in the chromatogram obtained with the test solution shall be similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).	Complies
Acid Value		
Acid Value	Maximum 2.0	0.21
Peroxide Value		
Peroxide value	Maximum 10.0	0.77
Assay		
BY HPLC	90.0 to 110.0 % of the labelled contents.	106.2 % of the labelled contents.

PREPARED BY	CHECKED BY	APPROVED BY
Kapil Kumar	Rajeev Kumar	Ravindra Srivastava
20.02.2019 11:55:57 IST +05:30	20.02.2019 16:23:06 IST +05:30	20.02.2019 16:40:17 IST +05:30
Sr. Executive - QC	Sr. Executive - QA	Sr. Manager-QA

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Page 1 of 2

Figure #34. Vitamin D3 COA.

```
Data File:  C:\CHEM32\1\DATA\VITAMIN D3 2021-09-08 10-05-23\091-2602.D
Sample Name:  BLANK ( Buffer + Ethanol)


    ========================================================================
    Acq. Operator   : Ramzi Muqedi                       Seq. Line : 26
    Acq. Instrument : Instrument 1                       Location :  Vial 91
    Injection Date  : 9/9/2021                           Inj :       2
    Injection Time:   4:06:49 AM             Inj. Volume  (Method): 100 µl
                                          Act. Inj. Vol. from Sequence: 100 µl

    Acq. Method   ->C:\Chem32\1\DATA\VITAMIN D3 2021-09-08 10-05-23\VITAMIN D3.M

    Analysis Method : C:\CHEM32\1\METHODS\VITAMIN D3\VITAMIN D3.M
    Last changed    : 10/11/ -> 10:18:28 AM
    Method Info:


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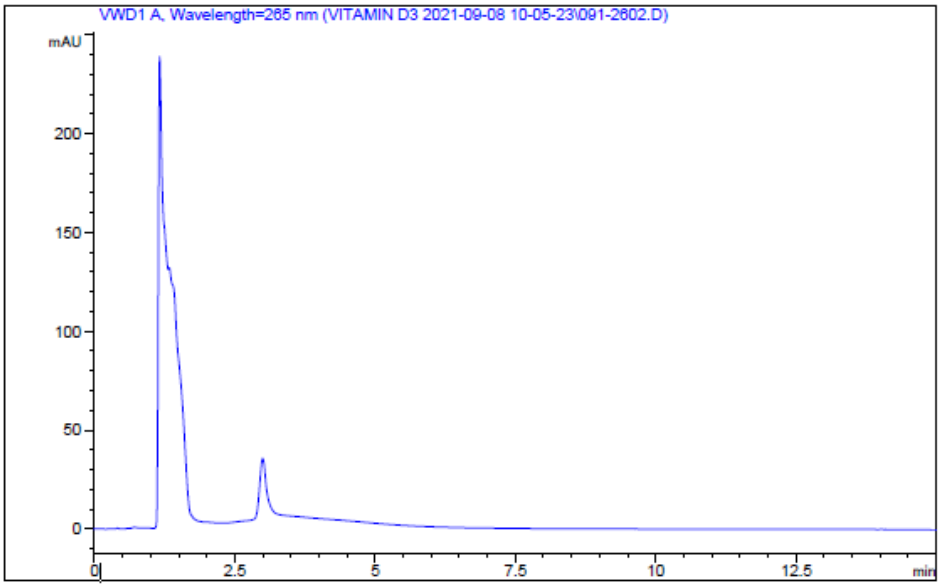


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    ========================================================================
    Customized  Report:  Performance  Report  per  Signal
    This report template has been designed for uncalibrated methods.
    ========================================================================

    Available Signals:
    VWD1 A, Wavelength=265 nm


    ========================================================================

    Signal:   VWD1 A, Wavelength=265 nm

    RetTime            Area      Halfh.      Peak      USP     Plates  Resolution
    [min]    Name      [mAU*s]   Width [min] Symmetry  Tail.
    ------|----------|---------|------------|--------|--------|--------|------->
    0.000  Vitamin D3   0.000                 0.000

    ========================================================================
```

