Industrial Pharmaceutical Technology Master Program.

MSc. Thesis.

Enhancement of Valsartan/ Hydrochlorothiazide permeability, and investigation of the effect of permeation enhancers using Permeapad membrane

تعزيز نفاذية الفالزارتان/والهيدروكلوروثيازايد والتحقق من تأثير معززات الاختراق باستخدام غشاء البيرمياباد

This thesis is submitted in partial fulfillment of the requirements for the degree of master in Industrial Pharmaceutical Technology from the Faculty of Graduated studies at Birzeit University, Palestine By:

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Supervisor: Dr. Hani Naseef Shtaya.

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Finally, I thank everyone who helped me and encouraged me through this research.

Palestine, February 2022

Walaa Jamil Issa

DECLARATION.

I certify that this thesis submitted for the degree of master is the result of my own research, except where otherwise acknowledged, and this thesis has not been submitted for the higher degree to any other university or institute.

Signed: ……

Walaa Jamil Issa

Date: 12 / 2 / 2022.

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LIST OF ABBREVIATIONS

API Active pharmaceutical ingredient.

ATP Adenosine triphosphate.

AUC_{24} Area under the curve during 24 hours.

BCS Biopharmaceutical classification system.

C Concentration.

C1 Concentration in the membrane in the donor compartment.

C2 Concentration in the membrane in the receiver compartment.

Cd Concentration in the donor compartment.

Cr Concentration in the receiver compartment.

Csa Concentration of sample.

C_{max} Maximal concentration.

D Diffusion coefficient.

EDTA Ethylenediaminetetraacetic acid.

ER Enhancement ratio.

FaSSIF Fast state simulate intestinal fluid.

FDC Franz diffusion cell.

FeSSIF Fed state simulate intestinal fluid.

GI Gastrointestinal.

GIT Gastrountestinal tract.

h membrane thichness.

HCT Hydrochlorothiazide.

HPLC High performance liquid chromatography.

ICH International council of harmonization

K Partition coefficient.

LCG Lauroyl carnitine chloride.

LOD Limit of detection.

LOQ Limit of quantification.

M the amount of material.

P Partition coefficient.

PAMPA Parallel artificial membrane permeability assay.

PBS Phosphate buffer saline.

PCPcys polycarbophyl-cycteine conjugate.

PCC Palmitoyl carnitine chloride.

PE Permeation enhancer.

PEG 4000 polyethylene glycol 4000.

Pgp P-glycoprotein.

PVPA Phospholipid vesicle based permeation assay.

PVP 30 polyvinylpyrrolidone 30.

Q Cumulative amount of drug penetrated

SIF Simulating intestinal fluid.

SLS sodium lauryl sulfate.

SNAC N-[8-(2-hydroxybenzoyl) amino]caprylate.

SNEDDS Self-nanoemulsifying drug delivery system.

STD Standard.

t_{max} Time at maximal concentration.

T time.

T_L Lag time.

USP United state pharmacopeia.

UV Ultra violet spectrophotometer.

VAL Valsartan.

VDC Vertical diffusion cell.

X The distance in cm of movement perpendicular to the surface of the barrier.

Zot Zonula occludens toxin.

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ABSTRACT

Valsartan and hydrochlorothiazide are two drugs that are combined in tablet drug dosage form used for management of hypertension. Valsartan is an angiotensin II receptor blocker, its bioavailability is about 25%. Hydrochlorothiazide is a thiazide diuretic which has bioavailability of less than 65%. Increasing bioavailability will help in lowering the amount of an administered drug necessary to achieve a therapeutic effect as a result this could decrease the risk of side effects and toxicity. Low bioavailability can result in insufficient efficacy and high Inter-individual differences and therefore can lead to unexpected response to a drug. Bioavailability can be increased through permeation enhancement by using permeation enhancers.

This thesis aimed to study the effect of different permeation enhancers on the permeability of valsartan hydrochlorothiazide combination through sandwiched dialysis membrane and Permeapad® membrane.

In the first stage of experiments, sandwiched dialysis membrane was used to test the permeation of VAL and HCT, also many enhancers were tested and compared with basic sample solution without using any enhancer.

In the second stage of experiments, a synthetic innovative membrane that mimics intestinal cell membrane properties called Permeapad® membrane was used instead of sandwiched dialysis membrane, to test the permeation of VAL and HCT. FaSSIF and FeSSIF were used to simulate the conditions in the intestine.

In-vitro study using Franz diffusion cell was performed to evaluate the permeability of the two drugs. In the first part the membrane was composed of one layer of nylon filter membrane soaked in octanol and sandwiched in between two layers of dialysis membrane that was previously

soaked in phosphate buffer pH= 7.4, The receiver compartment was filled with 20ml phosphate buffer pH= 7.4. The donor compartment contained 2 ml of the prepared sample solution. In the second part, an innovative membrane (Permeapad® membrane) was used to separate the donor and receiver compartments.

Samples of 1ml volume were taken from the acceptor compartment at half hour intervals for three hours, followed by one hour intervals for two hours for each experimental sample. The 1ml sample diluted with 2ml PBS and tested by UV to quantify VAL and HCT at λ = 248 and 271.5 nm by using simultaneous equations.

The penetration enhancers which investigation were citric acid, SLS, PEG 4000, Na acetate, sorbitol, PVP30, mannitol, EDTA, and tween80. The enhancers were added to VAL/HCT solution in the donor compartment. Diffusion parameters that was determined were cumulative, T_L, D, P, and K. The enhancement ratio ER was used as criteria for selecting the best penetration enhancer. No permeation was detected with PVP30, mannitol, EDTA, and tween80. The ER values obtained when sandwiched dialysis membrane was used, were found for VAL (in compare with basic sample solution without enhancer) to increase in the order of Na acetate > citric acid > PEG 4000 > SLS > sorbitol. And the ER values for HCT were in the following order (in compare with basic sample solution without enhancer): citric acid > Na acetate SLS > sorbitol >PEG4000.

In the second stage, further trials were performed using Permeapad membrane, citric acid and Na acetate were chosen for these trials. 2% citric acid was selected as suitable permeation enhancer for both VAL and HCT, taking in consideration that VAL has lower bioavailability.

PART ONE: IN TRODUCTION

1.1 Oral route for administration of drugs.

The most common route that of drug delivery is the oral route. Since it is convenient, economical no special system is needed to administer and patient can take the medicine safely reducing visits to the physician, and this increases benefits for both the patient and the physician[1], [2].

Site of action of most active pharmaceutical ingredients (APIs) are out of gastrointestinal tract (GIT), so APIs must be absorbed from the gastrointestinal (GI) gut to reach the systemic circulation and reach site of absorption[3]. Absorption of drugs from GIT is affected by conditions in GIT and physiochemical properties of the active ingredient [1],[2].

1.2 Small intestine.

Small intestine where major digestion and absorption take place, located at the abdomen. The small intestine which is 6-7m long and has 30 m^2 surface area, consists of three parts duodenum, jejunum, and ileum, where primary absorption takes place and to a lesser extent in the oral cavity, stomach, and large intestine[4], [5].

Physical and biological properties in small intestine segments are shown in table (1.1). The first section is the duodenum (20-25cm long), it is just after the stomach and receive partially digested content from it. In addition, it receives the pancreatic secretions, which digest protein, and bile enzymes that emulsify fats. Brunner's glands at the duodenum secrete alkaline secretion with bicarbonate that neutralize acid contents from the stomach[4],[5].

The middle segment is the jejunum (2.5m long) that connect the duodenum and the ileum. In this part, digested nutrients coming from the duodenum are absorbed. Jejunum contains circular folds

and villi that increase surface area for absorption. The absorbed nutrients enter the enterohepatic circulation in the liver[4],[5].

The ileum the final part of the small intestine (3m long), the main function is to absorb vitamin b12, bile salts and the remaining unabsorbed nutrients in the jejunum[4],[5].

Table 1.1*: Physical and biological properties in small intestine segments*[5]*:*

Small intestinal segments	Surface Area (m^2)	PH Value	Length (m)	Residence time (hr)	Catabolic enzymes
Duodenum	1.9	4.5- 5.5	0.35	0.5- 0.75	Peptidase,lipase, nuclease, polysaccharides, oligosaccharides.
Jejunum	184	5.5- 7	2.8	1.5- 2	Peptidase,lipase, oligosaccharides.
Ileum	276	7.0- 7.5	4.2	5- 7	Oligosaccharides, peptidase, nucleases, nucleotidase.

The cross section of the small intestine consist of four layers, they are illustrated in figure (1.1)[4]:

1. Mucosa: It consists of epithelial cells that secrete thick secretions that protect the wall; mucosa is responsible for absorption, protecting the body from poisons and has moisturizing effect[6].
2. Submucosa: It is a thin layer rich in collagen; it supports the mucosa and connect it to the muscular layer. Contains large blood vessels, lymphatic and nerves.

3. Muscular layer: This layer is responsible for the peristalsis and gut movement of the, due to its structure that compose of muscle tissue, circular layer and longitudinal layer.

4. Serosa and adventitia: A smooth muscle tissue composed of two layers, the mesothelium, and a parietal layer. It secretes serous fluid.

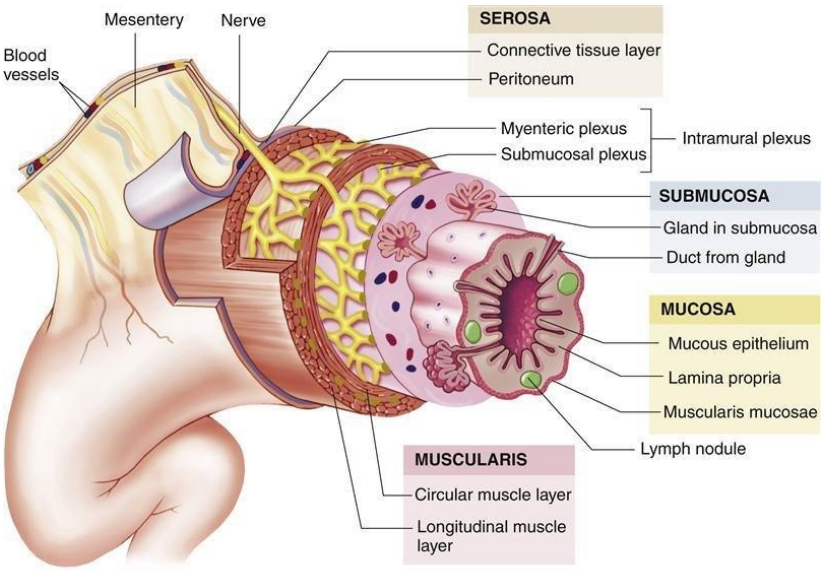

***Figure 1.1**: The cross section of intestinal wall*[7].

1.2.1 Drug penetration pathways

Pharmaceutical compounds can be pass cell membrane by several ways as illustrated in figure (1.2):

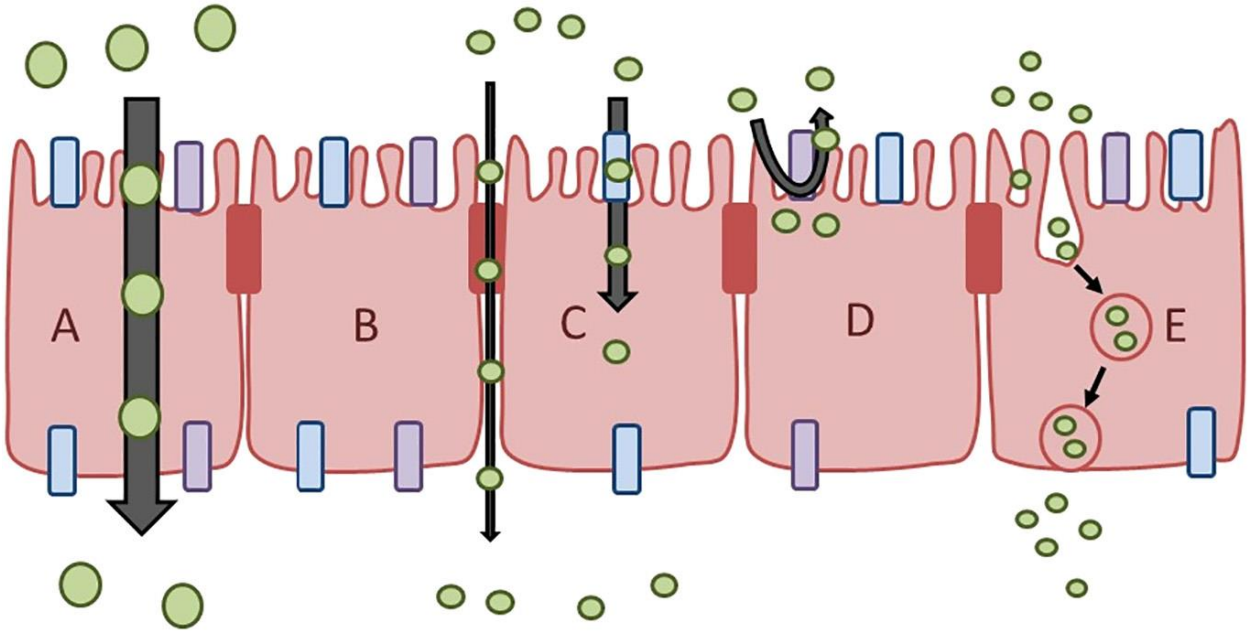

Figure 1.2 *:Schematic overview of different types of intestinal drug transport including passive transcellular diffusion (A), passive paracellular diffusion (B), active influx transport (C), active efflux transport (D) and transcytosis (E). Blue and purple boxes represent uptake and efflux transporters, respectively[8].*

1) **Transcellular passive transport:** pharmaceutical compounds, which passively cross biological cell membrane depends, primarily, on concentration gradient, they flow form high to lower concentration[9]. Drugs diffuse passively shows linear absorption kinetics. For a drug to passively cross the intestinal membrane must have appropriate physiochemical properties concerning lipophilicity, degree of ionization, and size. Also, the surface area of absorption. Lipophilicity is the most important one. Un-ionized lipophilic compounds that have high solubility in the lipid bilayer are rapidly absorbed. The unstirred water layer limits the permeation of highly lipophilic compound, the presence of bile salts greatly affects the permeation of these compound. Highly hydrophilic compounds cannot pass the cell membrane; due to low solubility in lipid bilayer.[5] In general, drug molecule must have a balance of, water solubility to dissolve in the unstirred

water layer, and lipid solubility to dissolve in lipid bilayer to diffuse through the cell membrane[10].

2) **Facilitated passive diffusion:** Low lipid soluble molecules cannot readily cross the cell membrane like nutrients such as monosaccharides, amino acids and di/tripeptides. Electrolytes, bile salts and some drugs need a carrier molecules to transport them[5]. In this case the carrier and compound act as one unit, the carrier facilitate the diffusion of the drug, then release it at interior surface of cell membrane[9]. This pathway is reversible and along the concentration gradient. The mechanism is limited by the number of carriers which binds to specific structure[5].
3) **Active transport:** Some hydrophilic drugs that are structurally similar to endogenous substances like ions, vitamins, sugars, and amino acids cannot diffuse passively through cell membrane. Other alternative transport mechanism is by active transport. It is against concentration gradient, shows non-linear absorption kinetics, and requires an energy supply: either by hydrolysis of ATP or by a coupled transport of usually Na+ or H+[5][9].
4) **Paracellular transport:** Small hydrophilic compounds can pass through pores formed between absorptive cells in the tight junction. Tight junctional transport depends on the molecular size and it is charge selective. It is a diffusion-controlled process and shows linear absorption kinetics[5].
5) **Pinocytosis:** In pinocytosis pathway, energy is required[9]. The drug engulfed by cell membrane, they combine together to form a vesicle which they separates at the end and goes to the interior of the cell. The mechanism shows non-linear absorption kinetics[5].

1.3 Potential absorption barriers.

Majority of drugs are weak acids or weak bases that is affected by different pH ranges along the small intestine affecting their solubility and ionization that influence drug absorption and bioavailability. Cell membrane, composed of a bio molecular lipid matrix, controls the passage of the drug. In mucous layers which coat epithelium, glycoproteins, enzymes, and electrolytes are present, they reduce the bioavailability of drug by interacting with them and form hydrogen or ionic bonds[5].

Potential barriers that affect drug absorption are listed below:

1) **Aqueous Stagnant Layer:**

Aqueous stagnant layer is considered as an absorption barrier especially for drugs that are absorbed rapidly. The unstirred water layer is rate limiting for the intestinal absorption of compounds with a high lipid-water partition coefficient, aromatic hydrocarbons**,** and long chain fatty acids. The effective thickness of the unstirred layer minimized by assumption of water, as water moves to the interface to be absorbed.[11]

2) **Mucus:**

Mucous layer adjacent to the apical surface, can be considered a part of the unstirred layer. Mucus is secreted by the goblet-cells[11], consisting of water, glycoproteins (mucins), electrolytes, proteins and nucleic acids. [12]: the mucous layer creates an acidic layer at the epithelial surface at pH=6. Binding of drug to the mucous layer will limit the extent of drug absorption[12]. The glycoprotein mucin which is a part of the mucous layer, is negatively charged due to the presence of sialic acid residues and sulfate groups[12]. So positively charged drugs like tetracycline and quaternary amines can bind to negatively charged mucin resulting in decrease in absorption of these drugs[11].

3) Apical cell membrane:

It is a 1µm brush border membrane, which consist of polar lipid molecules containing hydrophilic and hydrophobic part. Divalent ions are necessary to maintain membrane structure. Calcium ions chelates with negatively charged phospholipids, thus reduce membrane fluidity and permeability. Generally, transport the molecules across the phospholipid bilayer controlled with their lipid-water coefficient. It is an absorption limiting barrier for strongly hydrophilic substance, so these compounds need other pathway for transport other than transcellular transport like pores and carrier mediated transport[12].

4) Basal cell membrane:

It is a 7 to 9 nm phospholipid bilayer that contains proteins. The fluidity of this membrane is higher than that of apical membrane; due to less glycosphingolipids[12].

5) Basement membrane:

It is a bilayer just beyond the basal membrane touching it or they may be separated by a relatively narrow water-filled extracellular space between them[11]. It consists of glycoproteins and proteoglycans, and it has cationic sites that repels plasma proteins[12].

It has not been established to what extent drug absorption may be limited by the basement membrane[12].

6) Tight junctions:

They are regions of direct contact between the ends of apical cells. They are constructed of strands meshwork, the decrease of strands number, makes it leaky and increases the permeability of tight junctions. In this case, solutes, ions, and water can be passively transport through cells. The structure of tight junctions destabilize by Calcium depletion or exposure to hypertonic

solution. Tight junctions are cation selective. They are impermeable for cations with diameter more than 0.8nm or molecular weight more than 350. The tight junctional structure when exposed to hypertonic solutions and by Ca 2+ depletion can be destabilized[12].

7) Capillary walls:

It is located below basal cell membrane. It is a potential absorption barrier that must be crossed to reach blood flow. The thickness of the capillary wall is 1μm which is considered very thick compared to bilayer membrane. Contains large pores with a diameter of 40-50 nm, covered with a 2 to 4-nm-thick mucopolysaccharide membrane[12]. Therefore, they are not critical barriers for drug absorption. Strong hydrophilic compounds transported slowly when compared with hydrophobic ones[12].

1.4 Intestinal permeation enhancers.

Generally, a remarkable percentage of the drugs developed are of class III and IV[13] that have poor permeability through intestinal wall, where permeability is the rate-limiting step for absorption. Drugs that are hydrophilic, BCS class III drugs, small polar molecules, vaccines, hormones, peptides and proteins show low bioavailability because of low permeation and absorption through oral route. In pharmaceutical industry, the aim is to increase bioavailability of taken orally drugs through increasing permeation via alteration of intestinal wall properties reversibly. Recently researchers have been focused on intestinal permeation enhancers permeation enhancers (Pes) that can enhance the bioavailability and permeability of many drugs of the above mentioned classes[9].

Successfully improvement of permeation and bioavailability of certain drug needs the simultaneous delivery of the drug with effective concentration of a permeation enhancer to the site of absorption[14]. To achieve that get that many permeation enhancers (Pes) can be used,

including surfactants, medium chain fatty acids, bacterial toxins, chelating agents and bile salts which are proved to be effective [15],[9] [16].

1.4.1 Classification of permeation enhancers

1) Chelating agents: Chelating agents as EDTA, forms complexes with calcium and magnesium present between the epithelial cells of the intestinal membrane around the tight junctions leading to opening of the tight junctions and enhance permeation of present substances[15],[9].
2) Fatty acids and its derivatives: Long chains fatty acids as oleic acid (c18) and salts of medium chain fatty acids like capric acid (c10), lauric acid (c12) can increase paracellular permeation through increasing intracellular calcium ions by activation of phospholipase C in plasma membrane that result in contraction of calmodulin-dependent actin microfilaments, and dilation of tight junction and increase permeation[9]. The most studied fatty acid salt is sodium caprate which is the only enhancer included in marketed drugs. It can be added to the formulation of oral dosage form easily without the need of expensive special technique[17].
3) Chitosans and derivatives: Chitosan and its derivatives are biocompatible polymers, act by interaction with tight junction components leading to reduction in integrity of the tight junction and dilation in paracellular pathway and increase permeation. Also these molecules can tightly bind to epithelium cells leading to disruption of the F-actin and the TJ protein ZO-1 and increase paracellular permeation[15],[9]. They can enhance both low and large molecular weight drugs. Chitosan effect is pH dependent, it can work when it is protonated at 6.5 pH, but quaternized derivatives are able to overcome this problem and work at different

pHs. Chitosan are large molecules which are not absorbed from the gut so side effects are excluded.

4) Surfactants : Surfactants are able to increase permeability through disruption of epithelial cells of intestinal wall, leading to higher permeability through transcellular pathway[9]. They are shown to elevate membrane protein and phospholipid release, because they solubilize membrane components[14].
5) Bile salts and its derivatives: Bile salts are naturally produced in liver and excreted in the small intestine to promote fat digestion and absorption. Many examples have been used as permeation enhancers like sodium taurodeoxycholate, rsodeoxycholate, taurocholate and chenodeoxycholate. They exist as mixed micelles with lecithin, monoglycerides, fatty acids, and cholesterol. There permeation enhancing effect via transcellular pathway is achieved through solubilizing of phospholipids and proteins of intestinal membrane, which is correlated with mucosa damage. Studies showed that this damage is reversible[14]. Also bile salts can bind calcium ions found in the intestinal membrane and enhance permeation paracellularly[15].
6) Medium-Chain Glycerides: Medium chain glycerides are safe enhancers which improve the absorption of hydrophilic peptide compounds. They include both monoglycerides and diglycerides of caprylic and capric acid. Because they are lipophilic compound and poor soluble in water, so they combine with solubilizing agent which alters their enhancement role. When delivered orally as self-emulsifying W/O microemultion formulation, duodenal region can be targeted. They can be delivered by enteric coated technology in order to target lower intestine with an increase in their bioavailability compared with uncoated tablet[14],[15].

7) Acylcarnitines and Alkanoylcholines: They are medium and long chain fatty acid esters of carnitine and choline such as lauroyl carnitine chloride (LCC) and palmitoyl carnitine chloride (PCC), which has been shown to increase permeability of coated tablets of many drugs[18]. Their mechanism to enhance permeability is by dilation of paracelluar spaces and by modify the arrangement of lipid in intestinal brush membrane vesicles, which increase fluidity of membrane and so the absorption of drug[14].
8) N-Acetylated a-Amino Acids and N-Acetylated Non-a-Amino Acids: Some of these compounds have been used successfully as permeation enhancers. For example N-cyclohexanoylleucine, N-(phenylsulphonyl) leucine and 4-[4-[(2-ydroxybenzoyl) amino] phenyl] butyric acid. Recently studies have focused on N-[8-(2-hydroxybenzoyl)amino]caprylate (SNAC)[14].SNAC non covalently bind drug molecules making them more lipophilic and promote transcellular permeation. Another mechanism is specific for simaglutide SNAC combination that SNAC rise pH around the tablet and protect it from stomach pH[16]. Approved dietary supplement is present in the market that contains B12 and SNAC combination[18].
9) Secretory Transport Inhibitors: The epithelial cells of the intestine contain secretory system Pgp and MRP that transport certain compounds from cells to the lumen preventing absorption. Inhibition of this system can increase absorption and permeation of certain drugs. Many secretory transport inhibitors are used in order to improve intestinal permeability[14].
10) Cyclodextrin inclusion complex: Cyclodextrins are cyclic oligosaccharide, they have hydrophilic surface and hydrophobic core that make them able to hold poor water soluble drugs at the core and rise their apparent solubility and dissolution. Drugs of class II and

sometimes class IV can act like class I or class III when includes with cyclodextrin forming a complex[17].

11) Other enhancers

- Zonula occludens toxin (Zot)

  Vibrio choleram release its toxin zonula occludens which affect tight junction permeability reversibly. This toxin binds to specific receptors on epithelial cell surface followed by activation of intracellular cascade actions results in altering permeation .Paclitaxel, acyclovir, and cyclosporine and enamione anticonvulsants permeability was increased by this toxin in in vitro studies [9]. .

- Polycarbophyl-cysteine conjugate(PCPCys)

  They are thioated polymers where thiole group in cycteine is covalently bounded to the polycarbophyl polymer. This conjugate can reduce glutathione that is able to inhibit protein tyrosine phosphatase, resulting in more phosphorylated 13ccluding and open tight junction[9].

  The list of PEs we have used in our study were:

  - Citric acid.
  - Na acetate.
  - Sorbitol.
  - Tween 80.
  - PEG 4000.
  - PVP 30.
  - EDTA.
  - SLS.

- Mannitol.

Oramed Pharmaceuticals (USA) has tested oral and rectal formulations for insulin analogues, five formulations showed reduction in baseline serum glucose, Oramed's enteric-coated oral formats contain Na EDTA as absorption enhancer. Salts of EDTA enhance absorption by calcium chelation and affect tight junctions resulting in increase of paracellular permeation. They are considered strong to moderate enhancers. Sodium EDTA has widespread use in topical, oral and parenteral formulations at concentrations of 0.01–0.1% (w/v)[19]. In another study, EDTA was noticed to increase the absorption of PEG 4000 by 14 folds[20].

In a previous study, citric acid studied as permeation enhancer for chlortetracycline in turkey birds. The model indicated that the addition of citric acid increased the fraction of dose absorbed from 0.06 to 0.16[21]. Citric acid was used to prepare coamorphous system with amorphous loratidine for stabilizing amorphous loratadine and improving the dissolution and bioavailability The pharmacokinetic study in rats proved that coamorphous loratadine-citric acid system (1:1) could significantly improve absorption and bioavailability of loratadine over that of crystalline form. The improved stability of coamorphous loratadine-citricacid system could be the cause [22].

SLS was assessed to increase the bioavailability of low permeable drug amoxicillin, it was observed that SLS (0.2 mg/ml) increased the permeability of amoxicillin. The effect of SLS on the active secretion of amoxicillin was mainly attributed to the reversible cellular ATP depletion[23].

Results from a previous work, suggests that the preparation of fast dissolving ibuprofen solid despertions by low temperature melting method using polyethylene glycol 4000 (PEG 4000) as a meltable hydrophilic polymer carrier could be a promising approach to improve solubility, dissolution and absorption rate of ibuprofen. Quicker release of ibuprofen from solid despertions in rat intestine resulted in a significant increase in *AUC* and C_{max}, and a significant decrease in T_{max} over pure ibuprofen[24].

A previous study used tween 80 as an excipient to enhance the permeation of ganciclovir a BCS-III drug using everted gut sac model, and it was noticed that the permeability of ganiciclovir was significantly increased by tween 80[20].

One study demonstrated that sorbitol, when given by mouth in large quantities together with supraphysiological doses of B_{12}, enhanced absorption of B_{12} in intact animals[25].

PVP and tween 80 were components of FDA approved oral octreotide for acromegaly (2020). PVP was used in transient permeation enhancer technology in oral octreotide formulation. Transient permeation enhancer is an oily suspension of soluble hydrophilic microparticles of octreotide acetate, C8, and polyvinyl pyrrolidone (PVP) dispersed in an oil blend comprising glycerol monocaprylate and glycerol tricaprylate. Also, polysorbate 80 is present in the oily phase. Temporary mild membrane perturbation occured arising from the combination of C8 with PVP, polysorbate 80, and glycerides in the oily suspension. This drug is an evidence that peptides can be administered orally if formulated with selected intestinal permeation enhancers[26].

1.5 Valsartan and Hydrochlorothiazide in oral pharmaceutical preparations.

1.5.1 Valsartan

Valsartan (VAL) is a nonpeptide tetrazole derivative drug of angiotensin II receptor type 1 antagonist group, a potent orally drug that is used to lower blood pressure for hypertension, congestive heart failure, myocardial infarction and diabetes nephropathy. It was developed by Novartis and formulated alone or with other drugs like hydrochlorothiazide. Valsartan is 3-methyll-2-[pentanoyl-[[4-[2-(2*H*-tetrazoyl-5-yl)phenyl]phenyl]methyl]amino]-butanoic acid that has an empirical formula of $C_{24}H_{29}N_5O_3$, molecular weight of 435.5 g/mol, the chemical structure is shown in figure (1.3) available as white, microcrystalline powder with a melting range of (105-110) ˚C, The partition coefficient of is 0.033 (log P=1.499) showing that the drug has a relatively

hydrophilic character at physiological pH[27]. It is soluble in ethanol, methanol, acetonitrile and sparingly soluble in water. The solubility of valsartan is 0.18 g/L , and 16.8 g/L at 25˚C in water, and phosphate buffer pH 8.0 respectively [28],[29]. Valsartan is an acidic drug that is soluble in neutral pH range, solubility of VAL is pH dependent, when pH rises solubility increases and lipophilicity decreases as along small intestine in GIT[29],[30].

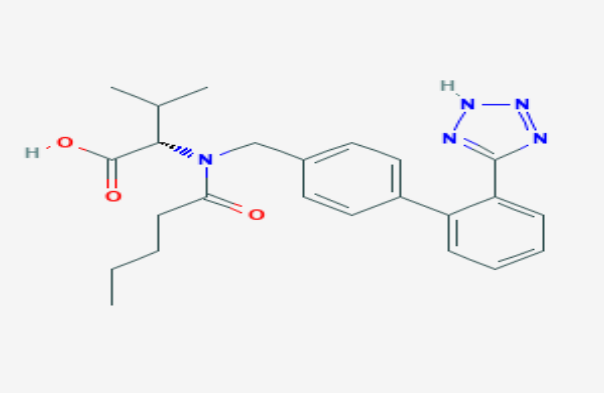

***Figure 1.3**: Chemical structure of valsartan*[29]

1.5.2 Hydrochlorothiazide.

Hydrochlorothiazide (HCT) is a diuretic which belongs to thiazide group, it is used in formulations with other agents to lower blood pressure [31],[9]. Hydrochlorothiazide is 6-chloro-1,1-dioxo-3,4-dihydro-2H-benzo[e][1,2,4]-thiadiazine-7-sulfonamide that has an empirical formula of $C_7H_8ClN_3O_4S_2$ [32], its chemical structure is shown in figure (1.4) molecular weight of 297.7g/mol , available as crystals or white powder with a melting range of (273-275 ℃)[31],[9]. HCT is very slightly soluble in water, the solubility is 722 mg/L at 25 ℃. It is soluble in ethanol and in acetone, freely soluble in sodium hydroxide solution, in n-butylamine and in dimethylformamide, sparingly soluble in alcohol, insoluble in ether, chloroform and in dilute mineral acids[33]. Solubility of HCTZ in aqueous solutions is low, in the pH range from 1.0 to

7.4, ranging from 0.0608 to 0.103 g /100 ml. Solubility in aqueous solutions within pH 10.2–11.6 changes to 1.79 and 2.2 g /100 ml[33]. The partition coefficient is −0.07, It has two pKa values, 7.9 and 9.2[34].

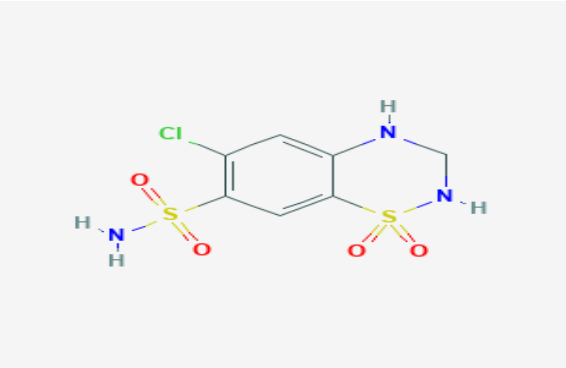

Figure 1.4: *Chemical structure of hydrochlorothiazide*[34]

1.5.3 Pharmacokinetic properties

1.5.3.1 Pharmacokinetic properties of Valsartan

After oral administration of Val, the peak plasma concentration (C_{max}) of 1.64 mg/L was achieved after 2-4 hours (t_{max}) with mean absolute bioavailability is 23%[29], [30],[35]. When 80mg VAL was orally administered the area under the plasma concentration-time curve from 0 to 24 hours (AUC_{24h}) was 8.54 mg · h/L[30].

Food decreases exposure to valsartan by about 40% and peak plasma concentration (C_{max}) by about 50%, although 8 hours after administration of Valsartan, the plasma concentration is similar for the fed and fast states.

It belongs to the BCS class III drug classified as low permeability and high solubility drug. Valsartan is absorbed by passive diffusion in the upper GIT due to higher acidity with about 25% bioavailability[29].

83% of Valsartan is mainly eliminated unchanged in feces, the other 13% is excreted unchanged in urine. The half-life of VAL is 6hrs.

1.5.3.2 Pharmacokinetic properties of Hydrochlorothiazide

Hydochlorothiazide has low bioavailability below 65% due to its poor solubility and permeability as it belongs to Class IV BCS classification[9],[31]. After oral dose administration of HCT the absorption is rapid, a C_{max} of 0.075 mg/L was achieved after 1.9 hours[30]. The increase in mean AUC is linear and dose proportional in the therapeutic range[29],[35]. Most of absorption take place in the duodenum and the upper jejunum[36].The gastrointestinal absorption of HCT is enhanced with food intake without alteration in AUC caused by decreased gastric emptying rate[37]. HCT is mainly eliminated unchanged in the urine with a half-life averaging 6 to 15 hours [29],[35].

1.5.4 Valsartan and hydrochlorothiazide combination in oral dosage forms

The combination of Valsartan/hydrochlorothiazide is compatible, and have synergistic effect in lowering blood pressure. Combination administration is more effective than monotherapy of either drugs [30].

Bioavailability of HCT is reduced by 30% when combined with valsartan, where valsartan bioavailability is not affected[30]. This does not impact the combination, since trials have shown a clear greater anti-hypertensive effect of the combination than either agents alone[35].When 25mg HCT was administered with 160mg VAL, the mean AUC_{24h}, C_{max} and t½ of HCT were reduced

by 22, 26 and 35%. The amount of hydrochlorothiazide excreted in the urine was reduced by 15%[30]. A comparison between VAL and HCT in some parameters, are listed in table (1.2)

Table 1.2: *Comparison between VAL and HCT*

	VAL	HCT
BCS class	class III	Class IV
bioavailability	Below 25%	Below 65%
T max	2-4hr	1.9hr
C max	1.64 mg/l	0.075 mg/l
AUC_{24}	8.5 mg.h/l	0.55 mg.h/l
Solubility in water	Sparingly soluble	Slightly soluble
Food intake	Delay absorption	Enhance absorption

1.6 Diffusion and permeability theory

Bioavailability of drugs are directly connected to their solubility and permeability. Permeability is studied at early stages of drug discovery which describes the ability of the drug to permeate through biological barriers according to chemicophysical properties[38]. It is expressed by Papp apparent permeability coefficient. In general, permeability assays measure the flux of drug molecules from a solution in a donor compartment through a barrier into an acceptor compartment. According to the type of barrier used to separate the two compartments, the available models for studying drug permeability can be divided into two models: cell-based models like the Caco- 2 assay and non-cell-based assay like the parallel artificial membrane permeability assay (PAMPA) and the phospholipid vesicle based permeation assay (PVPA)[39]. Franz cell diffusion

the most commonly employed apparatus for ex vivo permeability studies[39]. The problem with using cell and non cell assay models that is permeability of molecules can be affected by membrane type and excipients added like surfactants and co solvents. Another problem in using cell based assay method is using biomimetic media, the cell layer is highly affected by the biomimetic media, and need time for preparation. Cell layer has poor resistance to additives and short shelf life. This limited its usage in drug development research[39]. Therefore there is a need for an alternative artificial method that can overcome these problems. In this study we will use an innovative artificial barrier that is used in permeation studies, called Permeapad™. Two systems of synthetic membranes were used during experiments sandwiched dialysis membrane and Permeapad. Sandwiched dialysis membrane was used to predict the behavior of VAL and HCT during permeation and to give indications about PEs activity, so we can decrease the cost of using Permeapad membrane. It consist of a nylon filter layer between two layers of dialysis membrane, the nylon membrane was soaked in octanol to resemble the lipophicity of intestinal cell membrane. In a previous work, sandwiched dialysis membrane was used to investigate the effects of some penetration enhancers on permeation of orphenadrine citrate gel applied topically on skin[40]. Also, the dialysis membrane was used in a permeation study for evaluation of an emulgel containing calcipotriol for treatment of psoriasis[41].

1.6.1 Franz diffusion cell

Franz diffusion cell, the most common and efficient technique used to evaluate in vitro permeability of oral drug in early stages of development. It used to detect the permeability of active ingredients by using different membranes, the whole system is fixed and controlled only the membrane and compounds tested are the variables in each experiment [42].

Franz diffusion cell consists of two borosilicate glass chambers the donor chamber where the sample to be tested is placed, and the receptor chamber where a media that resemble body conditions with sink is placed. These two chambers are separated by a membrane through which the drug permeates to the receptor chamber. The chambers are connected together by pinch clamp. Sampling port is a tube connected to the receptor chamber used for taking samples. Taken samples should be replaced by fresh medium in accurate amount to maintain sink condition. During taking samples and replacing them, caution should be taken so bubbles will not be inserted, because it will adsorb on the filter and alter permeation process. During the test the donor chamber and sampling port are closed with parafilm to avoid evaporation. Degassing of the medium before inserting it in the cells play important rule in removing bubbles. The test is performed at 37 °C to simulate intestinal conditions, so water jacket with heat circulator is used to keep the temperature constant. A magnetic stirrer is placed in the receptor chamber to agitate the media to increase mixing efficiency and decrease boundary diffusion layer thickness to improve diffusion[42],[43].

There are three different designs for vertical diffusion cells as shown figures (1.5, 1.6, 1.7), but all have the same principle. . The main advantages of the vertical diffusion cell (VDC) are its ease of use, large sample size can be tested, which ensures consistent results[42].

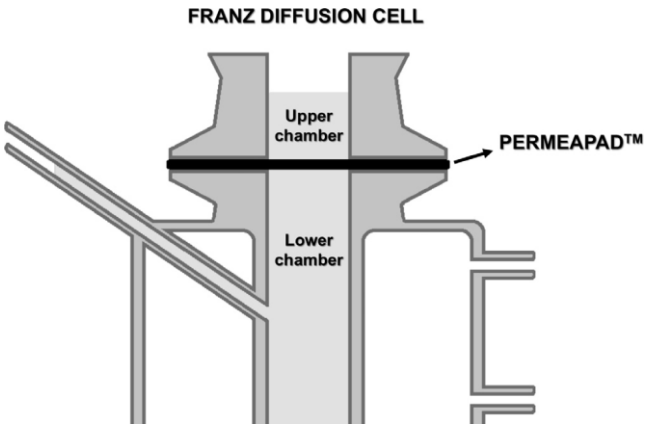

Figure 1.5 *Diagram of Franz diffusion cell and Permeapad™ barrier.*

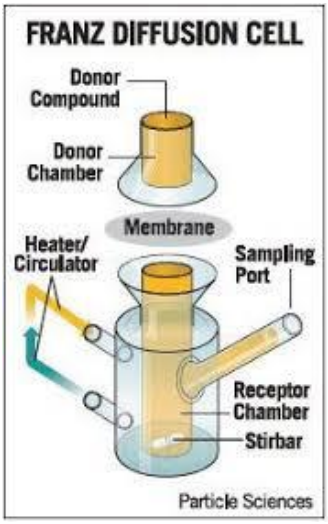

Figure 1.6 *: Franz diffusion cell* [42]

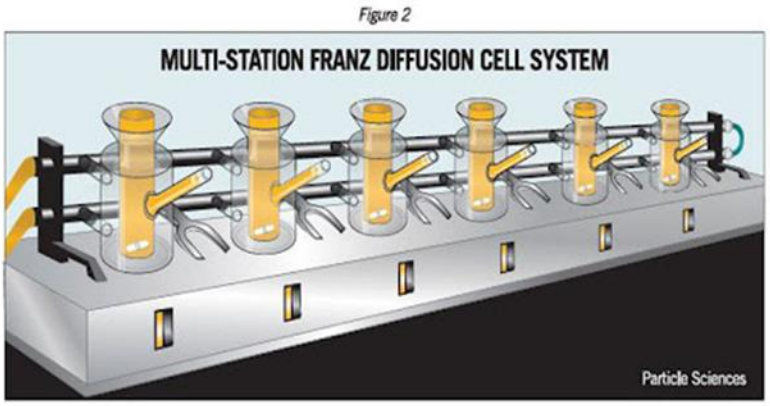

Figure 1.7 *:Multi-station franz diffusion system*[42]

1.6.2 Permeapad™

Permeapad is a biomemitic membrane with fully artificial phospholipids in layered structure. It is composed of soy bean phosphatidylcholine S-100 deposited between two support sheets[8]. Lipid crystals when get contact with water they swell generating within minutes a tightly packed layer of spheroids consisting of stacks of bilayers with intercalating water layers, which mimic the cell membrane[8], then the phospholipids fill the space between the support sheets and the vesicles are highly close to each other with similar morphology of tissue structure. The support layers protect the lipid layer from erosion and leakage. Permeapad membrane is available in ready to use form as shown in figure (1.8) , it is available as disk Permeapad and in the form of inserts for 6-well plates (Fig.1.4(B and C)), also high-throughput screening can be performed using 96-well plate Permeapad® Plate, Fig.1.4(D)[8].

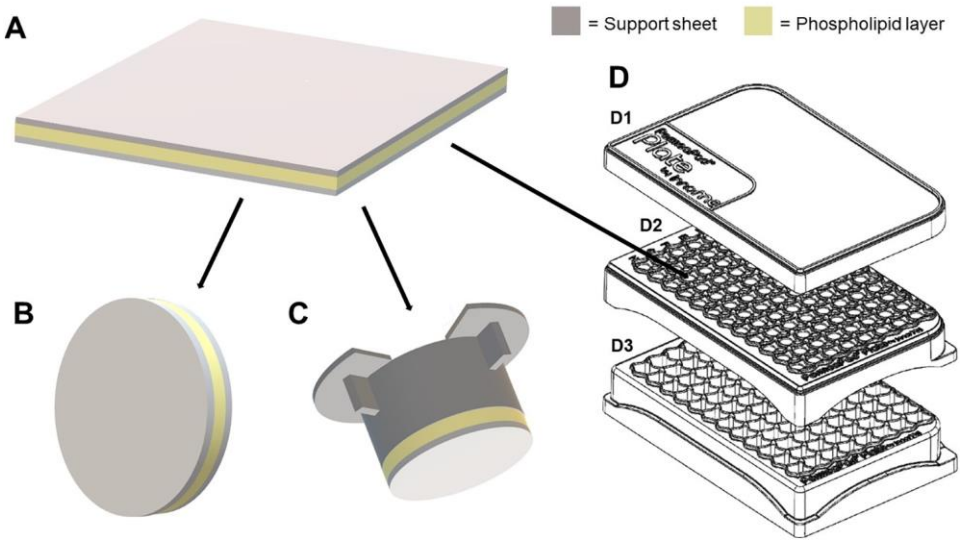

***Figure 1.8**: Schematic representation of the available formats of the Permeapad® barrier*[8].

Permeapad is expected to be more cost effective and easy to use in comparison to all other available models[39]**.** Permeapad was evaluated in the presence of many additives like surfactants, solvents, co-solvents, buffers with different pH values and different biomimetic medias[8]. It was found that Permeapad membrane is compatible, resistance to pH changes, and well suited for fast and reliable prediction of passive drug permeability[38]. In a previous work, Permeapad membrane was used to predict the absorption of metoprolol via buccal route. Results for the permeation study using Permeapad membrane were compared to published in vitro, ex vivo and in vivo studies for the same formulations. Results showed that the permeability of metoprolol using the Permeapad® barrier correlated very well to both in vitro and ex vivo studies. Results indicates that Permeapad membrane can withstand pH differences and can be used to mimic the buccal absorption of metoprolol as a faster and less laborious method[44].

