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A COMPARITIVE STUDY OF APPROACHES TO MODULATE THE PHARMACOTECHNICAL PROPERTIES OF BCS CLASS III DRUG

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1. INTRODUCTION

1.1 Anticoagulants[1]

Anticoagulants are medicines that facilitate stop formation of blood clots. They're given to individuals at a high risk of obtaining clots and to scale down their possibilities of developing serious conditions like strokes and heart attack. A blood clot acts as a seal created by the blood to prevent loss of blood from wounds. Whereas they're helpful in stopping bleeding, sometimes they may block blood vessels and reduce blood flowing to organs such as the brain, heart or lungs if they are generated within the wrong place. Anticoagulants work by interrupting the events concerned in the formation of blood clots. They're sometimes referred to as "blood-thinning" medicines, though they don't truly make the blood thinner. Though they're used for common purposes, anticoagulants differ completely from antiplatelet medicines, like low-dose Aspirin and clopidogrel. If flow of blood through a blood vessel is blocked by a clot, the affected part of the body will become short of oxygen and it will not work smoothly. Medical care involving use of anticoagulants is suggested within the case of stroke, deep vein thrombosis (DVT), pneumonic embolism, atrial fibrillation, etc. Treatment with anticoagulants could also be counselled if your doctor feels you're at an enhanced risk of developing one or more problems enlisted above. This may be as a result of your medical history of having blood clots within the past or a condition like atrial fibrillation may develop and trigger formation of blood clots. In case of vitamin K antagonists (VKA) like aspirin, warfarin dose is set on

a personal basis (no fix dose), whereas novel Non-vitamin K oral anticoagulants (NOACs) are administered in fixed doses, except in case of individuals with impaired functioning of liver or kidney. NOACs lead to direct inactivation of thrombin (FIIa) and prothrombinase (FXa) and therefore also known as direct oral anticoagulants or target anticoagulants APX falls within the class of NOACs. Clotting is initiated once the integrity of the endothelium is breached. Factor VIIa and tissue factor (TF) lead to formation of complex of the TF– factor VIIa, that subsequently activates factor X and factor IX. Thrombin is then converted to thrombin by Factor Xa. The little quantity of thrombin produced amplifies coagulation by activating factor V, antihaemophilic globulin and platelets. Factor VIIIa & factor IXa get together to form the factor IXa–factor VIIIa– phospholipid complex – a key activator of factor X. Factor Xa binds to negatively charged lipoid surfaces (e.g. activated platelets), and along with factor Va forms the coagulation factor advanced the central coagulation factor activator that converts prothrombin to thrombin (Figure 1.2). Thrombin contains a central role within the natural process of clotting, as well as changing soluble coagulation factor to fibrin, activating platelets and activating clotting factor to induce fibrin cross-linking. Blood-borne TF has been known on microparticles derived from leukocytes and different cell varieties that are concerned in the initiation of coagulation once vessel injury is proscribed to activation of endothelial tissues.

1.2 APIXABAN (APX)[2]

APX (Eliquis®) is a Factor Xa (FXa) inhibitor, with chemicals delineate as 1-(4 methoxyphenyl)-7-oxo-6-[4-(2-oxopiperidin-1-yl)phenyl]-4,5,6,7-tetrahydro-1H-pyrazolo[3,4c]pyridine-3- carboxamide. Its chemical formula is C25H25N5O4, that corresponds to a relative molecular mass of 459.5. Morphologically it is white to pale-yellow powder. At physiological pH scale (1.2- 6.8), APX doesn't ionize; it has an aqueous solubility of ~0.04 mg/mL throughout the physiological pH range **(Fig. 1.1)**.

Eliquis® tablets are available for oral administration in strengths of 2 mg and 5 mg of APX with the subsequent inactive ingredients: lactose anhydrous, Micro-crystalline cellulose, crosscarmellose sodium, sodium lauryl sulfate, and magnesium stearate. The components of film coating are as follows, lactose monohydrate, hypromellose, titanium dioxide, triacetin, and yellow iron oxide (2.5 mg tablets) or red iron oxide (5 mg tablets).

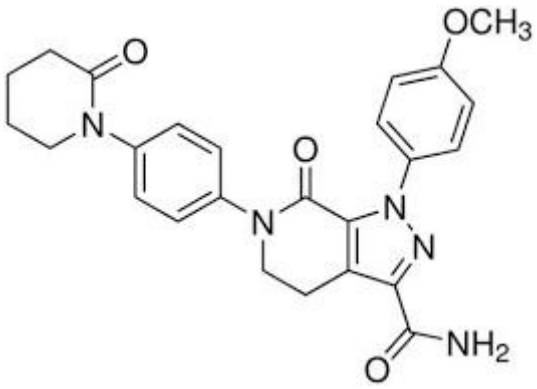


Fig. 1.1: Structure of APX.

1.3 MECHANISM OF ACTION[3]

The Factor Xa that is concerned within the blood clotting process is hindered by APX and thus averts clot formation. It has an effect on each factors Xa at intervals of the blood clot formation and within a pre-existent clot. Platelet aggregation is not affected by it. Both the extrinsic and intrinsic coagulation pathways lead to generation of Factor Xa which is liable for activating coagulation factor to thrombin. Factor Xa inhibitors have predictable decoagulant effects and don't need routine monitoring, not like another anticoagulants (Fig. 1.2).