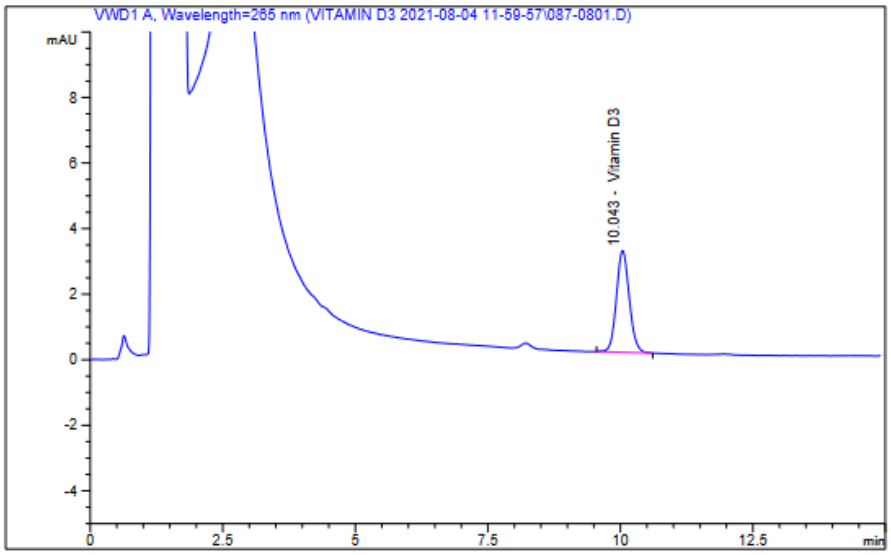
Figure #35. PBS: Ethanol (Blank) chromatogram.

Data File: C:\CHEM32\1\DATA\VITAMIN D3 2021-08-04 11-59-57\087-0801.D
Sample Name: STD7- Vitamin D3 Diffusion

Acq. Operator : Ramzi Muqedi Seq. Line : 8
Acq. Instrument : Instrument 1 Location : Vial 87
Injection Date : 8/4/2021 Inj : 1
Injection Time: 5:48:12 PM Inj. Volume (Method): 100 µl
Act. Inj. Vol. from Sequence: 100 µl

Acq. Method ->C:\Chem32\1\DATA\VITAMIN D3 2021-08-04 11-59-57\VITAMIN D3.M

Analysis Method : C:\CHEM32\1\METHODS\VITAMIN D3\VITAMIN D3.M
Last changed : 8/10/2021 8:50:07 AM (modified after loading)
Method Info:



Customized Report: Performance Report per Signal
This report template has been designed for uncalibrated methods.

Available Signals:
VWD1 A, Wavelength=265 nm

Signal: VWD1 A, Wavelength=265 nm

RetTime [min]	Name	Area [mAU*s]	Halfh. Width [min]	Peak Symmetry	USP Tail.	Plates	Resolution
10.043	Vitamin D3	52.185	0.260	0.885	1.073	8270	

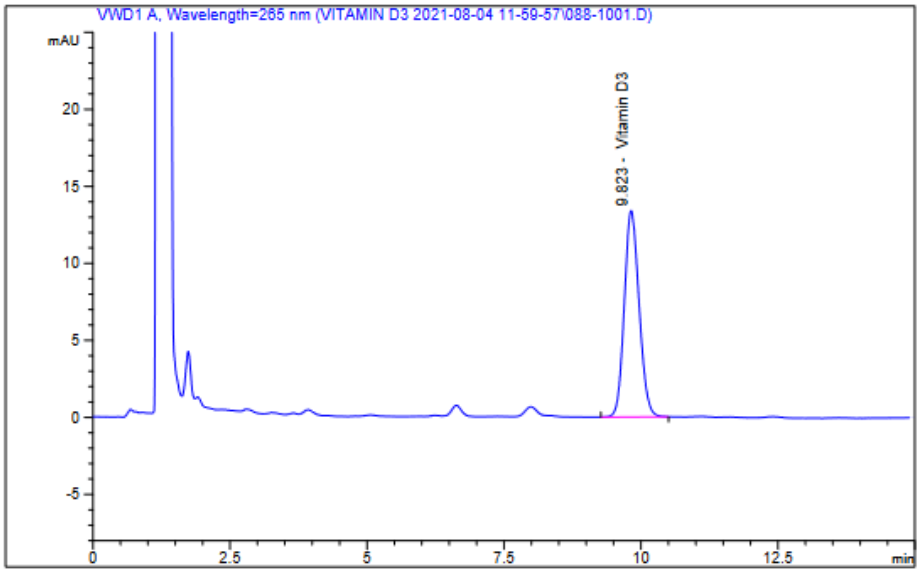
Figure #36. Example on chromatogram for one of the standards (STD7) used in the preparation of the calibration curve (for low vitamin D3 concentration).