1.6.3 Principles of diffusion through membrane

Simple diffusion laws can be used to describe the intestinal absorption process. Diffusion can be defined as transfer of individual molecules of a substance, which brought by random molecular motion and associated with concentration difference; the flow of a molecule through a membrane from the higher concentration to the lower one[45].

Fick's first law:

Flux, J is the flowing of the amount M of material through S a unit cross section, of a barrier in t unit time,

$$\boldsymbol{J = \frac{dM}{S.dt}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

In turn, the flux is proportional to concentration difference, dc/dx:

$$\boldsymbol{J = -D\ \frac{dC}{dx}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

D: diffusion coefficient of a penetrant in cm^2 /sec.

C: Concentration g/cm^3.

x : Distance in cm.

t: in seconds.

S in cm^2, diffusion is in the direction of decreasing the concentration, and this can indicates the negative sign[46].

Fick's second law represents the change of diffusion concentration with time at specific point in the system. Equation no.3 explains the mass transportation, i.e. the alteration of concentration with time at specific site. Instead of the mass diffusing across unit area of barrier in unit time.

$$\boldsymbol{\frac{\partial c}{\partial t} = -\frac{\partial J}{\partial x}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3)$$

Due to the concentration changes that are caused by variances in the output and input, alteration of penetrant concentration in the volume element occurs with time as the flux or amount diffusing differ with distance X.

Differentiating the 1st law expression from equation (2), with respect to X we get:

$$-\frac{\partial J}{\partial x} = \boldsymbol{D}\ \frac{\partial^2 c}{\partial x^2} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4)$$

Substituting from equation (3) in to equation (4) gives in Fick's 2nd law.

$$\frac{\partial C}{\partial t} = \boldsymbol{D}\frac{\partial^2 C}{\partial x^2} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (5)$$

In diffusion process, steady state is an important condition, equation (2) of Fick's 1st law gives the flux/ area in steady state conditions of the flow. The second low explains the change in concentration of diffusion with time at any distance, X. Fick's then may be written as follows [46] :

$$\boldsymbol{J} = \frac{\boldsymbol{dM}}{\boldsymbol{S.dt}} = \boldsymbol{D}\left(\frac{\boldsymbol{C1-C2}}{\boldsymbol{h}}\right) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (6)$$

where, S is the area of the membrane, h is the membrane thickness, $C_1$and C_2 represents the concentrations within the membrane boundaries, they are not recognized but they can be substituted by the partition coefficient K multiplied by the concentration of permeant in the donor phase Cd, or in the receiver Cr as follows,

$$\boldsymbol{K} = \frac{\boldsymbol{C1}}{\boldsymbol{Cd}} = \frac{\boldsymbol{C2}}{\boldsymbol{Cr}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (7)$$

So, from equation (6):

$$\frac{\boldsymbol{dM}}{\boldsymbol{dt}} = \frac{\boldsymbol{DSK(Cd-Cr)}}{\boldsymbol{h}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (8)$$

Cr=0, if sink conditions hold in the receptor phase. Resulting in the following equation:

$$\frac{\boldsymbol{dM}}{\boldsymbol{dt}} = \frac{\boldsymbol{DSK\ Cd}}{\boldsymbol{h}} = \boldsymbol{PSCd} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (9)$$

Where P is the permeability coefficient given by the next equation:

$$P = \frac{DK}{h}\ cm/hr. \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (10)$$

We measure the cumulative amount of diffusant, m, that passes per unit area through the membrane as a function of time and we obtain the plot shown in Figure 1.9.

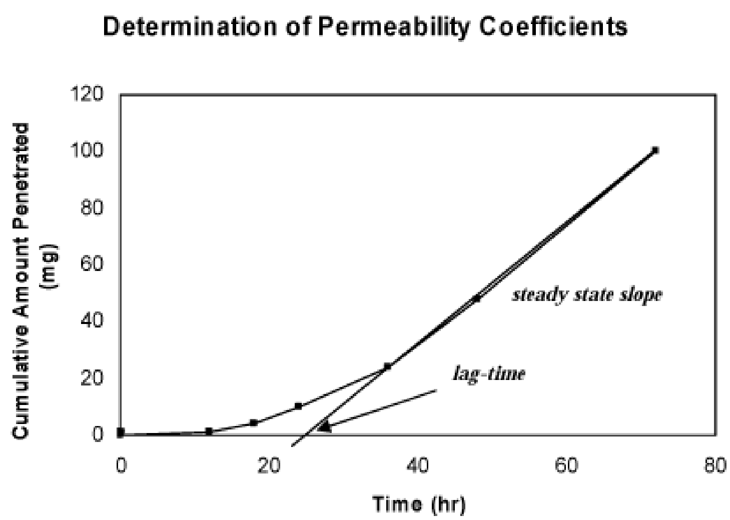

Figure 1.9: *Determination of steady state flux and lag-time.*

After prolonged times the plot has a straight line and a steady state flow is obtained. Intercept with x axis gives the lag time, TL which can be expressed by the following equation:

$$T_L = h^2/6D \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (11)$$

PART TWO: SIGNIFICANCE AND OBJECTIVES OF THE STUDY

2.1 Significance of the study

Valsartan has low permeability and hydrochlorothiazide has low solubility and low permeability that decrease their bioavailability, so multiple administration and more side effects are obtained. Permeation of drugs across the intestinal wall faces serious difficulties due to the nature of the intestinal wall and drug compounds. VAL and HCT that has low bioavailability, permeation of these drugs can be increased by adding intestinal permeation enhancer.

Classical membranes used for permeability assays have some limitations of poor reproducibility; additives like surfactants and co-solvents, buffers used and pH affect them. Some of old artificial membranes are expensive, difficult to use. So an innovative membrane that mimic biological membrane and is not affected by penetration enhancers and pH, is needed to be used in permeability assays.

The main objective of this research was to enhance the permeability of VAL/HTC by using different intestinal penetration enhancers. For this purpose, in this study many intestinal permeation enhancers were used. The permeation of VAL and HCT was evaluated, and the effects of different permeation enhancers and different systems of membranes were investigated. In this study sandwiched dialysis membrane and Permeapad™, an innovative artificial barrier that is used in permeation studies were used.

2.2 Scope and objectives:

- Enhance the permeability of VAL/HTC by using different intestinal penetration enhancers.
- Development and evaluation of analytical method to estimate the content of VAL/HCT in oral dosage form and solutions.

- Evaluation of VAL/HCT solubility in different simulated intestinal fluids.
- Evaluation of VAL/HCT stability in different simulated intestinal fluids
- Investigation of the effects of different penetration enhancers on drug permeation rate through sandwiched dialysis membrane
- Select the penetration enhancers with the higher effect on permeation of VAL/HTC through sandwiched dialysis membrane and investigation their effects on permeation of VAL/HTC by using Permeapad using a modified Franz- type diffusion cell.
- Evaluate the effect of the combination of the best penetration enhancers on the permeability of VAL/HCT combination of through Permeapad using Franz- type diffusion cell.
- Evaluate the effect of the concentration of the selected penetration enhancers on the permeability of VAL/HCT combination of through Permeapad using Franz- type diffusion cell.
- Collect samples and data analysis to determine the amount of the drug that penetrate the synthetic membrane.

PART THREE: RESEARCH METHODOLOGY

3.1 Materials and reagents:

All reagents used in this study were of analytical grade, and all materials were of pharmaceutical grade and listed in table (3.1) and (3.2). These materials and reagents were supplied from Birzeit University laboratories, Valsartan and hydrochlothiazide were gifted by Jerusalem Pharmaceutical Co, Ltd. Ramallah Al Bireh- Palestine.

Table 3.1: *The reagents and materials used in the study of valsartan and hydrochlorothiazide permeability enhancement (part 1).*

Purpose	**No**.	**Name of ingredient**	**Description**	**Source**	**Function**
Formula	1	Valsartan & hydrochlorothiazide	USP	KPS Chemicals & Pharmaceuticals	Active ingredient
	2	Sodium hydroxide	USP	DAEJUNG	pH adjustment
	3	Hydrochloric acid concentrated	USP	Fisher Scientific	pH adjustment
	4	Citric acid	USP	Fisher Scientific	Permeation enhancer
	5	SLS	USP	Kempex BV – Holland	Permeation enhancer
	6	EDTA	USP	Merck – Barcelona	Permeation enhancer
	7	Tween 80	USP	KOLB	Permeation enhancer
	8	PVP k30	USP	Glide chem. PVT	Permeation enhancer
	9	Na acetate	USP	Fisher Scientific	Permeation enhancer
	10	PEG 4000	USP	Acros Organics	Permeation enhancer
	11	Mannitol	USP	Wuxi Hexia Chemical	Permeation enhancer

Table 3.2: *The reagents and materials used in the study of valsartan and hydrochlorothiazide permeability enhancement (part 2).*

Purpose	**No**.	**Name of ingredient**	**Description**	**Source**	**Function**
analyticaL	1	KCL	USP	Fisher Scientific	Buffering agent
	2	Na_2HPO_4	USP	SIGMA-ALDRICH	Buffering agent
	3	KH_2PO_4	USP	SIGMA-ALDRICH	Buffering agent
	4	NaCL	USP	DAEJUNG	Buffering agent
	5	$Na_2HPO_{4.H2O}$	USP	SIGMA-ALDRICH	Buffering agent
	6	Glacial acetic acid	USP	Fisher Scientific	Buffering agent
	7	SIF powder	USP	Interchim®	Buffering agent
	8	Octanol 99%	USP	SIGMA-ALDRICH	Lipid layer simulator

3.2 Equipment and tools

Vials, pipettes, glassware, syringes, tubes and stands were supplied by Birzeit University laboratories table (3.3).

Table 3.3: *The equipment and tools used in the study of valsartan and hydrochlorothiazide permeability enhancement.*

Equipment.	Source/ Model.
U.V. Spectrophotometer.	PerkinElmer, Lambda 25.
Diffusion Cell Apparatus.	Orchid Scintific, Model No. : FDC-06.
pH meter.	HANNA instruments. pH/ ORP meter.
Hot Plate and magnetic stirrer.	Thermo scientific
Magnetic stirrer bar.	Large, Small, mini.
Bath Sonicator.	Elma, S 300 H, Elmasonic.
Centrifuge.	Centurion Scientific, Model: K2015R.
Water Bath shaker.	Mrc.
Refrigerator	beko®
Cellulose Nitrate Filter.	Sartorius Stedim Biotech, Pore size (µm): 0.45.
Polyamide Membrane Filters	Whatman, Pore size (µm): 0.45.
Dialysis tubing cellulose membrane.	SIGMA-ALDRICH.
Synthetic Membrane Permeapad	Innome, Pore size (µm): 0.45
Precision Balance	METTLER TOLEDO balance (5 digits), OHAUS®

3.3 Test method development.

Method of analysis for VAL / HTC combination was developed depending on the USP assay method.

Usually analysis of such components is done by HPLC and other chromatographic methods, because it is accurate, precise, and with good reproducibility. However, due to its high cost of

instruments and reagents there is a need to develop simpler and cheaper method with the same effectiveness as chromatographic methods for routine analysis. Therefore, UV method is a good choice.

Depending on the possibilities available in the lab, it was decided to analyze VAL and HCT by UV. Analyzing them, using UV needs to follow a method of UV spectrophotometric multicomponent analysis to obtain simultaneous equations that can estimate the concentration of them at the same solution[47].

There are different UV spectrophotometric multicomponent analysis methods that can be used to measure the concentration of two active ingredients at the same time. One of these methods is the simultaneous equation method[48]. In this method, two equations are constructed based upon the fact that at λ, the absorbance of the mixture is the sum of the individual absorbance of VAL and HCT.

To apply this method we need to know:

- Maximal wavelength for absorption (λ_{max}) of VAL and HCT.
- The absorptivity of VAL at λ_{max} of VAL (λ_1) and at λ_{max} of HCT (λ_2), ax_1 and ax2 respectively.
- The absorptivity of HCT at λ_{max} of VAL (λ_1) and at λ_{max} of HCT (λ_2), ay_1 and ay2 respectively.

3.3.1 Selection of an Appropriate Solvent System.

From the literature[49], it was found that 0.1NaOH can dissolve both VAL and HCT, it was suitable and stable.

3.3.2 Preparation of Stock Solutions.

Accurately weighed 25 mg of VAL & 25 mg of HCT was transferred to 25ml volumetric flask and dissolved separately in 0.1N NaOH, sonicated for three minutes, to give the standard stock solution of 1mg/ml for each. Then 10ml of each standard stock solution was transferred to 100ml volumetric flask separately and diluted with PBS to give the working solution of 100 µg/ml. Aliquots were prepared by using PBS in the increasing concentration range.

Dilutions that were made for VAL from the working solution:

1ml diluted in 100ml flask….1 µg/ml=0.0001g/100ml.

2ml diluted in 100ml flask…….2 µg/ml = 0.0002 g/100ml.

3ml diluted in 100ml flask….3 µg/ml=0.0003g/100ml.

4ml diluted in 100ml flask……4 µg/ml= 0.0004 g/100ml

6ml diluted in 100ml flask……6 µg/ml= 0.0006 g/100ml

15ml diluted in 100ml flask……15 µg/ml= 0.0015 g/100ml

10ml diluted in 50ml flask……20 µg/ml= 0.002 g/100ml

15ml diluted in 50ml flask……30 µg/ml= 0.003 g/100ml

10ml diluted in 25ml flask……40 µg/ml= 0.0040 g/100ml

Dilutions that were made for HCT from the working solution:

1ml diluted in 200ml flask…….0.5 µg/ml = 0.00005 g/100ml

1ml diluted in 100ml flask…….1 µg/ml = 0.0001 g/100ml

2ml diluted in 100ml flask……2 µg/ml= 0.0002 g/100ml

6ml diluted in 100ml flask……6 µg/ml= 0.0006 g/100ml

10ml diluted in 100ml flask……10 µg/ml= 0.001 g/100ml

6ml diluted in 50ml flask……12 µg/ml= 0.0012 g/100ml

8ml diluted in 50ml flask……16 µg/ml= 0.0016 g/100ml

3.3.3 Selection of analytical wavelength.

For selection of analytical wavelength, stock standard solutions of VAL and HCT were scanned separately from 400 to 200 nm. The overly spectra of both drugs were recorded. From overlay spectra, λ_{max} for VAL was 248 (λ_1) and λ_{max} for HCT was 271.5(λ_2). These wavelengths were selected for analysis of both drugs using simultaneous equation method.

3.3.4 Determination of absorptivity values of Drugs VAL and HCT at selected wavelengths

VAL and HCT solutions were prepared as mentioned in section 3.4.2. The concentrations have been chosen for VAL were 4, 6, 15, 20, 30, and 40 µg/ml. For HCT 1, 2, 6, 10 12, and 16 µg/ml. They were analyzed on UV and the absorbance was measured at 248 and 271.5 for each of VAL and HCT to determine the absorptivity of VAL at 248 and 271.5

Absorptivity values were calculated using the following formula:

A (1%, 1 cm) = Absorbance/Concentration (g/100ml) …… (12)

Where A (1%, 1 cm) is the absorptivity value.

3.3.5 Simultaneous equations[47].

$A_1 = ax_1 C_x + ay_1 C_y$ ……(13)

$A_2 = ax_2 C_x + ay_2 C_y$ ……(14)

where, C_X = Concentration of VAL; C_y = Concentration of HCT; A_1 = Absorbance of mixture at 248; A_2 = Absorbance of mixture at 271.5; ax_1 = Absorptivity of VAL at 248; ax_2 = Absorptivity of VAL at 271.5; ay_1 = Absorptivity of HCT at 248; ay_2 = Absorptivity of HCT at 271.5.

After obtaining the values of ax_1, ax_2, ay_1 and ay_2 we substitute them into the equation. After calculations and rearrangement, we can get the general formulas that can be used to determine the concentration of VAL and HCT in a mixture from the absorbance at λ_1 and λ_2.

3.3.6 Application of Proposed Method for Standard Mixture

Standard mixture of VAL and HCT was prepared by weighing accurately 80 mg of VAL and 12.5 mg of HCT accurately and dissolving them in 100ml 0.1N NaOH. 1ml of the solution was transferred to 100ml volumetric flask and diluted with PBS, to produce 8 and 1.25 μg/mL of VAL and HCT respectively. Then, it was analyzed.

3.3.7 Application of Proposed Method for Analysis of Tablets

Ten tablets were weighed, average weight determined, and finely powdered. A quantity of powder sample equivalent to 80 mg of VAL and 12.5 mg of HCT was transferred into 100mL volumetric flask containing 0.1 N NaOH, sonicated for 20 min; volume was adjusted to mark with same solvent and filtered through syringe filter with 45 μm pore size. Then 1ml of this solution was transferred and diluted with PBS in 100 volumetric flasks to produce 8 μg/mL of VAL and 1.25 μg/mL of HCT. It was analyzed against blank on UV.

3.3.8 Validation

The method was validated according to ICH guidelines to study linearity, specificity, accuracy, precision, robustness LOD and LOQ[48].

3.3.8.1 Linearity

It was evaluated by analyzing different concentrations of the standard solution of VAL and HCT in the concentration range 1-40 μg/ml and 0.5-16 μg/ml for VAL and HCT respectively. The Absorbance was plotted against the concentrations to obtain the calibration curves.

3.3.8.2 Specificity

For specificity assessment, two excipients were used, starch and lactose. 80mg VAL and 12.5mg HCT were weighed accurately and dissolved in 100ml 0.1N NaOH. 1ml of the stock solution was transferred to 100ml volumetric flask with 5ml of 80 μg/ml starch solution, and diluted with PBS. To another flask 1ml of the stock solution was transferred with 5ml of 120 μg/ml lactose solution, and diluted with PBS.

3.3.8.3 Accuracy

Accuracy of the method was assessed by percentage recovery experiments performed at three different levels, that is, 80, 100, and 120%. Known amounts of standard VAL and HCT solutions were added to the preanalyzed sample solutions; absorbances were recorded and reanalyzed by simultaneous equation method. To prepare the sample solutions, one tablet containing 80mg VAL and 12.5mg HCT was dissolved with 0.1N NaOH in 100ml volumetric flask, then 1ml was withdrawn and transferred to 100ml volumetric flask and diluted with PBS.

To prepare the standard solution, 80mg VAL and 12.5mg HCT were weighed accurately and dissolved with 0.1N NaOH in 100ml volumetric flask, then 1ml was withdrawn and transferred to 100ml volumetric flask and diluted with PBS.

Addition of standard solution to the sample was done as mentioned in table (3.4) and table (3.5):

Table 3.4*: Volume of STD added to sample solution in recovery study.*

Recovery level	Volume(ml) taken from Sample solution (8/1.25 µg/ml)	Volume(ml) taken from STD solution (8/1.25 µg/ml)	Final volume (ml)
80%	1	0.8	100
100%	1	1	100
120%	1	1.2	100

Table 3.5*: Initial concentration and final concentration after STD addition in recovery study.*

Recovery level	Initial amount (µg/ml)		Concentration of drug added (µg/ml)	
	VAL	HCT	VAL	HCT
80%	8	1.25	6.4	1
100%	8	1.25	8	1.25
120%	8	1.25	9.6	1.5

3.3.8.4 Precision

Intraday and interday precision were determined by analyzing three different standard solutions of VAL and HCT within the same day and three different days over a period of week. The standard solution was prepared by weighing 80mg VAL and 12.5mg HCT accurately and dissolving them in 100ml 0.1N NaOH. 1ml of the solution was transferred to 100ml volumetric flask and diluted with PBS. Then, they were analyzed.

3.3.8.5 Ruggedness

It was proved by analyzing a standard solution by two different analysts using the same experimental and environmental conditions. The standard solution was prepared by weighing 80mg VAL and 12.5mg HCT accurately and dissolving them in 100ml 0.1N NaOH. 1ml of the solution was transferred to 100ml volumetric flask and diluted with PBS. Then, it was analyzed.

3.3.8.6 Robustness

To prove the robustness of the method we used methanol instead of 0.1N NaOH as solvent for VAL and HCT using the same experimental conditions.

80mg VAL and 12.5mg HCT were weighed accurately and dissolved in 100ml 0.1N NaOH and 100ml ethanol separately. 1ml of each solution was transferred to 100ml volumetric flask separately, and diluted with PBS. Then, they were analyzed.

3.3.8.7 Limit of Detection and Limit of Quantitation LOD

Sensitivity of the method can be checked by the determination of LOD and LOQ. Based on the calibration curve and the standard deviation of y-intercepts of regression.

The detection limit (LOD) = 3.3 σ/S ……..(15)

The quantitation limit (LOQ) =10 σ/S……..(16)

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve.

3.4 Solubility study

To determine the valsartan and hydrochlorothizide solubility in different pH conditions and different solvents, Valsartan alone, hydrochlorothiazide alone and the combination of them was added in excess amount in separated flasks, containing:

- Water
- PBS 7.4 pH
- FaSSIF 6.5 pH
- FeSSIF 5 pH

The flasks were sealed and shaken for 24 hours at 25 C°, speed: 75 rpm. After 24 hours, a quantity of 10 ml was transferred from the content of each flask to plastic tubes. Each tube was

covered by Para film and transferred to the centrifuge machine at 3000 rpm/ 15 min, then the supernatant of each solvent was taken, about 1ml was taken from each solvent to be diluted, then the absorbance was measured using the U.V to find the concentration and by further calculation the solubility of each active ingredient in each media was determined.

To determine the solubility of VAL in different medias, after 24hrs shaking and obtaining the supernatant, dilutions were made as mentioned in table (3.6) (n=3):

Table 3.6: *Dilutions for the supernatant of each media that VAL was dissolved in, for solubility study of VAL.*

Media	Dilution
Water	1ml of the supernatant in 10ml PBS
PBS 7.4	1ml of the supernatant in 100ml PBS
FaSSIF 6.5	1ml of the supernatant in 100ml PBS
FeSSIF 5	1ml of the supernatant in 100ml PBS

After dilutions were made, the absorbance of the final solution was measured, and the concentration was determined by the linearity equation of VAL.

To determine the solubility of HCT in different medias, after 24hrs shaking and obtaining the supernatant, dilutions were made as mentioned in table (3.7) (n=3):

Table 3.7: *Dilutions for the supernatant of each media that HCT was dissolved in, for solubility study of HCT.*

Media	Dilution
Water	1ml of the supernatant in 100ml PBS
PBS 7.4	1ml of the supernatant in 100ml PBS
FaSSIF 6.5	1ml of the supernatant in 100ml PBS
FeSSIF 5	1ml of the supernatant in 100ml PBS

After dilutions were made, the absorbance of the final solution was measured, and the concentration was determined by the linearity equation of HCT.

To determine the solubility of VAL and HCT in a mixture in different medias, after 24hrs shaking and obtaining the supernatant, dilutions wer7e made as mentioned in table (3.8) (n=3):

Table 3.8: *Dilutions for the supernatant of each media that VAL and HCT was dissolved in, for solubility study of VAL and HCT.*

Media	Dilution
Water	1ml of the supernatant in 100ml PBS
PBS 7.4	1ml of the supernatant in 100ml PBS
FaSSIF 6.5	1ml of the supernatant in 100ml PBS
FeSSIF 5	1ml of the supernatant in 100ml PBS

After dilutions were made, the absorbance of the final solution was measured, and the concentration was determined by the simultaneous equations.

1L of PBS 7.4 pH:

It was be prepared by dissolving 8gr NaCl+0.2..gr KCl+ 1.44gr Na_2HPO_4+0.24gr KH_2PO_4 in 1L volumetric flask.

1L of FaSSIF:

Was prepared at PH= 6.5 by two steps. At first a blank buffer was prepared by dissolving 0.42gNaOH (pellets), 3.95gof $NaH_2PO_4*H_2O$ (monohydrate), and 6.19gof NaCl in 0.9L of purified water. The pH t is adjusted to 6.5 with either 1 N NaOH or by 1N HCl and make up to volume (1L) with purified water. In the second step 2.24g of SIF Powder was added to about 500mL of buffer at room temperature, stirred until SIF powder has dissolved and the volume was made up to 1L with the buffer. It was left for two hours to equilibrate then it was ready to use with slightly opalescent appearance.

1L of FeSSIF was prepared at pH= 5.0 by two steps. At first, a blank buffer was prepared by dissolving 4.04g of NaOH (pellets), 8.65g of glacial acetic Acid, and 11.87g of NaCl in 0.9L of purified water. The pH t is adjusted to 5 with either 1 N NaOH or by 1N HCl and make up to volume (1L) with purified water. In the second step 11.2g of SIF powder was added to about 500mL of buffer at room temperature, stirred until SIF Powder has dissolved and the volume was made up to 1L with the buffer, then it was ready to use with clear appearance.

3.5 Stability study.

To conduct the experiments, it must be approved that the APIs are stable during the experiment time, which is 5 hours. Therefore, the stability of valsartan hydrochlorothiazide combination was tested by dissolving 80mg valsartan and 12.5mg hydrochlorothiazide with 0.1N NaOH in 100ml volumetric flask. Then 1ml of this solution was transferred to three different 100ml flasks that contained:

- PBS 7.4 pH
- FaSSIF 6.5 pH
- FeSSIF 5pH

They were placed in water bath of 37 C°, for 8 hours. Samples were taken at zero time, after 3, 5, and 8 hours. The absorbance was measured and by using the simultaneous equations, concentrations and stability was determined.

3.6 Valzartan and Hydrochlorothiazide permeability behavior with different intestinal permeation enhancers tested using Franz Diffusion Cell (FDC) through synthetic membranes.

3.6.1 Description of diffusion apparatus

ORCHD diffusion cell apparatus was used to perform the experiments in this study. It consisted of three main parts:

- Six cells
- Water circulating pump
- Temperature controller

Each cell consist of two separated glass compartments, the upper one is the donor compartment, and the lower one is the receiver compartment. Upper and lower compartments are fixed using rubber rings between them and stainless steel clippers attaching them. Receptor volume of each cell is 20ml, 2mm mouth diameter. The cells are attached to water circulating pump with temperature controller in the range of 0 ºC- 60 ºC with accuracy ± 0.1 ºC. Each cell is jacketed by water jacket with inlet and outlet orifice that are connected with rubber tubes to water bath which contain water pump that help circulating the water from water bath through the rubber tubes to the water jacket and back to the water bath. Also the water bath contains a heater that control the temperature of the water. A magnetic bar is placed in the lower chamber for mixing to insure continuous diffusion.

3.6.2 Preparation of synthetic membrane

Two systems of synthetic membranes were used during experiments sandwiched dialysis membrane and Permeapad. In the first stage of the study we used two layers of dialysis membrane

and one layer of nylon filter membrane (pore size = 45 µm). Before half an hour of the beginning of the experiment, the two layers of the dialysis membrane were soaked in PBS where the nylon membrane was soaked in octanol to resemble the lipophilicity of the intestinal wall. After soaking, the nylon filter was sandwiched in between dialysis membrane layers. The thickness of the three membranes together is 0.03mm.

The second system of synthetic membranes which was used in the second stage of the study was the innovative synthetic membrane Permeapad membrane. Permeapad membrane is composed of thin layer of phosphatidylcholine (S-100) between two support sheets. The final barrier was composed by the support layer and a dry layer of lipid. It is flexible and resistant, with 0.01 thickness and can be cut to size by scissors. It is easy and ready to use[38],[39].

3.6.3 Diffusion procedure.

Phosphate buffer saline (PBS) with pH =7.4 was used in the acceptor compartment to mimic the natural of human blood. PBS receives the diffused particles of VAL and HCT from the donor compartment through the used membrane. PBS was prepared by mixing 8gr NaCl+0.2gr KCl+ 1.44gr Na_2HPO_4+0.24gr KH_2PO_4 in 1L volumetric flask. Before placing PBS in the acceptor chamber, it must be degassed; to get rid of air bubbles that may stick under the used membrane during the process due to stirring. Air bubbles that stick under the membrane, decrease the area for diffusion leading to decrease permeability and faulty results. Degassing was done by heating PBS using hot plate to 60 ºC, then degassing it on the sonicator while cooling it to 37 ºC, before the experiment directly. When PBS is ready, it is placed in the lower chambers with magnetic bar in each chamber. A previously prepared membrane is mounted carefully on the top of the acceptor

chamber making sure no air bubbles stick under it. To prevent any leakage rubber ring is placed above the membrane, then the donor chamber is mounted over the rubber ring. Parafilm and stainless steel clippers are used to close them tightly.

Now the donor and acceptor champers are totally separated by the membrane, 2ml of solution to be tested is placed in the donor chamber. The orifice of the donor chambers and the sampling ports were covered using parafilm to prevent any evaporation of the contents during the experiment. Magnetic induction is activated to 100%, speed of stirrer was fixed on 750 rpm. From the sampling port, 1ml is withdrawn from the acceptor chamber, this is done carefully and slowly to prevent air bubbles introduction. After every sampling from the acceptor chamber, the sample is replaced by an equal amount (1ml) of PBS; to keep the same volume in the acceptor chamber. The 1ml sample that has been withdrawn is diluted with 2ml PBS, and then it is analyzed by UV instrument. Every experiment is done in triplicate. Samples were withdrawn at half hour intervals for three hours and followed by one hour intervals for two hours (0.5, 1, 1.5, 2, 2.5, 3, 4, and 5 hours)

3.6.4 Sample preparation.

At first 80 mg VAL, 12.5 mg HCT were weighed and dissolved with 0.1NaOH in 10ml volumetric flask (stock solution 1). From this solution, 1ml was diluted with PBS in 10ml volumetric flask. 2ml sample from the final solution was transferred to the donor chamber for testing. After the first half an hour 1ml sample was taken from the sampling port and diluted with 2ml PBS, then it was analyzed by UV instrument, no absorbance was detected. The concentration was raised until the dilution was 5ml of the stock solution in 10 ml PBS, and no absorbance was detected.

Then, 160 mg VAL, 25 mg HCT were weighed and dissolved with 0.1NaOH in 10ml volumetric flask (stock solution (2)). From this solution 1ml was diluted with PBS in 10ml volumetric flask. 2ml sample from the final solution was transferred to the donor chamber for testing. After the first half an hour 1ml sample was taken from the sampling port and diluted with 2ml PBS, then it was analyzed by UV instrument, no absorbance was detected. The concentration was raised until the dilution was 4ml of the stock solution in 10 ml, an absorbance was detected and it was 0.012.

So the experiments were performed based on: 4ml of 16/2.5 mg/ml of VAL/HCT solution is diluted in 10ml volumetric flask with either PBS, FaSSIF, or FeSSIF. Experiments with their components were mentioned in tables (3.9) and (3.10).

When the experiment is performed with an enhancer, 10mg of the enhancer is added in the diluted solution to produce 1% solution. Enhancers used are mentioned in tables (3.9) and table (3.10). The details of each experiment, VAL and HCT concentration, the media used in donor chamber, media used in acceptor chamber, and enhancer added all are mentioned in table (3.9) and table (3.10).

***Table 3.9**: Experiments performed when sandwiched dialysis membrane with nylon membrane was used.*

Experiment No.	composition														
	VAL 6.4 mg/ml	HCT 1 mg/ml	PBS Donor	PBS Receptor	FaSSIF Donor	FeSSIF Donor	Citric acid 1%	SLS 1%	PEG 4000 1%	Na acetate 1%	Sorbitol 1%	PVP 30 1%	Mannitol 1%	EDTA 1%	Tween 80 1%
E1	X	X	X	X											
E2	X	X	X	X			X								
E3	X	X	X	X				X							
E4	X	X	X	X					X						
E5	X	X	X	X						X					
E6	X	X	X	X							X				
E7	X	X	X	X								X			
E8	X	X	X	X									X		
E9	X	X	X	X										X	
E10	X	X	X	X											X
E11	X	X		X	X		X								
E12	X	X		X		X	X								
E13	X	X		X	X					X					
E14	X	X		X		X				X					

Table 3.10*: Experiments performed when Permeapad membrane was used.*

Experiment No.	Composition									
	VAL 6.4 mg/ml	HCT 1mg/ml	PBS Donor	PBS Receptor	FaSSIF Donor	FeSSIF Donor	Na acetate 1%	Citric acid 1%	Citric acid 1.5%	Citric acid 2%
E15	X	X	X	X						
E16	X	X		X	X		X			
E17	X	X		X		X	X			
E18	X	X		X	X			X		
E19	X	X		X		X		X		
E20	X	X			X					
E21	X	X				X				
E22	X	X			X		X	X		
E23	X	X				X	X	X		
E24	X	X			X				X	
E25	X	X				X			X	
E26	X	X			X					X
E27	X	X				X				X

The cumulative amount of VAL and HCT is calculated according to the following equation:

Cumulative amount of penetrant at time

$$(t) = Ct \times V \sum_{t=0}^{t-0.5} \mathrm{Ct} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (17)$$

Where:

Ct: is the measured concentration of the penetrant at time t in the acceptor chamber in mg/ml.

V: is the volume of the solution in the acceptor chamber.

Calculation of diffusion parameters:

When a sample was withdrawn at every sampling time, it was diluted with 2ml PBS, and then analyzed using UV instrument. A cumulative amount of VAL and HCT through time is then drawn as flux per time, and the diffusion parameters will be calculated. The curve was then extrapolated using Excel 2016 to find the steady state line. The x intercept of the line will be the lag time.

According to equation (8):

$$\frac{dM}{dt} = \frac{DSK(Cd - Cr)}{h} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (8)$$

The slope = PSCd.

Where S is the area, P is the permeability coefficient; Cd is the concentration in the donor compartment. The permeability coefficient can be calculated as the slope. The area of membrane and concentration in donor compartment are known.

According to equation (11):

$$T_L = h^2/6D \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (11)$$

Where h is thickness of membrane that was measured during the experiment, T_L was calculated from the plot so D the diffusion coefficient is calculated.

According to equation no. (10):

The permeability coefficient: $P = \frac{DK}{h}$

Where h is thickness of membrane that was measured during the experiment, P is the permeability coefficient that was calculated previously, and thus the partition coefficient K is calculated.

Table (3.11) illustrate the diffusion parameters and their method of calculation.

Table 3.11: *Diffusion Parameter and their method of calculation.*

Slop	Lag time TL	Diffusion coefficient	Permeability coefficient	Partition coefficient.	Enhancement Ratio.
Calculated from the plot	Intercept with x axes.	$\frac{h^2}{6\,Tl}$	$slop/_{Cd}$	$\frac{P.h}{D}$	Permeability with enhancer/ permeability without enhancer.

3.7 Selecting the best permeation enhancer.

To study the effect of permeation enhancers on the permeability of VAL and HCT through a synthetic membrane, different PE were mixed with solution of the API and investigated for permeability using different synthetic membranes and FDC. The following permeation enhancers were used:

- Citric acid
- SLS
- PEG 4000
- Na acetate
- Sorbitol
- PVP 30
- Mannitol
- EDTA
- Tween80

PART FOUR: RESULTS AND DISCUSSION

4.1 Test method development

4.1.1 Selection of analytical wavelength.

The overly spectra of both drugs were recorded. From overlay spectra, λ_{max} for VAL was 248 (λ_1) as shown in figure (4.1), and λ_{max} for HCT was 271.5(λ_2) as shown in figure (4.2). These wavelengths were selected for analysis of both drugs using simultaneous equation method.

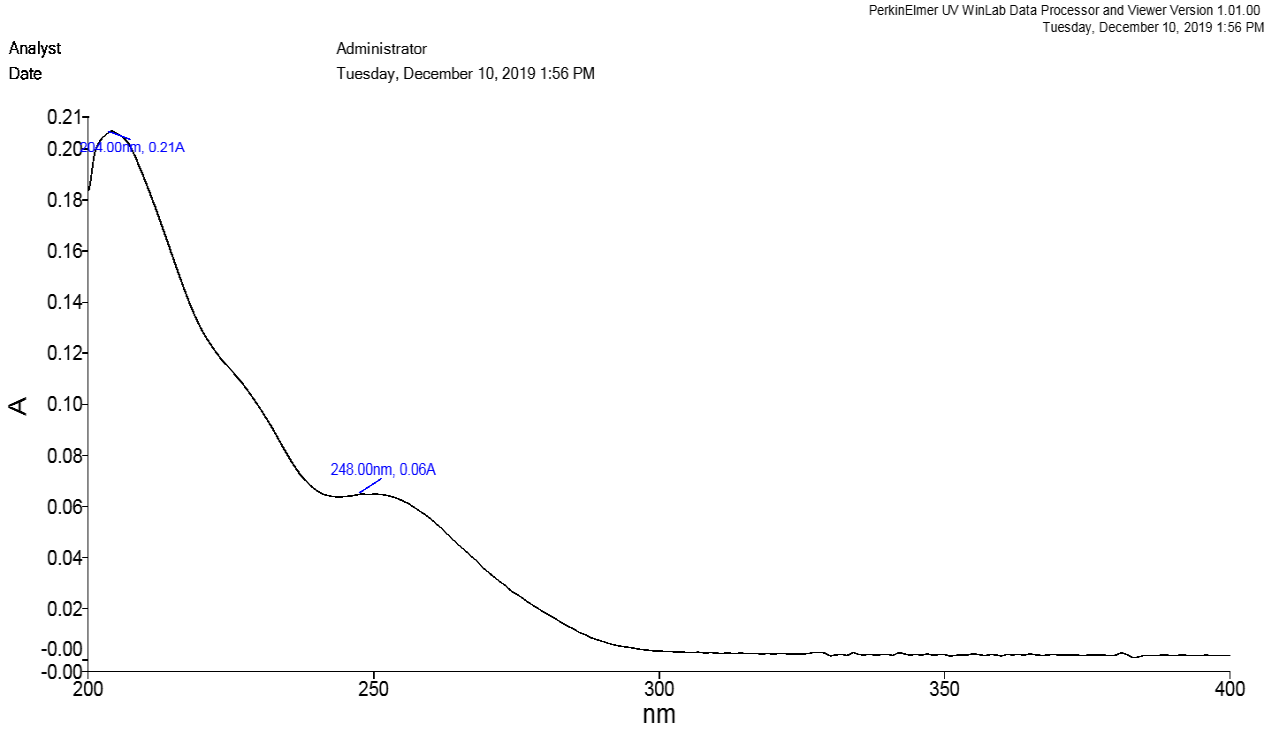

***Figure 4.1**: Overlay spectra of Valsartan*

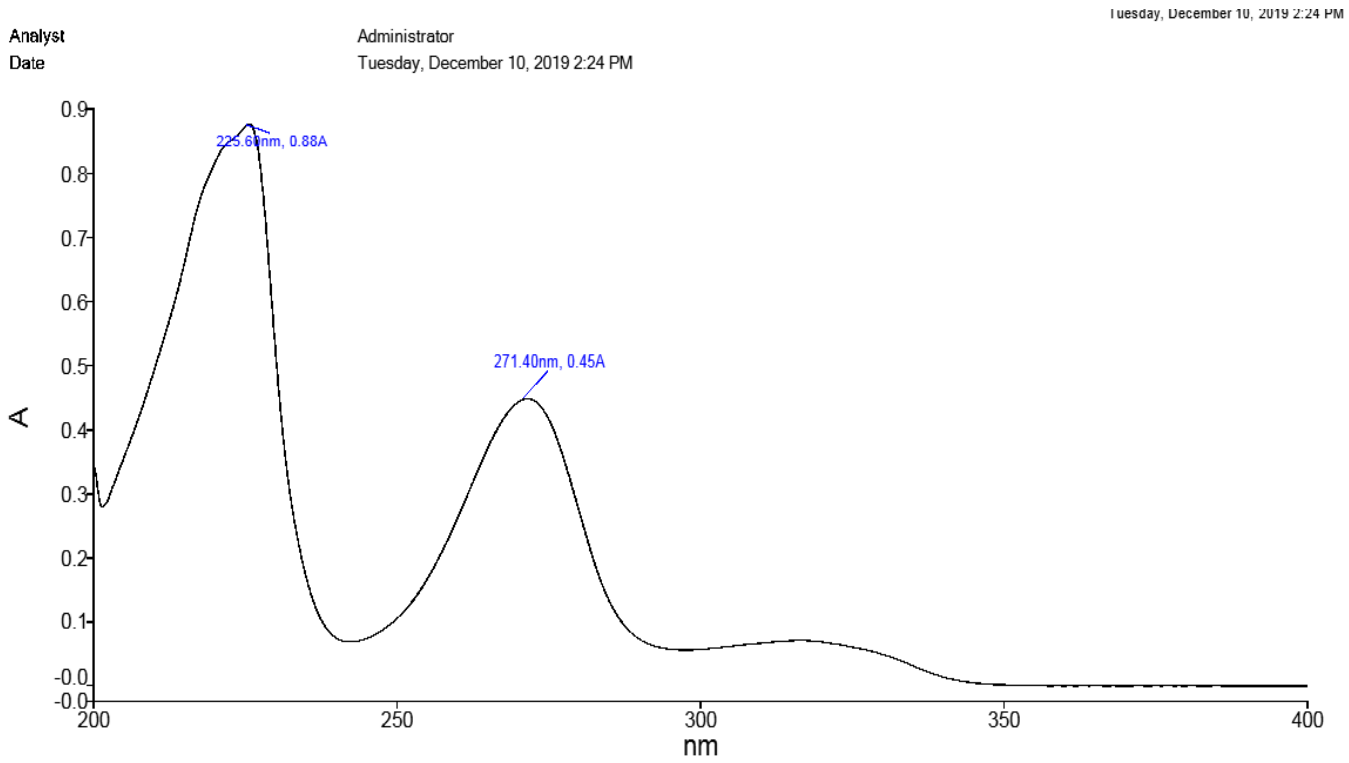

***Figure 4.2**: Overlay spectra of Hydrochlorothiazide.*

4.1.2 Determination of absorptivity values of Drugs VAL and HCT at selected wavelengths

After analyzing and measuring UV absorbance of prepared stock solutions as illustrated in section (3.4.2), results for VAL and HCT stock solutions were recorded in Table (4.1), (4.2) respectively. Absorptivity values for VAL and HCT at 248 and 271.5 were calculated and summarized in Table (4.3)

Table 4.1*: Absorbance of VAL solutions at 248, and 271.5 wavelength and absorptivity values calculated.*

conc.(g/100ml)			At (248)	At (271.5)
0.0004	absorbance of sample 1		0.134	0.068
	absorbance of sample 2		0.127	0.066
	absorbance of sample 3		0.129	0.0688
		mean	0.13	0.0676
		SD	0.00361	0.0014
		RSD%	2.7	2
		absorptivity	325	169
0.0006	absorbance of sample 1		0.183	0.099
	absorbance of sample 2		0.192	0.095
	absorbance of sample 3		0.1884	0.0976
		mean	0.1878	0.0972
		SD	0.00453	0.002
		RSD%	2.2	2
		absorptivity	313	162
0.0015	absorbance of sample 1		0.487	0.23
	absorbance of sample 2		0.481	0.24
	absorbance of sample 3		0.4855	0.2275
		mean	0.4845	0.2325
		SD	0.00312	0.0066
		RSD%	0.639	2
		absorptivity	323	155
0.002	absorbance of sample 1		0.656	0.312
	absorbance of sample 2		0.652	0.307
	absorbance of sample 3		0.66	0.305
		mean	0.656	0.308
		SD	0.004	0.0036
		RSD%	0.61	1.16
		absorptivity	328	154
0.003	absorbance of sample 1		0.934	0.499
	absorbance of sample 2		0.925	0.49
	absorbance of sample 3		0.931	0.496
		mean	0.93	0.495
		SD	0.00458	0.0046
		RSD%	0.48	0.92
		absorptivity	310	165
0.004	absorbance of sample 1		1.287	0.644
	absorbance of sample 2		1.28	0.649
	absorbance of sample 3		1.285	0.6455
		mean	1.284	0.6462
		SD	0.00361	0.0026
		RSD%	0.3	0.4
		absorptivity	321	161.54

***Table 4.2**: Absorbance of HCT solutions at 248, and 271.5 wavelength and absorptivity values calculated.*

conc.(g/100ml)			VAL(248)	HCT(271.5)
0.0001	absorbance of sample 1		0.0154	0.073
	absorbance of sample 2		0.012	0.068
	absorbance of sample 3		0.014	0.063
		mean	0.0138	0.068
		SD	0.00171	0.005
		RSD%		
		absorptivity	138	680
0.0002	absorbance of sample 1		0.029	0.137
	absorbance of sample 2		0.027	0.138
	absorbance of sample 3		0.0292	0.138
		mean	0.0284	0.1378
		SD	0.00122	0.0006
		RSD%	4.29	0.435
		absorptivity	142	688
0.0006	absorbance of sample 1		0.083	0.403
	absorbance of sample 2		0.078	0.402
	absorbance of sample 3		0.0802	0.4046
		mean	0.0804	0.4032
		SD	0.00251	0.0013
		RSD%	2.9	0.3
		absorptivity	134	672
0.001	absorbance of sample 1		0.14	0.678
	absorbance of sample 2		0.145	0.683
	absorbance of sample 3		0.147	0.673
		mean	0.144	0.678
		SD	0.00361	0.005
		RSD%	2.5	0.737
		absorptivity	144	678
0.0012	absorbance of sample 1		0.162	0.83
	absorbance of sample 2		0.168	0.829
	absorbance of sample 3		0.1668	0.825
		mean	0.1656	0.828
		SD	0.00317	0.0026
		RSD%	1.92	0.314
		absorptivity	138	690
0.0016	absorbance of sample 1		0.214	1.079
	absorbance of sample 2		0.21	1.076
	absorbance of sample 3		0.2096	1.0706
		mean	0.2112	1.0752
		SD	0.00243	0.0043
		RSD%	1.14	0.4
		absorptivity	132	672

***Table 4.3**: Summary of absorptivity values for VAL and HCT at 248 and 271.5.*

API	absorptivity values							mean	SD	RSD%
VAL	at 248	325	313	323	328	310	321	320	7.043	2.2
	at 271.5	169	162	155	154	165	161.54	161.09	5.766	3.5
HCT	at 248	138	142	134	144	138	132	138	4.56	3.3
	at 271.5	680	688	672	678	690	672	680	7.69	1.13

4.1.3 Simultaneous equations.

As obtained in table (4.3) of absorptivity values, they were substituted in the general formula (12)and (13) of simultaneous equation method as follows[47]:

$A_1 = ax_1 C_x + ay_1 C_y$ …… (13)

$A_2 = ax_2 C_x + ay_2 C_y$ …… (14)

where, C_X = Concentration of VAL; C_y = Concentration of HCT; A_1 = Absorbance of mixture at 248; A_2 = Absorbance of mixture at 271.5; ax_1 = Absorptivity of VAL at 248; ax_2 = Absorptivity of VAL at 271.5; ay_1 = Absorptivity of HCT at 248; ay_2 = Absorptivity of HCT at 271.5.