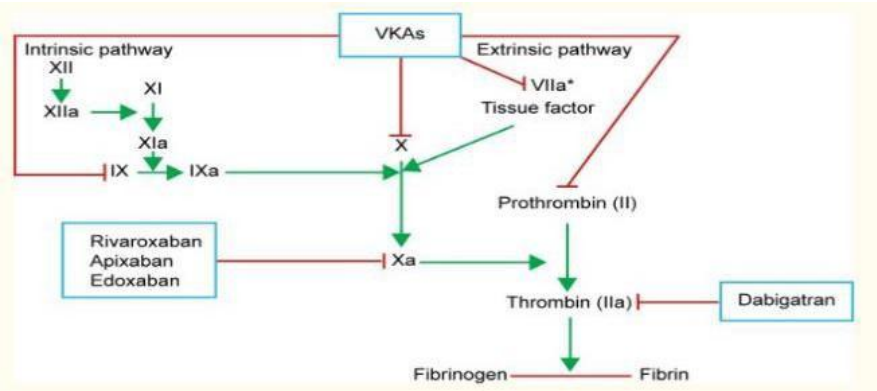


Fig. 1.2: Mechanism of action of APX.

1.4 BIOPHARMACEUTICAL CLASSIFICATION SYSTEM

Drug solubility improvement is a crucial challenge within the field of pharmaceutical formulation development. Among the 5 key physicochemical screens in early compound screening i.e. solubility, permeability, pKa, stability and lipophilicity, poor solubility surpasses the list of undesirable compound properties.[4] Molecules that have poor biopharmaceutical properties will offer variety of challenges in pharmaceutical development and should probably result in slow dissolution in biological fluids, inadequate and

inconsistent exposure to the systemic circulation.[5] Solubility as well as permeability are thus, key parameters for governing drug absorption. The biopharmaceutical classification system (BCS) is nothing but the scientific framework for classifying drug substances according to their solubility in aqueous solvents and permeability through intestine. It's a drug development tool that permits estimation of the contributions of 3 major factors: dissolution, solubility and intestinal permeability that have an effect on oral absorption of drugs. The BCS defines four categories counting on drug' solubility and permeability through the intestinal cell layer.

Table 1.1 BCS Classification.

BCS class	Solubility	Permeability
I	High	High
II	Low	High
III	High	Low
IV	Low	Low

A drug substance is alleged to be extremely soluble once the maximum dose strength is soluble in 250 ml water across a pH range of 1-7.5. A drug substance is taken into account highly permeable when extent of absorption in humans is set to be 90% of associate administered dose supported on mass-balance or as compared to reference dose administered intravenously. A drug product is considered as fast dissolving when 85% of the labelled quantity dissolves in 30 minutes in USP equipment I or II in 900 ml of buffer solutions.[6]

1.5 TECHNIQUES OF SOLUBILITY IMPROVEMENT

Diverse methodologies have been advised and applied practically to enhance the marketability of drug candidates whose development is proscribed by drug solubility as well as dissolution rate. The methods which could be used for this purpose may be generally classified into: Approaches based on formulation and Approaches based on structure. The previous one involves inclusion of varied solubility enhancers like lipophobic polymers, cyclodextrins, surfactants, oils and liposomes that turn out to be an aid to increase the solubility. The later approach involves modification either within the organic structure or chemical structure.[7,8] The varied techniques that aid in solubility improvement are briefed below.

1.5.1 Particle size reduction

Consistent with Noyes-Whitney equation, the dissolution rate of active pharmaceutical

ingredient may be inflated by reducing the scale to micro- or nano level enhance the surface area of drug particles.[9] The basic principle is to enhance the dissolution speed by improving the surface area of the drug powder. The principle is to increase the dissolution velocity by enlarging the surface area of the drug powder. The traditional approaches to generate ultrafine drug particles can be divided into top-down and bottom-up technologies.[10,11] Top-down techniques include jet-milling, colloidal milling and homogenization under high pressure. During this process, the majority of the drugs with the size of many hundred microns are comminuted into micro or nano-sized scale by the utilization of mechanical force. The foremost disadvantage of high pressure homogenizer is that the crystal structure of API may vary in some cases owing to the high pressure, which can lead to instability and may lead to quality control problems.

While in case of bottom-up technologies like precipitation, spray freezer, supercritical fluid (SCF) technology and so on, the ultrafine particles may be designed from the molecular state. These technologies are utilized to arrange many drugs in micro- or nano- scale, such as cephradine, cefuroxime axetil and nonsteroidal anti- inflammatory drugs.[12,13] Spray drying is another normally used methodology for drying liquid feed through a hot gas or air. Sensitive materials including prescribed drugs and solvents like ethyl alcohol need oxygen free drying and nitrogen is employed instead. The liquid feed varies counting on the material being dried which can be solution, suspension or a colloidal dispersion.

1.5.2 Drug dispersion in carriers

Molecular or close to molecular dispersions of poorly soluble drugs in carriers combines the advantages of increase within the solubility and improvement in the surface area of the compound that gets into the dissolution medium as the carrier dissolves. These dispersions are classified into the following: a] Eutectic mixtures b] Solid solutions which will be latter classified into i] Continuous solid solutions or ii] Discontinuous solid solutions, counting on their miscibility. A normal eutectic mixture consists of two compounds that are fully compatible within the liquid state however solely to a limited extent in the solid state. Once a combination of A and B with composition C is cooled, A and B crystallize out simultaneously. Solid eutectic mixtures are typically formulated by quick cooling of a co-melt of the 2 compounds in order to get a physical mixture of awfully fine crystals of the 2 elements. The enhanced surface area of the formulated suspension may lead to increased dissolution rate and thereby improved bioavailability.[14] Solid solutions could be compared to

liquid solutions, consisting of only one phase regardless of the quantity of components. They include the drug spread molecularly in an extremely water-soluble carrier. By even handed choice of the carrier, the dissolution rate of the drug may be inflated by upto many orders of magnitude. Discontinuous solid solutions are those within which the solubility of every component in the alternative is limited. In amorphous primary solid solutions, the solute is spread molecularly however its distribution amongst the amorphous solvent is irregular. With griseofulvin in citric acid, it had been demonstrated that the formation of an amorphous solid solution led to improvement in drug's dissolution properties.[15]