Data File: C:\CHEM32\1\DATA\VITAMIN D3 2021-08-04 11-59-57\088-1001.D
Sample Name: SA1- Acetone (4 month)

Acq. Operator : Ramzi Muqedi Seq. Line : 10
Acq. Instrument : Instrument 1 Location : Vial 88
Injection Date : 8/4/2021 Inj : 1
Injection Time: 7:15:09 PM Inj. Volume (Method): 100 µl
Act. Inj. Vol. from Sequence: 100 µl

Acq. Method ->C:\Chem32\1\DATA\VITAMIN D3 2021-08-04 11-59-57\VITAMIN D3.M

Analysis Method : C:\CHEM32\1\METHODS\VITAMIN D3\VITAMIN D3.M
Last changed : 8/10/2021 9:05:40 AM (modified after loading)
Method Info:



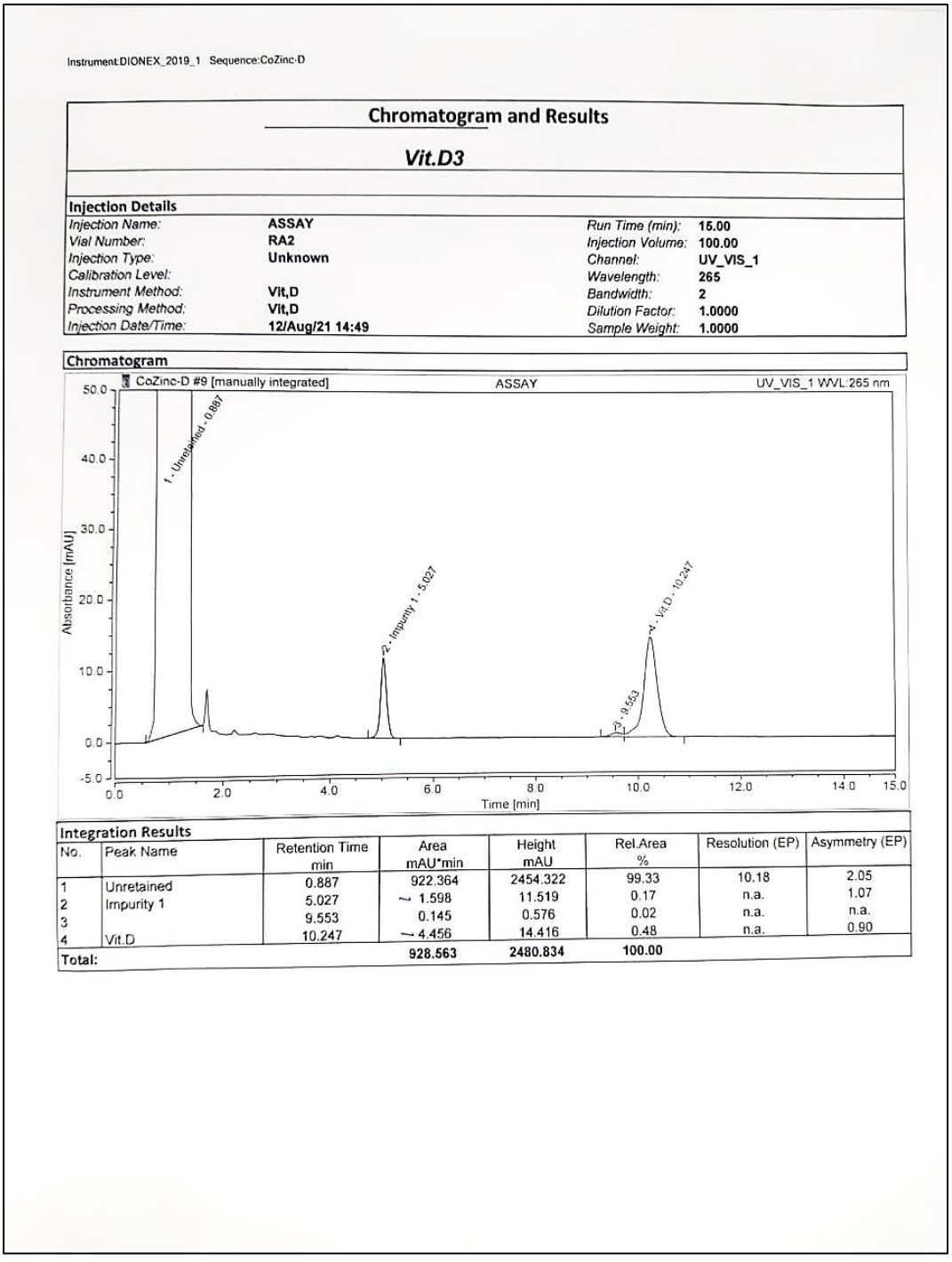
Customized Report: Performance Report per Signal
This report template has been designed for uncalibrated methods.