After substitution and further calculations, the final equations were:

$C_{VAL} = (A_{248} - 138\ C_{HCT})/320$ …… (18)

$C_{HCT} = (A_{271.5} - 0.478\ A_{248})/613.93$ …….. (19)

Where C_{VAL} and C_{HCT} are concentration of valsartan and hydrochlorothiazide respectively in the mixture, A_{248} and $A_{271.5}$ are absorbance of mixture at λ=248 and λ=271.5 respectively.

4.1.4 Application of Proposed Method for Standard Mixture

The concentrations of the two drugs (C_{VAL} and C_{HCT}) solution (8 and 1.25 μg/mL of VAL and HCT respectively) in standard mixture solution were determined, by using simultaneous equation method. Equations (13) and (14) were applied. Results for analysis of standard mixture (n=3) are shown in table (4.4) below:

***Table 4.4**: Absorbance values for standard mixture at 248 and 271.5, and the concentration of VAL and HCT calculated by simultaneous equation method.*

	VAL(248)	HCT(271.5)
absorbance of sample 1	0.275	0.208
absorbance of sample 2	0.274	0.207
absorbance of sample 3	0.277	0.21
mean	0.2753	0.20833
concentration(g/100ml)	0.0008	0.00012
concentration(µg/ml)	8.0652	1.24972
assay	1.0082	0.99978
SD	0.0015	0.00153
RSD%	0.545	0.153

4.1.5 Application of Proposed Method for Analysis of Tablets

The concentrations of the two drugs in sample solution (CVAL and CHCT) were determined, by using simultaneous equation method. Results for analysis of standard mixture (n=3) are shown in table (4.5):

***Table 4.5**: Absorbance values for sample solution at 248 and 271.5, and the concentration of VAL and HCT calculated by simultaneous equation method*

	VAL(248)	HCT(271.5)
absorbance of sample 1	0.281	0.213
absorbance of sample 2	0.28	0.211
absorbance of sample 3	0.283	0.214
mean	0.28133	0.2127
concentration(g/100ml)	0.00082	0.0001
concentration(µg/ml)	8.24243	1.2736
assay	1.0303	1.0189
SD	0.00153	0.0015
RSD%	0.544	0.7

4.1.6 Validation of analytical method

The method was validated in terms of linearity, specificity, accuracy, precision, ruggedness, and robustness, LOD, and LOQ.

4.1.6.1 Linearity

Stock solutions that have been prepared in section (3.4.2) were analyzed on UV. The absorbance values that were measured for VAL at 248 are shown in table (4.6). For HCT absorbance values of stock solution were measured at 271.5 and the results are shown in table (4.7). Calibration curve was constructed by plotting absorbance versus concentration in figure (4.3) and figure (4.4) for VAL and HTC respectively.

***Table 4.6**: Absorbance values of VAL stock solutions (n=3).*

Conc.(µg/ml)	Sample #1	Sample #2	Sample #3	Mean	SD	RSD%
1	0.03	0.029	0.03	0.02967	0.00058	1.955
2	0.058	0.061	0.064	0.061	0.003	4.918
3	0.086	0.088	0.09	0.088	0.002	2.27
4	0.132	0.131	0.132	0.13167	0.00058	0.440
6	0.186	0.186	0.187	0.18633	0.00058	0.311
15	0.478	0.478	0.479	0.47833	0.00058	0.121
20	0.633	0.634	0.633	0.63333	0.00058	0.0916
30	0.943	0.943	0.944	0.94333	0.00058	0.0615
40	1.261	1.262	1.263	1.262	0.001	0.0792

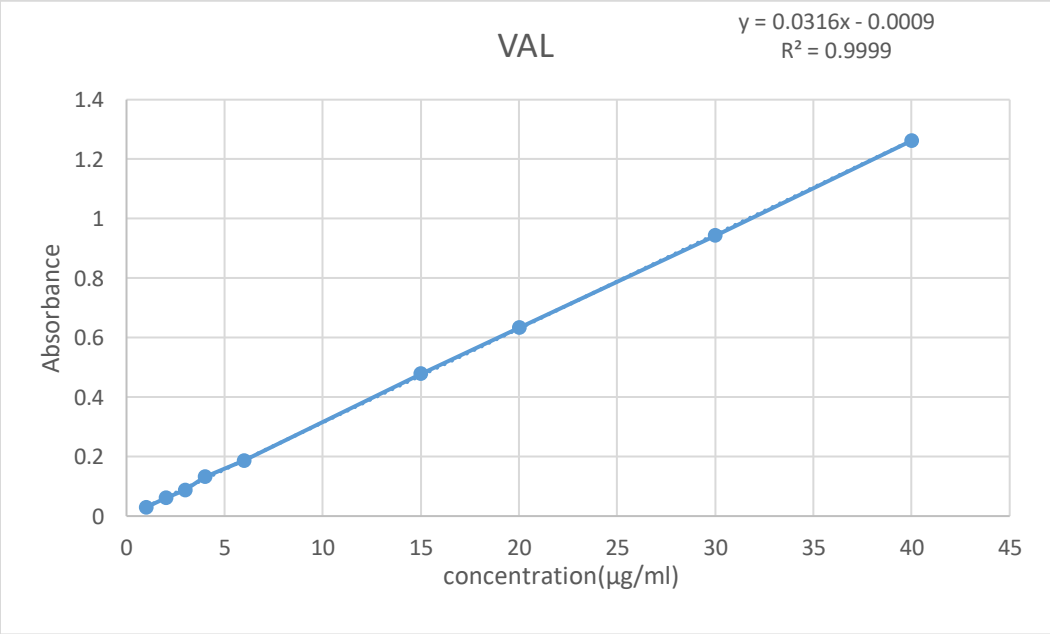

***Figure 4.3**: Calibration curve of VAL concentration (µg/ml) versus absorbance.*

***Table 4.7**: Absorbance values of HCT stock solutions (n=3).*

Conc.(µg/ml)	Sample #1	Sample #2	Sample #3	Mean	SD	RSD%
0.5	0.026	0.029	0.032	0.029	0.003	10.34
1	0.062	0.064	0.066	0.064	0.002	3.125
2	0.129	0.129	0.13	0.12933	0.0006	0.464
6	0.385	0.384	0.385	0.38467	0.0006	0.156
10	0.65	0.649	0.65	0.64967	0.0006	0.092
12	0.779	0.781	0.783	0.781	0.002	0.256
16	1.025	1.028	1.031	1.028	0.003	0.292

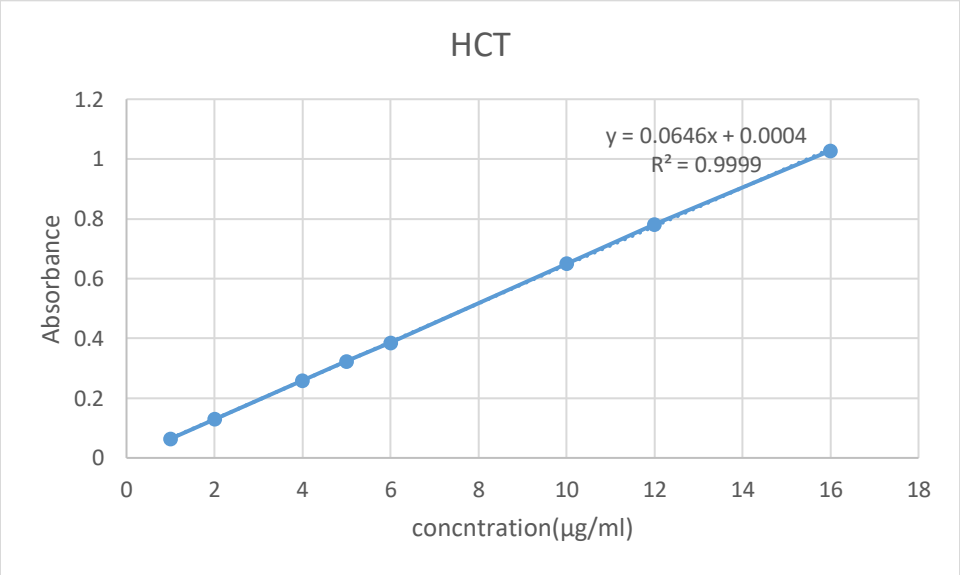

***Figure 4.4**: Calibration curve of HTC concentration (µg/ml) versus absorbance.*

4.1.6.2 Specificity

Results for absorbance of VAL/HCT mixture when starch and lactose were added separately are shown in table (4.8). The concentrations of VAL and HCT were calculated. From the results, it was noticed that the method has good specificity. When starch was added, the assay was 1.021 and

0.987 for VAL and HCT respectively. When lactose was added, the assay was 1.008 and 0.999 for VAL and HCT respectively. So added excipient didn't have an effect on the method of analysis.

Table 4.8: Absorbance values for sample solutions at 248 and 271.5 with addition of starch and lactose separately.

	With starch		With lactose	
	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)
absorbance of sample 1	0.277	0.208	0.275	0.208
absorbance of sample 2	0.279	0.209	0.274	0.207
absorbance of sample 3	0.28	0.21	0.277	0.21
mean	0.27867	0.209	0.27533	0.2083
concentration(g/100ml)	0.00082	0.0001235	0.00081	0.0001
concentration(µg/ml)	8.1759	1.234625	8.06523	1.2497
assay	1.02199	0.9877	1.00815	0.9998
SD	0.00153	0.001	0.00153	0.0015
RSD %	0.5490	0.478	0.555	0.720

4.1.6.3 Accuracy

Accuracy was estimated by recovery experiments at three levels, 80, 100, and 120%. Known amounts of standard VAL and HCT solutions were added to the preanalyzed sample solutions as illustrated in table (4.9); absorbances were recorded and reanalyzed in table (4.9) and (4.10). Recovery percentages were calculated using the equation (20), the results are shown in table (4.11).

% Recovery = (A-B)/C *100 …… (20)

Where A = total amount of drug estimated, B = amount of drug found on preanalyzed basis, and C = amount of bulk drug added.

From results, the recovered percentages of VAL and HCT at the three levels were within limits, the mean was 100.61% and 100.2% for VAL and HCT respectively. That's mean, the method has high accuracy.

Table 4.9: *Absorbance values of standard and sample solutions (n=3), and the concentrations calculated by simultaneous equation method.*

	STD		Sample(Tablet)	
	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)
absorbance of sample 1	0.274	0.207	0.279	0.21
absorbance of sample 2	0.269	0.205	0.28	0.211
absorbance of sample 3	0.276	0.209	0.283	0.214
mean	0.273	0.207	0.28067	0.2116667
concentration(g/100ml)	0.0008	0.0001246	0.00082	0.0001262
concentration(µg/ml)	7.99384	1.2461681	8.22638	1.2624892
assay	0.99923	0.9969345	1.0283	1.0099914
SD	0.00361	0.002	0.00208	0.0020817
RSD%	1.322344	0.966184	0.741084	0.98348

***Table 4.10**: Absorbance values of recovery experiments at three levels, and the concentrations obtained by simultaneous equation method.*

	80%		100%		120%	
	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)
absorbance of sample 1	0.495	0.375	0.556	0.418	0.609	0.46
absorbance of sample 2	0.493	0.374	0.56	0.419	0.611	0.463
absorbance of sample 3	0.5	0.377	0.558	0.418	0.613	0.462
mean	0.496	0.3753	0.558	0.4183333	0.611	0.4616667
concentration(g/100ml)	0.00145	0.0002	0.00164	0.0002469	0.00179	0.0002763
concentration(µg/ml)	14.5289	2.2518	16.3725	2.4694889	17.9023	2.7626711
SD	0.00361	0.0015	0.002	0.0005774	0.002	0.0015275
RSD%	0.727823	0.39968	0.358423	0.138024	0.327332	0.330866

***Table 4.11**: Results for recovery studies*

Recovery level	Initial amount (µg/ml)		Concentration of drug added (µg/ml)		%Recovery (n=3)	
	VAL	HCT	VAL	HCT	VAL	HCT
80%	8	1.25	6.4	1	98.78	99.35
100%	8	1.25	8	1.25	101.95	100.83
120%	8	1.25	9.6	1.5	101.1	100.41
mean					100.61	100.2

4.1.6.4 Precision

Intraday and interday precision were determined by analyzing three different standard solutions of VAL and HCT within the same day and three different days over a period of week. Results are shown in table (4.12) and (4.13).

Table 4.12: *Results for interday precision.*

	Sample 1		Sample 2		Sample 3	
	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)
absorbance of sample 1	0.272	0.206	0.274	0.207	0.273	0.206
absorbance of sample 2	0.276	0.209	0.278	0.211	0.276	0.209
absorbance of sample 3	0.273	0.207	0.276	0.209	0.273	0.207
mean	0.27367	0.2073333	0.276	0.209	0.274	0.2073333
concentration(g/100ml)	0.0008	0.0001246	0.00081	0.0001255	0.0008	0.0001244
concentration(µg/ml)	8.01457	1.246407	8.08361	1.2553874	8.02611	1.2438117
assay	1.00182	0.9971256	1.01045	1.0043099	1.00326	0.9950494
SD	0.00208	0.0015275	0.002	0.002	0.00173	0.0015275
RSD %	0.760	0.736	0.724	0.956	0.631	0.736

Table 4.13: *Results for intraday precision.*

	Day 1		Day 2		Day 3	
	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)
absorbance of sample 1	0.277	0.208	0.277	0.21	0.269	0.205
absorbance of sample 2	0.275	0.208	0.281	0.212	0.276	0.209
absorbance of sample 3	0.274	0.207	0.281	0.213	0.278	0.211
mean	0.27533	0.2076667	0.27967	0.2116667	0.27433	0.2083333
concentration(g/100ml)	0.00081	0.0001239	0.00082	0.000127	0.0008	0.0001258
concentration(µg/ml)	8.06991	1.23886	8.19178	1.2702751	8.03062	1.2575049
assay	1.00874	0.991088	1.02397	1.0162201	1.00383	1.0060039
SD	0.00153	0.0005774	0.00231	0.0015275	0.00473	0.0030551
RSD%	0.555	0.278	0.825	0.721	1.724	1.466

4.1.6.5 Ruggedness

Results for absorbance of VAL/HCT mixture, prepared and analyzed by two different analysts, are shown in table (4.14).

***Table 4.14**: Absorbance values of VAL/HCT mixture prepared and analyzed by two different analysts (n=3), and the concentrations calculated by simultaneous equation method.*

	Analyst 1		Analyst 2	
	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)
absorbance of sample 1	0.272	0.206	0.272	0.206
absorbance of sample 2	0.276	0.209	0.273	0.206
absorbance of sample 3	0.273	0.207	0.276	0.209
mean	0.27367	0.2073333	0.27367	0.207
concentration(g/100ml)	0.0008	0.0001246	0.0008	0.0001241
concentration(µg/ml)	8.01457	1.246407	8.01691	1.2409775
assay	1.00182	0.9971256	1.00211	0.992782
SD	0.00208	0.0015275	0.00208	0.0015275
RSD%	0.760039464	0.73673645	0.76003946	0.737922705

4.1.6.6 Robustness

Results for absorbance of VAL/HCT mixture when methanol was used as solvent for VAL/HCT mixture instead of 0.1N NaOH, are shown in table (4.15).

***Table 4.15**: Absorbance values of VAL/HCT mixture when methanol was used as solvent (n=3), and the concentrations calculated by simultaneous equation method.*

	methanol		0.1N NaOH	
	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)
absorbance of sample 1	0.281	0.212	0.272	0.206
absorbance of sample 2	0.281	0.213	0.276	0.209
absorbance of sample 3	0.28	0.212	0.273	0.207
mean	0.28067	0.2123333	0.27367	0.2073333
concentration(g/100ml)	0.00082	0.0001273	0.0008	0.0001246
concentration(µg/ml)	8.2217	1.2733482	8.01457	1.246407
assay	1.02771	1.0186786	1.00182	0.9971256
SD	0.00058	0.0005774	0.00208	0.0015275
RSD%	0.206648377	0.27193097	0.760039464	0.73673645

4.1.6.7 Limit of Detection and Limit of Quantitation LOD

LOD and LOQ can be calculated, based on the calibration curve and the standard deviation of y-intercepts of regression. Equation (15) and (16) were used.

The detection limit (LOD) = 3.3 σ/S .....….... (15)

The quantitation limit (LOQ) =10 σ/S…….. (16)

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve.

Excel 2016 and data analysis were used to obtain standard error of intercept and regression. Values of LOD and LOQ for VAL and HCT are summarized in table 4.16.

***Table 4.16**: Values of LOD and LOQ for VAL and HCT*

API	LOD (µg/ml)	LOQ (µg/ml)
VAL	0.248	0.753
HCT	0.1946	0.589

4.1.6.8 Summary of results and discussion of method development and validation

The analytical method has been developed for simultaneous estimation of VAL and HCT in combined pharmaceutical dosage form using simultaneous equation. In 0.1 N NaOH, VAL showed maximum absorbance at 248 nm and HCT at 271.5 nm. Linearity was observed in the range 4– 40 μg/mL ($R2$ = 0.999) of VAL and 1–16 μg/mL ($R2$ = 0.999) of HCT. The proposed method was applied for pharmaceutical formulation, and % label claim of VAL and HCT was found to be 103.0 and 101.87, respectively. The amount of drug estimated by proposed method was in good agreement with the label claim. Accuracy of the method was checked by the recovery studies at three different levels, which are, 80%, 100%, and 120%. The mean % recovery for VAL and HCT was found to be 100.61 and 100.2, respectively. The method was found to be precise as indicated by the interday and intraday analysis, showing that % R.S.D. is less than 2. The results did not show any statistical difference between operators suggesting that method developed was rugged. Also, there was no any statistical difference between different solvents suggesting that method was robust. The sensitivity of method was assessed by determining LOD and LOQ. For VAL, LOD and LOQ were found to be 0.248 and 0.753 μg/mL, respectively. For HCT, the LOD and LOQ

were found to be 0.1946 and 0.589 μg/mL, respectively. All validation parameters are summarized in table (4.17).

Table 4.17: *Summary of validation parameters.*

	VAL	HCT
λ_{max}	248	271.5
Linearity range(µg/ml)	4-40	1-16
Regression equation	y = 0.0316x - 0.0009	y = 0.0646x + 0.0004
Slope	0.0316	0.0646
Y- intercept	0.0009	0.0004
r^2	R^2 = 0.9999	R^2 = 0.9999
% Recovery (n=3)	100.61	100.2
LOD(µg/ml)	0.248	0.1946
LOQ(µg/ml)	0.753	0.589
Precision(% RSD)		
Intra- day (n=3)	0.948	0.821
Inter-day (n=3)	0.705	0.2076
Specificity (% RSD)		
Starch addition	0.549	0.478
Lactose addition	0.555	0.72
Ruggedness (% RSD)		
Analyst 1 (n=3)	0.76	0.76
Analyst 2 (n=3)	0.76	0.738
Robustness (% RSD)		
methanol	0.2066	0.2719
0.1N NaOH	0.76	0.7367

In a previous work, for simultaneous determination of VAL and HCT λ_{max} for VAL and HCT was 250 and 272 respectively[50], in other work they were 249, and 273[47]. Linearity range was 2-24 and 2-12 µg/ml for VAL and HCT respectively[47]. LOD was 0.0024 and .033 µg/ml for VAL and HCT, LOQ was 0.0063 and 0.036 µg/ml for VAL and HCT respectively[47] .In other work, LOD was 0.69 and 0.13 µg/ml for VAL and HCT respectively, where LOQ was 1.83 and 0.42 respectively[48].

4.2 Solubility Study Results

Solutions of VAL and HCT were prepared at different pHs as described previously (3.4 Solubility study). The concentrations of VAL and HTC were determined by UV and the responses were measured at 25°C. Results are shown in table (4.18).

Table 4.18*: Solubility of VAL and HCT in different media (n=3).*

	Water (mg/100ml)	PBS, 7.4 pH (mg/100ml)	FaSSIF, 6.5 pH (mg/100ml)	FeSSIF, 5pH (mg/100ml)
VAL alone	19.2	31.7	22.1	20.2
HCT alone	74.5	110.3	63.7	47.2
VAL in mixture	5.5	227.7	244.84	143.9
HCT in mixture	67.5	62.99	79.34	109.79

Valsartan is strongly pH dependent solubility. A rise from pH 4 to pH 6 increases the solubility of valsartan by a factor of about 1000. The increase in solubility could be as a result of an increase in the percentage of valsartan molecules ionizing at high pH values[51].. In previous work solubility of VAL was 197, 200 and 320 µg/ml at water, 6.5 and 7.4 pH respectively [52].

Also HCT solubility is pH dependent. Solubility of HCTZ in aqueous solutions is low, in the pH range from 1.0 to 7.4, ranging from 0.0608 to 0.103 g per 100 mL. Solubility in aqueous solutions within pH 10.2–11.6 changes to 1.79 and 2.2 g per 100 mL[33].

4.3 Stability Study

Results for absorbance of stability study samples in PBS, FaSSIF, and FeSSIF are shown in table (4.19), (4.20), and (4.21) respectively. From results, it was obtained that VAL/HCT mixture is stable in different media along the time of the diffusion experiment.

In literature, in a previous work it was noticed that after incubation of different aqueous solutions of valsartan at pH ranging from 2 to 12 and different incubation time from 1 to 8 days at 37°C there was a decrease in valsartan concentration in all tested pH at all time. However, the highest recovery rate was achieved with pH 6.8. The results may indicate that neutral and alkaline pH can protect or enhance stability of valsaran. VAL hydrolysis is pH dependent. Increase in temperature at low pH, decrease stability of VAL[53].

Stability of HCT is pH dependent it goes alkaline hydrolysis. The hydrolysis is complete at pH higher than 12. At pHs below 2.5 and above pH 12 degradation is linear and shows first-order dependence of H+ and OH– concentration. The degradation profile between pH 7 and 11.5 is probably the result of dissociation equilibrium. The pH is strongly influenced by the included excipients, and therefore the approaches to modify pH are useful for optimization of HCTZ stability[33].

In this stability study, it was noticed that incubation of VAL in PBS (7.4 pH) at 37°C doesn't show a remarkable decrease in recovery percentage, 95.31 and 95.33% was recovered after 5 and 8 hours of incubation respectively, so VAL was stable along experiment time (5 hours) at PBS.

In FaSSIF (6.5 pH), there was a decrease in VAL concentration with time, after 5 and 8 hours the amount recovered were 97.65 and 94.4 % respectively. Experiment can be performed along the five hours, VAL doesn't show remarkable decrease during this time.

In FeSSIF (5 pH), there was an increase in VAL concentration with time, after 5 and 8 hours the amount recovered was 104 and 106.18 % respectively. Experiment can be performed along the five hours, VAL doesn't show remarkable increase during this time.

For HCT, it was noticed that incubation of HCT in PBS (7.4 pH) at 37°C doesn't show a remarkable decrease in recovery percentage, 95.14 and 94.81% was recovered after 5 and 8 hours of incubation respectively, so HCT was stable along experiment time (5 hours) at PBS.

In FaSSIF (6.5 pH), there was a decrease in HCT concentration with time, after 5 and 8 hours the amount recovered was 97.84 and 94.48 % respectively. Experiment can be performed along the five hours, HCT doesn't show remarkable decrease during this time.

In FeSSIF (5 pH), there was an increase in HCT concentration with time, after 5 and 8 hours the amount recovered was 101.2 and 98.08 %. Experiment can be performed along the five hours, HCT doesn't show remarkable decrease during this time.

***Table 4.19**: Results for stability study for **VAL and HCT** (in mixture) in PBS (n=3).*

	VAL					HCT				
Time	VAL (248)	Concentration (µg/ml)	assay	SD	RSD%	HCT(271.5)	Concentration (µg/ml)	assay	SD	RSD%
at zero time	0.2703	7.914	100	0.0025	0.93	0.205	1.235	100	0.003	1.46
after 3hrs	0.267	7.84	99.064948	0.0025	0.94	0.2023	1.212	98.1377	0.0025	1.243
after 5hrs	0.2576	7.543	95.312105	0.0015	0.592	0.1953	1.175	95.1417	0.0025	1.288
after 8hrs	0.2576	7.545	95.337377	0.0011	0.448	0.195	1.171	94.8178	0.002	1.025

***Table 4.20**: Results for stability study for **VAL and HCT** (in mixture) in FaSSIF (n=3).*

	VAL					HCT				
Time	VAL(248)	concentration (µg/ml)	Assay%	SD	RSD%	HCT(271.5)	Concentration (µg/ml)	Assay%	SD	RSD%
at zero time	0.275	8.054	100	0.001	0.363	0.2083	1.252	100	0.003	1.46
after 3hrs	0.2723	7.982	99.106034	0.0005	0.212	0.2053	1.224	97.7636	0.0005	0.2811
after 5hrs	0.2686	7.865	97.65334	0.0005	0.214	0.2036	1.225	97.8435	0.0005	0.2834
after 8hrs	0.26	7.603	94.4	0.002	0.437	0.1986	1.183	94.4888	0.0011	0.581

***Table 4.21**: Results for stability study for **VAL and HCT** (in mixture) in FeSSIF (n=3).*

	VAL					HCT				
Time	VAL (248)	Concentration (µg/ml)	Assay%	SD	RSD%	HCT(271.5)	Concentration (µg/ml)	Assay%	SD	RSD%
at zero time	0.2806	8.234	100	0.0005	0.205	0.2103	1.241	100	0.0005	0.2744
after 3hrs	0.283	8.298	103.02955	0.001	0.3533	0.2116	1.266	101.118	0.0015	0.721665
after 5hrs	0.2856	8.379	104.03526	0.00115	0.404	0.2146	1.267	101.198	0.0005	0.269
after 8hrs	0.2906	8.552	106.18326	0.002	0.7161	0.2143	1.228	98.0831	0.0005	0.2693

4.4 Permeation study results using dialysis membrane.

The lag time (T_L) reflects the time required by the Active Pharmaceutical Ingredient API to pass through the intact membrane and reach the receiver compartment. Diffusion coefficient (D) measures the membrane resistance encountered by the diffusant. Permeability coefficient (P) gives an indication about the distance passed by the substance within specific period. The partition coefficient (K) gives an indication about the ability of API to partition between the oily phase and the aqueous phase, this parameter includes other diffusion parameters as previously shown in the calculation of diffusion parameter (part 3). Later on in this thesis, we will attempt to compare the enhancement ratio (ER) of various penetration enhancers (P after / P before). The greater the ER the greater the penetration enhancement ability of penetration enhancer used. For all the experiments samples were taken at half hour intervals for three hours and followed by one hour intervals for two hours and UV absorbance at 248 and 271.5 was presented in triplicates.

At first stage of experiments, we used sandwiched dialysis membrane with nylon membrane to test the permeation of VAL and HCT with and without intestinal permeation enhancer. List of experiments performed at this stage are listed in table (4.22).

At the second stage of experiments, Permeapad membrane was used to evaluate the permeation of VAL and HCT with and without intestinal enhancer. The permeation enhancers used are the best two enhancers established in stage one. List of experiments performed at this stage are listed in table (4.23).

The T_L was calculated by dividing the intercept of the equation of flux profile on the slop from the same equation. The diffusion parameter (D) was calculating using equation (11):

$$T_L = h^2/6D \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (11)$$

The permeability coefficient (P) of VAL and HCT was calculated by dividing the value of the slop of the flux profile by the concentration of VAL and HCT in the donor compartment (6.4mg/ml, 1mg/ml respectively). The permeability coefficient (P) of VAL and HCT obtained in this experiment were used as the main value in the comparison between the activity of different permeation enhancers for stage one experiments, since its value was obtained from all diffusion parameters as shown in equation (10).

$$K = (P.h)/D \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (10)$$

***Table 4.22**: list of samples used in permeation experiments to be tested using sandwiched dialysis membrane with nylon membrane.*

Experiment No.	Composition														
	VAL 6.4 mg/ml	HCT 1 mg/ml	PBS Donor	PBS Receptor	FaSSIF Donor	FeSSIF Donor	Citric acid 1%	SLS 1%	PEG 4000 1%	Na acetate 1%	Sorbitol 1%	PVP 30 1%	Mannitol 1%	EDTA 1%	Tween 80 1%
E1	X	X	X	X											
E2	X	X	X	X			X								
E3	X	X	X	X				X							
E4	X	X	X	X					X						
E5	X	X	X	X						X					
E6	X	X	X	X							X				
E7	X	X	X	X								X			
E8	X	X	X	X									X		
E9	X	X	X	X										X	
E10	X	X	X	X											X
E11	X	X		X	X		X								
E12	X	X		X		X	X								
E13	X	X		X	X					X					
E14	X	X		X		X				X					

***Table 4.23**: list of samples used in permeation experiments to be tested using Permeapad membrane.*

Experiment No.	Composition									
	VAL 6.4 mg/ml	HCT 1mg/ml	PBS Donor	PBS Receptor	FaSSIF Donor	FeSSIF Donor	Na acetate 1%	Citric acid 1%	Citric acid 1.5%	Citric acid 2%
E15	X	X	X	X						
E16	X	X		X	X		X			
E17	X	X		X		X	X			
E18	X	X		X	X			X		
E19	X	X		X		X		X		
E20	X	X			X					
E21	X	X				X				
E22	X	X			X		X	X		
E23	X	X				X	X	X		
E24	X	X			X				X	
E25	X	X				X			X	
E26	X	X			X					X
E27	X	X				X				X

4.4.1 Experiment no. E1, VAL/HCT solution in PBS without permeation enhancer using sandwiched dialysis membrane

The basic solution of VAL/HCT in PBS was prepared without using permeation enhancer as a control to constitute a base for comparison. Samples were taken from the sampling port of the acceptor compartment and analyzed by UV to measure the amount of VAL and HCT.

Tables (4.24), (4.25) illustrate the assay results of APIs permeated to the acceptor compartment by time. UV absorbance at 248 and 271.5 was presented in triplicates, and the cumulative amount of VAL and HCT permeated (Q) per unit of the membrane area was determined and plotted as a function of time (Fig. 4.5) and (Fig. 4.6) and the diffusion parameter were calculated for VAL and HCT and tabulated in table (4.26) and (4.27)

***Table 4.24**: Data obtained from E1, through sandwiched dialysis membrane with nylon membrane using Franz diffusion cell, without addition of permeation enhancer (part1).*

time	absorbance of sample 1 (n=3)		absorbance of sample 2 (n=3)		absorbance of sample 3 (n=3)		concentration of sample **I** mg/ml		concentration of sample **II** mg/ml		concentration of sample **III** mg/ml	
	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL	HCT	VAL	HCT	VAL	HCT
0.5	0.018	0.0153	0.0243	0.0196	0.017	0.015	0.0015	0.000327	0.0021	0.00039	0.00145	0.00034
1	0.0166	0.014	0.0173	0.0146	0.0156	0.015	0.0014	0.000296	0.0015	0.000309	0.0013	0.00037
1.5	0.011	0.009	0.012	0.01	0.0113	0.01	0.001	0.000183	0.001	0.000208	0.00096	0.00022
2	0.0153	0.013	0.0163	0.0134	0.0163	0.014	0.0013	0.000278	0.0014	0.000274	0.0014	0.0003
2.5	0.015	0.012	0.0143	0.011	0.0143	0.0113	0.0013	0.000236	0.0013	0.000204	0.00125	0.00022
3	0.017	0.012	0.017	0.013	0.018	0.014	0.0015	0.000189	0.0015	0.000238	0.00157	0.00026
4	0.0277	0.0205	0.025	0.019	0.027	0.021	0.0024	0.000355	0.0022	0.000345	0.00236	0.0004
5	0.035	0.026	0.0343	0.0253	0.0353	0.0276	0.0031	0.000453	0.003	0.000435	0.00308	0.00052

***Table 4.25**: Data obtained from E1, through sandwiched dialysis membrane with nylon membrane using Franz diffusion cell, without addition of permeation enhancer (part2).*

Q: cumulative amount released **I** (mg)		Q: cumulative amount released **II** (mg)		Q: cumulative amount released **III** (mg)		m: cumulative amount released per area **I** (mg/cm2)		m: cumulative amount released per area **II** (mg/cm2)		m: cumulative amount released per area **III** (mg/cm2)		**mean amount (mg/cm2)**		**SD**		**%RSD**	
VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT
0.031	0.007	0.042	0.008	0.029	0.007	0.01	0.002	0.0134	0.002	0.009	0.002	0.0108	0.002	0.002	0.00022	20.97	9.719
0.061	0.013	0.074	0.014	0.056	0.014	0.019	0.004	0.0236	0.005	0.018	0.005	0.0203	0.004	0.003	0.0003	14.29	6.681
0.082	0.017	0.096	0.019	0.077	0.019	0.026	0.005	0.0307	0.006	0.025	0.006	0.0271	0.006	0.003	0.00043	11.84	7.416
0.109	0.022	0.126	0.025	0.106	0.026	0.035	0.007	0.04	0.008	0.034	0.008	0.0361	0.008	0.003	0.0005	9.318	6.494
0.136	0.027	0.152	0.029	0.132	0.03	0.043	0.009	0.0484	0.009	0.042	0.01	0.0446	0.009	0.003	0.00044	7.439	4.774
0.168	0.032	0.183	0.034	0.165	0.036	0.053	0.01	0.0583	0.011	0.053	0.011	0.0548	0.011	0.003	0.00067	5.657	6.283
0.218	0.039	0.228	0.041	0.214	0.044	0.069	0.012	0.0728	0.013	0.068	0.014	0.0701	0.013	0.002	0.00082	3.418	6.226
0.282	0.048	0.291	0.05	0.278	0.055	0.09	0.015	0.0927	0.016	0.088	0.017	0.0904	0.016	0.002	0.00108	2.399	6.661

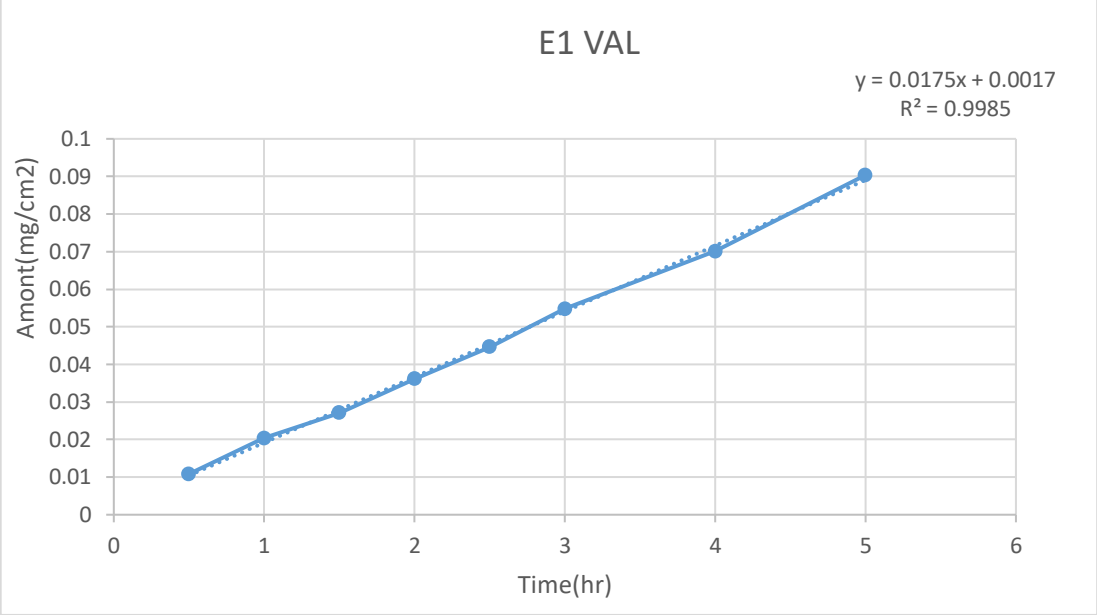

***Figure 4.5**: In vitro permeation profile for the cumulative amount of VAL permeated per unit area of sandwiched dialysis membrane with nylon membrane (mg/cm²) for experiment E1, without addition of permeation enhancers.*

The best linear line for VAL and HCT is determined in Figure (4.5) and Figure (4.6) by Excel 2016, from which the linear line equation is determined. The equation helps in determining the slop and the x intercept, these were used for further calculation of diffusion parameters. The diffusion parameters are calculated according to the equations presented on table (3.10). The diffusion parameters for VAL and HCT are shown in table (4.26) and (4.27).

***Table 4.26:** Diffusion parameters for VAL E1, without addition of PE.*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	**ER**
E1	0.0175	0.0017	10.2941	0.00146	0.00273	0.056296	1

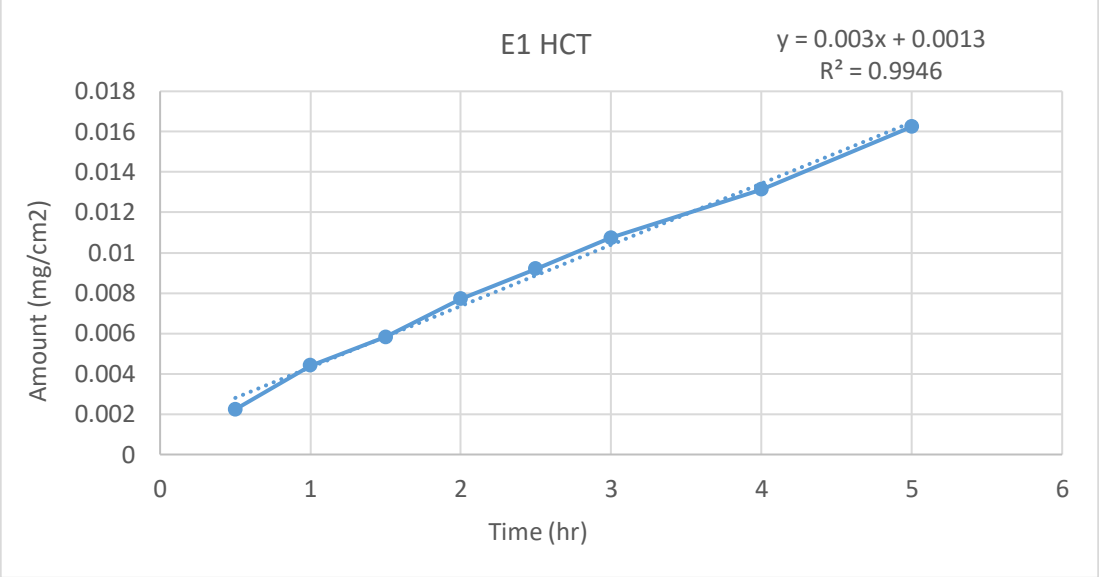

***Figure 4.6**: In vitro permeation profile for the cumulative amount of HCT permeated per unit area of sandwiched dialysis membrane with nylon membrane (mg/cm²) for experiment E1, without addition of permeation enhancers.*

***Table 4. 27:** Diffusion parameters for HCT E1, without addition of PE.*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	**ER**
E1	0.003	0.0013	2.30769	0.0065	0.003	0.013846	1

The permeability coefficient (P) and other parameters of VAL and HCT permeation obtained in this experiment were used as the main value in the comparison between the activity of different permeation enhancers for stage one experiments.

4.4.2 Experiment no. E2, VAL/HCT solution in PBS with Citric acid through sandwiched dialysis membrane.

Citric acid is chelating agent, buffering agent and anti-oxidant,it is a tribasic acid, with pK_a values of 3.128, 4.761, and 6.396 at 25 °C. I.t can be used as chelating agent to enhance permeation. It forms complexation of calcium and magnesium ions present in between intestinal epithelial cells and ultimately leads to opening of tight junctions and thereby increasing permeability for exogenous substances[54].

In a previous works, citric acid was used to improve dissolution and bioavailability of loratidine [22], and it was used with oral peptides and proteins to enhance permeation, it inhibits small intestinal serine proteases[55].

Basic formulation of VAL and HCT with 1% of citric acid was prepared according to general method described in section (3.7.4). The permeability results of drug through sandwiched dialysis membrane with nylon membrane are shown in tables (4.28), (4.29).

The cumulative amount of VAL and HCT permeated through unit area of membrane was then calculated as mentioned before in section 3.6.4 , the linear section, i.e. the steady state flux was plotted versus time (Figure (4.7) and Figure (4.8)).