1.5.3 Complexation

Complexation could be explained as an association between two or more molecules to create a non-bonded entity with a well-defined stoichiometry. It depends on comparatively weak forces like London forces, H - bonding and hydrophobic interactions. The category of inclusion complexes called inclusion or occlusion compounds result a lot of from the design of molecules than from their chemical affinity. One of the constituents of the complex is confined within the open lattice or cage-like crystal structure of the opposite to yield a stable arrangement. The dissolution rate of famotidine, a potent drug used in the treatment of ulcers and of an oral antidiabetic tolbutamide are enhanced by complexation with CD.[16,17]

1.5.4 Co-crystallization[18,19]

A co-crystal could be a crystalline material that consists of 2 or a lot of (electrically neutral) species brought together by non-covalent forces. The term covers a full range of complexes including molecular complexes, solvates, inclusion compounds, channel compounds and clathrates. Pharmaceutical co-crystallisation is rising as an emerging variant to polymorphs, salts and solvates in the modification of an API throughout the dosage form design. Co-crystals primarily composed of 2 substances that are solids at room temperature. They're a product of a lot of rational design than solvates and are more stable. Whereas co-crystals are outlined by one section (miscible) multi-component system within the crystalline state, in the amorphous state they are cited as molecular dispersions with interactions between the elements differentiating them from solid dispersions. Co-crystals may be formulated by evaporating a heteromeric solution or by grinding the components together. The essential parameter in formation of co-crystals is that the correct selection of co-formers and solvent for recrystallization. The pharmaceutical trade is incredibly critical of the solvent selection and mentions an inventory of safe solvents within the ICH Q3C guidelines.[20]

1.6 Techniques for permeability improvement

Drugs having high solubility and low permeability fall under BCS Class III. This makes it vital to enhance permeability across biological membranes. For this class of drug both the rate and extent of drug absorption could also be variable, as in case of fast dissolution, variability may be attributed to GI transit and contents and membrane permeability. The bioavailability of BCS class III molecules could be improved either by improving permeability of the drug or by controlling the drug release from the formulation which will subsequently allow the drug to be present in the physiological system for longer period of time.[21] Few of the techniques to enhance permeability are explained below Lipidic drug delivery systems.

The utilization of natural and artificial lipids has generated a lot of interest as a prospective formulation strategy for enhancing the oral bioavailability of drugs having poor aqueous solubility. Lipidic formulations not solely enhance however normalize drug absorption, that is especially useful for low therapeutic index drugs. These formulations may boost drug absorption by variety of accessory mechanisms, together with inhibition of P-glycoprotein-mediated drug outflow and pre-absorbtive metabolism by gut membrane- bound haemoprotein enzymes or by promoting lymphatic transport, that delivers the API directly into the systemic circulation whereas avoiding first-pass metabolism & enhancing permeability across GI membrane.[22]

1.6.1 Niosomes

Niosome is represented as a category of molecular cluster shaped by self-association of non-ionic surfactants in an aqueous phase, their distinctive structure presents an effective novel drug delivery system with potential of loading each hydrophobic and lipophobic medications[23] Niosomes could turn out to be potential delivery system in improving oral bioavailability of BCS category III drugs. Attai et al, formulated Acyclovir Niosomes that were unilamellar spherical in shape, the nonionic wetting agent vesicles were prepared by the traditional thin film hydration method. It's reported that formulated niosomes flaunted considerably retarded release compared to the free drug wherein the pharmacokinetic study performed by them unconcealed that the niosomal dispersion drastically improved the oral bioavailability of acyclovir by almost 2-fold compared to the free drug solution.[24]

1.6.2 Solid Lipid nanoparticles (SLN)[25,26]

Formulation and development of lipid vesicular drug delivery and polymer-based nanoparticles was followed by solid lipid nanoparticles (SLN) that were introduced within the

early Nineteen Nineties as natural lipids that are solid at body temperature, physiological lipids and biocompatible. Surfactants are normally used to prepare SLN dispersions. In one of the literatures, solid dispersion of beta-adrenergic blocker was developed with fatty excipients to alter the release and enhance enteric permeability of the drug. The outcomes of in-vitro permeability unconcealed that drug- phosphotidylcholine solid dispersion considerably increased permeation as compared with the pure drug, which might be attributed to higher lipophilicity obtained by incorporation of the drug inside the solid lipid dispersion. it's conjointly reported that because the quantity of phospholipids inflated relative thereto of drug, the percent of penetrated drug was also increased.[27]

1.7 Novel techniques for solubility and permeability improvement

Each of the above-named techniques for solubility and permeability improvement require use of multiple chemical compound and lipid excipients. Theses excipients not solely raise the value of development however also will increase method complexity.