Available Signals:
VWD1 A, Wavelength=265 nm

Signal: VWD1 A, Wavelength=265 nm

RetTime [min]	Name	Area [mAU*s]	Halfh. Width [min]	Peak Symmetry	USP Tail.	Plates	Resolution
9.823	Vitamin D3	251.440	0.291	0.835	1.136	6308	

Figure #37. Vitamin D3 chromatogram after 4 month incubation in acetone during compatibility test.



Instrument:DIONEX_2019_1 Sequence:CoZinc-D

Chromatogram and Results

Vit.D3

Injection Details

Injection Name:	**ASSAY**	*Run Time (min):*	**15.00**
Vial Number:	**RA2**	*Injection Volume:*	**100.00**
Injection Type:	**Unknown**	*Channel:*	**UV_VIS_1**
Calibration Level:		*Wavelength:*	**265**
Instrument Method:	**Vit,D**	*Bandwidth:*	**2**
Processing Method:	**Vit,D**	*Dilution Factor:*	**1.0000**
Injection Date/Time:	**12/Aug/21 14:49**	*Sample Weight:*	**1.0000**

Chromatogram

Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Rel.Area %	Resolution (EP)	Asymmetry (EP)
1	Unretained	0.887	922.364	2454.322	99.33	10.18	2.05
2	Impurity 1	5.027	1.598	11.519	0.17	n.a.	1.07
3		9.553	0.145	0.576	0.02	n.a.	n.a.
4	Vit.D	10.247	4.456	14.416	0.48	n.a.	0.90
Total:			**928.563**	**2480.834**	**100.00**		

Figure #38. Vitamin D3 chromatogram after 4 month incubation in acetone during compatibility test done by Jerusalem Pharmaceutical Company.

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الملخص

أصبحت مكملات فيتامين د مهمة جدًا لتلافي وعلاج العديد من الحالات المتعلقة بنقصه, يتوفرهذا الفيتامين بأشكال صيدلانية تؤخذ عن طريق الفم والحقن, ولكن هذه الطرق تعاني من العديد من السلبيات, وبالتالي فإن توفير طرق أخرى بديلة لإعطاء فيتامين د مثل إعطائه عن طريق الجلد يعتبر مفيداً.

الهدف من هذه الدراسة هو تحضير و فحص فيتامين د على شكل محلول قابل لتشكيل غشاء يُمكِّن من نفاذية فيتامين د عبر الجلد, يتمتع فيتامين د ببعض الخصائص التي تجعله مرشحًا جيدًا للنفاذ عبر الجلد, ويتكون المحلول القابل لتشكيل غشاء من الدواء والمكونات الأخرى المذابة في مذيبات شديدة التطاير وأخرى وغير متطايرة, بعد وضع المحلول القابل لتشكيل غشاء على الجلد ، تتبخر منه المذيبات شديدة التطاير وتترك وراءها غشاءً رقيقًا وشفافًا ومريحًا يمكن إزالته بسهولة.

في هذه الدراسة قمنا بتحضير عدد من المحاليل القابلة لتشكيل غشاء, والتي استوفت الشروط التي وضعناها كي يعتبر الغشاء المتكون مقبولا, وهي أن يكون زمن جفاف الغشاء اقل من 5 دقائق ويكون غير دبق وقابل للالتصاق بالجلد ومرن ويُشَكِّل غشاء صافي وشفاف ناعم. كشفت دراسات النفاذية عبر الجلد الصناعي (Strat M®) الشبيه بالجلد عن تمكن فيتامين د من النفاذ بكميات مُعتبرة (ما يقارب 800 IU وحدة دولية) بعد 24 ساعة. يعتبر الوصول لوضع فوق الإشباع هو القوة الأساسية لدفع فيتامين د عبر الجلد الصناعي بعد تبخر المذيبات شديدة التطاير. لم يؤدي استخدام الليمونين (limonene) و PVP من زيادة النفاذية بكمية مُعتبرة, بينما أدى استخدام حمض الأوليك و خليط سهل الانصهار (eutectic mixture) من تقليل نفاذية فيتامين د الى النّصف.