Table 4.28: Data obtained from E2, through sandwiched dialysis membrane with nylon membrane using Franz diffusion cell, with addition of citric acid (part1).

time	absorbance of sample 1 (n=3)		absorbance of sample 2 (n=3)		absorbance of sample 3 (n=3)		concentration of sample **I** mg/ml		concentration of sample **II** mg/ml		concentration of sample **III** mg/ml	
	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL	HCT	VAL	HCT	VAL	HCT
0.5	0.0406	0.04	0.042	0.041	0.0446	0.0416	0.00337	0.00101	0.0035	0.001	0.0038	0.00099
1	0.0406	0.039	0.0406	0.061	0.044	0.0573	0.00339	0.00096	0.0029	0.002	0.0034	0.00177
1.5	0.0583	0.0856	0.0596	0.1043	0.0583	0.0933	0.00425	0.00282	0.004	0.0037	0.0041	0.0032
2	0.0726	0.146	0.0696	0.125	0.071	0.141	0.00446	0.00544	0.0046	0.0045	0.0044	0.00523
2.5	0.0866	0.155	0.0833	0.1783	0.087	0.166	0.00572	0.00555	0.0049	0.0068	0.0055	0.00608
3	0.0986	0.2146	0.0976	0.2136	0.1	0.1946	0.00571	0.00818	0.0056	0.0082	0.0063	0.00717
4	0.142	0.2816	0.1426	0.3073	0.1416	0.2813	0.00881	0.01044	0.0083	0.0117	0.0088	0.01044
5	0.149	0.311	0.181	0.383	0.166	0.343	0.00892	0.01172	0.0107	0.0145	0.01	0.01288

***Table 4.29**: Data obtained from E2, through sandwiched dialysis membrane with nylon membrane using Franz diffusion cell, with addition of citric acid (part 2).*

Q: cumulative amount released **I** (mg)		Q: cumulative amount released **II** (mg)		Q: cumulative amount released **III** (mg)		m: cumulative amount released per area **I** (mg/cm2)		m: cumulative amount released per area **II** (mg/cm2)		m: cumulative amount released per area **III** (mg/cm2)		**mean amount (mg/cm2))**		**SD**		**%RSD**	
VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT
0.067	0.0201	0.0699	0.0204	0.0751	0.0198	0.0215	0.0064	0.022	0.0065	0.0239	0.0063	0.02255	0.0064	0.0012	0.0001	5.4961	1.5605
0.139	0.0403	0.132	0.0621	0.146	0.0563	0.0442	0.0128	0.042	0.0198	0.0465	0.0179	0.04424	0.0168	0.0022	0.0036	5.0492	21.373
0.227	0.0977	0.2148	0.1382	0.2311	0.122	0.0723	0.0311	0.068	0.044	0.0736	0.0388	0.07144	0.038	0.0027	0.0065	3.8036	17.121
0.321	0.2093	0.3106	0.2316	0.3232	0.2298	0.1021	0.0666	0.099	0.0738	0.1029	0.0732	0.10131	0.0712	0.0021	0.00395	2.0942	5.554
0.439	0.3257	0.413	0.3714	0.4383	0.3566	0.14	0.1037	0.132	0.1183	0.1396	0.1136	0.13703	0.1119	0.0048	0.00743	3.4789	6.639
0.559	0.4949	0.5305	0.5413	0.5695	0.5062	0.1782	0.1576	0.169	0.1724	0.1814	0.1612	0.17617	0.1637	0.0064	0.00771	3.6584	4.7087
0.741	0.712	0.7027	0.7832	0.7512	0.7221	0.2361	0.2268	0.224	0.2494	0.2392	0.23	0.23305	0.2354	0.0082	0.01227	3.5026	5.213
0.929	0.9568	0.9255	1.0847	0.9601	0.9902	0.2957	0.3047	0.295	0.3454	0.3058	0.3154	0.29874	0.3218	0.0061	0.02112	2.0458	6.5625

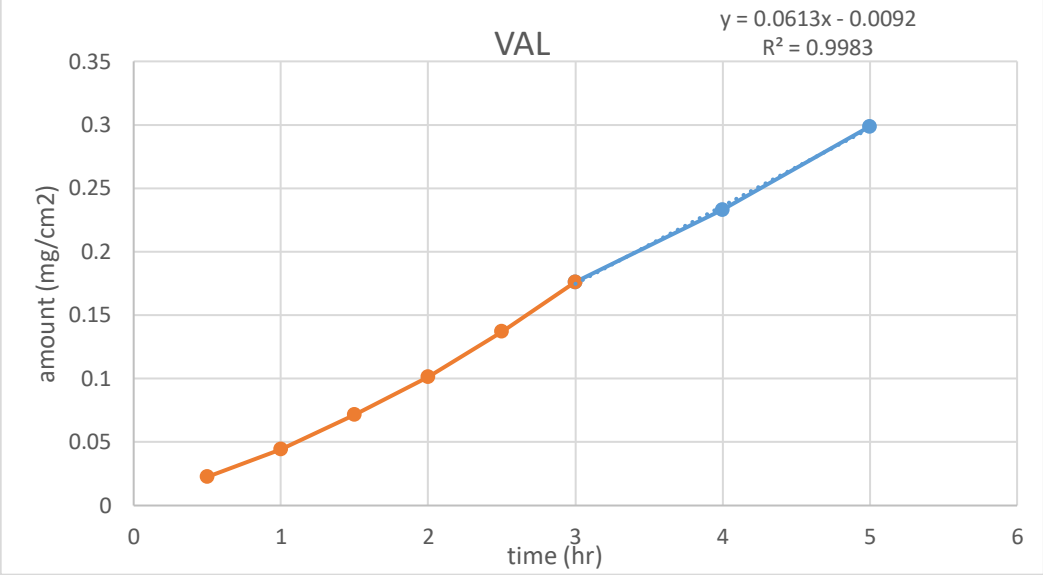

***Figure 4.7**: In vitro permeation profile for the cumulative amount of VAL permeated per unit area of sandwiched dialysis membrane with nylon membrane (mg/cm²) for experiment E2, with citric acid.*

The diffusion parameters for VAL were calculated in table (4.30) and the enhancement ratio was determined.

***Table 4.30:** Diffusion parameters for VAL E2, with citric acid.*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	**ER**
E2	0.0613	0.0092	6.663	0.0023	0.0096	0.1276	3.503

The rate of diffusion of VAL in the presence of 1% citric acid is faster than when it was alone, this is indicated that a higher value of permeability coefficient (P) and an enhancement ratio (ER) of 3.503 as showed in table (4.30).

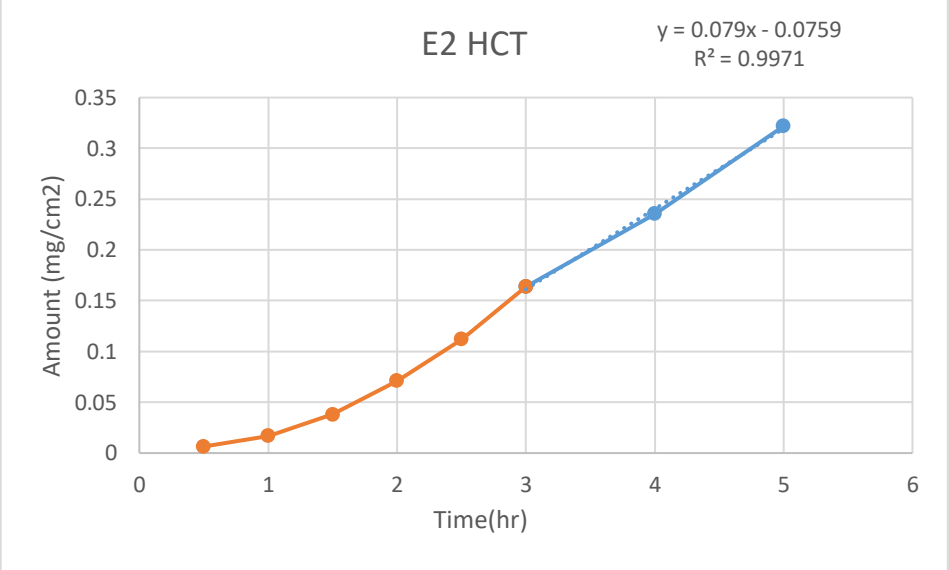

***Figure 4.8**: In vitro permeation profile for the cumulative amount of HCT permeated per unit area of sandwiched dialysis membrane with nylon membrane (mg/cm²) for experiment E2, with citric acid.*

The diffusion parameters for HCT were calculated in table (4.31), and the enhancement ratio was determined.

***Table 4.31:** Diffusion parameters for HCT E2, with citric acid.*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	**ER**
E2	0.079	0.0759	1.040843	0.014411	0.079	0.164453	26.33333

The rate of diffusion of HCT in the presence of citric acid was faster than when it was alone; this is indicated by a higher value of permeability coefficient (P) and an enhancement ratio (ER) of 26.33, as showed in table (4.31).

4.4.3 Experiment no. E3, VAL/HCT solution in PBS with SLS through sandwiched dialysis membrane

Sodium lauryl sulfate (SLS) is an anionic surfactant, emulsifying agent, detergent, skin penetrant, wetting agent and lubricant. It can be used as intestinal permeation enhancer due to its emulsifying properties, it enhance partitioning by reducing the surface tension between the vehicle and the membrane surface and by influencing the barrier potential of the membrane and the tight junctions. It may also disrupt the barrier layers of the membrane[54].

In previous works, SLS was used to enhance permeation of amoxicillin[23] , and progesterone [56].

Basic formulation of VALand HCT and 1% of SLS was prepared according to general method described in section (3.7.4). The permeability results of drug through sandwiched dialysis membrane with nylon membrane are shown in tables (4.32), (4.33).

The cumulative amount of VAL and HCT permeated through unit area of membrane was then calculated as mentioned before, the linear section, i.e. the steady state flux was plotted versus time (Figure (4.9) and Figure (4.10)).

***Table 4.32**: Data obtained from E3, through sandwiched dialysis membrane with nylon membrane using Franz diffusion cell, with addition of SLS (part1).*

time	absorbance of sample 1 (n=3)		absorbance of sample 2 (n=3)		absorbance of sample 3 (n=3)		concentration of sample **I** mg/ml		concentration of sample **II** mg/ml		concentration of sample **III** mg/ml	
	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL	HCT	VAL	HCT	VAL	HCT
0.5	0.0123	0.011	0.0136	0.0126	0.0156	0.0146	0.001	0.00025	0.0011	0.0003	0.00131	0.00035
1	0.0123	0.0113	0.0146	0.0136	0.0173	0.0183	0.001	0.00026	0.0012	0.00032	0.00141	0.00049
1.5	0.023	0.0196	0.0223	0.021	0.0213	0.02	0.002	0.00042	0.0019	0.00051	0.00179	0.00048
2	0.0143	0.013	0.0163	0.0143	0.0156	0.015	0.0012	0.0003	0.0014	0.00032	0.0013	0.00037
2.5	0.0156	0.0136	0.0166	0.0143	0.017	0.015	0.0013	0.0003	0.0014	0.00031	0.00145	0.00034
3	0.0286	0.0246	0.027	0.025	0.0253	0.0246	0.0025	0.00053	0.0023	0.00059	0.00211	0.00061
4	0.0456	0.0406	0.0466	0.0416	0.0476	0.043	0.0039	0.00092	0.004	0.00094	0.00404	0.00099
5	0.0433	0.0373	0.0436	0.036	0.043	0.0376	0.0037	0.00081	0.0038	0.00074	0.00367	0.00083

***Table 4.33**: Data obtained from E3, through sandwiched dialysis membrane with nylon membrane using Franz diffusion cell, with addition of SLS*

Q: cumulative amount released **I** (mg)		Q: cumulative amount released **II** (mg)		Q: cumulative amount released **III** (mg)		m: cumulative amount released per area **I** (mg/cm2)		m: cumulative amount released per area **II** (mg/cm2)		m: cumulative amount released per area **III** (mg/cm2)		**mean amount(mg/cm2)**		**SD**		**%RSD**	
VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT
0.021	0.005	0.023	0.006	0.026	0.007	0.007	0.002	0.007	0.002	0.008	0.002	0.007	0.002	0.0009	0.0003	11.5	16.5
0.043	0.011	0.049	0.013	0.056	0.017	0.014	0.003	0.015	0.004	0.018	0.005	0.016	0.004	0.0021	0.0011	13.3	24.9
0.083	0.019	0.087	0.023	0.093	0.027	0.027	0.006	0.028	0.007	0.03	0.009	0.028	0.007	0.0016	0.0013	5.55	17.2
0.109	0.026	0.117	0.03	0.121	0.035	0.035	0.008	0.037	0.01	0.038	0.011	0.037	0.01	0.0018	0.0015	5	15.5
0.137	0.032	0.147	0.037	0.151	0.042	0.044	0.01	0.047	0.012	0.048	0.013	0.046	0.012	0.0022	0.0016	4.87	13.8
0.188	0.043	0.194	0.049	0.195	0.055	0.06	0.014	0.062	0.016	0.062	0.017	0.061	0.016	0.0012	0.0019	2	12
0.268	0.062	0.275	0.068	0.278	0.075	0.085	0.02	0.088	0.022	0.088	0.024	0.087	0.022	0.0016	0.0021	1.89	9.69
0.346	0.079	0.355	0.084	0.355	0.093	0.11	0.025	0.113	0.027	0.113	0.03	0.112	0.027	0.0017	0.0022	1.49	8.17

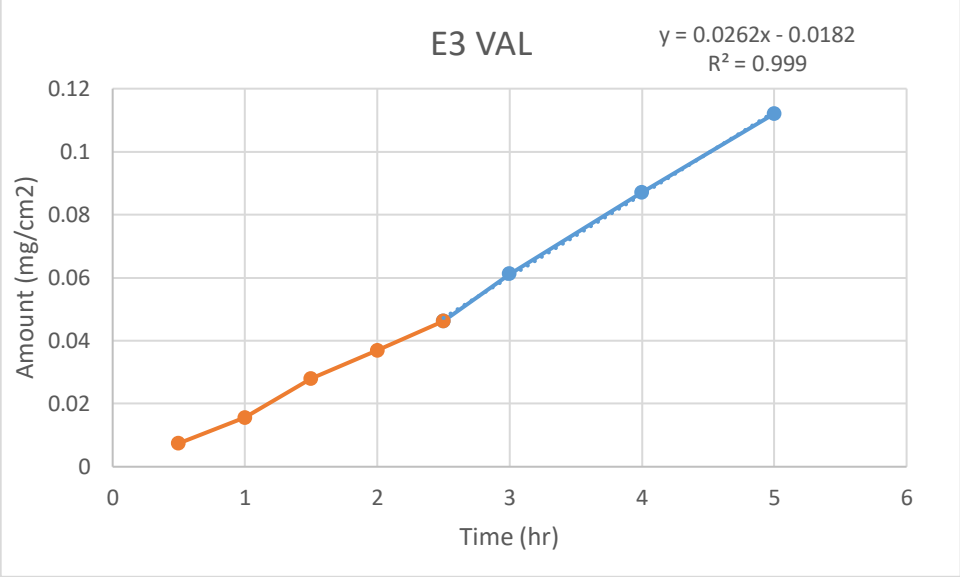

***Figure 4.9**: In vitro permeation profile for the cumulative amount of VAL permeated per unit area of sandwiched dialysis membrane with nylon membrane (mg/cm²) for experiment E3, with SLS.*

The diffusion parameters for VAL were calculated in table (4.34) and the enhancement ratio was determined.

***Table 4.34:** Diffusion parameters for VAL E3, with SLS.*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	**ER**
E3	0.0262	0.0182	1.43956	0.01042	0.004094	0.011786	1.497143

The rate of diffusion of VAL in the presence of SLS was faster than when it was alone; this is indicated by a higher value of permeability coefficient (P) and an enhancement ratio (ER) of 1.497.

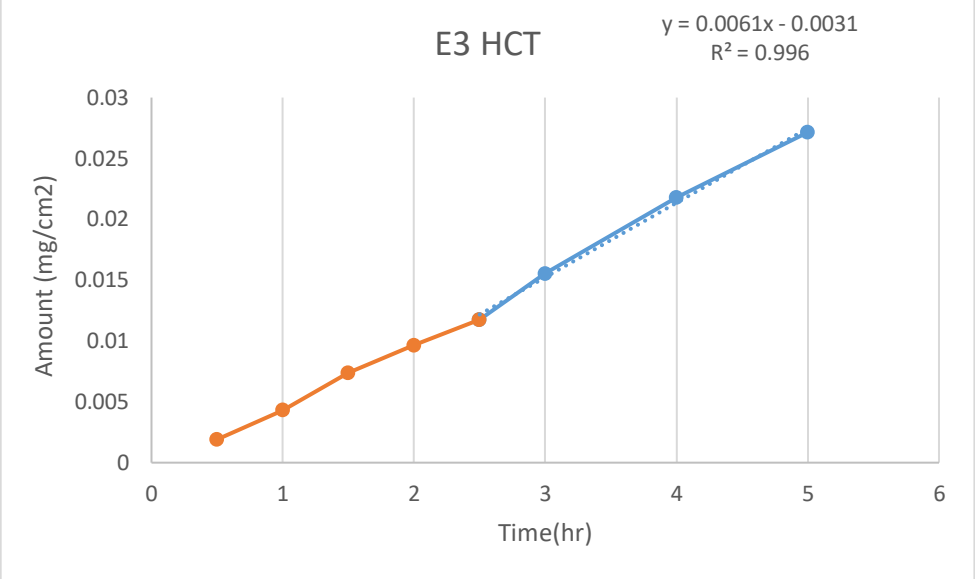

***Figure 4.10**: In vitro permeation profile for the cumulative amount of HCT permeated per unit area of sandwiched dialysis membrane with nylon membrane (mg/cm²) for experiment E3, with SLS.*

The diffusion parameters for HCT were calculated in table (4.35) and the enhancement ratio was determined.

***Table 4.35:** Diffusion parameters for HCT E3, with SLS.*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	**ER**
E3	0.0061	0.0031	1.967742	0.007623	0.0061	0.024006	2.033333

The rate of diffusion of HCT in the presence of SLS was faster than when it was alone; this is indicated by a higher value of permeability coefficient (P) and an enhancement ratio (ER) of 2.033.

4.4.4 Experiment no. E4, VAL/HCT solution in PBS with PEG 4000 through sandwiched dialysis membrane

Polyethylene glycols (PEGs) are polyether compound derived from petroleum with many applications widely used in a variety of pharmaceutical formulations, they are stable, hydrophilic substances that are essentially nonirritant to the skin. Solid grades are generally employed in topical ointments, with the consistency of the base being adjusted by the addition of liquid grades of polyethylene glycol.

Polyethylene glycol 4000 PEG4000 is a water soluble linear polymer formed by the addition reaction of ethylene oxide. It is used as an inactive ingredient in pharmaceutical industry as solvent, plasticizer, surfactant, ointment and suppository base, and tablet and capsule lubricant. It has low toxicity with systemic absorption less than 0.5% [54]. PEG 4000 was used as a hydrophilic carrier that improves dissolution and absorption of ibuprofen[24].

A formulation of VAL and HCT and with 1% of PEG 4000 was prepared according to general method described in section (3.7.4). The permeability results of drug through sandwiched dialysis membrane with nylon membrane are shown in tables (4.36), (4.37).

The cumulative amount of VAL and HCT permeated through unit area of membrane was then calculated as mentioned before, the linear section, i.e. the steady state flux was plotted versus time (Figure (4.11) and Figure (4.12)).

***Table 4.36**: Data obtained from E4, through sandwiched dialysis membrane with nylon membrane using Franz diffusion cell, with addition of PEG4000 (part1)*

time	absorbance of sample 1 (n=3)		absorbance of sample 2 (n=3)		absorbance of sample 3 (n=3)		concentration of sample **I** mg/ml		concentration of sample **II** mg/ml		concentration of sample **III** mg/ml	
	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL	HCT	VAL	HCT	VAL	HCT
0.5	0.02033	0.026	0.02066	0.0276	0.01966	0.02633	0.0016	0.0008	0.00156	0.0009	0.00149	0.00083
1	0.02133	0.0166	0.0226	0.01566	0.02066	0.01733	0.0019	0.0003	0.00202	0.0002	0.00178	0.00036
1.5	0.0223	0.0113	0.0213	0.010966	0.0226	0.01933	0.0021	3E-05	0.00198	4E-05	0.00194	0.00042
2	0.02166	0.0163	0.0203	0.0143	0.02233	0.015	0.0019	0.0003	0.00181	0.0002	0.002	0.00021
2.5	0.0306	0.0213	0.03103	0.02136	0.03086	0.021203	0.0027	0.0003	0.00277	0.0003	0.00276	0.00032
3	0.02566	0.0176	0.02733	0.017	0.0253	0.01733	0.0023	0.0003	0.00248	0.0002	0.00226	0.00026
4	0.0293	0.0194	0.02903	0.0196	0.02893	0.0197	0.0026	0.0003	0.0026	0.0003	0.00259	0.00029
5	0.0396	0.0283	0.03836	0.02853	0.0403	0.02813	0.0035	0.0005	0.00338	0.0005	0.00359	0.00043

Table 4.37*: Data obtained from E4, through sandwiched dialysis membrane with nylon membrane using Franz diffusion cell, with addition of PEG4000 (part2).*

Q: cumulative amount released **I** (mg)		Q: cumulative amount released **II** (mg)		Q: cumulative amount released **III** (mg)		m: cumulative amount released per area **I** (mg/cm2)		m: cumulative amount released per area **II** (mg/cm2)		m: cumulative amount released per area **III** (mg/cm2)		**mean amount(mg/cm2)**		**SD**		**%RSD**	
VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT
0.0313	0.016	0.0313	0.0173	0.03	0.017	0.01	0.0051	0.01	0.0055	0.0095	0.005	0.0098	0.00528	0.0003	0.0002	2.884	4.2538
0.0701	0.023	0.0732	0.0229	0.067	0.025	0.0223	0.0073	0.0233	0.0073	0.0213	0.008	0.0223	0.00749	0.001	0.0003	4.536	4.1971
0.1135	0.024	0.1148	0.0239	0.107	0.033	0.0362	0.0076	0.0366	0.0076	0.0342	0.011	0.0356	0.00862	0.0013	0.0017	3.545	20.127
0.1537	0.03	0.1529	0.0285	0.149	0.038	0.049	0.0095	0.0487	0.0091	0.0476	0.012	0.0484	0.01021	0.0007	0.0016	1.522	16.133
0.2102	0.037	0.2101	0.0351	0.206	0.045	0.0669	0.0116	0.0669	0.0112	0.0658	0.014	0.0665	0.01233	0.0007	0.0016	1.008	13.118
0.2588	0.042	0.2625	0.0392	0.254	0.05	0.0824	0.0134	0.0836	0.0125	0.081	0.016	0.0823	0.01394	0.0013	0.0018	1.547	12.675
0.3137	0.048	0.317	0.045	0.309	0.056	0.0999	0.0152	0.1009	0.0143	0.0983	0.018	0.0997	0.01578	0.0014	0.0018	1.363	11.514
0.3867	0.057	0.3872	0.0553	0.383	0.065	0.1231	0.0182	0.1233	0.0176	0.122	0.021	0.1228	0.01881	0.0007	0.0016	0.603	8.6726

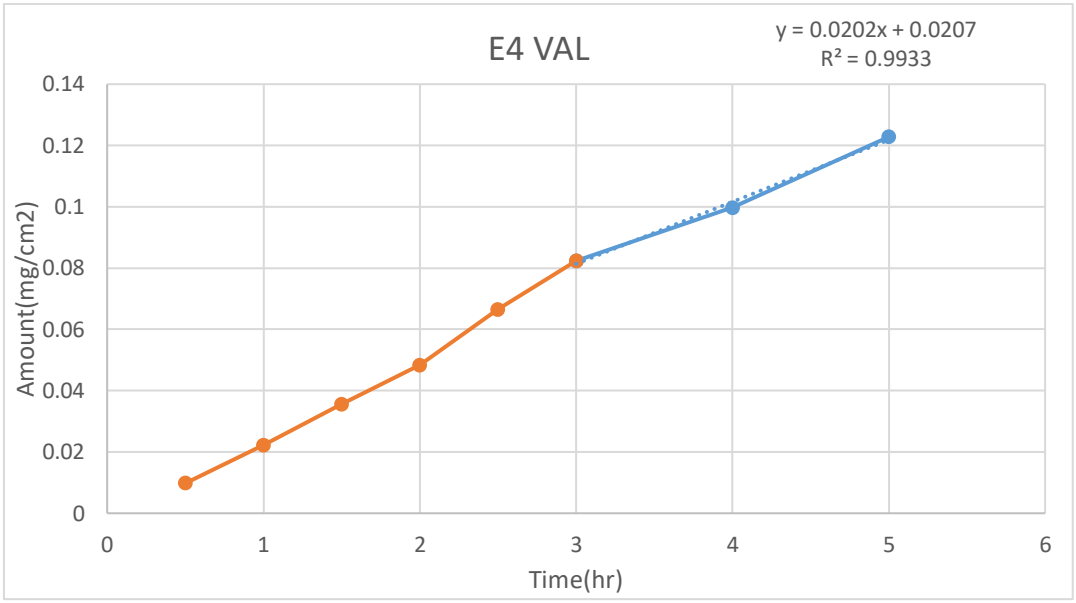

***Figure 4.11**: In vitro permeation profile for the cumulative amount of VAL permeated per unit area of sandwiched dialysis membrane with nylon membrane (mg/cm²) for experiment E4, with PEG4000.*

The diffusion parameters for VAL were calculated in table (4.38) and the enhancement ratio was determined.

***Table 4.38:** Diffusion parameters for VAL E4, with PEG4000.*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	ER
E4	0.0202	0.0207	0.975845	0.015371	0.003156	0.00616	1.154286

The rate of diffusion of VAL in the presence of PEG4000 was faster than when it was alone; this is indicated by a higher value of permeability coefficient (P) and an enhancement ratio (ER) of 1.154.

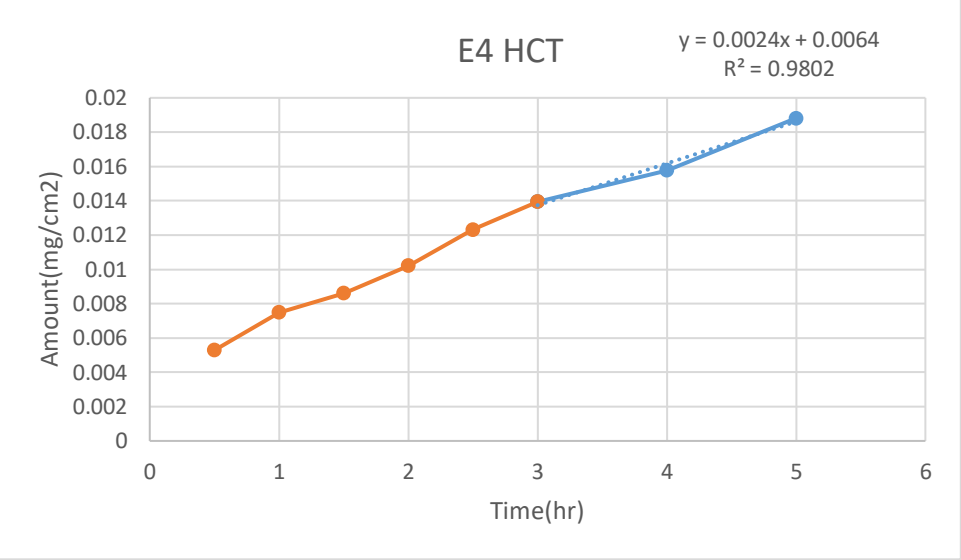

***Figure 4.12**: In vitro permeation profile for the cumulative amount of HCT permeated per unit area of sandwiched dialysis membrane with nylon membrane (mg/cm²) for experiment E4, with PEG4000.*

The diffusion parameters for HCT were calculated in table (4.39) and the enhancement ratio was determined.

***Table 4.39:** Diffusion parameters for HCT E4, with PEG4000.*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	**ER**
E4	0.0024	0.0064	0.375	0.04	0.0024	0.0018	0.8

The rate of diffusion of HCT in the presence of PEG4000 was slower than when it was alone; this is indicated by a lower value of permeability coefficient (P) and an enhancement ratio (ER) of 0.8.

4.4.5 Experiment no. E5, VAL/HCT solution in PBS with Na acetate through sandwiched dialysis membrane

Na acetate is a sodium salt of acetic acid. It is a hygroscopic powder very soluble in water. It is antimicrobial preservative; buffering agent; flavoring agent, stabilizing agent. Sodium acetate is used as part of a buffer system when combined with acetic acid in various intramuscular, intravenous, topical, ophthalmic, nasal, oral, otic, and subcutaneous formulations. It is used to enhance permeation by adjusting pH and control ionization[54].

A formulation of VAL and HCT with 1% of Na acetate was prepared according to general method described in section (3.7.4). The permeability results of drug through sandwiched dialysis membrane with nylon membrane are shown in tables (4.40) and (4.41).

The cumulative amount of VAL and HCT permeated through unit area of membrane was then calculated as mentioned before, the linear section, i.e. the steady state flux was plotted versus time (Figure (4.13) and Figure (4.14)).

***Table 4.40**: Data obtained from E5, through sandwiched dialysis membrane with nylon membrane using Franz diffusion cell, with addition of Na acetate (part 1).*

time	absorbance of sample 1 (n=3)		absorbance of sample 2 (n=3)		absorbance of sample 3 (n=3)		concentration of sample **I** mg/ml		concentration of sample **II** mg/ml		concentration of sample **III** mg/ml	
	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL	HCT	VAL	HCT	VAL	HCT
0.5	0.0373	0.0306	0.0383	0.0313	0.0356	0.0303	0.0032	0.00062	0.0033	0.0006	0.0031	0.00065
1	0.0436	0.0303	0.0406	0.0326	0.044	0.0336	0.0039	0.00046	0.0035	0.0006	0.0039	0.00061
1.5	0.0626	0.0446	0.0606	0.0433	0.0596	0.0426	0.0056	0.00072	0.0054	0.0007	0.0053	0.00069
2	0.0793	0.0556	0.0803	0.0543	0.0776	0.0566	0.0071	0.00086	0.0072	0.0008	0.0069	0.00095
2.5	0.0953	0.0703	0.0993	0.0736	0.0986	0.0726	0.0084	0.00121	0.0088	0.0013	0.0087	0.00124
3	0.0946	0.0696	0.0903	0.0656	0.0956	0.0703	0.0084	0.00119	0.008	0.0011	0.0084	0.0012
4	0.122	0.0896	0.121	0.0903	0.12	0.0916	0.0108	0.00153	0.0107	0.0016	0.0105	0.00167
5	0.11	0.0736	0.103	0.0743	0.106	0.0766	0.0099	0.00103	0.0091	0.0012	0.0094	0.00127

***Table 4.41**: Data obtained from E5, through sandwiched dialysis membrane with nylon membrane using Franz diffusion cell, with addition of Na acetate (part2).*

Q: cumulative amount released **I** (mg)		Q: cumulative amount released **II** (mg)		Q: cumulative amount released **III** (mg)		m: cumulative amount released per area **I** (mg/cm2)		m: cumulative amount released per area **II** (mg/cm2)		m: cumulative amount released per area **III** (mg/cm2)		**mean amount (mg/cm2))**		**SD**		**%RSD**	
VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT
0.065	0.012	0.066	0.013	0.061	0.013	0.021	0.004	0.021	0.004	0.019	0.004	0.02	0.004	0.0008	8E-05	4.115	1.975
0.146	0.022	0.14	0.026	0.141	0.026	0.046	0.007	0.045	0.008	0.045	0.008	0.045	0.008	0.0009	0.0007	1.964	8.674
0.261	0.037	0.251	0.041	0.251	0.04	0.083	0.012	0.08	0.013	0.08	0.013	0.081	0.013	0.0017	0.0006	2.139	5.089
0.407	0.055	0.401	0.057	0.394	0.06	0.13	0.018	0.128	0.018	0.125	0.019	0.128	0.018	0.0022	0.0008	1.719	4.298
0.583	0.08	0.583	0.083	0.575	0.086	0.186	0.026	0.186	0.027	0.183	0.027	0.185	0.026	0.0015	0.0009	0.815	3.437
0.758	0.105	0.752	0.107	0.752	0.111	0.241	0.034	0.239	0.034	0.24	0.035	0.24	0.034	0.0012	0.001	0.489	2.889
0.982	0.137	0.973	0.139	0.971	0.146	0.313	0.044	0.31	0.044	0.309	0.046	0.311	0.045	0.0019	0.0015	0.607	3.246
1.19	0.159	1.166	0.166	1.17	0.173	0.379	0.051	0.371	0.053	0.372	0.055	0.374	0.053	0.0042	0.0022	1.119	4.163

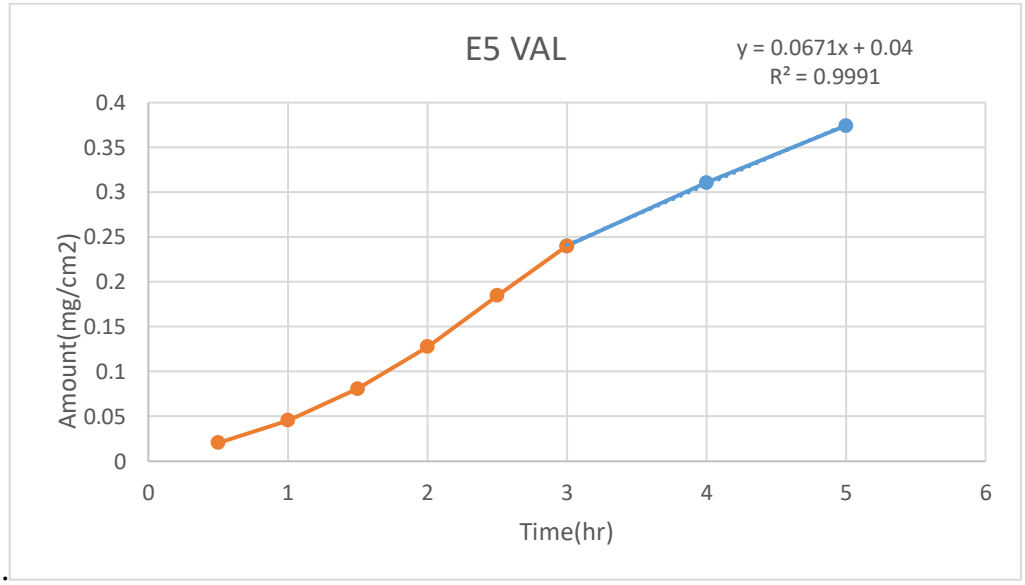

***Figure 4.13**: In vitro permeation profile for the cumulative amount of VAL permeated per unit area of sandwiched dialysis membrane with nylon membrane (mg/cm²) for experiment E5, with Na acetate.*

The diffusion parameters for VAL were calculated in table (4.42) and the enhancement ratio was determined.

***Table 4.42:** Diffusion parameters for VAL E5, with Na acetate.*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	ER
E5	0.067	0.04	1.675	0.008955	0.010469	0.03507	3.828571

The rate of diffusion of VAL in the presence of Na acetate was faster than when it was alone; this is indicated by a higher value of permeability coefficient (P) and an enhancement ratio (ER) of 3.83.

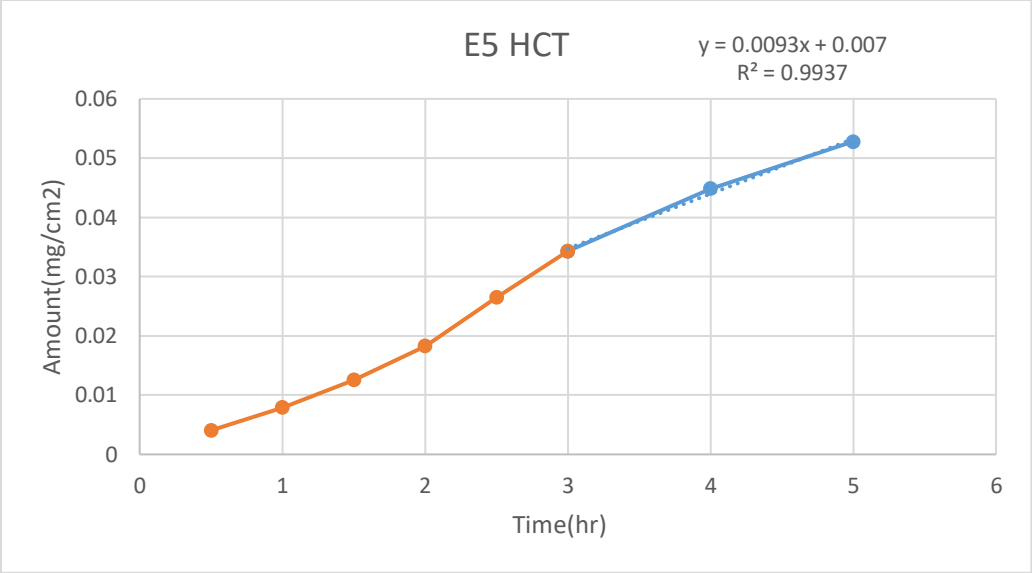

***Figure 4.14**: In vitro permeation profile for the cumulative amount of HCT permeated per unit area of sandwiched dialysis membrane with nylon membrane (mg/cm²) for experiment E5, with Na acetate.*

The diffusion parameters for HCT were calculated in table (4.43) and the enhancement ratio was determined.

***Table 4.43:** Diffusion parameters for HCT E5, with Na acetate.*

sample #	slope	intercept	T_L	D	P	K	ER
E5	0.0093	0.007	1.328571	0.01129	0.0093	0.024711	3.1

The rate of diffusion of VAL in the presence of Na acetate was faster than when it was alone; this is indicated by a higher value of permeability coefficient (P) and an enhancement ratio (ER) of 3.1.

4.4.6 Experiment no. E6, VAL/HCT solution in PBS with sorbitol through sandwiched dialysis membrane

Sorbitol is D-glucitol. It is a hexahydric alcohol related to mannose and is isomeric with mannitol. Sorbitol is available in a wide range of grades and polymorphic forms, such as granules, flakes, or pellets. It functions as a humectant, plasticizer, stabilizing agent, sweetening agent, and tablet and capsule diluent. Sorbitol is a nonionic surfactant, that seem to affect membranes by solubilizing membrane components and thus enhance permeation[54].

In previous work, the effect of sorbitol was investigated as intestinal permeation enhancer. It was obtained that sorbitol decreased the absorption of metoprolol and ranitidine[57].

A sample of VAL and HCT with 1% of sorbitol was prepared according to general method described in section (3.7.4). The permeability results of drug through sandwiched dialysis membrane with nylon membrane are shown in tables (4.44) and (4.45).

The cumulative amount of VAL and HCT with 1% of sorbitol permeated through unit area of membrane was then calculated as mentioned before, the linear section, i.e. the steady state flux was plotted versus time (Figure (4.15) and Figure (4.16)).

***Table 4.44**: Data obtained from E6, through sandwiched dialysis membrane with nylon membrane using Franz diffusion cell, with addition of sorbitol (part 1).*

time	absorbance of sample 1 (n=3)		absorbance of sample 2 (n=3)		absorbance of sample 3 (n=3)		concentration of sample **I** mg/ml		concentration of sample **II** mg/ml		concentration of sample **III** mg/ml	
	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL	HCT	VAL	HCT	VAL	HCT
0.5	0.0143	0.014	0.01453	0.0143	0.01426	0.014	0.00119	0.00035	0.00121	0.00036	0.0012	0.00035
1	0.0216	0.0183	0.0213	0.018	0.021	0.0186	0.00186	0.00039	0.00183	0.00038	0.0018	0.00042
1.5	0.0196	0.0169	0.019	0.017	0.01896	0.0168	0.00168	0.00037	0.00161	0.00039	0.0016	0.00038
2	0.0196	0.017	0.01986	0.017	0.019633	0.01683	0.00168	0.00037	0.0017	0.00037	0.0017	0.00036
2.5	0.023	0.025	0.0243	0.0246	0.0233	0.025	0.00186	0.00068	0.002	0.00063	0.0019	0.00068
3	0.0246	0.0206	0.0236	0.02	0.02396	0.0203	0.00212	0.00043	0.00203	0.00043	0.0021	0.00043
4	0.0246	0.0243	0.0243	0.024	0.02403	0.024	0.00204	0.00061	0.00202	0.00061	0.002	0.00061
5	0.0286	0.0306	0.02796	0.0313	0.02753	0.03093	0.00232	0.00083	0.00224	0.00088	0.0022	0.00087

***Table 4.45**: Data obtained from E6, through sandwiched dialysis membrane with nylon membrane using Franz diffusion cell, with addition of sorbitol (part 2).*

Q: cumulative amount released **I** (mg)		Q: cumulative amount released **II** (mg)		Q: cumulative amount released **III** (mg)		m: cumulative amount released per area **I** (mg/cm2)		m: cumulative amount released per area **II** (mg/cm2)		m: cumulative amount released per area **III** (mg/cm2)		**mean amount(mg/cm2)**		**SD**		**%RSD**	
VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT
0.0238	0.007	0.0241	0.007	0.024	0.007	0.008	0.002	0.008	0.002	0.008	0.002	0.008	0.002	7E-05	3E-05	0.965	1.447
0.0621	0.015	0.062	0.015	0.061	0.016	0.02	0.005	0.02	0.005	0.019	0.005	0.02	0.005	0.0003	0.0001	1.312	2.155
0.0976	0.023	0.0961	0.023	0.095	0.024	0.031	0.007	0.031	0.007	0.03	0.008	0.031	0.007	0.0004	0.0001	1.464	1.766
0.1328	0.031	0.1318	0.031	0.13	0.031	0.042	0.01	0.042	0.01	0.041	0.01	0.042	0.01	0.0004	0.0001	1.056	1.05
0.1717	0.045	0.1736	0.044	0.17	0.045	0.055	0.014	0.055	0.014	0.054	0.014	0.055	0.014	0.0006	0.0002	1.177	1.344
0.2159	0.054	0.2162	0.053	0.213	0.055	0.069	0.017	0.069	0.017	0.068	0.017	0.068	0.017	0.0006	0.0002	0.917	1.279
0.2589	0.067	0.2585	0.066	0.254	0.067	0.082	0.021	0.082	0.021	0.081	0.021	0.082	0.021	0.0008	0.0002	0.951	1.149
0.3074	0.084	0.3054	0.084	0.301	0.085	0.098	0.027	0.097	0.027	0.096	0.027	0.097	0.027	0.0011	0.0002	1.15	0.903

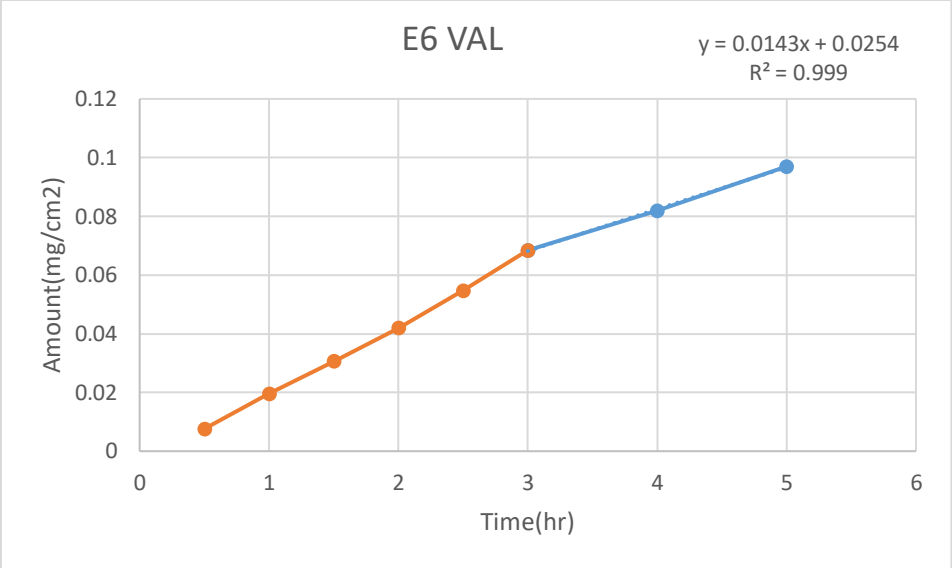

***Figure 4.15**: In vitro permeation profile for the cumulative amount of VAL permeated per unit area of sandwiched dialysis membrane with nylon membrane (mg/cm²) for experiment E6, with sorbitol.*

The diffusion parameters for VAL were calculated in table (4.46) and the enhancement ratio was determined.

***Table 4.46:** Diffusion parameters for VAL E6, with sorbitol.*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	ER
E6	0.0143	0.0254	0.562992	0.026643	0.002234	0.002516	0.817143

The rate of diffusion of in the presence of sorbitol was slower than when it was alone; this is indicated by a lower value of permeability coefficient (P) and an enhancement ratio (ER) of 0.817.

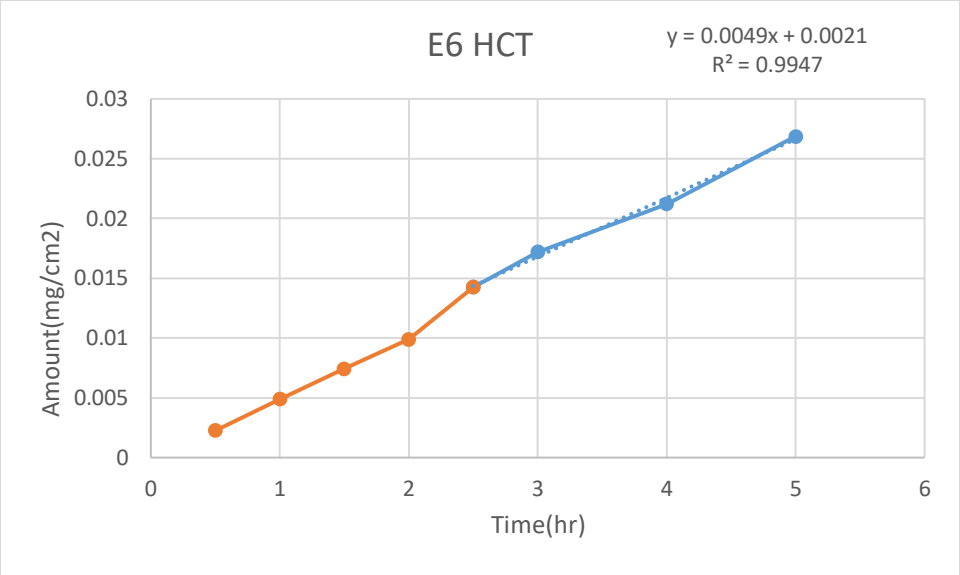

***Figure 4.16**: In vitro permeation profile for the cumulative amount of HCT permeated per unit area of sandwiched dialysis membrane with nylon membrane (mg/cm²) for experiment E6, with sorbitol.*

The diffusion parameters for HCT were calculated in table (4.47) and the enhancement ratio was determined.

***Table 4.47:** Diffusion parameters for HCT E6, with sorbitol.*

sample #	slope	intercept	T_L	D	P	K	ER
E6	0.0049	0.0021	2.333333	0.006429	0.0049	0.022867	1.633333

The rate of diffusion of HCT in the presence of sorbitol was faster than when it was alone; this is indicated by a higher value of permeability coefficient (P) and an enhancement ratio (ER) of 1.63.

4.4.7 Experiments no. E7, E8, E9, and E10 VAL/HCT solution in PBS with PVP 30, Mannitol, EDTA and Tween 80 through sandwiched dialysis membrane

When PVP 30, Mannitol, EDTA and Tween 80 were used as permeation enhancers, to enhance VAL and HCT permeation, no permeation was detected along five hours of the experiment. So they were excluded from the study.