Recently, use of one excipient system having a porous structure has been explored for enhancing solubility and permeability of BCS category IV drugs.[28,29]

1.7.1 Porous carriers

Numerous drug delivery systems including lipid vesicular systems, micelles, emulsions, polymeric nanoparticles are proven to be a promising approach for controlled and targeted drug delivery. Among these systems porous materials are rising as a brand new class of host/guest systems. Higher attention has been targeted on the event of porous materials as controlled drug delivery matrices as a result of possessing many alternatives options such as stable uniform porous structure, high surface area and tunable pore sizes with well outlined surface properties. As a result of wide variety of helpful properties, porous carriers are utilized in prescribed drugs for several functions together with development of novel drug delivery systems like floating drug delivery system, sustained drug delivery system, etc. These carriers possess large number of nanopores that permit the confinement of drugs. These characteristics let them take up drugs and release them in quite a consistent and predictable manner. The utilization of mesoporous, microporous and nanoporous carriers employed in delivering the drug will be a part of growing research. Once a porous hydrophobic compound drug delivery system is placed within the acceptable dissolution medium, delivery of drug to medium should be preceded by the drug dissolution within the water filled pores or from surface and by diffusion through the water filled channels. Drug release from the porous

carrier could also be complete at interval of ten min or be incomplete even after hours or days. Solvent polarity and surface characteristics play a very important role in the surface assimilation and release from the porous carriers.[30]

Varieties of porous carriers

As stated by International Union for Pure and Applied Chemistry (IUPAC), porous material are classified mesoporous, microporous and macroporous as given within the **Table 1.2**.

Table 1.2 Types of silica based upon pore dimension.

Types of pores	Pore dimensions(width)
Microporous	< 2 nm
Mesoporous	2-50nm
Macroporous	> 50 nm

Various porous minerals are used together with artificial zeolite, silicon dioxide xerogel materials, porous vacant silica nanoparticle, porous hydroxyapatite, porous silica–calcium phosphate composite, porous carbonate microparticle and different porous ceramics. These materials carry large number of nanopores that permit the inclusion of API within them.[31] An excellent deal of analysis has recently centered on the use of porous silica (SiO2)-based materials to boost the solubility of poorly soluble drugs and to regulate the delivery of the loaded substances. There's a huge choice of porous silica material designed for numerous use. In addition, it's conjointly listed in the. Inactive Ingredient database (IIG) of US FDA.[32] In practice, the porous silicon dioxide and silicon materials diverge in their formulation techniques; porous silica materials are synthesize through a bottom-up approach, whereas porous silicon materials are made by a so-called top-down approach. Within the field of drug research, MCM-41 and SBA-15 are the foremost studied ordered mesoporous silica materials, that contain highly unidirectional and uniform pore channel structures. surface chemistries of the ordered and disordered silica materials are alike, comprising of siloxane groups (–Si O–Si–) having an oxygen on the surface, and of three forms of silanol groups (–Si–OH).[30,33]

1.7.1.1 Loading strategies into porous silica[30]

Loading of drug into silica is achieved by numerous technique involving solvents or solvent free techniques. The selection of method depends upon the kind of molecule, charge on the molecule, its pKa, molecular size, pore size distribution of the porous silica particle and interaction happening between silica and drug molecule. Also, the loading technique even has

a bearing on whether or not the drug is adsorbed on the surface (monolayer sorption) or onto the walls of the pores.

Solvent immersion technique

Organic solvent drug loading strategies are extensively outlined within the literature by many analysis groups. The foremost commonly employed technique is an immersion method involving adsorption from organic solution followed by filtration to recuperate the drug-loaded mesoporous silica. Low yield is obtained in most of the cases owing to confinement of adsorbed drug to a monolayer on the surface. Furthermore, it's necessary to get rid of the solvent to acceptable limits outlined within the International Conference on Harmonization (ICH) guideline Q3C.[20] Drug loading is influenced by interactions involving drug, silicon dioxide and solvent. Extremely polar solvents will contend with drug molecules for sorption sites. Charnay et al. found that polar solvents similar to dimethyl sulfoxide (DMSO), dimethylformamide (DMF) and dimethylacetamide (DMA) resulted in a very low degree of NSAID loading onto MCM-41. In contrast, alcohol and dissolving agent like n-hexane (nonpolar solvents) showed comparatively high drug loading concentrations. The optimum drug concentration in solvent would even have to be determined before drug loading. If the concentration is just too high, drug molecules adsorb onto the surface quickly and block the mesopores that reduces the potential particle surface area available out there for loading drug.

Incipient wetness impregnation technique

During this process, a high degree of loading is obtained employing a highly concentrated drug solution (generally on the point of the saturation solubility of the drug). This method utilizes the massive pore volume of the mesoporous silica. The drug's solubility within the loading solvent is very important because it affects the drug loading potency and release. Compared to the solvent immersion method, the long filtration step can be excluded as a loading solution with a volume some up to the pore volume is used. The biggest advantage of this technique is that it's a productive method and therefore the quantity of drug loaded into the carrier is simply controlled, rendering it appropriate for loading costly drug molecules. A normally used single-step, incipient wetness impregnation involves formulating a concentrated drug solution followed by its drop-wise incorporation into the mesoporous silica. The powder is later on dried with the aid of air for twenty-four hours and placed under vacuum for 48 hours at a temperature of 40°C. The mechanism of loading into the pores is hypothesized to be the movement of drug molecules along side the solvent molecules and

succeeding evaporation of the solvent ends up in formation of a film of the API within the pore walls. This technique ensures that the drug truly goes into the pores.

Melt method

The drug-loaded mesoporous system are heated in the melt process beyond the melting temperature of the drug and kept at that temperature so that liquefied drug diffuses into the pores. During this technique, the consistency of the molten drug is very important for the drug to truly diffuse through and access the pores. This method may end up into complete amorphization of the drug to boost the solubility and hence, the dissolution rate. The apparent disadvantage of opting this method is that the drug may degrade at higher temperatures that makes it unsuitable for thermally unstable drugs.

Supercritical fluid loading method

The application of SCF drug loading techniques offers several benefits by exploiting the solvent power variation that may be achieved by manipulation of the fluid pressure and temperature within the critical region. CO2 is the most typically used SCF as a result of it's lower critical point (7.4 MPa, 31.2°C), and also it is non- flammable, recyclable, environmentally benign and inexpensive. Several prescription drugs have reported solubility in supercritical CO2 (SCCO2). Another advantage of utilizing SCF for drug loading is that the ultimate product is completely solvent-free post fluid evacuation. Studies have compared an SCF method directly with a solvent method. The results showed that SC-CO2 made an analogous degree of drug loading to impregnation of the drug utilizing n-hexane as a solvent.

1.7.1.2 Application of porous silicon dioxide carrier[34–36]

The pores inside the mesoporous materials will solely play a part in slow release under traditional physiological conditions, however they can't block the drug molecules. In comparison, the tempering of responsive molecules to the surface of the mesoporous material not only blocks the channels, averts the premature delivery of the drug molecules, but conjointly has the ability of stimulating the delivery of the drug molecules in response to the environment. Currently, considering the mesoporous silica nanoparticles, the researchers have formulated a series of modified release systems that respond to pH, light, enzyme, and so on

pH controlled release

In general, human tissue, cancer sites and different parts of body have different pH values. To

attain targeted drug delivery and enhanced drug utilization, this pH distinction might be applied within the drug carrier design. Zhang et. al. have satisfactorily designed and synthesized Fe3O4/silica core/shell nanocarrier, and pH sensitive amide bond was coupled on the particle surface. The experiment revealed that these particles have a decent pH response to regulate release performance. The release control system of pH response is an intracellular self-responsive release system, that is highly effective than different response systems.

Light controlled release

The basic principle is to regulate the light exposure time and area of light to interrupt the chemical bond, which ends up in opening of the mesoporous structure. Jiang et al have restructured the carboxy- functional spiropyran on the surface of the mesoporous silicon dioxide containing the drug, and therefore the drug is encapsulated in the channel owing to the lipophillic property of the chemical group.[37] Once irradiated with ultraviolet light, spiropyran structure changes, leading to drug release to attain the required effect.

Enzyme controlled release

Compared with different response systems, the enzyme controlled release system is extra specific, more accurate & more appropriate for human environment. Since some human medical conditions are subject to the abnormal rise in some enzyme indicators, the detection of those enzymes and response to the deliverance of these enzymes have a crucial clinical significance. An esterase responsive mesoporous silicon dioxide system was developed by Song et al by altering the mesopores with a rotaxane moiety that was gated with an adamantane component conjugated through an ester link.[38] Once the esterase was present, the ester bond on the cluster was hydrolyzed, delivering the drug.

1.8 Soluplus® as a novel excipient for bioavailability enhancement

Soluplus® is a polyvinyl caprolactam–polyvinyl acetate– polyethylene glycol graft copolymer, which could be effectively used for improving the oral bioavailability by enhancing the intestinal drug absorption.[39] In contrast to conventional polymers such as PVP and HPMC, Soluplus® is bifunctional and can act as a polymeric carrier for solid dispersions and as a solubilizer by forming micelles in water, which could be useful for the production of highly water-soluble formulations. the polymer has an inherent binding site that enables hydrogen bonds or van der Waal interactions with drug molecules, which can lead to a stabilization of the drug molecule in the matrix. Soluplus® exhibits amphipathic characteristics and thus forms micelles when comes in contact with water and aids in

improving the solubility and being a p-gp efflux inhibitor in enhances the permeability of p-gp substrate drugs as well.[40–42]

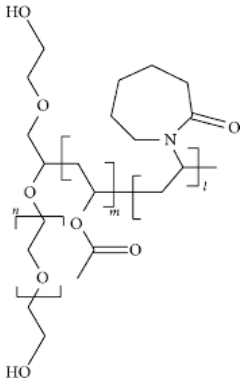


Fig 1.3 Structure of Soluplus®.

2. LITERATURE SURVEY

Richard G., Pfizer and Bristol-Myers Squibb (WO 2010/147978 A1) have a joint patent on various formulations of Apixaban with improved solubility and strategies to treat DVT and pulmonic embolism. The patent covers every type of dosage forms like tablets together with layered tablets, osmotic dosage forms; multi particulate dosage forms, lipidic vehicle formulations like emulsion and controlled drug release formulations.[43]

Frost et. al., Pfizer and Bristol-Myers Squibb (2016) (US 9,326,945 B2) possess a joint patent covering Apixaban formulations. The patent covers aspects of wet and dry granulation formulation with in- vivo pharmacokinetic profiles of the formulated formulations.[44]

Kinnari et. al. (2011) compared the ordered and non-ordered mesoporous silica as a carrier for itraconzole and demonstrated that the 2 grades of silica showed identical enhancement in dissolution rate and chemical properties of particles and also the drug loading procedure was accountable for changes in drug unleash mechanism and drug loading.[45]

Patel et. al. (2013) evaluated the colloidal solid dispersions for physiochemical parameters and in-vitro release profile of Sulfathiazole and reported that porous silica confined into the traditional solid dispersion acted as a carrier to disperse the complex and improve the dissolution rate.[33]