4.4.8 Selecting the best penetration enhancer from sandwiched dialysis membrane

Table (4.48) summarizes the diffusion parameters of VAL for the previous six experiments.

Table 4.48: *Summary of diffusion parameters for VAL in different sample solutions and comparison between all the enhancement ratio of sample without enhancer and other samples with different enhancers.*

sample #	slope	intercept	T_L	D	P	K	ER
E1	0.018	0.002	10.294	0.001	0.003	0.056	1
E2	0.0613	0.0092	6.663	0.0024	0.0096	0.1276	3.503
E3	0.026	0.018	1.44	0.01	0.004	0.012	1.497
E4	0.0202	0.021	0.975	0.0154	0.0032	0.0062	1.154
E5	0.067	0.04	1.675	0.009	0.0105	0.0351	3.828
E6	0.0143	0.025	0.563	0.0266	0.0022	0.0025	0.817

The cumulative amount of VAL permeated per unit area during the six experiments, are shown in figure (4.17)

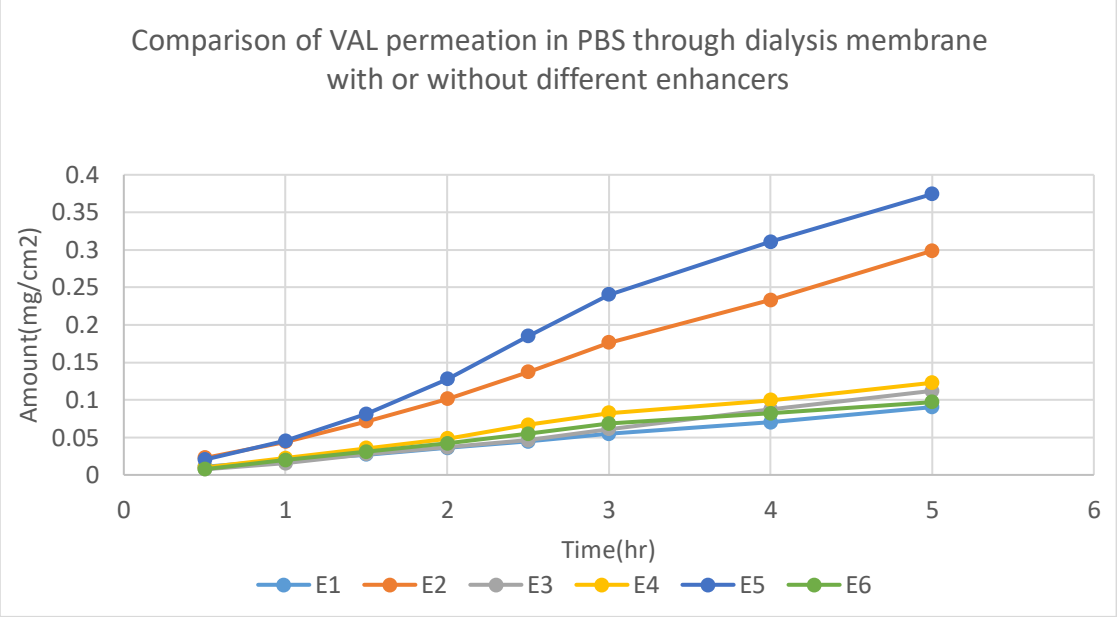

***Figure 4.17**: Comparison of VAL permeation in PBS through dialysis membrane between sample without permeation PE and other samples that contain different PEs. Where E1: without PE, E2: with citric acid, E3: with SLS, E4: with PEG, E5: with Na acetate, E6: with sorbitol.*

The results revealed that for VAL the highest ER (3.62) and highest cumulative permeation per unit area when combined with Na acetate, followed by citric acid with (3.82) ER. SLS enhance VAL permeation nearly the same as PEG 4000 with ER of (1.497) and (1.154) respectively. On the contrary, Sorbitol has decreased VAL permeation with respect to basic sample E1 that was performed without permeation enhancer. Na acetate may control pH and enhance permeation, absorption of VAL is in the upper GIT due to higher acidity with about 25% bioavailability[29].

Table (4.49) summarizes the diffusion parameters of HCT for the previous six experiments.

Table 4.49: *Summary of diffusion parameters for HCT in different sample solutions and comparison between all the enhancement ratio of sample without enhancer and other samples with different enhancers.*

sample #	slope	intercept	T_L	D	P	K	ER
E1	0.003	0.001	2.3077	0.007	0.003	0.014	1
E2	0.079	0.0759	1.0408	0.0144	0.079	0.1645	26.33
E3	0.0061	0.0031	0.508	0.0076	0.006	0.024	2.033
E4	0.0024	0.0064	2.667	0.04	0.002	0.002	0.8
E5	0.334	0.0765	0.753	0.0004	0.334	8.749	3.1
E6	0.0049	0.0021	0.429	0.0064	0.005	0.023	1.63

The cumulative amount of VAL permeated per unit area during the six experiments, are shown in figure (4.18).

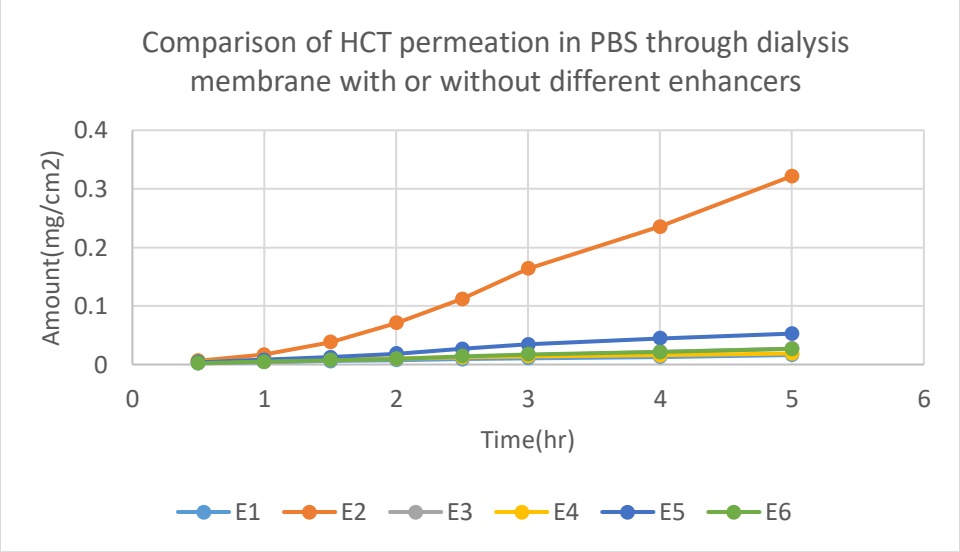

Figure 4.18*: Comparison of HCT permeation in PBS through dialysis membrane between sample without permeation PE and other samples that contain different PEs. Where E1: without PE, E2: with citric acid, E3: with SLS, E4: with PEG, E5: with Na acetate, E6: with sorbitol***.**

The results indicated that HCT has the highest ER (26.34) and highest cumulative permeation per unit area when combined with citric acid, followed by Na acetate with (3.1) ER. However, SLS and sorbitol enhance HCT permeation but in less degree compare to citric acid and sodium

acetate, with ER of (2.033), (1.63) respectively. PEG 4000 has decreased HCT permeation with respect to basic sample E1 that was performed without permeation enhancer. Citric acid may increase stability of HCT and improve permeation.

It has been shown it has been shown that Na acetate and citric acid gave the higher best enhancement ratio for VAL and HTC. Specifically, for VAL, Na acetate had the higher enhancement ratio followed by citric acid. On the other hand for HTC, citric acid had the higher enhancement ratio followed by Na acetate.

Based on these results, Na acetate and citric acid were used for further study as permeation enhancers using the solutions FaSSIF and FeSSIF through sandwiched dialysis membrane.

4.5 Permeation study results using sandwiched dialysis membrane, samples are prepared in FaSSIF and FeSSIF

4.5.1 Experiment no. E11, VAL/HCT in FaSSIF with citric acid through sandwiched dialysis membrane

A sample of VAL and HCT with 1% of citric acid was prepared according to general method described in section (3.7.4). The permeability results of drug through sandwiched dialysis membrane with nylon membrane are shown in tables (4.50), and (4.51).

The cumulative amount of VAL and HCT permeated through unit area of membrane was then calculated, the linear section, i.e. the steady state flux was plotted versus time (Figure (4.19) and Figure 4.20)).

***Table 4.50**: Data obtained from E11, in FaSSIF through sandwiched dialysis membrane with nylon membrane using Franz diffusion cell, with addition of citric acid (part 1).*

time	absorbance of sample 1 (n=3)		absorbance of sample 2 (n=3)		absorbance of sample 3 (n=3)		concentration of sample **I** mg/ml		concentration of sample **II** mg/ml		concentration of sample **III** mg/ml	
	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL	HCT	VAL	HCT	VAL	HCT
0.5	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0
1.5	0.0423	0.0796	0.0406	0.0786	0.0443	0.0813	0.003	0.0029	0.003	0.003	0.003	0.0029
2	0.1433	0.1413	0.1393	0.1386	0.1373	0.1356	0.012	0.0036	0.012	0.004	0.011	0.0034
2.5	0.218	0.1903	0.2153	0.2093	0.2176	0.1846	0.019	0.0042	0.018	0.005	0.019	0.0039
3	0.3326	0.2783	0.3356	0.304	0.3344	0.2896	0.029	0.0058	0.028	0.007	0.029	0.0063
4	0.4383	0.3866	0.4446	0.407	0.4196	0.3983	0.037	0.0087	0.038	0.01	0.035	0.0097
5	0.4943	0.4506	0.5013	0.4716	0.5106	0.4753	0.042	0.0105	0.042	0.011	0.043	0.0113

Table 4.51*: Data obtained from E11, in FaSSIF through sandwiched dialysis membrane with nylon membrane using Franz diffusion cell, with addition of citric acid (part 2).*

Q: cumulative amount released **I** (mg)		Q: cumulative amount released **II** (mg)		Q: cumulative amount released **III** (mg)		m: cumulative amount released per area **I** (mg/cm2)		m: cumulative amount released per area **II** (mg/cm2)		m: cumulative amount released per area **III** (mg/cm2)		**mean amount (mg/cm2)**		**SD**		**%RSD**	
VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
0.054	0.058	0.051	0.058	0.058	0.059	0.017	0.018	0.0163	0.018	0.018	0.019	0.017	0.019	0.001	0.0002	6.02	0.83
0.295	0.132	0.285	0.131	0.289	0.13	0.094	0.042	0.0906	0.042	0.092	0.041	0.092	0.042	0.002	0.0003	1.82	0.76
0.679	0.22	0.655	0.239	0.674	0.212	0.216	0.07	0.2086	0.076	0.215	0.068	0.213	0.071	0.004	0.0043	1.92	6.07
1.271	0.341	1.242	0.384	1.265	0.343	0.405	0.108	0.3954	0.122	0.403	0.109	0.401	0.113	0.005	0.0078	1.24	6.88
2.047	0.52	2.022	0.581	1.997	0.543	0.652	0.165	0.6439	0.185	0.636	0.173	0.644	0.174	0.008	0.0099	1.24	5.69
2.921	0.738	2.901	0.817	2.892	0.778	0.93	0.235	0.924	0.26	0.921	0.248	0.925	0.248	0.005	0.0127	0.51	5.13

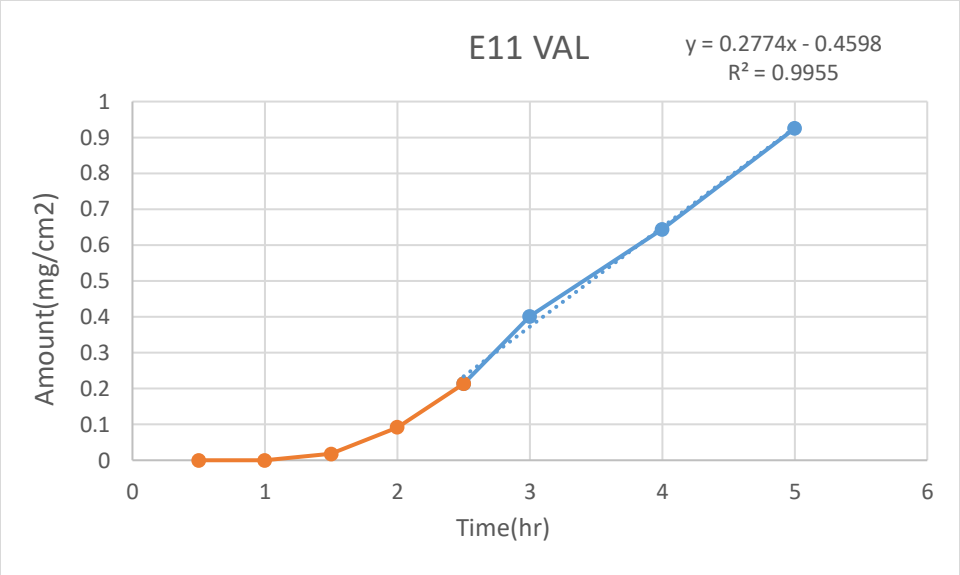

***Figure 4.19**: In vitro permeation profile for the cumulative amount of VAL in FaSSIF permeated per unit area of sandwiched dialysis membrane with nylon membrane (mg/cm²) for experiment E11, with citric acid.*

The diffusion parameters for VAL were calculated in table (4.52) and the enhancement ratio was determined.

From the results, VAL showed lower and delayed permeation when FaSSIF media was used instead of PBS. P was 0.0611cm/hr and 0.0433cm/hr for PBS and FaSSIF respectively. That is closer to the real conditions.

***Table 4.52:** Diffusion parameters for VAL E11, with citric acid.*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	ER
E11	0.2774	0.4598	0.603306	0.024863	0.043344	0.052299	4.373739

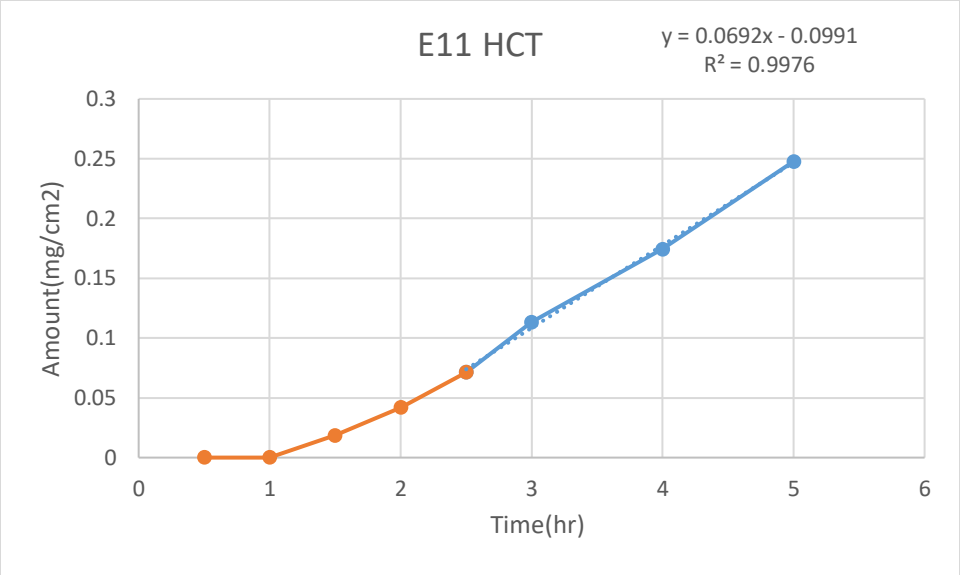

***Figure 4.20**: In vitro permeation profile for the cumulative amount of HCT in FaSSIF permeated per unit area of sandwiched dialysis membrane with nylon membrane (mg/cm²) for experiment E11, with citric acid.*

The diffusion parameters for HCT were calculated in table (4.53) and the enhancement ratio was determined.

From the results, HCT showed lower and permeation when FaSSIF media was used instead of PBS. P was 0.079 cm/hr and 0.0692 cm/hr for PBS and FaSSIF respectively. That is closer to the real conditions.

***Table 4.53:** Diffusion parameters for HCT E11, with citric acid.*

sample #	slope	intercept	T_L	D	P	K	ER
E11	0.0692	0.0991	0.698285	0.021481	0.0692	0.096643	0.875949

4.5.2 Experiment no. E12, VAL/HCT in FeSSIF with citric acid through sandwiched dialysis membrane

A sample of VAL and HCT with 1% of citric acid was prepared according to general method described in section (3.7.4). The permeability results of drug through sandwiched dialysis membrane with nylon membrane are shown in tables (4.54), and (4.55).

The cumulative amount of VAL and HCT permeated through unit area of membrane was then calculated, the linear section, i.e. the steady state flux was plotted versus time (Figure (4.21) and Figure (4.22)).

***Table 4.54**: Data obtained from E12, in FeSSIF through sandwiched dialysis membrane with nylon membrane using Franz diffusion cell, with addition of citric acid (part 1).*

time	absorbance of sample 1 (n=3)		absorbance of sample 2 (n=3)		absorbance of sample 3 (n=3)		concentration of sample **I** mg/ml		concentration of sample **II** mg/ml		concentration of sample **III** mg/ml	
	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL	HCT	VAL	HCT	VAL	HCT
0.5	0	0.1273	0	0.1256	0	0.1293	0	0.006	0	0.006	0	0.0063
1	0	0.1383	0	0.1346	0	0.1363	0	0.007	0	0.007	0	0.0067
1.5	0	0.1416	0	0.147	0	0.1486	0	0.007	0	0.007	0	0.0073
2	0.1903	0.2686	0.1876	0.2676	0.1896	0.272	0.0141	0.009	0.0138	0.009	0.014	0.0089
2.5	0.3476	0.352	0.3403	0.367	0.3426	0.373	0.0287	0.009	0.0276	0.01	0.028	0.0102
3	0.3776	0.444	0.3853	0.4436	0.3796	0.4436	0.0298	0.013	0.0307	0.013	0.03	0.0128
4	0.8336	0.7986	0.8356	0.7993	0.8373	0.7976	0.0697	0.02	0.0699	0.02	0.07	0.0194
5	0.8476	0.8683	0.8503	0.8676	0.8466	0.8693	0.0697	0.023	0.07	0.023	0.07	0.0227

***Table 4.55**: Data obtained from E12, in FeSSIF through sandwiched dialysis membrane with nylon membrane using Franz diffusion cell, with addition of citric acid (part 2).*

Q: cumulative amount released **I** (mg)		Q: cumulative amount released **II** (mg)		Q: cumulative amount released **III** (mg)		m: cumulative amount released per area **I** (mg/cm2)		m: cumulative amount released per area **II** (mg/cm2)		m: cumulative amount released per area **III** (mg/cm2)		**mean amount (mg/cm2))**		**SD**		**%RSD**	
VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT
0	0.124	0	0.123	0	0.126	0	0.04	0	0.039	0	0.04	0	0.04	0	0.0006		1.454
0	0.266	0	0.26	0	0.266	0	0.085	0	0.083	0	0.085	0	0.084	0	0.001		1.183
0	0.411	0	0.411	0	0.418	0	0.131	0	0.131	0	0.133	0	0.132	0	0.0013		0.975
0.282	0.591	0.277	0.592	0.279	0.602	0.0898	0.188	0.088	0.188	0.089	0.192	0.089	0.19	0.0008	0.002	0.93	1.037
0.869	0.782	0.843	0.8	0.847	0.816	0.2769	0.249	0.268	0.255	0.27	0.26	0.272	0.255	0.0046	0.0054	1.687	2.122
1.495	1.048	1.483	1.064	1.476	1.082	0.4761	0.334	0.472	0.339	0.47	0.345	0.473	0.339	0.003	0.0054	0.643	1.585
2.919	1.452	2.912	1.467	2.909	1.483	0.9297	0.463	0.927	0.467	0.926	0.472	0.928	0.467	0.0017	0.0049	0.185	1.054
4.383	1.925	4.382	1.937	4.37	1.957	1.3959	0.613	1.396	0.617	1.392	0.623	1.394	0.618	0.0022	0.0052	0.161	0.836

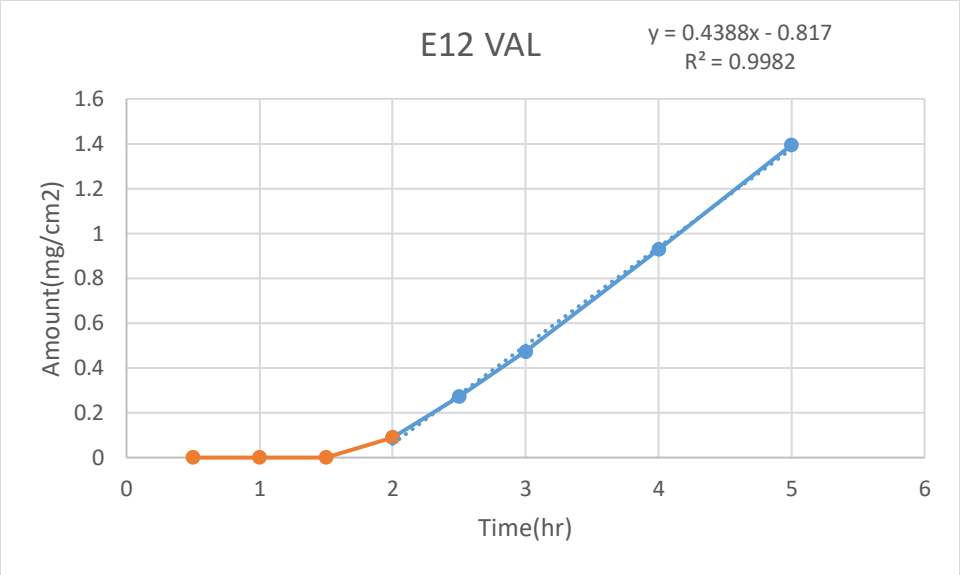

***Figure 4.21**: In vitro permeation profile for the cumulative amount of VAL in FeSSIF permeated per unit area of sandwiched dialysis membrane with nylon membrane (mg/cm²) for experiment E12, with citric acid.*

The diffusion parameters for VAL were calculated in table (4.56) and the enhancement ratio was determined.

***Table 4.56:** Diffusion parameters for VAL E12, with citric acid.*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	ER
E12	0.4388	0.817	0.537087	0.027928	0.068563	0.073648	6.918517

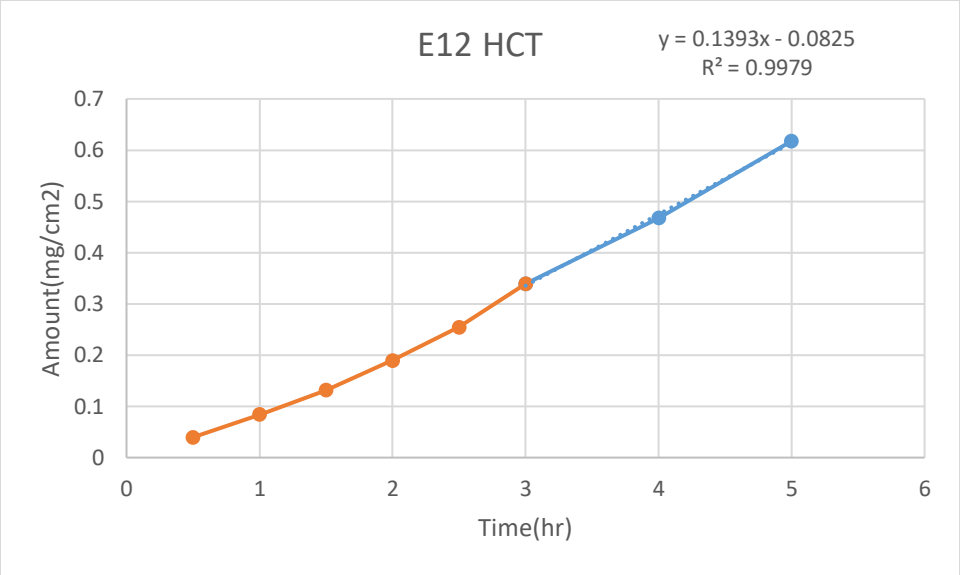

***Figure 4.22**: In vitro permeation profile for the cumulative amount of VAL in FeSSIF permeated per unit area of sandwiched dialysis membrane with nylon membrane (mg/cm²) for experiment E12, with citric acid.*

The diffusion parameters for HCT were calculated in table (4.57) and the enhancement ratio was determined.

***Table 4.57:** Diffusion parameters for HCT E12, with citric acid.*

sample #	slope	intercept	T_L	D	P	K	ER
E12	0.1339	0.0825	1.62303	0.009242	0.1339	0.434648	1.694937

4.5.3 Experiment no. E13, VAL/HCT in FaSSIF with Na acetate through sandwiched dialysis membrane

A sample of VAL and HCT with 1% of Na acetate was prepared according to general method described in section (3.7.4). The permeability results of drug through sandwiched dialysis membrane with nylon membrane are shown in tables (4.58), and (4.59).

Cumulative amount of VAL and HCT permeated through unit area of membrane was then calculated, the linear section, i.e. the steady state flux was plotted versus time (Figure (4.23) and Figure (4.24)).

***Table 4.58**: Data obtained from E13, in FaSSIF through sandwiched dialysis membrane with nylon membrane using Franz diffusion cell, with addition of Na acetate (part 1).*

time	absorbance of sample 1 (n=3)		absorbance of sample 2 (n=3)		absorbance of sample 3 (n=3)		concentration of sample **I** mg/ml		concentration of sample **II** mg/ml		concentration of sample **III** mg/ml	
	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL	HCT	VAL	HCT	VAL	HCT
0.5	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0
1.5	0.025	0.1136	0.028	0.115	0.023	0.112	0.0002	0.005	0.00048	0.005	2.8E-05	0.0049
2	0.03	0.13	0.033	0.134	0.028	0.127	0.00038	0.0057	0.0006	0.0058	0.00023	0.0056
2.5	0.036	0.154	0.039	0.159	0.032	0.15	0.00049	0.0067	0.0007	0.0069	0.00016	0.0066
3	0.038	0.175	0.035	0.171	0.04	0.178	0.00026	0.0077	3E-05	0.0075	0.0004	0.0078
4	0.049	0.228	0.046	0.225	0.052	0.233	0.00028	0.01	3.4E-05	0.0099	0.00049	0.0102
5	0.055	0.257	0.059	0.262	0.052	0.253	0.00029	0.0113	0.0006	0.0114	6.7E-05	0.0111

***Table 4.59**: Data obtained from E13, in FaSSIF through sandwiched dialysis membrane with nylon membrane using Franz diffusion cell, with addition of Na acetate (part 2).*

Q: cumulative amount released **I** (mg)		Q: cumulative amount released **II** (mg)		Q: cumulative amount released **III** (mg)		m: cumulative amount released per area **I** (mg/cm2)		m: cumulative amount released per area **II** (mg/cm2)		m: cumulative amount released per area **III** (mg/cm2)		**mean amount (mg/cm2)**		**SD**		**%RSD**	
VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
0.004	0.099	0.01	0.099	0.0006	0.099	0.0013	0.032	0.0031	0.032	0.0002	0.031	0.008	0.032	0.0015	0.00011	17.91	0.357
0.0117	0.217	0.022	0.22	0.0052	0.215	0.0037	0.069	0.0071	0.07	0.0017	0.068	0.024	0.069	0.0027	0.00082	11.47	1.181
0.022	0.357	0.037	0.363	0.0087	0.352	0.007	0.114	0.0117	0.116	0.0028	0.112	0.045	0.114	0.0045	0.00174	10.06	1.527
0.0276	0.517	0.038	0.52	0.0169	0.514	0.0088	0.165	0.0121	0.166	0.0054	0.164	0.056	0.165	0.0034	0.00106	6.036	0.645
0.0335	0.724	0.039	0.726	0.027	0.725	0.0107	0.231	0.0124	0.231	0.0086	0.231	0.068	0.231	0.0019	0.00034	2.761	0.147
0.0397	0.96	0.051	0.965	0.0289	0.958	0.0126	0.306	0.0162	0.307	0.0092	0.305	0.08	0.306	0.0035	0.00111	4.363	0.363

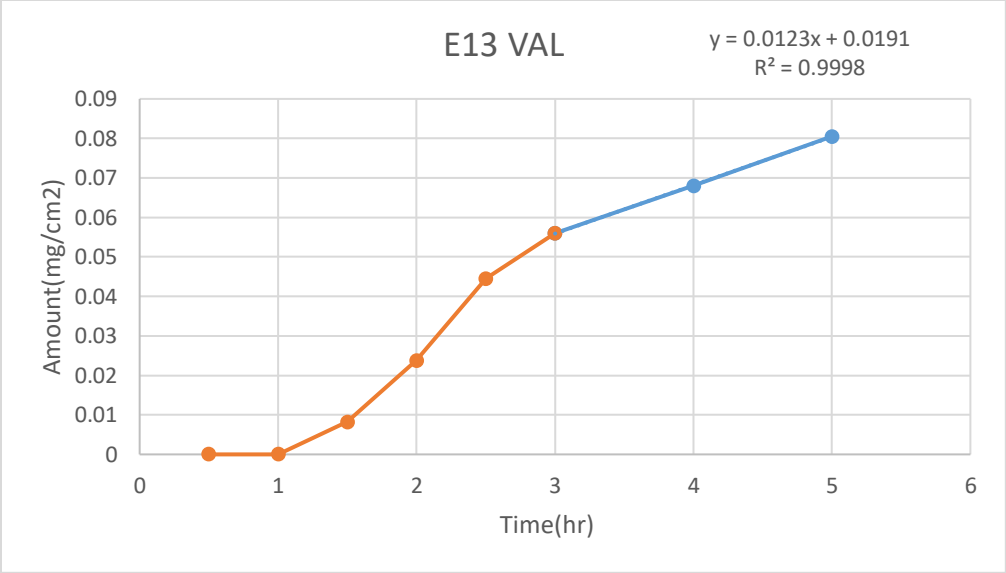

***Figure 4.23**: In vitro permeation profile for the cumulative amount of VAL in FaSSIF permeated per unit area of sandwiched dialysis membrane with nylon membrane (mg/cm²) for experiment E13, with Na acetate.*

The diffusion parameters for VAL were calculated in table (4.60) and the enhancement ratio was determined.

***Table 4.60:** Diffusion parameters for VAL E13, with Na acetate.*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	ER
E13	0.0123	0.0191	0.644	0.023	0.002	0.002	0.1836

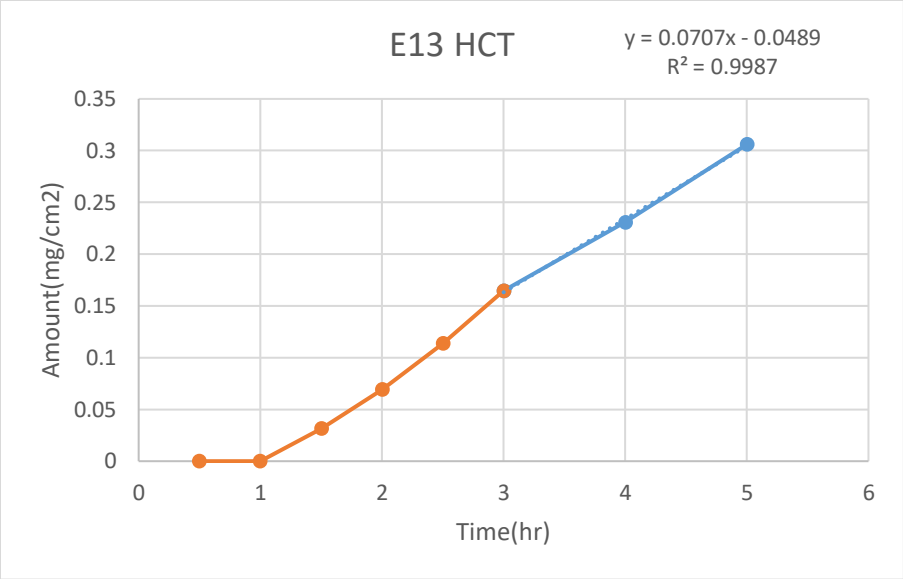

***Figure 4.24**: In vitro permeation profile for the cumulative amount of HCT in FaSSIF permeated per unit area of sandwiched dialysis membrane with nylon membrane (mg/cm²) for experiment E13, with Na acetate.*

The diffusion parameters for HCT were calculated in table (4.61) and the enhancement ratio was determined.

***Table 4.61:** Diffusion parameters for HCT E13, with Na acetate.*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	ER
E13	0.071	0.0489	1.446	0.0104	0.071	0.204	0.895

4.5.4 Experiment no. E14, VAL/HCT in FeSSIF with Na acetate through sandwiched dialysis membrane.

A sample of VAL and HCT with 1% of Na acetate was prepared according to general method described in section (3.7.4). The permeability results of drug through sandwiched dialysis membrane with nylon membrane are shown in tables (4.62), and (4.63).

The cumulative amount of VAL and HCT permeated through unit area of membrane was then calculated, the linear section, i.e. the steady state flux was plotted versus time (Figure (4.25) and Figure (4.26)).

***Table 4.62**: Data obtained from E14, in FeSSIF through sandwiched dialysis membrane with nylon membrane using Franz diffusion cell, with addition of Na acetate (part 1).*

time	absorbance of sample 1 (n=3)		absorbance of sample 2 (n=3)		absorbance of sample 3 (n=3)		concentration of sample **I** mg/ml		concentration of sample **II** mg/ml		concentration of sample **III** mg/ml	
	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL	HCT	VAL	HCT	VAL	HCT
0.5	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0.0663	0	0.0676	0	0.0696	0	0.0032	0	0.003	0	0.0034
1.5	0	0.1393	0	0.1376	0	0.1366	0	0.0068	0	0.007	0	0.0067
2	0.1683	0.2653	0.1693	0.2656	0.1686	0.2656	0.012	0.009	0.012	0.009	0.012	0.009
2.5	0.236	0.334	0.2356	0.3366	0.23596	0.3363	0.017	0.0108	0.0174	0.011	0.017	0.0109
3	0.2603	0.3573	0.2703	0.3543	0.2656	0.3556	0.019	0.0114	0.0206	0.011	0.02	0.0112
4	0.416	0.5043	0.4186	0.5053	0.4183	0.5053	0.033	0.0149	0.0328	0.015	0.033	0.0149
5	0.4926	0.5736	0.4893	0.5756	0.4903	0.5763	0.039	0.0165	0.0387	0.017	0.039	0.0167

***Table 4.63**: Data obtained from E14, in FeSSIF through sandwiched dialysis membrane with nylon membrane using Franz diffusion cell, with addition of Na acetate (part 2)*

Q: cumulative amount released **I** (mg)		Q: cumulative amount released **II** (mg)		Q: cumulative amount released **III** (mg)		m: cumulative amount released per area **I** (mg/cm2)		m: cumulative amount released per area **II** (mg/cm2)		m: cumulative amount released per area **III** (mg/cm2)		**mean amount (mg/cm2)**		**SD**		**%RSD**	
VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
0	0.065	0	0.0661	0	0.068	0	0.021	0	0.021	0	0.022	0	0.0211	0	0.00052		2.4506
0	0.204	0	0.2038	0	0.205	0	0.065	0	0.0649	0	0.065	0	0.0651	0	0.00018		0.2697
0.238	0.392	0.24	0.3911	0.2382	0.392	0.076	0.125	0.076	0.1245	0.0758	0.125	0.0759	0.1247	0.0003	0.00022	0.425	0.1733
0.599	0.617	0.599	0.619	0.5983	0.62	0.191	0.196	0.191	0.1971	0.1905	0.197	0.1907	0.197	0.0001	0.0005	0.0579	0.2525
1.006	0.855	1.028	0.8499	1.0173	0.854	0.32	0.272	0.327	0.2707	0.324	0.272	0.324	0.2717	0.0035	0.0009	1.084	0.3327
1.677	1.165	1.705	1.1592	1.693	1.164	0.534	0.371	0.543	0.3692	0.5392	0.371	0.5388	0.3703	0.0045	0.001	0.8343	0.2693
2.491	1.511	2.511	1.5081	2.501	1.513	0.793	0.481	0.8	0.4803	0.7965	0.482	0.7965	0.4811	0.0033	0.00078	0.4136	0.1618

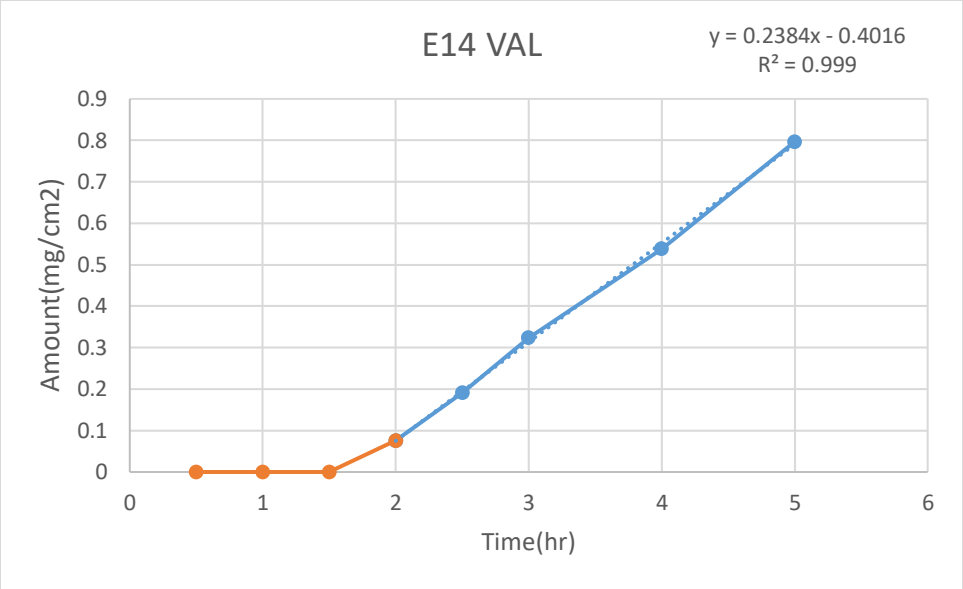

***Figure 4.25**: In vitro permeation profile for the cumulative amount of VAL in FeSSIF permeated per unit area of sandwiched dialysis membrane with nylon membrane (mg/cm²) for experiment E11, with Na acetate.*

The diffusion parameters for VAL were calculated in table (4.64) and the enhancement ratio was determined.

***Table 4.64:** Diffusion parameters for HCT E14, with Na acetate.*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	ER
E14	0.2384	0.4016	0.593625	0.025268	0.03725	0.044225	3.557784

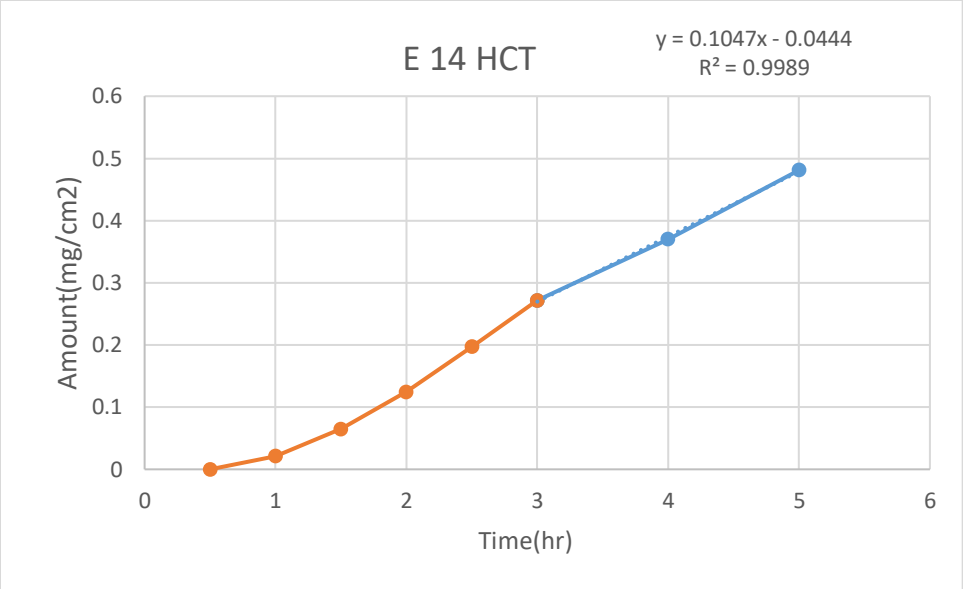

***Figure 4.26**: In vitro permeation profile for the cumulative amount of HCT in FeSSIF permeated per unit area of sandwiched dialysis membrane with nylon membrane (mg/cm²) for experiment E11, with Na acetate.*

The diffusion parameters for HCT are calculated in table (4.65) and the enhancement ratio is determined.

***Table 4.65:** Diffusion parameters for HCT E14, with Na acetate.*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	ER
E14	0.1047	0.0444	2.358108	0.006361	0.1047	0.493788	1.325316

From the results, VAL showed lower and delayed permeation when prepared with 1% citric acid in FaSSIF media instead of PBS. P was 0.0611cm/hr and 0.0433cm/hr for PBS and FaSSIF respectively. On the contrary, it showed higher and delayed permeation when prepared in FeSSIF, P was 0.0686cm/hr. When the sample was prepared with Na acetate, VAL showed lower permeation in FaSSIF (P=0.002cm/hr) and higher permeation in FeSSIF (P=0.37cm/hr). VAL had P=0.009cm/hr when the sample was prepared in PBS.

From the results, HCT showed lower permeation when prepared with 1% citric acid in FaSSIF media instead of PBS. P was 0.079cm/hr and 0.0692cm/hr for PBS and FaSSIF respectively. On the contrary, it showed higher and delayed permeation when prepared in FeSSIF, P was 0.1339cm/hr. When the sample was prepared with Na acetate, HCT showed lower permeation in FaSSIF (P=0.071cm/hr) and FeSSIF (P=0.1047cm/hr). VAL had P=0.334cm/hr when the sample was prepared in PBS.

Na acetate enhanced the permeation of VAL, where citric acid enhanced the permeation of HCT.

4.6 Permeation study results using Permeapad membrane, samples are prepared in FaSSIF and FeSSIF.

Permeapad is a biomemitic membrane with fully artificial phospholipids in layered structure [8]. Permeapad membrane is available in ready to use form[8]. In literature, many works prove that Permeapad™ appears to be a promising tool for fast, cost effective and reliable screening of passive permeability of drugs and chemical entities[58],[59],[60]. Permeapad was evaluated in the presence of many additives like surfactants, solvents, co-solvents, buffers with different pH values and different biomimetic medias. It was found that Permeapad membrane is compatible, resistance to pH changes, and well suited for fast and reliable prediction of passive drug permeability[38].
In a previous study, Permeapad membrane was used to investigate metoprolol absorption via buccal route. Results showed that the permeability of metoprolol using the Permeapad® barrier correlated very well to both *in vitro* and *ex vivo* studies[61].

In this stage, Na acetate and citric acid will be used as PEs, and investigate the effect of Permeapad membrane on the permeation of VAL and HCT instead of sandwiched dialysis membrane. Na acetate enhanced the permeation of VAL, where citric acid enhanced the permeation of HCT, when samples were prepared in FaSSIF and FeSSIF through sandwiched dialysis membrane.

4.6.1 Experiment no. E15, VAL/HCT in PBS through Permeapad.

A sample of VAL and HCT and prepared according to general method described in section (3.7.4). The permeability results of drug through Permeapad membrane are shown in tables (4.66), and table (4.67).

The cumulative amount of VAL and HCT permeated through unit area of membrane was then calculated, the linear section, i.e. the steady state flux was plotted versus time (Figure (4.27) and (Figure (4.28)).