Uejo et. al. (2013) Prepared matrix formulation exploiting Fenofibrate and confined into mesoporous silicon oxide by employing a novel melting technique and reported that up to

33% of drug concentration might be loaded with success assuring amorphous nature and enhancement in dissolution rate.[46]

McCarthy et. al. (2015) reviewed the various ways for incorporating drug into the porous element and additionally the influence of pore size, pore volume, surface area, of silicon influences the quantity of drug loaded and also the drug molecular size and mass may be a major decisive parameter whether or not the loading might occur on the surface or into the mesopores. The review also includes the kinetic side of drug release and furthermore the future prospects of porous elements in drug delivery application.[30]

Chen et. al. (2016) formulated the acid cocrystals of Apixaban. The results complete that cocrystals improved the solubility 2.1 folds Associate in Nursingd in-vivo pharmacokinetic study in hound dog dogs showed an improvement in AUC upto 2.7 folds as compared to pure drug.[47]

Bahirat et. al. (2016) Studied the advances in oral bioavailability of Valsartan by loading into non ordered mesoporous silicon oxide and reportable complete amorphization within the mesopores and indicated that pill preparation of drug loaded silicon oxide are will be future application for oral drug delivery.[48]

Nel et. al. (US 2016/0008283 A1) developed lipid bilayer coated MS with Gemcitabine for the treatment of human exocrine gland carcinoma. The loading of GEM into the MSNs was found to be around 20% w/w. The delivery system showed increased uptake in neoplasms showing a major reduction in tumor volume. To prolong the circulation time, the MSNs were coated with PEI/PEG.[49]

Meka et. al. (2018) studied the loading of BCS category IV drug Vorinostat into MCM-41 mesoporous silicon oxide nanoparticles and evaluated for permeableness and cell toxicity studies MCM-41 formulation showed higher permeability than pure drug and cell lines studies indicated biocompatibility of MS.[50]

Waters et. al. (2018) studied the dissolution rate improvement of anti-inflammatory loaded into 3 totally different grades of Syloid. it had been found that drug unleash from Syloid AL1 FP was poor and incomplete (78.6%) compared to Syloid XDP thirty50 that showed highest and virtually complete release at intervals 30 min (99.6%).[51]

Xi et. al. (2020) co-loaded nonsteroidal anti-inflammatory with precipitation inhibitors in MCM-41 sort mesoporous silica and ascertained improved dissolution profile of co-loaded formulation. In- vivo bio imaging studies discovered presence of MS within the tiny intestine. The formulation containing HPMC E5 as precipitation inhibitors showed the very best improvement in bioavailability.[52]

Liong et. al. developed MS to include water insoluble drug Camptothecin and Paclitaxel for exocrine gland carcinoma. They controlled the discharge of medication by adding oxide nanocrystals within MS. Uptake inside the duct gland was improved by surface functionalization of vitamin Bc moiety. Hepa-1 cell lines studies showed improved uptake and antitumour activity.[53]

Soluplus® has wide been utilized in varied pharmaceutical dose kind developments. Oral solid formulations supported Angelica gigas Nakai (AGN) Associate in Nursing flavourer drug and Soluplus were ready by the hot-melting extrusion (HME) technique. the utilization of Soluplus resultively contributed into the increasing solubilization therefore increasing bioavailability.[54]

Sumit et. al. developed an amorphous solid dispersion of Soluplus with Rivaroxaban that established to be an economical method to reinforce the solubility, circumvent the p-gp effect and improve the bioavailability.[41]

Michael et. al. developed physical mixtures and solid solutions of Soluplus with BCS category II medication fenofibrate, antimycotic and danazole which showed substantial increase in the plasma AUC and Caco -2 permeation.[39]

3. NEED OF WORK

- Apixaban (APX) is an oral selective direct reversible factor Xa inhibitor.
- According to biopharmaceutical classification system (BCS) APX is a BCS class III drug having an aqueous solubility of 0.028 mg/ml and log P value of 1.65.[55]
- APX exhibits polymorphism, and only form N-1 is constantly used in the marketed product as it is the thermodynamically stable anhydrous form.
- According to literature, APX is a substrate for P-glycoprotein (P- gp) an efflux transport protein which can results into low oral bioavailability (~ 45% for a single 10 mg dose).[56]
- Although, APX is classified under BCS Class III drug for a single 5 mg dose, a higher

strength (>7 mg) could not be formulated as it will shift the drug from BCS III to BCS Class IV.

- Hence, solubility enhancement and p-gp inhibition turn out to be prior requirement for improvement of oral bioavailability.
- Also, literature survey reveals that not much work is carried out to modulate release of APX.
- Therefore, the present study was aimed towards solubility improvement and modulation of drug release using mesoporous silica-based drug delivery system and improving the bioavailability of APX using novel p-gp efflux inhibitor such as Soluplus®.

AIM AND OBJECTIVES

Aim: Preparation and optimization of APX loaded mesoporous silica and Soluplus® complex.

Objectives

- To perform the preformulation studies of APX.
- Screening of various methods to load APX on mesoporous silica matrix.
- Selection of an appropriate method of preparation for APX loaded MS carrier based on the desired properties.
- Selection of optimized ratio of APX:Soluplus® physical mixture based on the desired properties.
- Characterization of prepared complexes
- In-vitro and in-vivo comparison of the developed optimized products with marketed formulation (Eliquis®)
- To perform the stability studies of optimized formulation as per ICH guideline.