***Table 4.66**: Data obtained from E15, in PBS through Permeapad membrane using Franz diffusion cell, without enhancer (part 1).*

time	absorbance of sample 1 (n=3)		absorbance of sample 2 (n=3)		absorbance of sample 3 (n=3)		concentration of sample **I** mg/ml		concentration of sample **II** mg/ml		concentration of sample **III** mg/ml	
	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL	HCT	VAL	HCT	VAL	HCT
0.5	0.043	0.049	0.042	0.048	0.044	0.048	0.0034	0.001	0.0033	0.0014	0.0036	0.0013
1	0.1126	0.1456	0.112	0.146	0.113	0.1463	0.0086	0.004	0.0086	0.0045	0.0086	0.0045
1.5	0.1676	0.2913	0.1656	0.291	0.1683	0.2916	0.0113	0.01	0.0111	0.0104	0.0113	0.0103
2	0.2046	0.2813	0.2053	0.282	0.2043	0.281	0.0153	0.009	0.0154	0.009	0.0153	0.009
2.5	0.26	0.355	0.2606	0.3556	0.2596	0.3546	0.0195	0.011	0.0196	0.0113	0.0195	0.0113
3	0.323	0.435	0.3243	0.4356	0.32	0.434	0.0244	0.014	0.0245	0.0137	0.0241	0.0137
4	0.4333	0.5686	0.432	0.567	0.4336	0.569	0.033	0.018	0.0329	0.0176	0.033	0.0177
5	0.5763	0.7353	0.575	0.735	0.5783	0.734	0.0443	0.022	0.0442	0.0225	0.0446	0.0224

Table 4.67*: Data obtained from E15, in PBS through Permeapad membrane using Franz diffusion cell, without enhancer.*

Q: cumulative amount released **I** (mg)		Q: cumulative amount released **II** (mg)		Q: cumulative amount released **III** (mg)		m: cumulative amount released per area **I** (mg/cm2)		m: cumulative amount released per area **II** (mg/cm2)		m: cumulative amount released per area **III** (mg/cm2)		**mean amount (mg/cm2)**		**SD**		**%RSD**	
VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT
0.069	0.0278	0.067	0.0273	0.071	0.026	0.022	0.0089	0.0213	0.009	0.0227	0.0084	0.0219	0.009	0.0007	0.00023	3.034	2.6982
0.245	0.1189	0.2414	0.119	0.248	0.118	0.078	0.0379	0.0769	0.038	0.0789	0.0375	0.0779	0.038	0.001	0.0002	1.29	0.5324
0.478	0.3298	0.4711	0.3306	0.483	0.329	0.152	0.105	0.15	0.105	0.1538	0.1047	0.1521	0.105	0.0019	0.00029	1.243	0.2795
0.796	0.5194	0.7896	0.5206	0.8	0.518	0.253	0.1654	0.2515	0.166	0.2548	0.165	0.2532	0.165	0.0017	0.00038	0.657	0.2293
1.201	0.7539	1.1963	0.7554	1.205	0.752	0.383	0.2401	0.381	0.241	0.3837	0.2396	0.3824	0.24	0.0014	0.00046	0.362	0.1936
1.708	1.0394	1.7056	1.0409	1.706	1.038	0.544	0.331	0.5432	0.331	0.5433	0.3307	0.5435	0.331	0.0005	0.0004	0.088	0.1211
2.393	1.4064	2.3882	1.4069	2.391	1.406	0.762	0.4479	0.7606	0.448	0.7613	0.4477	0.7613	0.448	0.0007	0.0002	0.097	0.0452
3.313	1.8734	3.3053	1.8743	3.315	1.871	1.055	0.5966	1.0526	0.597	1.0557	0.5957	1.0544	0.596	0.0016	0.00062	0.153	0.1045

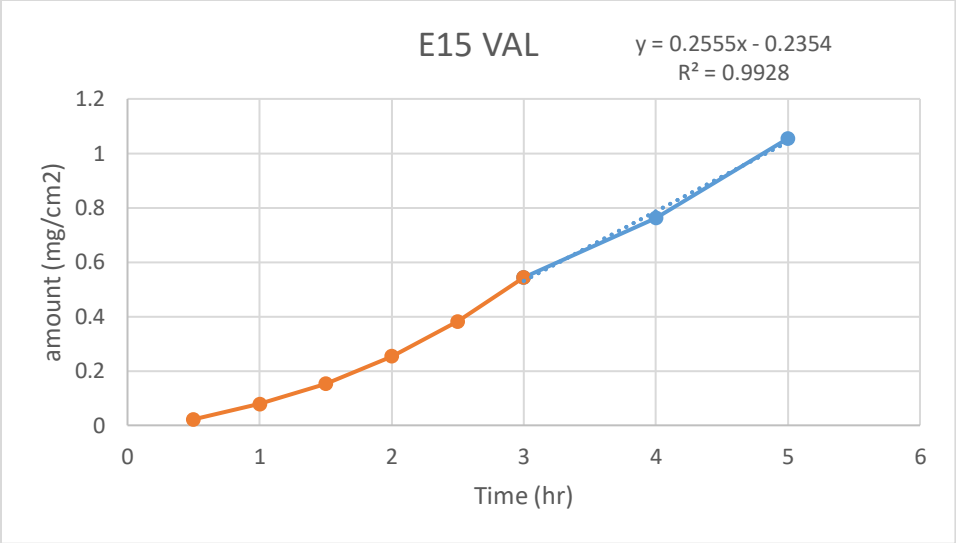

***Figure 4.27**: In vitro permeation profile for the cumulative amount of VAL in PBS permeated per unit area of Permeapad (mg/cm²) for experiment E15, without enhancer.*

The diffusion parameters for VAL were calculated in table (4.68) and the enhancement ratio was determined.

***Table 4.68:** Diffusion parameters for VAL E15, without enhancer.*

sample #	slope	intercept	T_L	D	P	K
E15	0.2555	0.2354	1.085387	0.001536	0.039922	0.259984

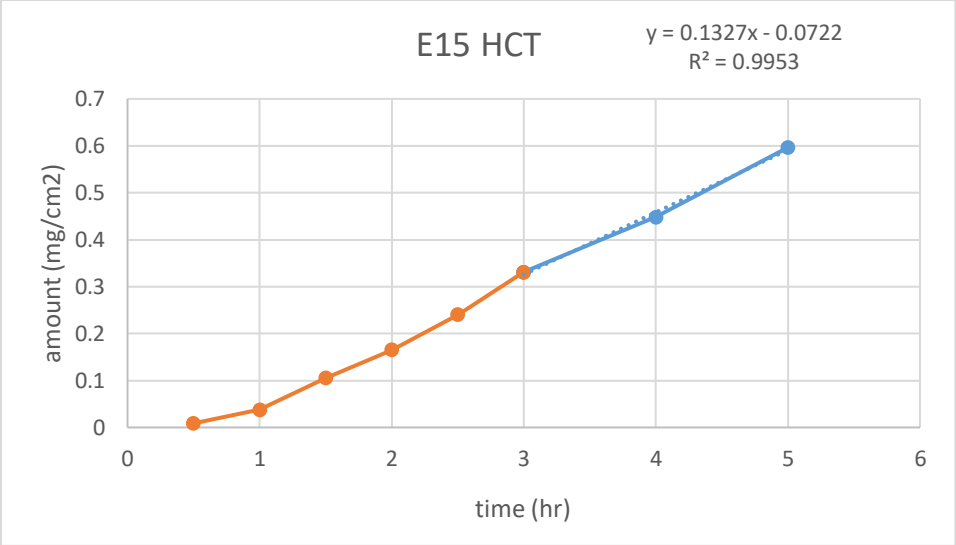

***Figure 4.28**: In vitro permeation profile for the cumulative amount of HCT in PBS permeated per unit area of Permeapad (mg/cm²) for experiment E15, without enhancer.*

The diffusion parameters for HCT were calculated in table (4.69) and the enhancement ratio was determined.

***Table 4.69:** Diffusion parameters for HCT E15, without enhancer.*

sample #	slope	intercept	TL	D	P	K
E15	0.1327	0.0722	1.83795	0.000907	0.1327	1.463376

4.8.2 Experiment no. E16, VAL/HCT in FaSSIF through Permeapad.

The sample VAL/HCT solution in FaSSIF was prepared without using permeation enhancer as a control to constitute a base for comparison according to general method described in section (3.7.4). The permeability results of drug through Permeapad membrane are shown in tables (4.70), and table (4.71).

The cumulative amount of VAL and HCT permeated through unit area of membrane was then calculated, the linear section, i.e. the steady state flux was plotted versus time (Figure (4.29) and Figure (4.30)).

***Table 4.70**: Data obtained from E16, in FaSSIF through Permeapad membrane using Franz diffusion cell, without enhancer (part 1).*

time	absorbance of sample 1 (n=3)		absorbance of sample 2 (n=3)		absorbance of sample 3 (n=3)		concentration of sample **I** mg/ml		concentration of sample **II** mg/ml		concentration of sample **III** mg/ml	
	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL	HCT	VAL	HCT	VAL	HCT
0.5	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0
1.5	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0.091	0	0.094	0	0.089	0	0.0044	0	0.0046	0	0.0043
2.5	0	0.14	0	0.144	0	0.138	0	0.0068	0	0.007	0	0.0067
3	0.045	0.216	0.047	0.219	0.044	0.0214	0.00012	0.0095	0.0003	0.0096	0.0041	2E-05
4	0.136	0.376	0.139	0.377	0.133	0.374	0.0062	0.0152	0.0065	0.0152	0.0059	0.0152
5	0.259	0.526	0.262	0.529	0.256	0.525	0.01581	0.0197	0.0161	0.0197	0.0155	0.0197

Table 4.71*: Data obtained from E16, in FaSSIF through Permeapad membrane using Franz diffusion cell, without enhancer.*

Q: cumulative amount released **I** (mg)		Q: cumulative amount released **II** (mg)		Q: cumulative amount released **III** (mg)		m: cumulative amount released per area **I** (mg/cm2)		m: cumulative amount released per area **II** (mg/cm2)		m: cumulative amount released per area **III** (mg/cm2)		**mean amount (mg/cm2)**		**SD**		**%RSD**	
VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
0	0.089	0	0.092	0	0.087	0	0.028	0	0.029	0	0.028	0	0.028	0	8E-04		2.7554
0	0.23	0	0.237	0	0.226	0	0.073	0	0.076	0	0.072	0	0.074	0	0.002		2.4067
0.0024	0.427	0.0053	0.436	0.0823	0.233	0.0008	0.136	0.002	0.139	0.026	0.074	0.01	0.116	0.014	0.037	151.1	31.36
0.1265	0.741	0.1353	0.749	0.205	0.537	0.0403	0.236	0.043	0.239	0.065	0.171	0.05	0.215	0.014	0.038	27.66	17.813
0.4488	1.149	0.4629	1.159	0.5212	0.945	0.1429	0.366	0.147	0.369	0.166	0.301	0.152	0.345	0.012	0.038	8.045	11.118

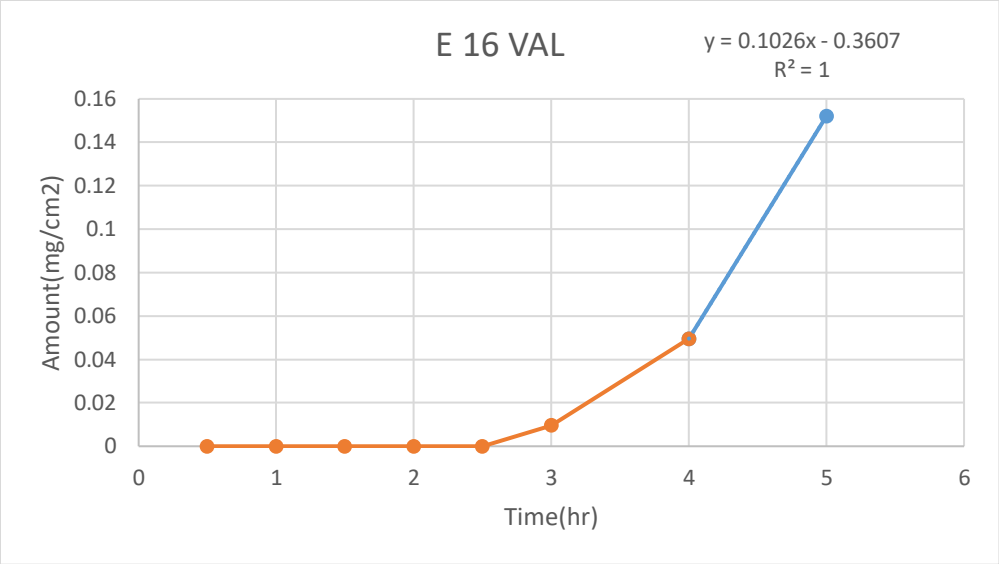

***Figure 4.29**: In vitro permeation profile for the cumulative amount of VAL in FaSSIF permeated per unit area of Permeapad (mg/cm²) for experiment E16, without enhancer.*

The diffusion parameters for VAL were calculated in table (4.72) and the enhancement ratio was determined.

***Table 4.72:** Diffusion parameters for VAL E16, without enhancer.*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	**ER**
E16	0.1026	0.3607	0.284447	0.005859	0.01603125	0.02736	1

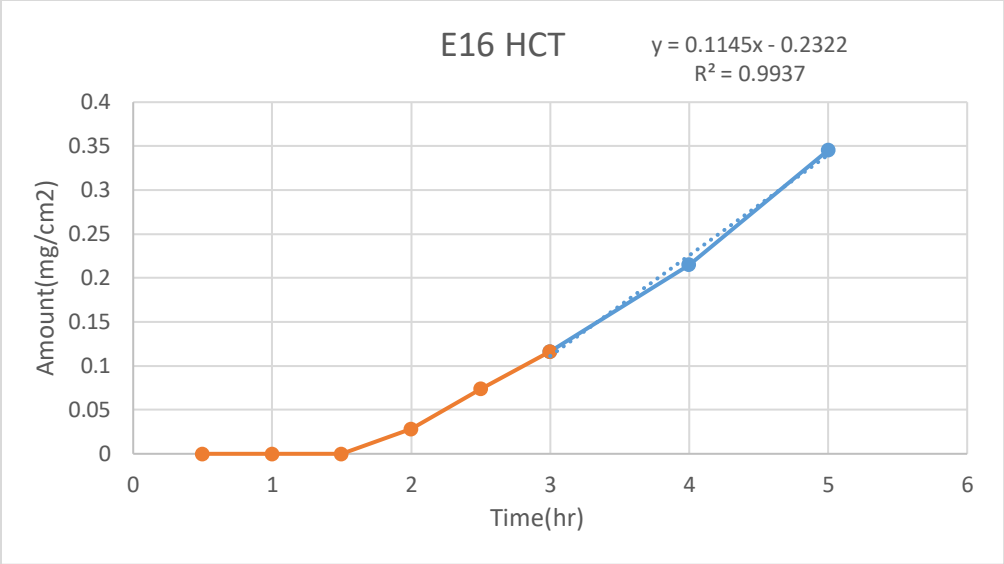

***Figure 4.30**: In vitro permeation profile for the cumulative amount of HCT in FaSSIF permeated per unit area of Permeapad (mg/cm²) for experiment E16, without enhancer.*

The diffusion parameters for HCT were calculated in table (4.73) and the enhancement ratio was determined.

***Table 4.73:** Diffusion parameters for HCT E16, without enhancer.*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	**ER**
E16	0.1145	0.2322	0.493109	0.00338	0.1145	0.338766	1

4.8.3 Experiment no. E17, VAL/HCT in FaSSIF through Permeapad.

The basic VAL/HCT solution in FaSSIF was prepared without using permeation enhancer as a control to constitute a base for comparison according to general method described in section (3.7.4). The permeability results of drug through Permeapad membrane are shown in tables (4.74), and table (4.75).

The cumulative amount of VAL and HCT permeated through unit area of membrane was then calculated, the linear section, i.e. the steady state flux was plotted versus time Figure (4.31) and Figure (4.32)).

***Table 4.74**: Data obtained from E17, in FeSSIF through Permeapad membrane using Franz diffusion cell, without enhancer (part 1).*

time	absorbance of sample 1 (n=3)		absorbance of sample 2 (n=3)		absorbance of sample 3 (n=3)		concentration of sample **I** mg/ml		concentration of sample **II** mg/ml		concentration of sample **III** mg/ml	
	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL	HCT	VAL	HCT	VAL	HCT
0.5	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0.028	0	0.03	0	0.025	0	0	0	0.001	0	0.001
1.5	0	0.018	0	0.02	0	0.015	0	9E-04	0	1E-03	0	7E-04
2	0	0.114	0	0.115	0	0.112	0	0.006	0	0.006	0	0.005
2.5	0	0.202	0	0.205	0	0.2	0	0.01	0	0.01	0	0.01
3	0	0.297	0	0.3	0	0.294	0	0.015	0	0.015	0	0.014
4	0.1	0.476	0.11	0.478	0.098	0.474	0.0004	0.021	0.0013	0.021	0.0002	0.021
5	0.17	0.611	0.19	0.609	0.14	0.612	0.0048	0.026	0.0069	0.025	0.0016	0.027

Table 4.75*: Data obtained from E17, in FeSSIF through Permeapad membrane using Franz diffusion cell, without enhancer.*

Q: cumulative amount released **I** (mg)		Q: cumulative amount released **II** (mg)		Q: cumulative amount released **III** (mg)		m: cumulative amount released per area **I** (mg/cm2)		m: cumulative amount released per area **II** (mg/cm2)		m: cumulative amount released per area **III** (mg/cm2)		**mean amount (mg/cm2)**		**SD**		**%RSD**	
VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
0	0	0	0.029	0	0.024	0	0	0	0.009	0	0.008	0	0.0057	0	0.005		87.67
0	0.0176	0	0.05	0	0.04	0	0.006	0	0.016	0	0.013	0	0.0115	0	0.005		46.5
0	0.1299	0	0.164	0	0.151	0	0.041	0	0.052	0	0.048	0	0.0471	0	0.005		11.51
0	0.3329	0	0.37	0	0.351	0	0.106	0	0.118	0	0.112	0	0.1119	0	0.006		5.237
0	0.633	0	0.673	0	0.649	0	0.202	0	0.214	0	0.207	0	0.2075	0	0.006		3.085
0.007	1.066	0.027	1.103	0.0037	1.08	0.002	0.339	0.0086	0.351	0.0012	0.344	0.004	0.345	0.004	0.006	100.01	1.737
0.103	1.6046	0.1662	1.631	0.0367	1.634	0.033	0.511	0.0529	0.519	0.0117	0.52	0.0325	0.5169	0.021	0.005	63.541	0.987

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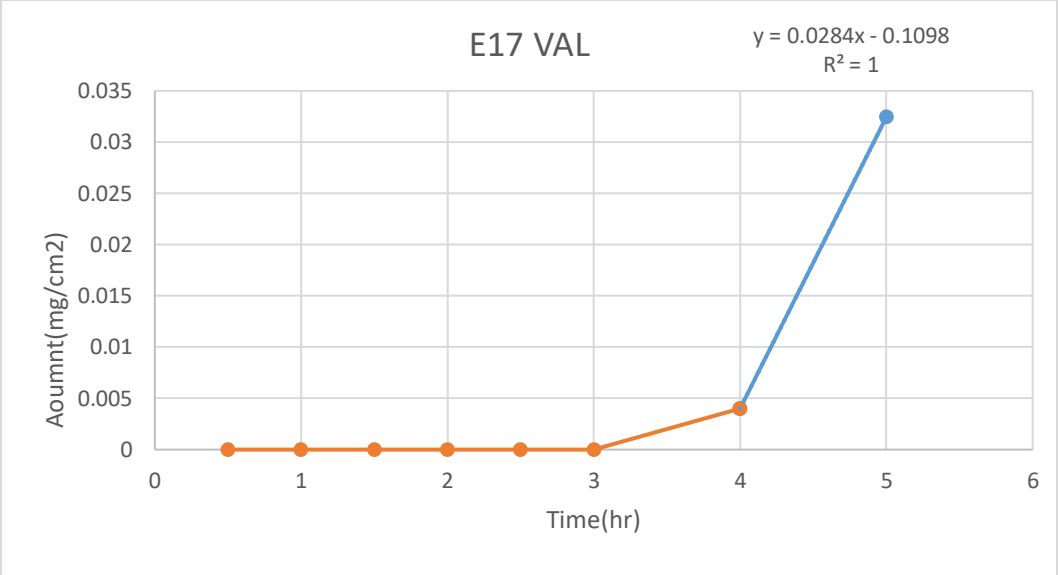

***Figure 4.31**: In vitro permeation profile for the cumulative amount of VAL in FeSSIF permeated per unit area of Permeapad (mg/cm²) for experiment E17, without enhancer.*

The diffusion parameters for VAL were calculated in table (4.76) and the enhancement ratio was determined.

***Table 4.76:** Diffusion parameters for HCT E17, without enhancer.*

slope	**intercept**	**T_L**	**D**	**P**	**K**	ER
0.0284	0.1098	0.258652	0.006444	0.0044375	0.006887	1

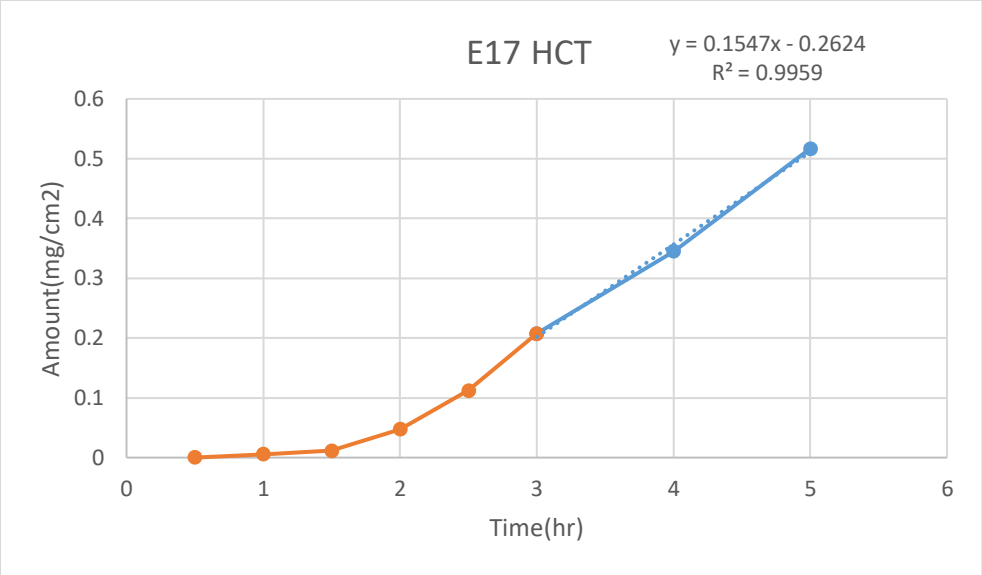

***Figure 4.32**: In vitro permeation profile for the cumulative amount of HCT in FeSSIF permeated per unit area of Permeapad (mg/cm²) for experiment E17, without enhancer.*

The diffusion parameters for HCT were calculated in table (4.77) and the enhancement ratio was determined.

***Table 4.77:** Diffusion parameters for HCT E17, without enhancer.*

slope	intercept	T_L	D	P	K	ER
0.1547	0.2624	0.589558	0.002827	0.1547	0.547228	1

4.8.4 **Experiment no. E18, VAL/HCT in FaSSIF with Na acetate through Permeapad**.

A sample of VAL and HCT was prepared with Na acetate according to general method described in section (3.7.4). The permeability results of drug through Permeapad membrane are shown in tables (4.78), and table (4.79).

The cumulative amount of VAL and HCT permeated through unit area of membrane was then calculated, the linear section, i.e. the steady state flux was plotted versus time (Figure (4.33) and Figure (4.34)).

***Table 4.78**: Data obtained from E18, in FaSSIF with Na acetae through Permeapad membrane using Franz diffusion cell (part1).*

time	absorbance of sample 1 (n=3)		absorbance of sample 2 (n=3)		absorbance of sample 3 (n=3)		concentration of sample **I** mg/ml		concentration of sample **II** mg/ml		concentration of sample **III** mg/ml	
	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL	HCT	VAL	HCT	VAL	HCT
0.5	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0.08	0	0.082		0.0796	0	0.004	0	0.004	0	0.004
1.5	0	0.183	0	0.186	0	0.18	0	0.009	0	0.009	0	0.009
2	0	0.271	0	0.2706	0	0.2733	0	0.013	0	0.013	0	0.013
2.5	0.071	0.3433	0.0723	0.3423	0.0733	0.3436	0.0001	0.015	3E-04	0.015	4E-04	0.015
3	0.124	0.459	0.1246	0.46	0.1236	0.4583	0.0032	0.02	0.003	0.02	0.003	0.02
4	0.232	0.5983	0.2313	0.597	0.2333	0.56	0.0115	0.024	0.011	0.024	0.012	0.022
5	0.369	0.774	0.371	0.776	0.3676	0.7766	0.022	0.029	0.022	0.029	0.022	0.029

***Table 4.79**: Data obtained from E18, in FaSSIF with Na acetae through Permeapad membrane using Franz diffusion cell (part 2).*

Q: cumulative amount released **I** (mg)		Q: cumulative amount released **II** (mg)		Q: cumulative amount released **III** (mg)		m: cumulative amount released per area **I** (mg/cm2)		m: cumulative amount released per area **II** (mg/cm2)		m: cumulative amount released per area **III** (mg/cm2)		**mean amount (mg/cm2)**		**SD**		**%RSD**	
VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
0	0.078	0	0.08	0	0.078	0	0.025	0	0.026	0	0.025	0	0.025	0	0.0004		1.597
0	0.261	0	0.266	0	0.258	0	0.083	0	0.085	0	0.082	0	0.083	0	0.0013		1.602
0	0.535	0	0.539	0	0.533	0	0.17	0	0.172	0	0.17	0	0.171	0	0.001		0.589
0.003	0.85	0.006	0.853	0.007	0.848	9E-04	0.271	0.002	0.272	0.002	0.27	0.002	0.271	8E-04	0.0008	44.46	0.299
0.067	1.256	0.071	1.26	0.071	1.254	0.021	0.4	0.023	0.401	0.023	0.399	0.022	0.4	8E-04	0.001	3.502	0.249
0.3	1.752	0.303	1.755	0.323	1.711	0.095	0.558	0.096	0.559	0.103	0.545	0.098	0.554	0.004	0.0077	4.066	1.394
0.751	2.36	0.758	2.364	0.771	2.321	0.239	0.752	0.241	0.753	0.246	0.739	0.242	0.748	0.003	0.0076	1.35	1.014

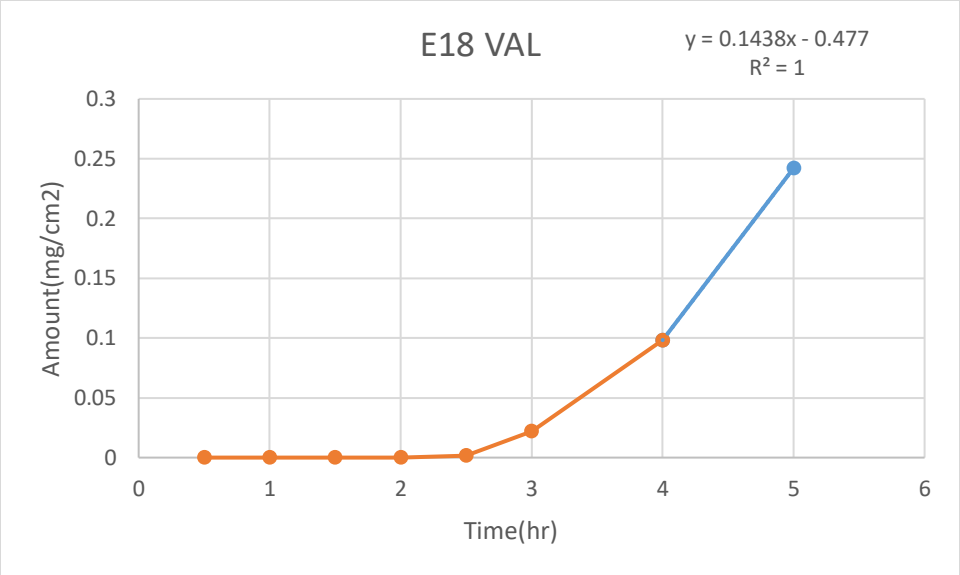

***Figure 4.33**: In vitro permeation profile for the cumulative amount of VAL in FaSSIF with Na acetate permeated per unit area of Permeapad (mg/cm²) for experiment E18.*

The diffusion parameters for VAL were calculated in table (4.80) and the enhancement ratio was determined.

***Table 4.80:** Diffusion parameters for VAL E18, with Na acetate.*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	ER
E18	0.1438	0.477	0.301468	0.005529	0.022469	0.040642	1.401559

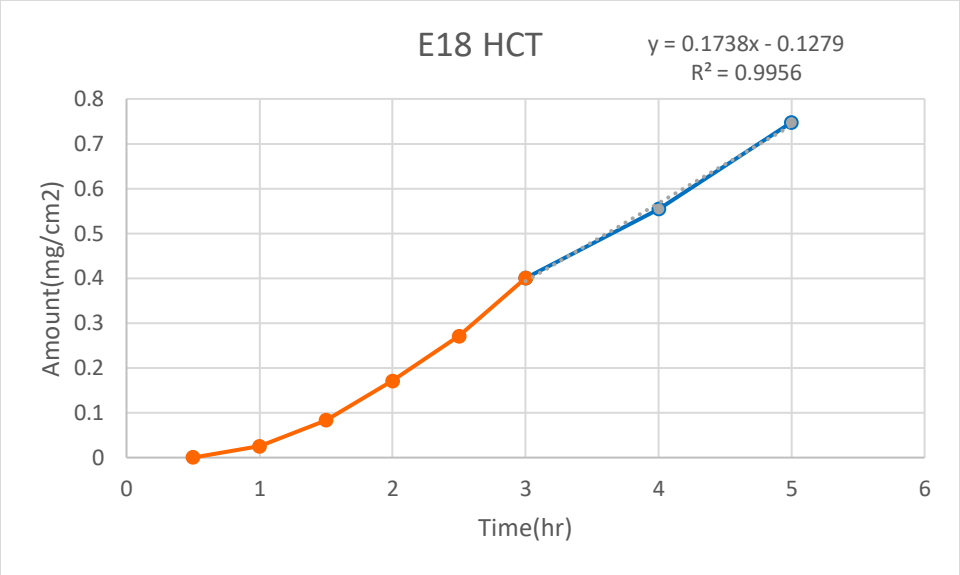

***Figure 4.34**: In vitro permeation profile for the cumulative amount of HCT in FaSSIF with Na acetate permeated per unit area of Permeapad (mg/cm²) for experiment E18.*

The diffusion parameters for HCT were calculated in table (4.81) and the enhancement ratio was determined.

***Table 4.41:** Diffusion parameters for HCT E18, with Na acetate.*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	ER
E18	0.1738	0.1279	1.358874	0.001227	0.1738	1.417034	1.517904

4.8.5 Experiment no. E19, VAL/HCT in FeSSIF with Na acetate through Permeapad.

A sample of VAL and HCT was prepared with Na acetate according to general method described in section (3.7.4). The permeability results of drug through Permeapad membrane are shown in tables (4.82), and table (4.83).

The cumulative amount of VAL and HCT permeated through unit area of membrane was then calculated, the linear section, i.e. the steady state flux was plotted versus time (Figure (4.35) and Figure (4.36)).

***Table 4.82**: Data obtained from E19, in FeSSIF with Na acetae through Permeapad membrane using Franz diffusion cell (part 1).*

time	absorbance of sample 1 (n=3)		absorbance of sample 2 (n=3)		absorbance of sample 3 (n=3)		concentration of sample **I** mg/ml		concentration of sample **II** mg/ml		concentration of sample **III** mg/ml	
	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL	HCT	VAL	HCT	VAL	HCT
0.5	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0.091	0	0.0906	0	0.0916	0	0.004	0	0.004	0	0.004
1.5	0	0.213	0	0.2103	0	0.216	0	0.01	0	0.01	0	0.011
2	0	0.338	0	0.3403	0	0.3396	0	0.017	0	0.017	0	0.017
2.5	0.1	0.454	0.1033	0.4573	0.1056	0.4533	8E-04	0.02	0.0011	0.02	0.0014	0.02
3	0.12	0.558	0.1223	0.556	0.1236	0.557	7E-04	0.024	0.001	0.024	0.0011	0.024
4	0.202	0.727	0.2036	0.7286	0.2016	0.7266	0.006	0.031	0.0058	0.031	0.0056	0.031
5	0.278	0.948	0.2753	0.9463	0.2736	0.9486	0.009	0.04	0.0086	0.04	0.0084	0.04

***Table 4.83**: Data obtained from E19, in FeSSIF with Na acetae through Permeapad membrane using Franz diffusion cell (part 2).*

Q: cumulative amount released **I** (mg)		Q: cumulative amount released **II** (mg)		Q: cumulative amount released **III** (mg)		m: cumulative amount released per area **I** (mg/cm2)		m: cumulative amount released per area **II** (mg/cm2)		m: cumulative amount released per area **III** (mg/cm2)		**mean amount (mg/cm2)**		**SD**		**%RSD**	
VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
0	0.089	0	0.089	0	0.09	0	0.028	0	0.028	0	0.029	0	0.028	0	0.0002		
0	0.302	0	0.298	0	0.305	0	0.096	0	0.095	0	0.097	0	0.096	0	0.0011		1.094
0	0.642	0	0.641	0	0.648	0	0.205	0	0.204	0	0.206	0	0.205	0	0.0011		0.519
0.016	1.056	0.022	1.057	0.028	1.058	0.005	0.336	0.007	0.337	0.009	0.337	0.007	0.337	0.0019	0.0003	27.01	0.097
0.031	1.565	0.042	1.563	0.052	1.564	0.01	0.498	0.014	0.498	0.016	0.498	0.013	0.498	0.0033	0.0003	24.53	0.067
0.145	2.206	0.159	2.204	0.165	2.204	0.046	0.702	0.051	0.702	0.053	0.702	0.05	0.702	0.0033	0.0002	6.629	0.034
0.328	3.033	0.338	3.031	0.339	3.034	0.105	0.966	0.108	0.965	0.108	0.966	0.107	0.966	0.0019	0.0005	1.754	0.054

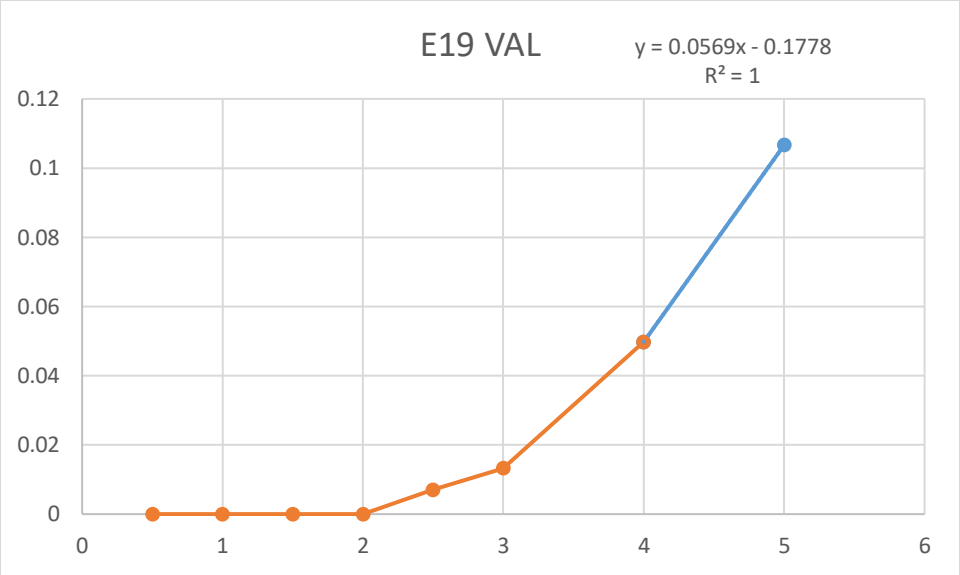

***Figure 4.35**: In vitro permeation profile for the cumulative amount of VAL in FeSSIF with Na acetate permeated per unit area of Permeapad (mg/cm²) for experiment E19.*

The diffusion parameters for VAL were calculated in table (4.84) and the enhancement ratio was determined.

***Table 4.84:** Diffusion parameters for VAL E19, with Na acetate.*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	ER
E19	0.0569	0.1778	0.320022	0.005208	0.008891	0.017071	2.003521

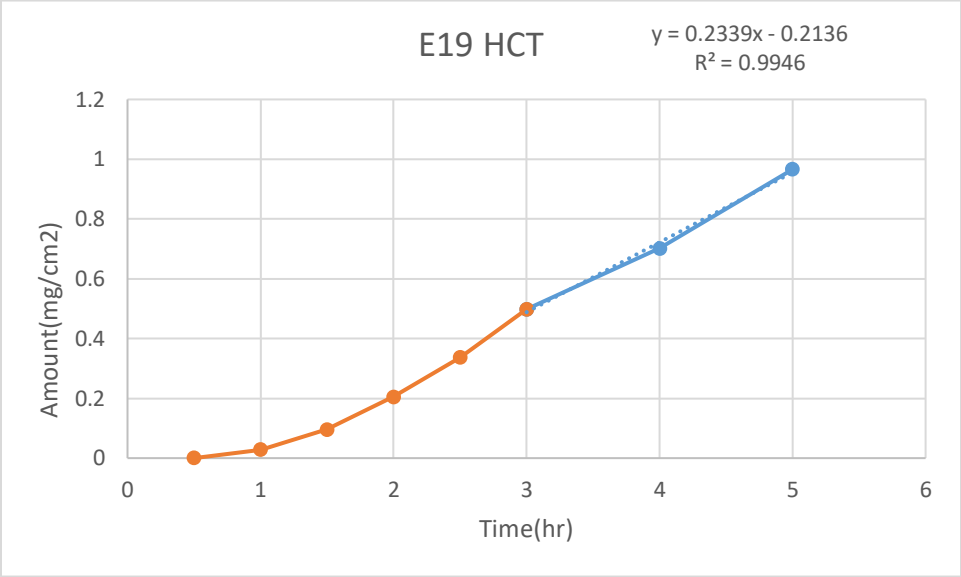

***Figure 4.36**: In vitro permeation profile for the cumulative amount of HCT in FeSSIF with Na acetate permeated per unit area of Permeapad (mg/cm²) for experiment E19.*

The diffusion parameters for HCT were calculated in table (4.85) and the enhancement ratio was determined.

***Table 4.85:** Diffusion parameters for HCT E19, with Na acetate.*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	**ER**
E19	0.2339	0.2136	1.095037	0.001522	0.2339	1.536776	1.511959

4.6.6 Experiment no. E20, VAL/HCT in FaSSIF with 1% citric acid through Permeapad.

A sample of VAL and HCT was prepared with 1% citric acid according to general method described in section (3.7.4). The permeability results of drug through Permeapad membrane are shown in tables (4.86), (4.87).

The cumulative amount of VAL and HCT permeated through unit area of membrane was then calculated, the linear section, i.e. the steady state flux was plotted versus time (Figure (4.37) and Figure (4.38)).

***Table 4.86**: Data obtained from E20, in FaSSIF with citric acid through Permeapad membrane using Franz diffusion cell (part 1).*

time	absorbance of sample 1 (n=3)		absorbance of sample 2 (n=3)		absorbance of sample 3 (n=3)		concentration of sample **I** mg/ml		concentration of sample **II** mg/ml		concentration of sample **III** mg/ml	
	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL	HCT	VAL	HCT	VAL	HCT
0.5	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0.0746	0	0.0733	0	0.0783	0	0.004	0	0.004	0	0.004
1.5	0	0.1856	0	0.1863	0	0.1893	0	0.009	0	0.009	0	0.009
2	0.062	0.287	0.0616	0.286	0.063	0.2866	4E-04	0.013	4E-04	0.013	5E-04	0.013
2.5	0.1386	0.3953	0.1383	0.394	0.1383	0.3946	0.006	0.016	0.006	0.016	0.006	0.016
3	0.2586	0.538	0.258	0.5376	0.2593	0.5393	0.016	0.02	0.015	0.02	0.016	0.02
4	0.4246	0.7326	0.426	0.7343	0.424	0.7333	0.029	0.026	0.029	0.026	0.029	0.026
5	0.6113	0.938	0.6106	0.9376	0.6123	0.9393	0.044	0.032	0.044	0.032	0.044	0.032

***Table 4.87**: Data obtained from E20, in FaSSIF with citric acid through Permeapad membrane using Franz diffusion cell (part 2).*

Q: cumulative amount released **I** (mg)		Q: cumulative amount released **II** (mg)		Q: cumulative amount released **III** (mg)		m: cumulative amount released per area **I** (mg/cm2)		m: cumulative amount released per area **II** (mg/cm2)		m: cumulative amount released per area **III** (mg/cm2)		**mean amount (mg/cm2)**		**SD**		**%RSD**	
VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
0	0.073	0	0.072	0	0.077	0	0.023	0	0.023	0	0.0244	0	0.023	0	0.0008		3.441
0	0.258	0	0.257	0	0.265	0	0.082	0	0.082	0	0.0845	0	0.083	0	0.0014		1.721
0.008	0.519	0.007	0.517	0.01	0.525	0.002	0.165	0.002	0.165	0.003	0.1673	0.003	0.166	0.00046	0.0014	17.03	0.836
0.129	0.853	0.129	0.85	0.131	0.859	0.041	0.272	0.041	0.271	0.042	0.2735	0.041	0.272	0.00043	0.0014	1.033	0.525
0.446	1.274	0.444	1.271	0.449	1.281	0.142	0.406	0.141	0.405	0.143	0.4079	0.142	0.406	0.00073	0.0016	0.512	0.397
1.034	1.812	1.035	1.81	1.035	1.82	0.329	0.577	0.329	0.576	0.33	0.5795	0.33	0.578	0.00023	0.0017	0.069	0.289
1.937	2.469	1.936	2.467	1.94	2.478	0.617	0.786	0.617	0.786	0.618	0.789	0.617	0.787	0.00059	0.0018	0.096	0.232

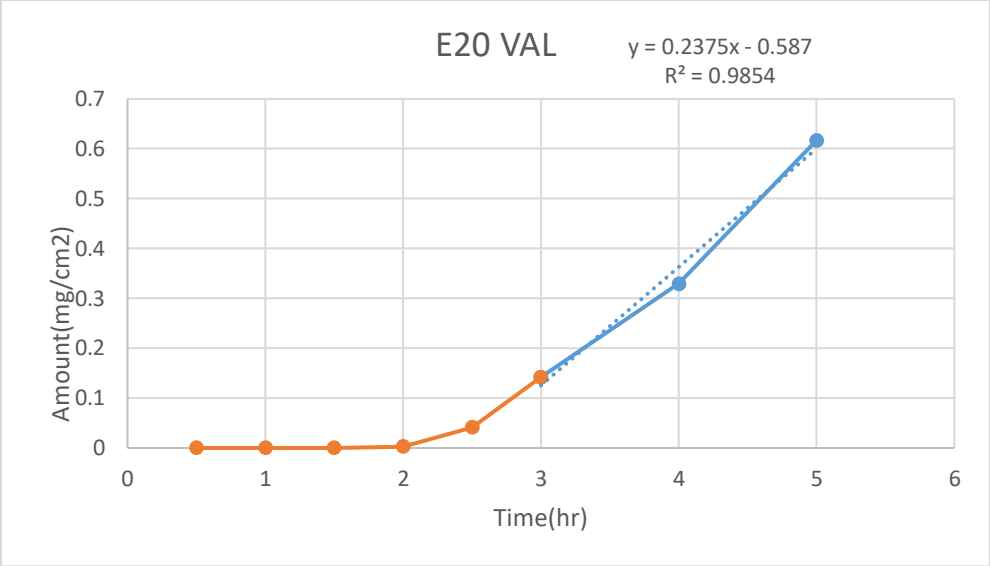

***Figure 4.37**: In vitro permeation profile for the cumulative amount of VAL in FaSSIF with citric acid permeated per unit area of Permeapad (mg/cm²) for experiment E20.*

The diffusion parameters for VAL were calculated in table (4.88) and the enhancement ratio was determined.

***Table 4.88:** Diffusion parameters for VAL E20, with citric acid.*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	**ER**
E20	0.2375	0.587	0.4046	0.004119	0.037109	0.090087	2.314815

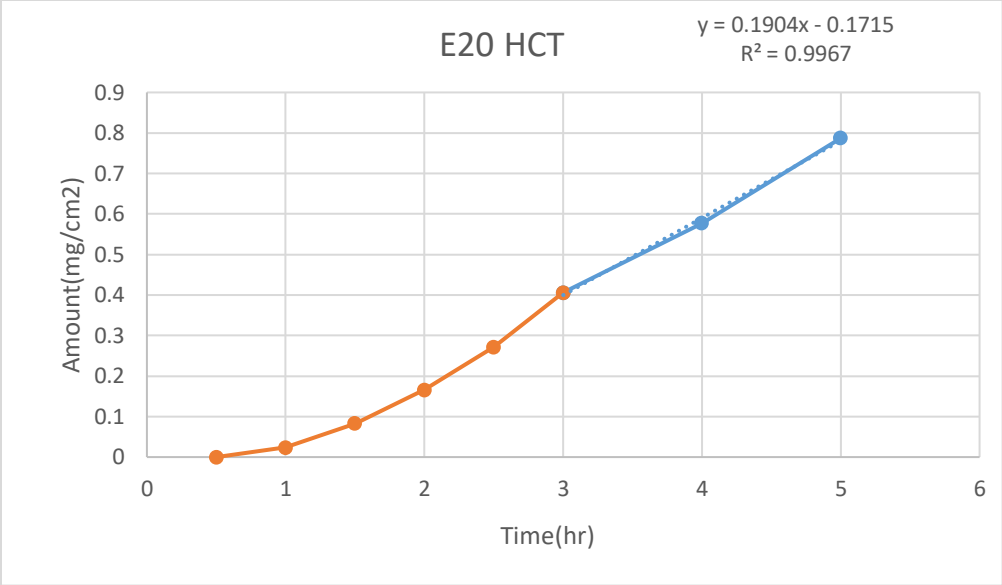

***Figure 4.38**: In vitro permeation profile for the cumulative amount of HCT in FaSSIF with citric acid permeated per unit area of Permeapad (mg/cm²) for experiment E20.*

The diffusion parameters for HCT were calculated in table (4.89) and the enhancement ratio was determined.