5. PLAN OF WORK

5.1 Literature Survey

5.2 Procurement of Drug and Excipients

5.3 Pre formulation Studies

- Identification of drug
- Calibration curve

5.4 Solubility Improvement

- Screening and selection of suitable method for loading APX into porous silica

- Preparation of Physical mixture of Drug and Soluplus ®

5.5 FTIR, XRD, DSC Analysis of prepared formulations

5.6 SEM analysis of APX-SYL complex

5.7 Comparison of dissolution profile of prepared formulation with marketed formulation (Eliquis®)

5.8 Comparison of permeability profiles of prepared formulations with marketed formulation (Eliquis®)

5.9 In-vivo pharmacokinetic study

5.10 Stability Studies

6. DRUG AND EXCIPIENTS PROFILE

6.1 DRUG PROFILE

Apixaban (APX)

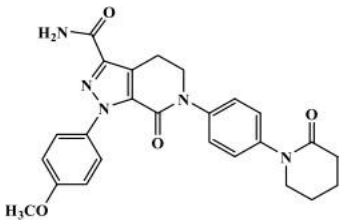
Description: APX is an anticoagulant drug indicated to reduce the risk of stroke and systemic embolism in patients with nonvalvular atrial fibrillation. APX is an oral, direct, and highly selective factor Xa (FXa) inhibitor of both free and bound FXa, as well as prothrombinase, independent of antithrombin III for the prevention and treatment of thromboembolic diseases. It's marketed by the brand name Eliquis® in the form of a film coated tablet in the dose strengths of 2.5 and 5mg by Bristol Myers Squibb. APX was approved by the FDA on December 28, 2012. It has low water solubility of 24.7 µg/ml and low bioavailability of 50%.[1]

- **Drug profile**[2,55]

Appearance: A white to off –white powder.

IUPAC name: 1-(4-methoxyphenyl)-7-oxo-6-[4-(2-oxopiperidin-1-yl) phenyl]-1H,4H,5H,6H,7H-pyrazolo[3,4-c]pyridine-3-carboxamide

Structural Formula



Molecular formula: C25H25N5O4 **Molecular weight**: 459.497 g/mol **Melting point**: 237-238°C

Solubility: insoluble in water (24.7 µg/mL)

Log P: 1.65

pKa: 13.1 (acidic), -1.6(basic)

Indication: To reduce risk of stroke and systemic embolism in patients who have nonvalvular atrial fibrillation, prophylaxis of deep vein thrombosis (DVT) leading to pulmonary embolism (PE) in patients after a hip or knee replacement surgery, and treatment of DVT and PE to reduce the risk of recurrence.

- **Pharmacokinetic profile**

Absorption: Absorbed majorly from the distal SI and ascending colon after oral administration with a bioavailability of 50%. APX is absorbed throughout the gastrointestinal (GI) tract, with about 55% of absorption occurring in the distal small bowel and ascending colon.

Metabolism: 50% is metabolized unchanged, 25% of the dose is excreted as O-dimethyl apixaban sulfate. **t1/2** :12.7 ± 8.55 hrs. **Plasma protein binding**: 87%

Excretion: 56% of an orally administered dose is recovered in the feces and 24.5-28.8% of the dose is recovered in the urine.

- **Clinical Pharmacology**

Legal status: RX prescription only

Routes: oral

- **Mechanism of action**: It is highly selective factor Xa (FXa) inhibitor of both free and bound FXa, as well as prothrombinase, independent of antithrombin III.
- **Marketed dosage form**: Tablet (2.5 and 5 mg)
- **Contraindication and prevention:** APX is contraindicated in patients having artificial heart valve, patients undertaking NSAIDs.

6.2 EXCIPIENTS

6.2.1 Syloid XDP3150[57]

Synonym: Porous silica gel, Fumed silica, fumed silicon dioxide.

Functional category and applications: Adsorbent; anticaking agent; emulsion stabilizer; glidant; suspending agent; tablet disintegrant; thermal stabilizer; viscosity-increasing agent. Colloidal silicon dioxide is widely used in pharmaceuticals, cosmetics, and food products. Its small particle size and large specific surface area give it desirable flow characteristics that are

exploited to improve the flow properties of dry powders in a number of processes such as tableting.

Appearance: White or creamy-white powder**.**

Description: It is a submicroscopic fumed silica with a particle size of about 150 µm. It is a light, loose, white colored, odorless, tasteless, non-gritty amorphous powder.

Molecular formula: (--O-Si-O-)n Molecular weight: 60.08 g/mol

Solubility: Practically insoluble in water, organic solvents and acids, except hydrofluoric acid. Soluble in hot solution of alkali hydroxide.

Pore volume: 1.7cc/g.

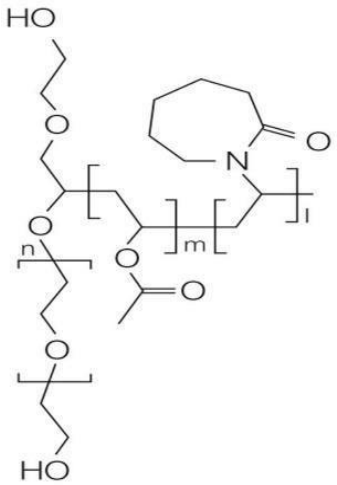
Regulatory status: Available in FDAs Inactive Ingredient list. Maximum strength permitted per unit dose orally is 138.5 mg for tablets and 100mg or capsules.[58]

6.2.2 Soluplus®[59]

Soluplus® is a polymeric solubilizer with an amphiphilic chemical structure, which was particularly developed for solid solutions**.**

Appearance: It is a free-flowing white to slightly yellowish granule with a faint characteristic odour.