***Table 4.89:** Diffusion parameters for HCT E20, with citric acid.*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	**ER**
E20	0.1904	0.1715	1.110204	0.001501	0.1904	1.268297	1.662882

4.6.7 Experiment no. E21, VAL/HCT in FeSSIF with 1%citric acid through Permeapad.

A sample of VAL and HCT was prepared with 1%citric acid according to general method described in section (3.7.4). The permeability results of drug through Permeapad membrane are shown in tables (4.90), (4.91).

The cumulative amount of VAL and HCT permeated through unit area of membrane was then calculated, the linear section, i.e. the steady state flux was plotted versus time Figure (4.39) and Figure (4.40)).

***Table 4.90**: Data obtained from E21, in FeSSIF with citric acid through Permeapad membrane using Franz diffusion cell (part 1).*

time	absorbance of sample 1 (n=3)		absorbance of sample 2 (n=3)		absorbance of sample 3 (n=3)		concentration of sample **I** mg/ml		concentration of sample **II** mg/ml		concentration of sample **III** mg/ml	
	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL	HCT	VAL	HCT	VAL	HCT
0.5	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0.0786	0	0.0803	0	0.0816	0	0.004	0	0.004	0	0.004
1.5	0	0.1896	0	0.1903	0	0.1893	0	0.009	0	0.009	0	0.009
2	0	0.3226	0	0.3273	0	0.3306	0	0.016	0	0.016	0	0.016
2.5	0	0.4296	0	0.4303	0	0.4323	0	0.021	0	0.021	0	0.021
3	0.14	0.6312	0.138	0.629	0.143	0.635	0.0012	0.028	0.0011	0.028	0.0015	0.028
4	0.196	0.842	0.193	0.84	0.199	0.845	0.0026	0.037	0.0023	0.037	0.0029	0.037
5	0.331	0.9936	0.329	0.991	0.334	0.996	0.0134	0.041	0.0133	0.041	0.0137	0.041

***Table 4.91**: Data obtained from E21, in FeSSIF with citric acid through Permeapad membrane using Franz diffusion cell (part 2).*

Q: cumulative amount released **I** (mg)		Q: cumulative amount released **II** (mg)		Q: cumulative amount released **III** (mg)		m: cumulative amount released per area **I** (mg/cm2)		m: cumulative amount released per area **II** (mg/cm2)		m: cumulative amount released per area **III** (mg/cm2)		**mean amount (mg/cm2)**		**SD**		**%RSD**	
VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
0	0.077	0	0.078	0	0.08	0	0.024	0	0.025	0	0.025	0	0.025	0	0.0005		1.877
0	0.266	0	0.268	0	0.269	0	0.085	0	0.085	0	0.086	0	0.085	0	0.0005		0.566
0	0.591	0	0.598	0	0.601	0	0.188	0	0.19	0	0.191	0	0.19	0	0.0017		0.904
0	1.026	0	1.034	0	1.04	0	0.327	0	0.329	0	0.331	0	0.329	0	0.0022		0.662
0.025	1.599	0.021	1.605	0.029	1.615	0.008	0.509	0.007	0.511	0.009	0.514	0.008	0.512	0.0013	0.0026	15.7	0.502
0.078	2.357	0.069	2.364	0.088	2.375	0.025	0.751	0.022	0.753	0.028	0.756	0.025	0.753	0.003	0.0029	11.9	0.38
0.349	3.21	0.337	3.215	0.364	3.229	0.111	1.022	0.107	1.024	0.116	1.028	0.112	1.025	0.0044	0.0031	3.92	0.304

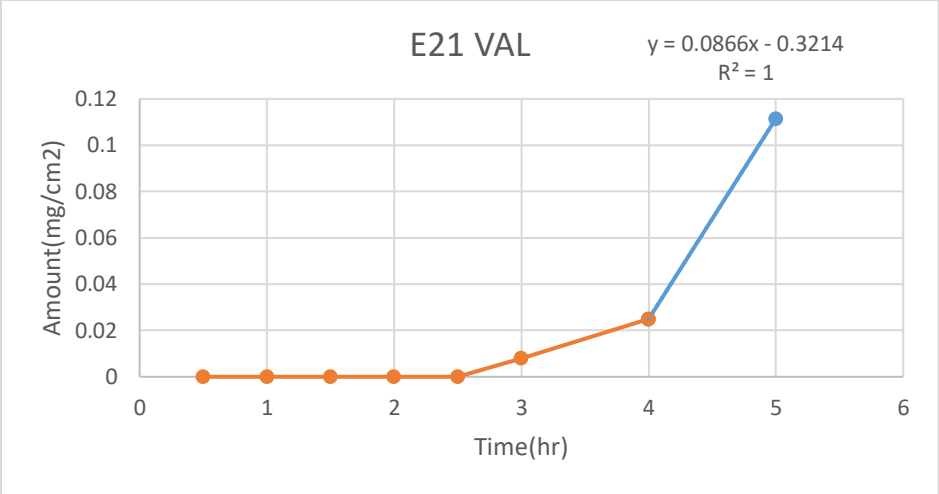

***Figure 4.39**: In vitro permeation profile for the cumulative amount of VAL in FeSSIF with citric acid permeated per unit area of Permeapad (mg/cm²) for experiment E21.*

The diffusion parameters for VAL were calculated in table (4.92) and the enhancement ratio was determined.

***Table 4.92:** Diffusion parameters for VAL E21, with citric acid.*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	ER
E21	0.0866	0.3272	2.646699	0.00063	0.014	0.022	3.049296

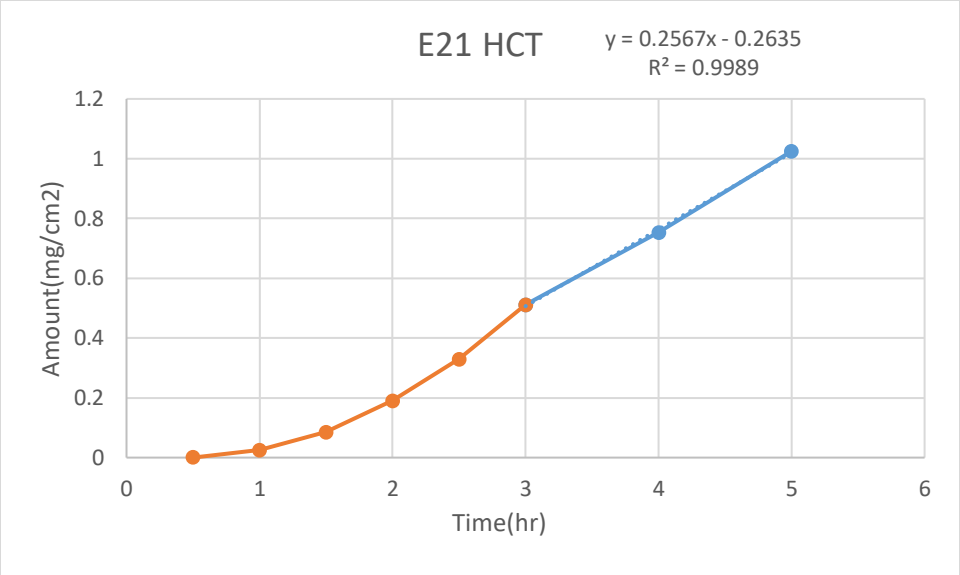

***Figure 4.40**: In vitro permeation profile for the cumulative amount of HCT in FeSSIF with citric acid permeated per unit area of Permeapad (mg/cm²) for experiment E21.*

The diffusion parameters for HCT were calculated in table (4.93) and the enhancement ratio was determined.

***Table 4.93:** Diffusion parameters for HCT E21, with citric acid.*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	**ER**
E21	0.2567	0.2635	0.974194	0.001711	0.2567	1.500453	1.659341

At first when 1% citric acid and 1% Na acetate were added to prepared samples separately, from figures (4.55) and (4.56) it seems that 1% citric acid enhances permeation of VAL in both FaSSIF and FeSSIF. Also Na acetate enhance permeation of VAL when prepared in FeSSIF the same as 1% citric acid. In figures (4.57) and (4.58) permeation of HCT was enhanced by similarly by citric acid and Na acetate when prepared in FaSSIF. However, 1% Na acetate enhances permeation of HCT more than 1% citric acid. So to cover the benefit of both of 1%citric acid and

1% Na acetate, we decided to add a combination of enhancers including 1% citric acid and 1% Na acetate.

4.6.8 Experiment no. E22, VAL/HCT in FeSSIF with 1% citric acid and 1% Na acetate through Permeapad.

A sample of VAL and HCT was prepared with a combination of 1% citric acid and 1% Na acetate according to general method described in section (3.7.4). The permeability results of drug through Permeapad membrane are shown in tables (4.94), (4.95).

The cumulative amount of VAL and HCT permeated through unit area of membrane was then calculated, the linear section, i.e. the steady state flux was plotted versus time (Figure (4.41) and Figure (4.42)).

***Table 4.94**: Data obtained from E22, in FaSSIF with citric acid and Na acetate through Permeapad membrane using Franz diffusion cell (part 1).*

time	absorbance of sample 1 (n=3)		absorbance of sample 2 (n=3)		absorbance of sample 3 (n=3)		concentration of sample **I** mg/ml		concentration of sample **II** mg/ml		concentration of sample **III** mg/ml	
	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL	HCT	VAL	HCT	VAL	HCT
0.5	0	0.028	0	0.017	0	0.023	0	0.001368	0	0.000831	0	0.001124
1	0	0.095	0	0.097	0	0.092	0	0.004642	0	0.00474	0	0.004496
1.5	0	0.169	0	0.173	0	0.165	0	0.008258	0	0.008454	0	0.008063
2	0.041	0.201	0.043	0.203	0.042	0.202	2.1E-05	0.008864	0.000187	0.008915	0.000104	0.00889
2.5	0.129	0.44	0.12	0.4	0.134	0.46	0.004121	0.018488	0.004029	0.016743	0.004219	0.019348
3	0.222	0.554	0.218	0.545	0.23	0.56	0.011374	0.021886	0.011148	0.02154	0.012078	0.021992
4	0.38	0.734	0.35	0.729	0.351	0.71	0.023985	0.026991	0.020976	0.027448	0.02148	0.026496
5	0.539	0.916	0.534	0.912	0.342	0.919	0.036658	0.032171	0.036223	0.032092	0.016141	0.036919

***Table 4.95**: Data obtained from E22, in FaSSIF with citric acid and Na acetate through Permeapad membrane using Franz diffusion cell (part 2).*

Q: cumulative amount released **I** (mg)		Q: cumulative amount released **II** (mg)		Q: cumulative amount released **III** (mg)		m: cumulative amount released per area **I** (mg/cm2)		m: cumulative amount released per area **II** (mg/cm2)		m: cumulative amount released per area **III** (mg/cm2)		**mean amount (mg/cm2)**		**SD**		**%RSD**	
VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT
0	0.027	0	0.017	0	0.022	0	0.009	0	0.005	0	0.007	0	0.007	0	0.002		24.3
0	0.122	0	0.112	0	0.114	0	0.039	0	0.036	0	0.036	0	0.037	0	0.002		4.37
0	0.291	0	0.286	0	0.279	0	0.093	0	0.091	0	0.089	0	0.091	0	0.002		2.13
4E-04	0.477	0.004	0.473	0.002	0.465	1E-04	0.152	0.001	0.151	7E-04	0.148	7E-04	0.15	0.0005	0.002	79.74	1.27
0.083	0.856	0.085	0.817	0.087	0.861	0.026	0.272	0.027	0.26	0.028	0.274	0.027	0.269	0.0006	0.008	2.185	2.87
0.314	1.312	0.312	1.264	0.332	1.32	0.1	0.418	0.099	0.403	0.106	0.42	0.102	0.414	0.0036	0.01	3.528	2.33
0.806	1.873	0.742	1.835	0.774	1.872	0.257	0.597	0.236	0.584	0.247	0.596	0.246	0.592	0.0101	0.007	4.094	1.18
1.563	2.544	1.488	2.504	1.118	2.637	0.498	0.81	0.474	0.797	0.356	0.84	0.443	0.816	0.0758	0.022	17.12	2.66

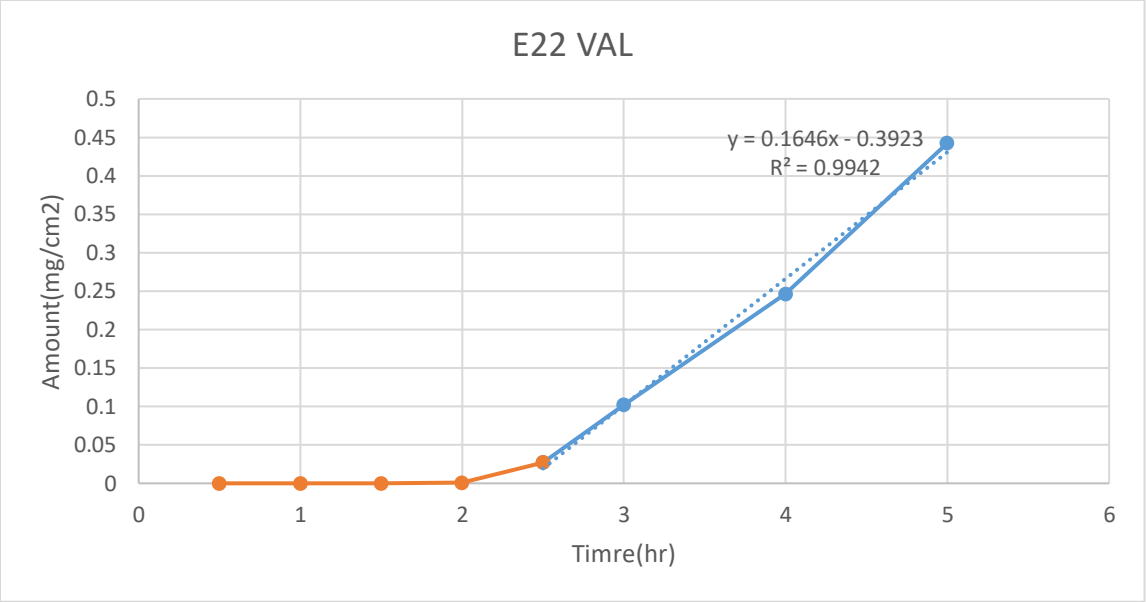

***Figure 4.41**: In vitro permeation profile for the cumulative amount of VAL in FaSSIF with citric acid and Na acetate permeated per unit area of Permeapad (mg/cm²) for experiment E22.*

The diffusion parameters for VAL were calculated in table (4.96) and the enhancement ratio was determined.

***Table 4.96:** Diffusion parameters for VAL E22, with citric acid and Na acetate*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	ER
E22	0.1646	0.3923	0.4196	0.004	0.026	0.065	1.607

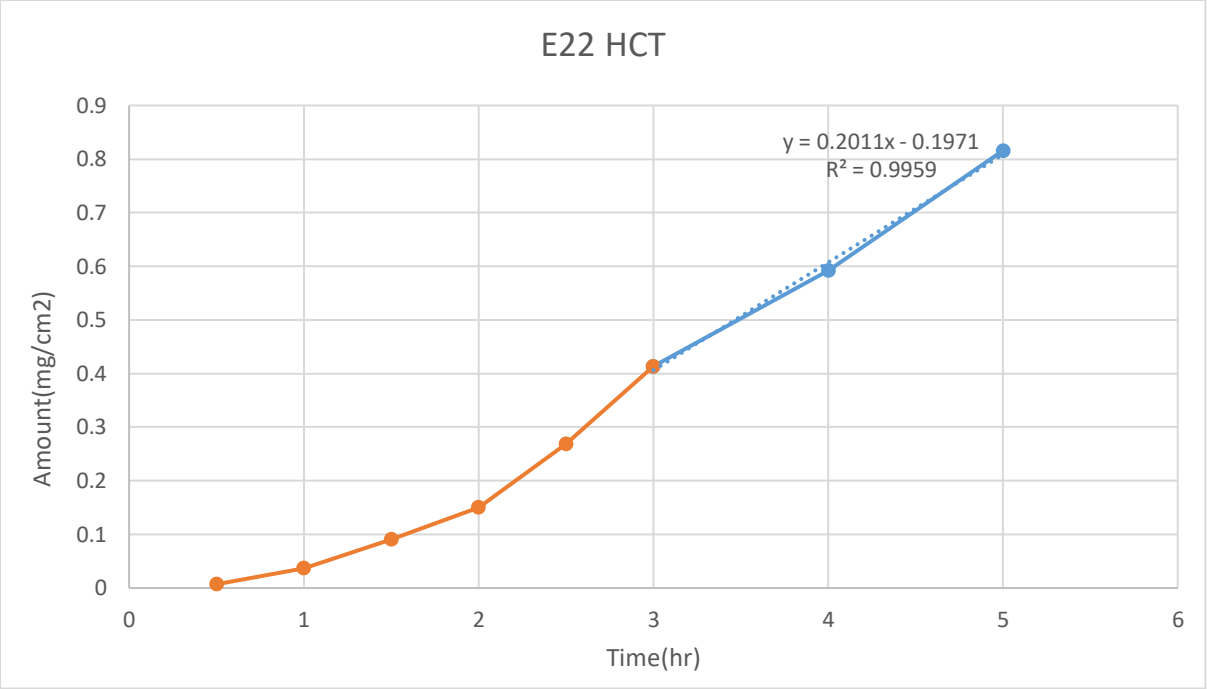

***Figure 4.42**: In vitro permeation profile for the cumulative amount of HCT in FaSSIF with citric acid and Na acetate permeated per unit area of Permeapad (mg/cm²) for experiment E22.*

The diffusion parameters for HCT were calculated in table (4.97) and the enhancement ratio was determined.

***Table 4.97:** Diffusion parameters for HCT E22, with citric acid and Na acetate.*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	**ER**
E22	0.201	0.197	1.02	0.002	0.2011	1.231	1.749

4.6.9 Experiment no. E23, VAL/HCT in FeSSIF with 1%citric acid and 1%Na acetate through Permeapad.

A sample of VAL and HCT was prepared with a combination of 1%citric acid and 1% Na acetate according to general method described in section (3.7.4). The permeability results of drug through Permeapad membrane are shown in tables (4.98), (4.99).

The cumulative amount of VAL and HCT permeated through unit area of membrane was then calculated, the linear section, i.e. the steady state flux was plotted versus time (Figure (4.43) and Figure (4.44)).

***Table 4.98**: Data obtained from E22, in FeSSIF with citric acid and Na acetate through Permeapad membrane using Franz diffusion cell (part 1).*

time	absorbance of sample 1 (n=3)		absorbance of sample 2 (n=3)		absorbance of sample 3 (n=3)		concentration of sample **I** mg/ml		concentration of sample **II** mg/ml		concentration of sample **III** mg/ml	
	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL	HCT	VAL	HCT	VAL	HCT
0.5	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0.097	0	0.092	0	0.099	0	0.00474	0	0.004496	0	0.004838
1.5	0	0.258	0	0.267	0	0.262	0	0.012607	0	0.013047	0	0.012803
2	0	0.338	0	0.345	0	0.335	0	0.016517	0	0.016859	0	0.01637
2.5	0	0.448	0	0.44	0	0.439	0	0.021892	0	0.021501	0	0.021452
3	0	0.57	0	0.555	0	0.562	0	0.027853	0	0.02712	0	0.027462
4	0.152	0.745	0.155	0.749	0.151	0.742	8.15E-05	0.032854	0.000309	0.03298	4.09E-05	0.032731
5	0.198	0.916	0.192	0.91	0.199	0.918	0.001254	0.040136	0.000757	0.039983	0.001316	0.04021

***Table 4.99**: Data obtained from E22, in FeSSIF with citric acid and Na acetate through Permeapad membrane using Franz diffusion cell (part 2).*

Q: cumulative amount released **I** (mg)		Q: cumulative amount released **II** (mg)		Q: cumulative amount released **III** (mg)		m: cumulative amount released per area **I** (mg/cm2)		m: cumulative amount released per area **II** (mg/cm2)		m: cumulative amount released per area **III** (mg/cm2)		**mean amount (mg/cm2)**		**SD**		**%RSD**	
VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
0	0.095	0	0.09	0	0.097	0	0.03	0	0.029	0	0.031	0	0.03	0	0.001		3.756
0	0.352	0	0.355	0	0.358	0	0.112	0	0.113	0	0.114	0	0.113	0	1E-03		0.847
0	0.695	0	0.706	0	0.698	0	0.221	0	0.225	0	0.222	0	0.223	0	0.002		0.804
0	1.149	0	1.152	0	1.143	0	0.366	0	0.367	0	0.364	0	0.366	0	0.001		0.404
0	1.728	0	1.716	0	1.714	0	0.55	0	0.547	0	0.546	0	0.548	0	0.002		0.435
0.0016	2.413	0.006	2.403	8E-04	2.396	0.0005	0.768	0.002	0.765	3E-04	0.763	0.0009	0.766	0.0009	0.003	100.42	0.352
0.0268	3.248	0.022	3.236	0.027	3.233	0.0085	1.035	0.007	1.03	0.009	1.03	0.008	1.032	0.001	0.003	12.282	0.255

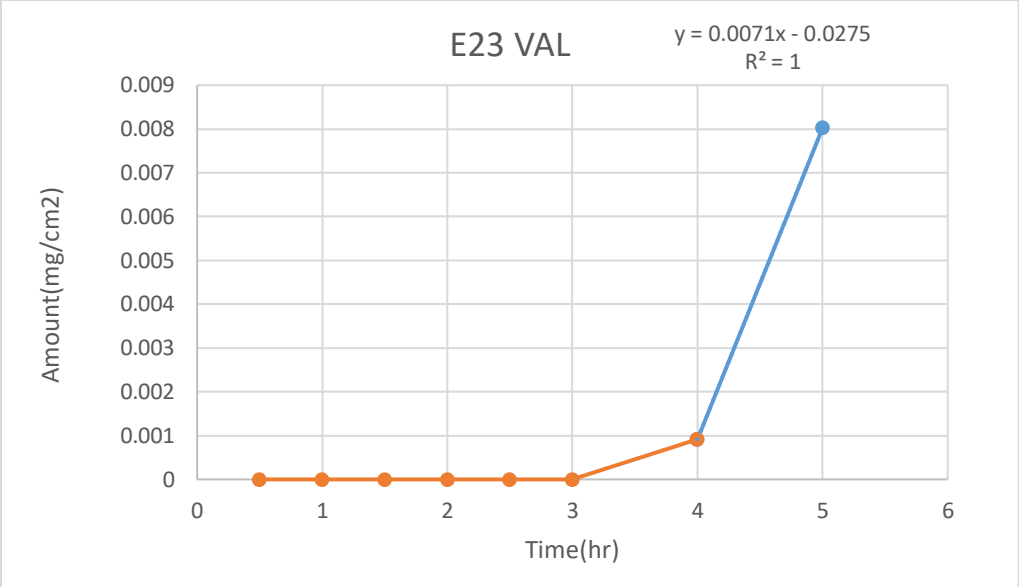

***Figure 4.43**: In vitro permeation profile for the cumulative amount of VAL in FeSSIF with citric acid and Na acetate permeated per unit area of Permeapad (mg/cm²) for experiment E23.*

The diffusion parameters for VAL were calculated in table (4.100) and the enhancement ratio was determined.

***Table 4.100**: Diffusion parameters for VAL E23, with citric acid and Na acetate*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	ER
E23	0.0071	0.0275	0.2582	0.006	0.001	0.002	0.25

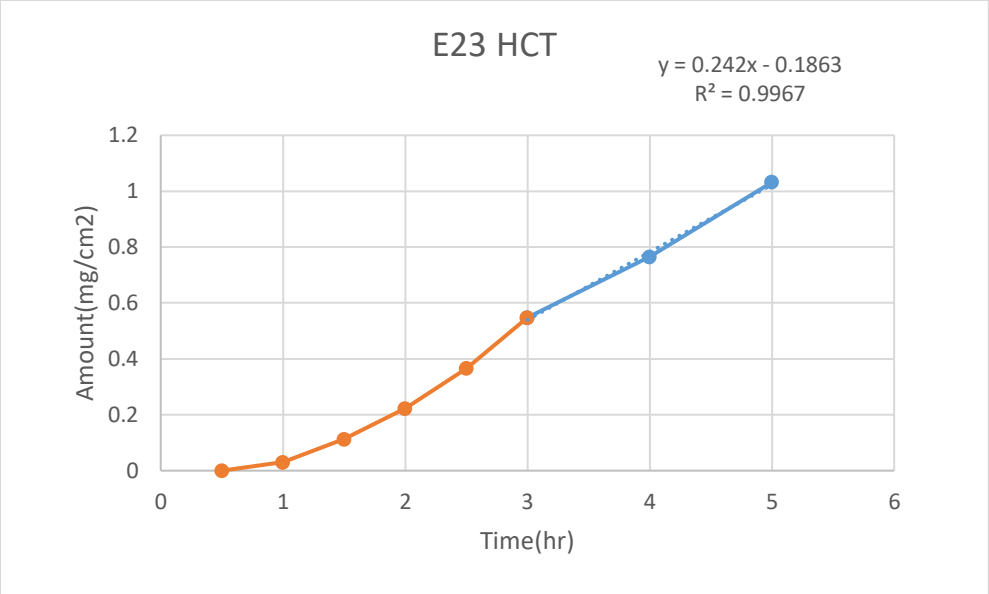

***Figure 4.44**: In vitro permeation profile for the cumulative amount of HCT in FeSSIF with citric acid and Na acetate permeated per unit area of Permeapad (mg/cm²) for experiment E23.*

The diffusion parameters for HCT were calculated in table (4.101) and the enhancement ratio was determined.

***Table 4.101:** Diffusion parameters for HCT E23, with citric acid and Na acetate.*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	**ER**
E23	0.242	0.186	1.299	0.001	0.242	1.8861	1.564

Results for the combination of 1% citric acid and 1% Na acetate were not as expected, the combination gives good enhancement for VAL but less than 1%citric acid when placed in FaSSIF, on the contrary of FeSSIF the combination compromise the permeation. The combination of 1% citric acid and Na acetate enhances the permeation of HCT when prepared in both FaSSIF and FeSSIF. To avoid the effect of combination on VAL we excluded this choice and decided to increase the concentration of citric acid from 1% to 1.5% and 2%.

4.6.10 Experiment no. E24, VAL/HCT in FaSSIF with citric acid 1.5% through Permeapad.

A sample of VAL and HCT was prepared with 1.5%citric acid according to general method described in section (3.7.4). The permeability results of drug through Permeapad membrane are shown in tables (4.102), (4.103).

The cumulative amount of VAL and HCT permeated through unit area of membrane was then calculated, the linear section, i.e. the steady state flux was plotted versus time Figure (4.45) and Figure (4.46)).

Table 4.102*: Data obtained from E24, in FaSSIF with citric acid 1.5% through Permeapad membrane using Franz diffusion cell (part 1).*

time	absorbance of sample 1 (n=3)		absorbance of sample 2 (n=3)		absorbance of sample 3 (n=3)		concentration of sample **I** mg/ml		concentration of sample **II** mg/ml		concentration of sample **III** mg/ml	
	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL	HCT	VAL	HCT	VAL	HCT
0.5	0	0.068	0	0.064	0	0.05	0	0.0033	0	0.0031	0	0.0024
1	0.034	0.164	0.033	0.162	0.036	0.166	7E-05	0.0072	1E-05	0.0071	0.0002	0.0073
1.5	0.143	0.2796	0.148	0.284	0.14	0.275	0.009	0.0103	0.0094	0.0104	0.0087	0.0102
2	0.245	0.403	0.251	0.408	0.24	0.4	0.0169	0.014	0.0175	0.0141	0.0165	0.0139
2.5	0.404	0.5836	0.409	0.589	0.4	0.54	0.0296	0.0191	0.0301	0.0192	0.0301	0.017
3	0.531	0.726	0.526	0.721	0.538	0.732	0.0398	0.0231	0.0394	0.0229	0.0404	0.0232
4	0.714	0.91	0.709	0.87	0.719	0.96	0.055	0.0278	0.0553	0.026	0.0544	0.0301
5	0.914	1.25	0.92	1.255	0.909	1.2	0.0686	0.0397	0.0691	0.0398	0.0691	0.0374

Table 4.103*: Data obtained from E24, in FaSSIF with citric acid 1.5% through Permeapad membrane using Franz diffusion cell (part 2).*

Q: cumulative amount released **I** (mg)		Q: cumulative amount released **II** (mg)		Q: cumulative amount released **III** (mg)		m: cumulative amount released per area **I** (mg/cm2)		m: cumulative amount released per area **II** (mg/cm2)		m: cumulative amount released per area **III** (mg/cm2)		**mean amount (mg/cm2)**		**SD**		**%RSD**	
VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT
0	0.066	0	0.063	0	0.049	0	0.021	0	0.02	0	0.016	0	0.019	0	0.003		15.58
0.0015	0.214	0.0002	0.209	0.005	0.197	5E-04	0.068	8E-05	0.066	0.002	0.063	7E-04	0.066	7E-04	0.003	108.2	4.315
0.1806	0.428	0.1879	0.424	0.18	0.407	0.058	0.136	0.06	0.135	0.057	0.13	0.058	0.134	0.001	0.003	2.424	2.602
0.5285	0.718	0.5465	0.716	0.518	0.696	0.168	0.229	0.174	0.228	0.165	0.222	0.169	0.226	0.005	0.004	2.685	1.669
1.1383	1.113	1.165	1.115	1.138	1.051	0.363	0.355	0.371	0.355	0.362	0.335	0.365	0.348	0.005	0.012	1.354	3.317
1.9646	1.594	1.9834	1.593	1.977	1.532	0.626	0.508	0.632	0.507	0.629	0.488	0.629	0.501	0.003	0.011	0.481	2.24
3.1035	2.173	3.1283	2.135	3.105	2.158	0.988	0.692	0.996	0.68	0.989	0.687	0.991	0.686	0.004	0.006	0.444	0.883
4.5295	2.995	4.565	2.958	4.542	2.936	1.443	0.954	1.454	0.942	1.446	0.935	1.448	0.944	0.006	0.01	0.397	1.008

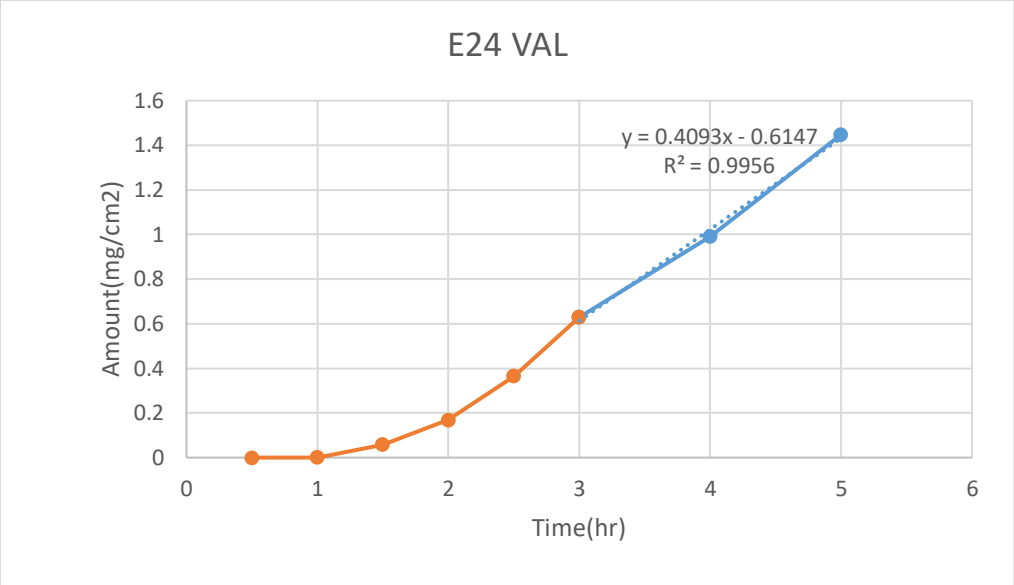

***Figure 4.45**: In vitro permeation profile for the cumulative amount of VAL in FaSSIF with citric acid 1.5% permeated per unit area of Permeapad (mg/cm²) for experiment E24.*

The diffusion parameters for VAL were calculated in table (4.104) and the enhancement ratio was determined.

***Table 4.104:** Diffusion parameters for VAL E24, with citric acid 1.5%.*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	ER
E24	0.4093	0.6147	0.6659	0.003	0.064	0.256	3.997

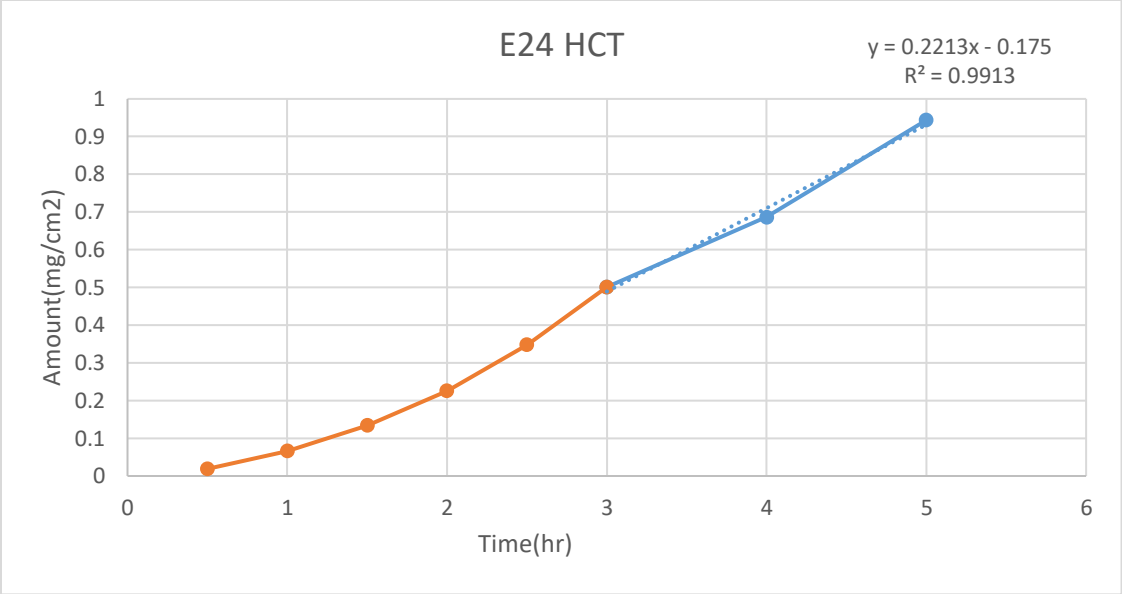

***Figure 4.46**: In vitro permeation profile for the cumulative amount of HCT in FaSSIF with citric acid 1.5% permeated per unit area of Permeapad (mg/cm²) for experiment E24.*

The diffusion parameters for HCT were calculated in table (4.105) and the enhancement ratio was determined.

***Table 4.105:** Diffusion parameters for HCT E24, with citric acid 1.5%.*

sample #	**slope**	**intercept**	**TL**	**D**	**P**	**K**	**ER**
E24	0.221	0.175	1.265	0.001	0.221	1.679	1.924

4.6.11 Experiment no. E25, VAL/HCT in FeSSIF with 1.5% citric acid through Permeapad.

A sample of VAL and HCT was prepared with 1.5% citric acid according to general method described in section (3.7.4). The permeability results of drug through Permeapad membrane are shown in tables (4.106), (4.107).

The cumulative amount of VAL and HCT permeated through unit area of membrane was then calculated, the linear section, i.e. the steady state flux was plotted versus time (Figure (4.47) and Figure (4.48)).

***Table 4.106**: Data obtained from E25, in FeSSIF with citric acid 1.5% through Permeapad membrane using Franz diffusion cell (part1).*

time	absorbance of sample 1 (n=3)		absorbance of sample 2 (n=3)		absorbance of sample 3 (n=3)		concentration of sample **I** mg/ml		concentration of sample **II** mg/ml		concentration of sample **III** mg/ml	
	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL	HCT	VAL	HCT	VAL	HCT
0.5	0	0.018	0	0.021	0	0.015	0	9E-04	0	0.001	0	7E-04
1	0	0.131	0	0.137	0	0.128	0	0.006	0	0.007	0	0.006
1.5	0	0.276	0	0.282	0	0.271	0	0.013	0	0.014	0	0.013
2	0	0.371	0	0.375	0	0.368	0	0.018	0	0.018	0	0.018
2.5	0	0.526	0	0.532	0	0.521	0	0.026	0	0.026	0	0.025
3	0	0.554	0	0.548	0	0.559	0	0.027	0	0.027	0	0.027
4	0.156	0.76	0.162	0.765	0.159	0.763	0.00018	0.033	0.0007	0.034	0.0004	0.034
5	0.245	0.916	0.251	0.92	0.239	0.911	0.00613	0.039	0.00667	0.039	0.0056	0.039

***Table 4.107**: Data obtained from E25, in FeSSIF with citric acid 1.5% through Permeapad membrane using Franz diffusion cell (part 2).*

Q: cumulative amount released **I** (mg)		Q: cumulative amount released **II** (mg)		Q: cumulative amount released **III** (mg)		m: cumulative amount released per area **I** (mg/cm2)		m: cumulative amount released per area **II** (mg/cm2)		m: cumulative amount released per area **III** (mg/cm2)		**mean amount (mg/cm2)**		**SD**		**%RSD**	
VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT
0	0.018	0	0.021	0	0.015	0	0.006	0	0.007	0	0.0047	0	0.006	0	0.0009		16.67
0	0.146	0	0.155	0	0.14	0	0.047	0	0.05	0	0.0447	0	0.047	0	0.0024		5.102
0	0.423	0	0.438	0	0.412	0	0.135	0	0.139	0	0.1311	0	0.135	0	0.0042		3.095
0	0.799	0	0.818	0	0.784	0	0.254	0	0.261	0	0.2498	0	0.255	0	0.0054		2.102
0	1.331	0	1.356	0	1.312	0	0.424	0	0.432	0	0.4177	0	0.425	0	0.0071		1.679
0	1.898	0	1.918	0	1.883	0	0.604	0	0.611	0	0.5998	0	0.605	0	0.0055		0.909
0.0036	2.595	0.014	2.617	0.009	2.582	0.0012	0.826	0.004	0.833	0.0027	0.8223	0.0028	0.827	0.0016	0.0055	59.372	0.669
0.1265	3.409	0.148	3.432	0.121	3.394	0.0403	1.086	0.047	1.093	0.0386	1.081	0.042	1.087	0.0045	0.006	10.77	0.556

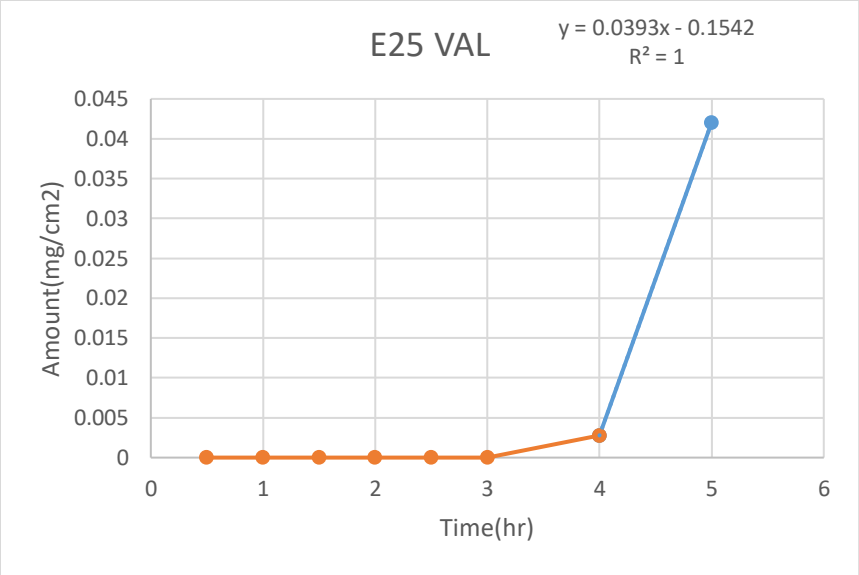

***Figure 4.47**: In vitro permeation profile for the cumulative amount of VAL in FeSSIF with citric acid 1.5% permeated per unit area of Permeapad (mg/cm²) for experiment E25.*

The diffusion parameters for VAL were calculated in table (4.108) and the enhancement ratio was determined.

***Table 4.108:** Diffusion parameters for VAL E25, with citric acid 1.5%.*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	ER
E25	0.0393	0.1542	0.2549	0.007	0.006	0.009	1.384

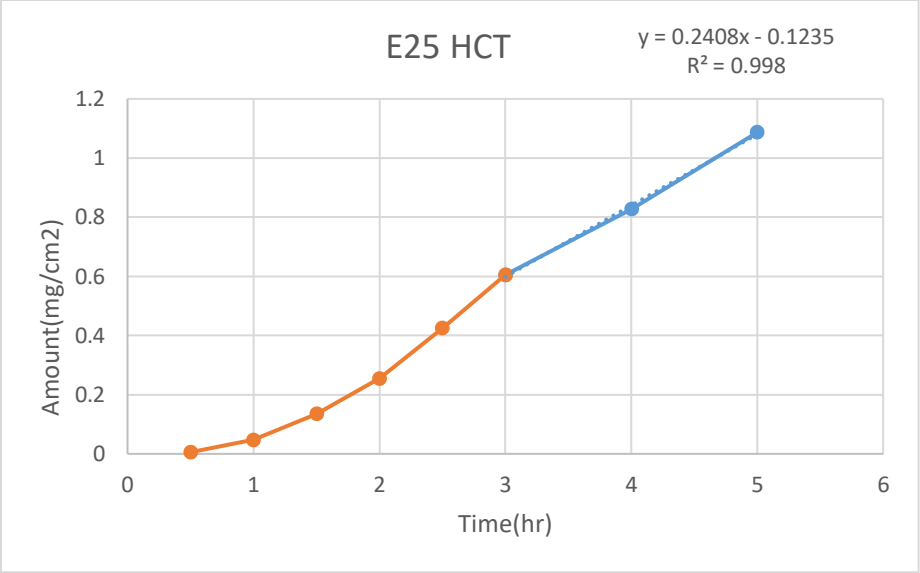

***Figure 4.48**: In vitro permeation profile for the cumulative amount of HCT in FeSSIF with citric acid 1.5% permeated per unit area of Permeapad (mg/cm²) for experiment E25.*

The diffusion parameters for VAL were calculated in table (4.109) and the enhancement ratio was determined.

***Table 4.109:** Diffusion parameters for HCT E25, with citric acid 1.5%.*

sample #	**slope**	**intercept**	**T_L**	**D**	**P**	**K**	**ER**
E25	0.241	0.124	1.95	0.0009	0.2408	2.8171	1.557

4.6.12 Experiment no. E26, VAL/HCT in FaSSIF with 2% citric acid through permeapad.

A sample of VAL and HCT was prepared with 2% citric acid according to general method described in section (3.7.4). The permeability results of drug through Permeapad membrane are shown in tables (4.110), (4.111).

The cumulative amount of VAL and HCT permeated through unit area of membrane was then calculated, the linear section, i.e. the steady state flux was plotted versus time (Figure (4.49) and Figure (4.50)).

***Table 4.110**: Data obtained from E26, in FaSSIF with citric acid 2% through Permeapad membrane using Franz diffusion cell (part 1).*

time	absorbance of sample 1 (n=3)		absorbance of sample 2 (n=3)		absorbance of sample 3 (n=3)		concentration of sample **I** mg/ml		concentration of sample **II** mg/ml		concentration of sample **III** mg/ml	
	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL	HCT	VAL	HCT	VAL	HCT
0.5	0	0.043	0	0.046	0	0.05	0	0.0021	0	0.002	0	0.002
1	0	0.125	0	0.129	0	0.121	0	0.0061	0	0.006	0	0.006
1.5	0.077	0.278	0.072	0.284	0.08	0.275	0.0021	0.0118	0.001	0.012	0.003	0.012
2	0.184	0.397	0.188	0.401	0.181	0.395	0.0107	0.0151	0.011	0.015	0.01	0.015
2.5	0.332	0.565	0.329	0.561	0.337	0.568	0.0226	0.0199	0.022	0.02	0.023	0.02
3	0.472	0.712	0.478	0.719	0.468	0.709	0.034	0.0238	0.034	0.024	0.034	0.024
4	0.735	0.97	0.74	0.974	0.73	0.967	0.0559	0.0302	0.056	0.03	0.055	0.03
5	0.951	1.208	0.945	1.215	0.955	1.205	0.0733	0.0368	0.073	0.037	0.074	0.037

Table 4.111*: Data obtained from E26, in FaSSIF with citric acid 2% through Permeapad membrane using Franz diffusion cell (part 2).*

Q: cumulative amount released **I** (mg)		Q: cumulative amount released **II** (mg)		Q: cumulative amount released **III** (mg)		m: cumulative amount released per area **I** (mg/cm2)		m: cumulative amount released per area **II** (mg/cm2)		m: cumulative amount released per area **III** (mg/cm2)		**mean amount (mg/cm2)**		**SD**		**%RSD**	
VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT
0	0.042	0	0.045	0	0.049	0	0.013	0	0.014	0	0.016	0	0.014	0	0.0011		7.58
0	0.166	0	0.173	0	0.17	0	0.053	0	0.055	0	0.054	0	0.054	0	0.0011		2.06
0.043	0.408	0.03	0.424	0.05	0.407	0.014	0.13	0.009	0.135	0.016	0.13	0.013	0.131	0.0033	0.003	25.227	2.244
0.26	0.722	0.253	0.74	0.262	0.72	0.083	0.23	0.08	0.236	0.083	0.229	0.082	0.232	0.0016	0.0035	1.8913	1.503
0.722	1.134	0.71	1.15	0.733	1.133	0.23	0.361	0.226	0.366	0.233	0.361	0.23	0.363	0.0036	0.003	1.5575	0.822
1.424	1.629	1.422	1.649	1.429	1.627	0.454	0.519	0.453	0.525	0.455	0.518	0.454	0.521	0.0011	0.0038	0.2392	0.731
2.576	2.258	2.583	2.279	2.571	2.255	0.82	0.719	0.823	0.726	0.819	0.718	0.821	0.721	0.0019	0.0042	0.2352	0.583
4.097	3.024	4.089	3.055	4.101	3.016	1.305	0.963	1.302	0.973	1.306	0.961	1.304	0.966	0.0019	0.0065	0.149	0.676

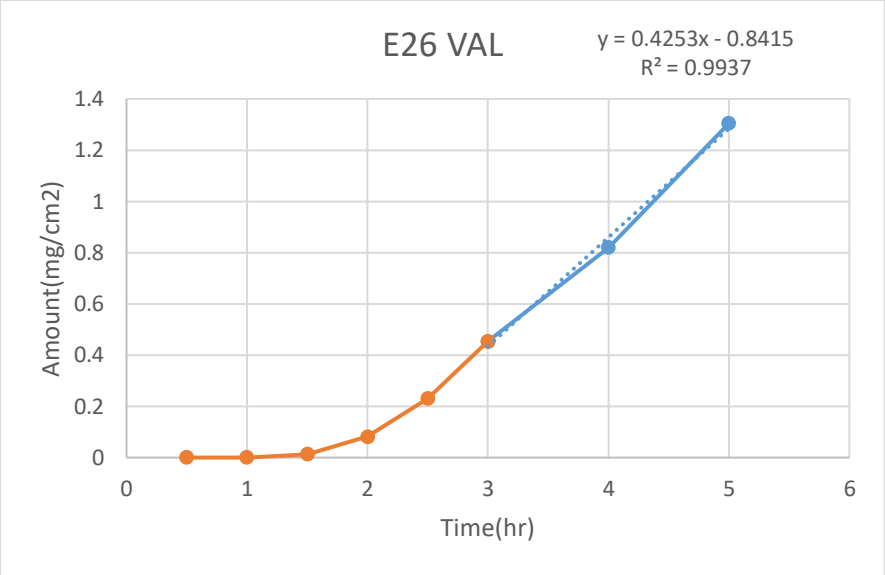

***Figure 4.49**: In vitro permeation profile for the cumulative amount of VAL in FaSSIF with citric acid 2% permeated per unit area of Permeapad (mg/cm²) for experiment E26.*

The diffusion parameters for VAL were calculated in table (4.112) and the enhancement ratio was determined.

***Table 4.112:** Diffusion parameters for VAL E26, with citric acid 2%.*

sample #	slope	intercept	T_L	D	P	K	ER
E26	0.4253	0.8416	0.505347	0.003	0.066	0.201	4.15

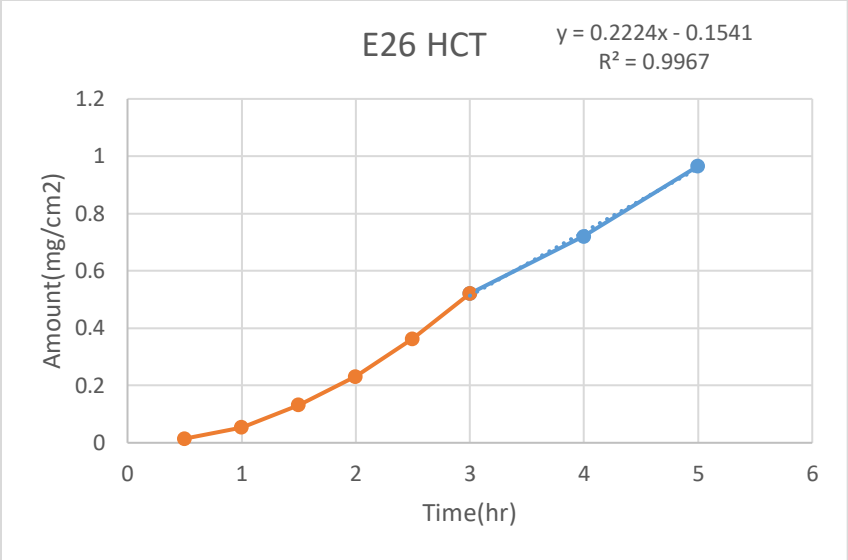

***Figure 4.50**: In vitro permeation profile for the cumulative amount of HCT in FaSSIF with citric acid 2% permeated per unit area of Permeapad (mg/cm²) for experiment E26.*

The diffusion parameters for HCT are calculated in table (4.113) and the enhancement ratio is determined.

***Table 4.113:** Diffusion parameters for HCT E26, with citric acid 2%.*

sample #	slope	intercept	T_L	D	P	K	ER
E26	0.222	0.154	1.443	0.001	0.222	1.926	1.934

4.6.13 Experiment no. E27, VAL/HCT in FeSSIF with 2% citric acid through Permeapad.

A sample of VAL and HCT was prepared with 2% citric acid according to general method described in section (3.7.4). The permeability results of drug through Permeapad membrane are shown in tables (4.114), (4.115).

The cumulative amount of VAL and HCT permeated through unit area of membrane was then calculated, the linear section, i.e. the steady state flux was plotted versus time (Figure (4.51) and Figure (4.52)).

***Table 4.114**: Data obtained from E27, in FeSSIF with citric acid 2% through Permeapad membrane using Franz diffusion cell (part 1).*

time	absorbance of sample 1 (n=3)		absorbance of sample 2 (n=3)		absorbance of sample 3 (n=3)		concentration of sample **I** mg/ml		concentration of sample **II** mg/ml		concentration of sample **III** mg/ml	
	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL(248)	HCT(271.5)	VAL	HCT	VAL	HCT	VAL	HCT
0.5	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0.081	0	0.086	0	0.078	0	0.004	0	0.004	0	0.004
1.5	0	0.227	0	0.23	0	0.225	0	0.011	0	0.011	0	0.011
2	0	0.331	0	0.338	0	0.327	0	0.016	0	0.017	0	0.016
2.5	0	0.522	0	0.527	0	0.518	0	0.026	0	0.026	0	0.025
3	0.082	0.681	0.089	0.689	0.078	0.678	0	0.031	0	0.032	0	0.031
4	0.334	0.99	0.339	0.998	0.329	0.986	0.0138	0.041	0.014	0.041	0.0134	0.04
5	0.828	1.203	0.835	1.208	0.824	1.2	0.0606	0.039	0.061	0.04	0.0603	0.039

***Table 4.115**: Data obtained from E27, in FeSSIF with citric acid 2% through Permeapad membrane using Franz diffusion cell (part 2).*

Q: cumulative amount released **I** (mg)		Q: cumulative amount released **II** (mg)		Q: cumulative amount released **III** (mg)		m: cumulative amount released per area **I** (mg/cm2)		m: cumulative amount released per area **II** (mg/cm2)		m: cumulative amount released per area **III** (mg/cm2)		**mean amount (mg/cm2)**		**SD**		**%RSD**	
VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
0	0.079	0	0.084	0	0.076	0	0.025	0	0.027	0	0.024	0	0.025	0	0.001		4.949
0	0.305	0	0.313	0	0.3	0	0.097	0	0.1	0	0.096	0	0.097	0	0.002		2.159
0	0.64	0	0.655	0	0.631	0	0.204	0	0.208	0	0.201	0	0.204	0	0.004		1.897
0	1.166	0	1.186	0	1.153	0	0.371	0	0.378	0	0.367	0	0.372	0	0.005		1.441
0	1.819	0	1.844	0	1.804	0	0.579	0	0.587	0	0.575	0	0.58	0	0.006		1.096
0.276	2.662	0.283	2.692	0.268	2.645	0.088	0.848	0.09	0.857	0.085	0.843	0.088	0.849	0.003	0.008	2.854	0.892
1.502	3.491	1.522	3.524	1.486	3.474	0.478	1.112	0.485	1.122	0.473	1.106	0.479	1.113	0.006	0.008	1.198	0.724

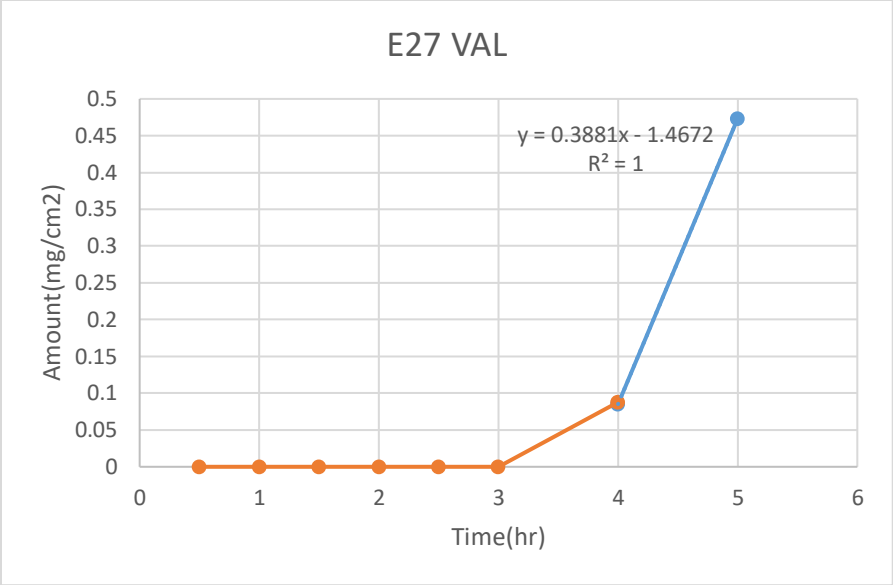

Figure 4.51*: In vitro permeation profile for the cumulative amount of VAL in FeSSIF with citric acid 2% permeated per unit area of Permeapad (mg/cm²) for experiment E27.*

The diffusion parameters for VAL were calculated in table (4.116) and the enhancement ratio was determined.

Table 4.116: *Diffusion parameters for VAL E27, with citric acid 2%.*

sample #	slope	intercept	T_L	D	P	K	ER
E27	0.388	1.467	0.265	0.006	0.061	0.096	13.67

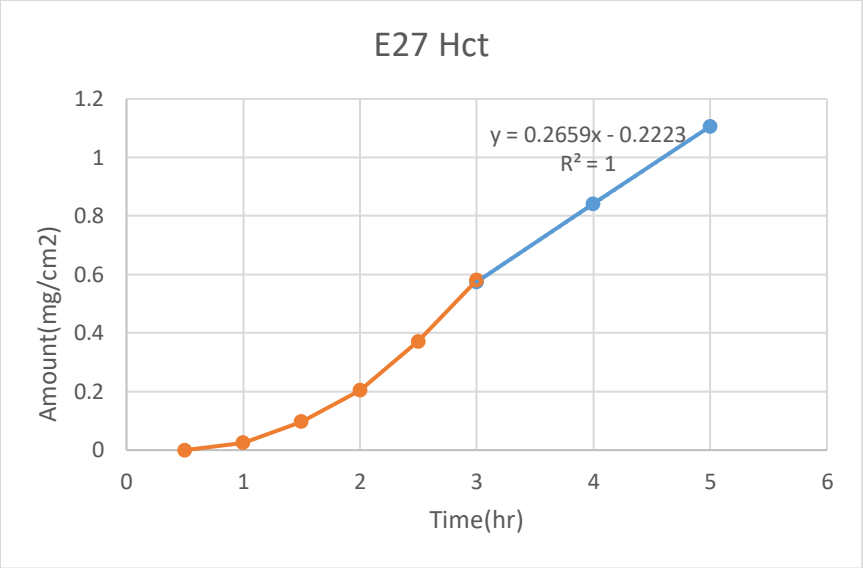

***Figure 4.52**: In vitro permeation profile for the cumulative amount of HCT in FeSSIF with citric acid 2% permeated per unit area of Permeapad (mg/cm²) for experiment E27.*

The diffusion parameters for HCT were calculated in table (4.117) and the enhancement ratio was determined.

***Table 4.117:** Diffusion parameters for HCT E27, with citric acid 2%.*

sample #	slope	intercept	T_L	D	P	K	ER
E27	0.266	0.222	1.196	0.0014	0.2659	1.908	1.719

4.7 **Comparison of VAL/HCT permeation with and without enhancer in different medias through Permeapad membrane.**

Table (4.118) and (4.119) summarize the diffusion parameters of VAL with and without enhancer in FaSSIF and FeSSIF media respectively through Permeapad membrane.

Table 4.118: *Summary of diffusion parameters for VAL when prepared in FaSSIF and comparison between all the enhancement ratio of sample without enhancer and other samples with different enhancers through Permeapad.*

sample	slope	intercept	T_L	D	P	K	ER
E16	0.1026	0.3607	0.2844	0.0059	0.016	0.0274	1
E18	0.1438	0.477	0.301468	0.005529	0.022	0.041	1.402
E20	0.238	0.587	0.4046	0.004119	0.037109	0.090087	2.314815
E22	0.1646	0.3923	0.42	0.004	0.026	0.065	1.607
E24	0.4093	0.6147	0.6659	0.003	0.064	0.256	3.997
E26	0.4253	0.8416	0.505347	0.003	0.066	0.201	4.153

Table 4.119: *Summary of diffusion parameters for VAL when prepared in FeSSIF and comparison between all the enhancement ratio of sample without enhancer and other samples with different enhancers through Permeapad.*

sample	slope	intercept	T_L	D	P	K	ER
E17	0.0284	0.11	0.2587	0.0064	0.004	0.0069	1
E19	0.057	0.1778	0.32	0.0052	0.009	0.017071	2.003521
E21	0.0866	0.3272	2.647	0.000619	0.014	0.022	3.049
E23	0.0071	0.0275	0.2582	0.006	0.001	0.002	0.25
E25	0.0393	0.1542	0.2549	0.007	0.006	0.009	1.384
E27	0.388	1.467	0.265	0.006	0.061	0.096	13.67

Table (4.120) and (4.121) summarize the diffusion parameters of HCT with and without enhancer in FaSSIF and FeSSIF media respectively through Permeapad membrane.

Table 4.120: *Summary of diffusion parameters for HCT when prepared in FaSSIF and comparison between all the enhancement ratio of sample without enhancer and other samples with different enhancers through Permeapad.*

sample	slope	intercept	T_L	D	P	K	ER
E16	0.1145	0.2322	0.4931	0.003	0.1145	0.339	1
E18	0.174	0.128	1.359	0.001	0.174	1.417	1.518
E20	0.19	0.172	1.11	0.002	0.19	1.268	1.663
E22	0.201	0.197	1.02	0.002	0.201	1.231	1.749
E24	0.221	0.175	1.265	0.001	0.221	1.679	1.924
E26	0.222	0.154	1.443	0.001	0.222	1.926	1.934

Table 4.121: *Summary of diffusion parameters for HCT when prepared in FeSSIF and comparison between all the enhancement ratio of sample without enhancer and other samples with different enhancers through Permeapad.*

sample	slope	intercept	T_L	D	P	K	ER
E17	0.155	0.2624	0.59	0.0028	0.1547	0.547	1
E19	0.234	0.214	1.095	0.002	0.234	1.537	1.512
E21	0.257	0.264	0.974	0.002	0.257	1.5	1.659
E23	0.242	0.186	1.299	0.001	0.242	1.8861	1.564
E25	0.241	0.124	1.95	0.0009	0.2408	2.8171	1.557
E27	0.266	0.222	1.196	0.0014	0.2659	1.908	1.719

The cumulative amount of VAL permeated per unit area when prepared in FaSSIF and FeSSIF without enhancer during experiments from E16 and E17, are shown in figure (4.53).

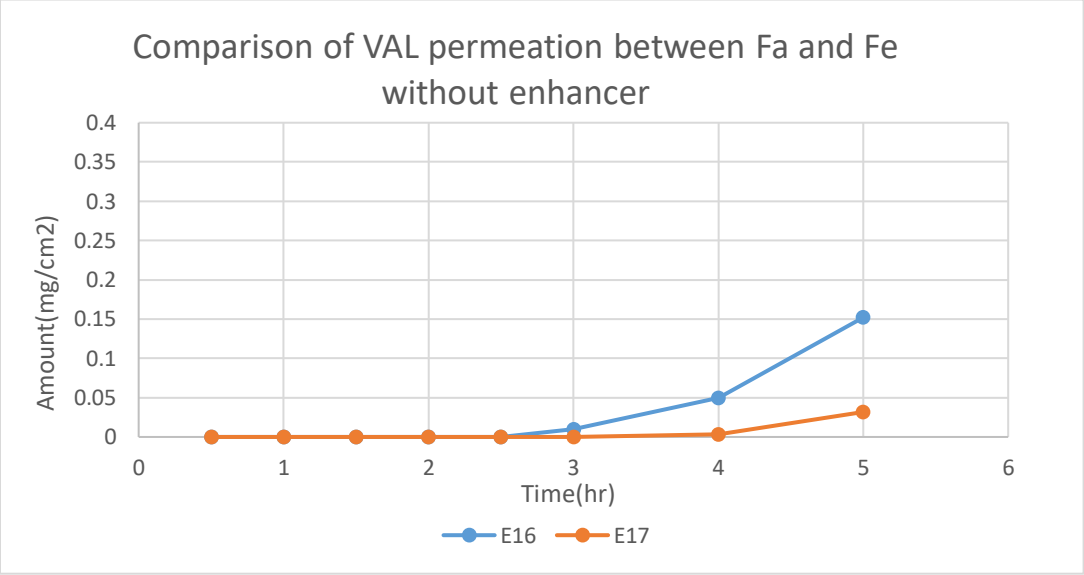

***Figure 4.53**: Comparison between VAL permeation through Permeapad membrane in FaSSIF and FeSSIF, where, E16: In FaSSIF without PE, E17: In FeSSIF without PE.*

The cumulative amount of HCT permeated per unit area when prepared in FaSSIF and FeSSIF without enhancer during experiments from E16 and E17, are shown in figure (4.54).

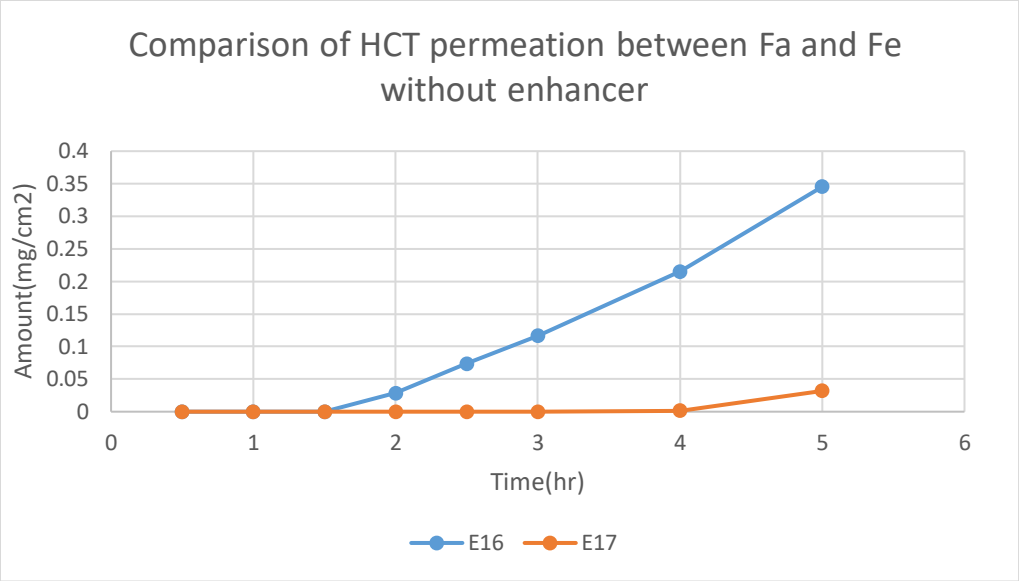

***Figure 4.54**: comparison between HCT permeation through Permeapad membrane in FaSSIF and FeSSIF, where, E16: In FaSSIF without PE, E17: In FeSSIF without PE*

It was noticed from figure (4.53) and figure (4.54) that generally VAL and HCT have higher permeation when placed in FaSSIF than in FeSSIF.

To compare the permeation enhancement ability between 1% citric acid and 1%Na acetate for VAL and HCT when placed in FaSSIF and FeSSIF, cumulative amount of VAL permeated per unit area when placed in FaSSIF and FeSSIF with and without permeation enhancers, they are plotted versus time, see figures (4.55) and (4.56) for VAL, and see figures (4.57) and (4.58)

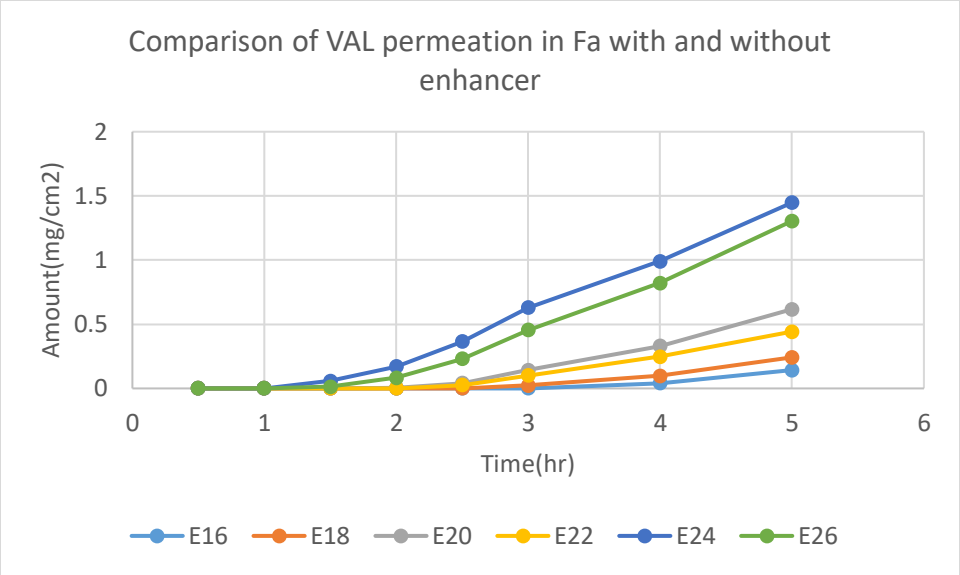

***Figure 4.55**: Comparison of VAL permeation in FaSSIF through Permeapad with and without PE, where E16: In FaSSIF without PE, E18: In FaSSIF with Na acetate, E20: In FaSSIF with 1% citric acid, E22: In FaSSIF with citricacid+Na acetate, E24: In FaSSIF with 1.5% citric acid, E26: In FaSSIF with 2% citric acid.*

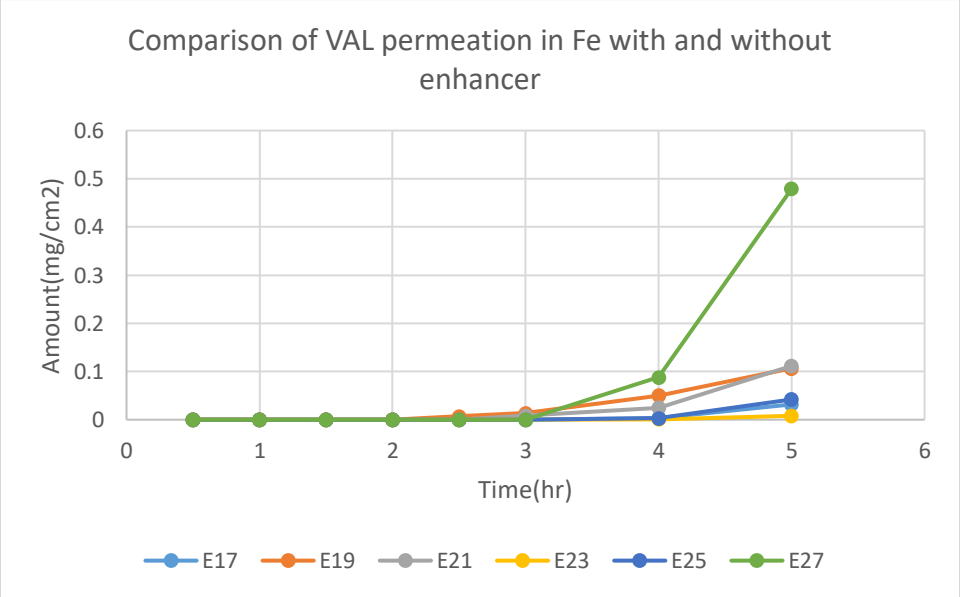

***Figure 4.56**: Comparison of VAL permeation in FeSSIF through Permeapad with and without PE, whereE17: In FeSSIF without PE, E19: In FeSSIF with Na acetate, E21:In FeSSIF with 1% citric acid, E23: In FeSSIF with citricacid+Na acetate, E25: In FeSSIF with 1.5% citric acid, E27: In FeSSIF with 2% citric acid.*

To compare cumulative amount of HCT permeated per unit area when placed in FaSSIF and FeSSIF with and without permeation enhancers, they are plotted versus time, see figure (4.57) and figure (4.58).

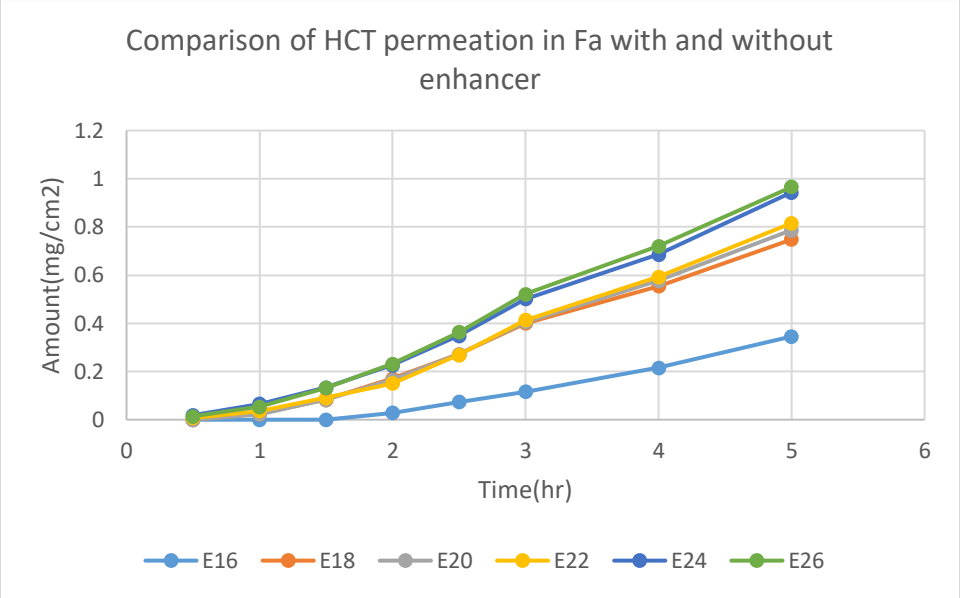

Figure 4.57*: Comparison of HCT permeation in FaSSIF through Permeapad with and without PE, where E16: In FaSSIF without PE, E18: In FaSSIF with Na acetate, E20: In FaSSIF with 1% citric acid, E22: In FaSSIF with citricacid+Na acetate, E24: In FaSSIF with 1.5% citric acid, E26: In FaSSIF with 2% citric acid.*

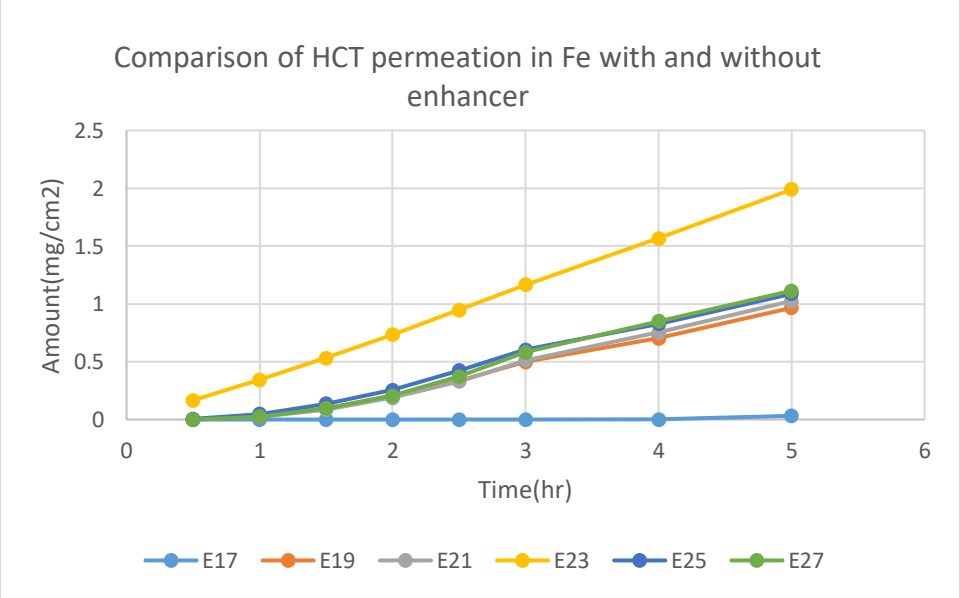

Figure 4.58: *Comparison of HCT permeation in FeSSIF through Permeapad with and without PE, whereE17: In FeSSIF without PE, E19: In FeSSIF with Na acetate, E21: In FeSSIF with 1% citric acid, E23: In FeSSIF with citricacid+Na acetate, E25: In FeSSIF with 1.5% citric acid, E27: In FeSSIF with 2% citric acid.*

Permeation experiments are typically designed to meet sink conditions. The acceptor concentration should be less than 10% of the donor concentration. A decay of compound in donor compartment may occur during experiment. A decrease up to 10 % is commonly considered as compatible with the sink conditions. , the steady state flux (J) of a drug across a permeation barrier can be derived from the linear part of the curve obtained when plotting the cumulative permeated amount versus time[60].During experiments, the highest concentration of VAL and HCT in the acceptor compartment was 0.227 mg/ml and 0.174 mg/ml, which is less than 10% of the saturated concentration, that insures the presence of sink conditions for continuous permeation.

At first when 1% of citric acid and 1% Na acetate were added to prepared samples separately, from figure (4.55) and (4.56) it indicates that 1% citric acid enhances permeation of VAL in both FaSSIF and FeSSIF. Also Na acetate enhance permeation of VAL when prepared in FeSSIF the same as 1% citric acid. In figures (4.57) and (4.58) permeation of HCT was enhanced similarly by citric acid and Na acetate when prepared in FaSSIF. However, Na acetate enhances permeation of HCT more than 1% citric acid, so to cover the benefit of both of 1%citric acid and Na acetate, we decided to add a combination of enhancers including 1%citric acid and Na acetate. But results were not as expected, the combination gives good enhancement for VAL but less than 1%citric acid when placed in FaSSIF, on the contrary of FeSSIF the combination compromise the permeation. The combination of 1% citric acid and Na acetate enhances the permeation of HCT when prepared in both FaSSIF and FeSSIF. To avoid the effect of combination on VAL we excluded this choice and decided to increase the concentration of citric acid from 1% to 1.5% and 2%.

From figure (4.55) and (4.56) it seems that 1.5% citric acid enhancer increase permeation of VAL more than 2% citric acid in fast state, but the difference is small. While in fed state 2% citric acid has the best enhancement amount. Na acetate and 1% citric acid increase permeation more than 1.5% citric acid, so we can choose 2%citric acid to enhance permeation of VAL to cover both fast and fed state.

From figure (4.57) and (4.58) it seems that, 2% and 1.5% citric acid enhancer increase permeation of HCT when prepared in FaSSIF media and gives the best permeation enhancement. But when the sample was prepared in FeSSIF media 1.5 and 2% citric acid gives permeation enhancement less than the combination of citric acid and Na acetate. But 2% citric acid still gives good enhancement compared to the basic sample solution without enhancer in E17. In general, 2% citric acid gave significant permeation enhancement for VAL compared with 1% citric acid in both FaSSIF and FeSSIF. For HCT, 2% citric acid gave higher permeation enhancement than 1% citric acid in both FaSSIF and FeSSIF. And because we are concerned about VAL which has lower bioavailability 2% citric acid is a good choice for both VAL and HCT.

4.8 A comparison between sandwiched dialysis membrane and Permapad membrane according to the permeation of VAL and HCT.

In a previous work, Permeapad membrane was used for prediction of buccal absorption. Metoprolol was used at different pH values. It was confirmed that Permeapad® can withstand these conditions. Results showed that the permeability of metoprolol using the Permeapad® barrier correlated very well to both *in vitro* and *ex vivo* studies. Results indicate that Permeapad® can be used to mimic the buccal absorption of metoprolol as a faster and less laborious method as compared to any of the other mentioned methods[44]. In other study that investigate the permeation of set of drugs on a 96-well plate comprising the Permeapad® membrane. The

Permeapad® 96-well plate was found suited to distinguish high and low absorption drugs, it is a promising new tool for rapid and reproducible passive permeability profiling. Permeapad®, in contrast to PAMPA, appears to allow the minor passage of drug compounds with paracellular absorption pathway, which may serve as a first indication for the presence of water-filled pores across Permeapad®, under microscope Permeapad® barrier after swelling appears with large phospholipid vesicles and myelin-structures[58].Another study investigated the functional stability of Permeapad during the lipolysis of self-nanoemulsifying drug delivery system (SNEDDS). The lipolysis medium, and digestion process of SNEDDS, both are harsh to the permeation barrier. So permeation study was not included into the models due to the harsh conditions of lipid digestion compromising permeation barriers. In this study when Permaepad was used, Permeapad was able to maintain its permeation properties in the presence of the SNEDDS formulation, the lipolysis medium, and the lipolysis medium while digesting the SNEDDS. Results obtained from a model formulation of cinnarizine in a SNEDDS showed significantly higher permeability of cinnarizine, when lipolysis was combined with permeation[62].

According to the results, in E1 and E15 when sample solution consisted of VAL and HCT only prepared in PBS, and the variable was the membrane only, VAL and HCT showed higher permeation (P= 0.043cm/hr and 0.133cm/hr respectively) through Pearmeapad membrane than sandwiched dialysis membrane (P=0.003cm/hr and 0.0027cm/hr for VAL and HCT respectively). When Permeapad was used instead of sandwiched dialysis membrane to investigate the permeation behavior of VAL and HCT when prepared in FaSSIF and FeSSIF with citric acid or Na acetate, VAL showed lower P values and longer delay for onset of permeation. On the contrary, HCT showed higher P values and shorter delay for onset of permeation. These results were close to the

real conditions and the nature of intestinal membrane. VAL which has low bioavailability would show lower P values through Permeapad membrane compared to sandwiched dialysis membrane, and needs more time to permeate. HCT which has higher bioavailability would show higher P values and need less time to permeate if we compare between sandwiched dialysis membrane and Permeapad membrane.

PART FIVE: CONCLUSION

Conclusion

In this work, according to the validation of method development for simultaneous determination of VAL and HCT, this method found to be simple, sensitive, accurate, precise, reproducible, specific, robust, and economical, 0.1N NaOH was used as solvent which is cheap. It can be used for the routine simultaneous estimation of VAL and HCT in pharmaceutical formulations.

The solubility of VAL and HCT was determined in different medias, solubility of VAL and HCT was found to be pH dependent, as pH increases solubility increases.

During experiments, the highest concentration of VAL and HCT in the acceptor compartment was 0.227 mg/ml and 0.174 mg/ml, which is less than 10% of the saturated concentration, that insures the presence of sink conditions for continuous permeation.

The influence of selected penetration enhancer included in VAL/HCT solution was investigated through synthetic membrane, poly amide filter membrane soaked and saturated in octanol and was sandwiched in between 2 layers of dialysis membrane presoaked with phosphate buffer pH= 7.4. The enhancement ratio was calculated for each penetration enhancer and found to be in the following order for VAL (in compare with basic sample solution without enhancer):

Na acetate > citric acid > PEG 4000 > SLS > sorbitol.

FOR HCT they were in the following order (in compare with basic sample solution without enhancer):

Citric acid > Na acetate SLS > sorbitol >PEG4000.

So citric acid and Na acetate the first two enhancers that enhance permeation for both VAL and HCT. They were chosen for further experiments using Permeapad membrane.

When Permeapad membrane was used longer lag time was detected. With sandwiched dialysis membrane lag time for VAL and HCT was (0.028hr) and (0.433hr) respectively. When Permeapad membrane was used lag time for VAL and HCT was (1.08hr), (1.8hr) respectively. Also, shorter lag time was detected when FaSSIF and FeSSIF media were used instead of PBS, for VAL in FaSSIF and FeSSIF it was (0.28), (0.25) respectively. And for HCT in FaSSIF and FeSSIF it was (0.493), and (0.59). Permeation coefficient was higher when Permeapad membrane was used Instead of sandwiched dialysis membrane, for VAL and HCT through sandwiched dialysis membrane it was (0.003cm/hr), and (0.0027cm/hr) respectively, and through Permeapad it was (0.0399cm/hr) and (0.133cm/hr) respectively. Diffusion coefficient was lower when Permeapad was used instead of sandwiched dialysis membrane, for VAL and HCT through sandwiched dialysis membrane it was (0.00153cm^2/hr), and (0.0065cm^2/hr) respectively, and through Permeapad it was (0.00146 cm^2/hr) and (0.0009 cm^2/hr) respectively. When FaSSIF and FeSSIF media were used instead of PBS, diffusion coefficient for VAL in FaSSIF and FeSSIF it was (0.0059 cm^2/hr) and (0.0064 cm^2/hr) respectively. And for HCT in FaSSIF and FeSSIF it was (0.003 cm^2/hr), and (0.0028 cm^2/hr). The results that is closer to real conditions suggests that Permeapad mimic intestinal membrane.

At first stage of experiments, Na acetate and citric acid were found to have the best permeation enhancement. At the second stage, and further development and improvement 2% citric acid was the suitable choice to enhance permeation of both VAL and HCT taking in mind that we have to focus on VAL because it has the lower bioavailability.

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عزيز نفاذية الفالزارتان/والهيدروكلوروثيازايد والتحقق من تأثير معززات الاختراق باستخدام غشاء البيرمياباد.

اعداد: ولاء جميل

المشرف: د.هاني اشتيه.

ملخص

فالزارتان وهيدروكلوروثيازايد هما مادتان دوائيتان مركبتان معا في القرص الدوائي، يستخدمان للسيطرة على ارتفاع ضغط الدم. فالزارتان هو حاصر لمستقبلات أنجيوتينسن 2. التوافر الحيوي له يقارب 25%. هيدروكلوروثيازايد هو من نوع مدرات الثيوزايد، يمتلك توافر حيوي أقل من 65%. زيادة التوافر الحيوي للدوائين سيساعد في تقليل كمية الدواء المعطاة اللازمة للحصول على تأثير دوائي، كنتيجة من الممكن تقليل مخاطر الآثار الجانبية والسمية للدواء. توافر حيوي منخفض من الممكن أن ينتج عنه فعالية غير كافية، وفروقات فردية مرتفعة تؤدي إلى استجابة غير متوقعة للدواء. من الممكن زيادة التوافر الحيوي من خلال تعزيز النفاذية باستخدام محسنات الاختراق.

هذه الدراسة تهدف إلى دراسة تأثير محسنات اختراق مختلفة على نفاذية الفالزارتان والهيدروكلوروثيازايد من خلال غشائين، الأول غشاء مركب من طبقتين من غشاء الديناليسيز بينهما غشاء من النايلون، والثاني غشاء البيرمياباد.

في المرحلة الأولى من التجارب، استخدم غشاء الديناليسيز المحشو لاختبار نفاذية الفالزارتان والهيدروكلوروثيازايد. وأيضا العديد من محسنات الاختراق اختبرت وقورنت مع محلول للعينة الأساسية المحضرة بدون استخدام أي محسن للاختراق.

في المرحلة الثانية من التجارب، تم استخدام غشاء صناعي مبتكر يحاكي خصائص الغشاء الخلوي المعوي يسمى غشاء البيرمياباد، استخدم بدلا من غشاء الديناليسيز المحشو لاختبار معدل نفاذية الفالزارتان والهيدروكلوروثيازايد. تم استخدام محلول مماثل للسائل المعوي في حالة التغذية وبدون تغذية، لمحاكاة الظروف في الأمعاء.

تم اجراء دراسة داخل المختبر باستخدام خلية فرانز للنشر، لتقييم نفاذية الدوائين. في المرحلة الأولى كان الغشاء مكون من طبقة واحدة من غشاء النايلون المنقوع في الاوكتناول وموجود بين طبقتين من غشاء الديناليسيز الذي كان منقوع مسبقا في محلول

الفوسفات المتعادل ذو درجة حموضة تعادل 7.4. تمتلئ حجرة الاستقبال ب 20 مل من محلول الفوسفات المتعادل ذو درجة حموضة تعادل 7.4. الحجرة المانحة تحتوي على 2 مل من محلول للعينة المحضرة. في المرحلة الثانية من التجارب تم استخدام غشاء مبتكر (Permeapad™ membrane) استخدم لفصل الحجرة المانحة عن الحجرة المستقبلة.

تم أخذ عينات من حجرة الاستقبال بحجم 1مل بعد نصف ساعة وكل نصف ساعة لمدة 3 ساعات، ثم كل ساعة لمدة ساعتين لكل عينة تجريبية. هذه العينة (1مل) خففت ب 2مل من محلول الفوسفات المتعادل، واختبرت بواسطة مقياس الطيف الضوئي للأشعة الفوق بنفسجية على الموجة الضوئية 248 و 271.5 نانوميتر، لتقدير كمية الفالزارتان والهيدروكلوروثيازايد، بواسطة المعادلات المتزامنة.

محسنات الاختراق التي تم التحقق منها، هي: سيتريك أسيد، صوديوم لوريل سلفات، بولي ايثيلين جلايكول 4000، صوديوم أسيتيت، سوربيتول، بولي فينيل بايروليدون 30، مانيتول، ايديتا, وتوين 80. تم اضافة كل محسن اختراق لمحلول من الفالزارتان والهيدروكلوروثيازايد في الحجرة المانحة. معلمات الانتشار T_L, D, P, K كانت تراكمية. تم استخدام نسبة التعزيز ER كمعيار لاختبار أفضل محسن للاختراق.

لم يتم الحصول على أي نفاذية باستخدام بولي فينيل بايروليدون 30، مانيتول، ايديتا, وتوين 80. نسبة التعزيز للفالزارتان (بالمقارنة مع محلول العينة الأساسية المحضرة بدون محسن للاختراق) كانت تزداد بالترتيب التالي:

صوديوم أسيتيت> سيتريك أسيد> بولي ايثيلين جلايكول 4000> صوديوم لوريل سلفات> سوربيتول.

نسبة التعزيز للهيدروكلوروثيازايد(بالمقارنة مع محلول العينة الأساسية المحضرة بدون محسن للاختراق) كانت تزداد بالترتيب التالي:

سيتريك أسيد> صوديوم أسيتيت> صوديوم لوريل سلفات> سوربيتول> بولي ايثيلين جلايكول 4000.

في المرحلة الثانية، أجريت المزيد من التجارب باستخدام غشاء البيرمياباد، تم اختيارسيتريك أسيد وصوديوم أسيتيت للقيام بهذه التجارب. تم اختيارسيتريك أسيد 2% كمحسن مناسب للاختراق لكلا الفالزارتان والهيدروكلوروثيازايد، مع الأخذ بعين الاعتبار أن الفالزارتان يمتلك توافر حيوي أقل.