Structure



Chemical name: Polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft co-polymer (PCL-PVAc-PEG)

Molecular weight: 1, 18,000 g/mol

Glass transition temperature (Tg): ~70℃

Density: 1.082 g/cm3

HLB: ~14

Regulatory Status: Soluplus® is approved in generics in Taiwan and Argentina

Solubility: Freely soluble in water.

Applications: Preparation of Solid dispersions, Hot melt Extrusion, Drug layering, Spray drying, binder in both dry and wet granulation.

7. MATERIALS AND INSTRUMENTS

The following materials were used for the study.

Sr. No.	Material
1.	Apixaban
2.	Syloid® XDP 3150
3.	Soluplus®
4.	Microcrystalline cellulose
5.	Lactose monohydrate
6.	Croscarmellose sodium
7.	Magnesium stearate
8.	Sodium lauryl sulfate
9.	Sodium dihydrogen phosphate
10.	Disodium hydrogen phosphate
11.	Sodium lauryl sulfate
12.	N,N-Dimethyl formamide
13.	Isopropyl alcohol
14.	Acetonitrile (HPLC grade)

The following instruments were used for the study

Sr. No.	Instrument
1.	Tablet Compression
3.	Electronic Balance
4.	Tap Density Tester (USP)
6.	Friabilator
7.	Tablet Hardness Tester
8.	Dissolution Test Apparatus USP type II
9.	UV-Visible Spectrophotometer
10.	FTIR
11.	Differential Scanning Calorimetry
13.	X-ray Diffraction
14.	Melting apparatus
15.	pH Meter
16.	HPLC
17.	Centrifuge
18.	Vortex mixer

8. EXPERIMENTAL WORK

8.1 Pre-formulation studies

Pre-formulation studies were conducted before the formulation development on API as it was necessary to find out drug characteristics, identification and its stability in the formulation.

8.1.1 Drug analysis

8.1.1.1 Description

Drug was visually inspected for any change in colour, odour, etc.

8.1.1.2 FT-IR analysis

APX sample was analyzed by infrared spectroscopy to know the purity and to characterize the probable structure modification of drug sample. Potassium bromide (KBr) was allowed for activation in hot air oven for 15-20 min. Drug sample was mixed with KBR in the ration of 1:9 and filled in the cavity along with blank KBR. A blank KBR spectrum was run to minimize the interference of peaks due to functional groups present in KBr. Sample spectrum was run in the range of 4000 to 400 cm-1 using Fourier transform Infra-red spectrophotometer.

8.1.1.3 Determination of wavelength

Preparation of standard stock solution

The stock solution was prepared by dissolving 10mg of APX in DMF upto 10ml. Using distilled water 1 ml of the stock solution was further diluted upto 10 ml to obtain 100µg/ml i.e. 100ppm solution. The solution was analyzed using UV visible spectrophotometer (Jasco) in triplicates at 276 nm.

Determination of wavelength of maximum amplitude (R2 value) of APX

1 ml of the stock (100 ppm) was diluted to 10 ml with distilled water to get 10 ppm of concentration. The UV spectrum of final solution was scanned in the range of 400-200 nm against water as blank.

8.1.1.4 Standard Curve of APX

A range of final concentration of 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 25 and 27µg/ml was prepared by pipetting out 0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6, 1.8, 2, 2.5 and 2.7ml solution from the standard stock solution respectively and diluting them to 10 ml with distilled water. The graph of concentration (µg/ml) was plotted against the absorbance (nm) to obtain calibration curve using UV spectrophotometer.

8.1.1.5 Drug and excipient compatibility studies

Drug-excipients compatibility is an early risk reduction strategy. It could help to exclude the use of excipients which might interact with the drug substance. Each excipient was mixed with APX in the ratio 1:1. The samples were stored for 4 weeks 40°C/75% RH. These mixtures were kept under observation to check any changes in the physical appearance. The drug-excipient compatibility was confirmed by subjecting the samples to FTIR analysis.

8.2 Solubility studies

The solubility of pure APX was determined in all dissolution media (Distilled water, 0.1 N HCl, acetate buffer pH 4.5, phosphate buffer pH 6.8, phosphate buffer 6.8 with 0.05%SLS). Briefly, excess amount of APX powder was added to beakers containing 50 mL of the media. The beakers were placed in a shaking water bath rotating at 50 rpm and maintained at 37 ± 1°C. After 24 hours, the dispersion was filtered through a 22µ syringe filter and analyzed using UV-Spectrophotometer. The saturation solubility of drug was performed in different pH media in order to check possibility of pH dependent solubility.

8.3 Formulation strategies to improve pharmaco-technical properties of APX

8.3.1 MSN based drug delivery

Pharmaco-technical properties of APX were modulated by using MSN as a carrier. The approach was to incorporate APX into pores of silica to improve the solubility and permeability. Based on the literature, Syloid XDP 3150® was chosen as a carrier as it allows easy incorporation and uniform dispersion of actives. Also, the pore volume of 1.7 cc/gm allows easy confinement of active inside the pores. It effectively contributes to release of the active and could be used to modify the drug release. The APX was incorporated into MSN by following methods.

Melt method

A previously reported method with slight modifications were used.[46] The amorphous solid dispersion was prepared by melting the drug (APX) over a hot plate and incorporating the Mesoporous silica (Syloid® XDP 3150) into the melted drug and mixing it properly. To achieve high drug loading, the APX & XDP 3150 were mixed in 1:1 ratio (weight ratio). The prepared solid dispersion was triturated using a glass mortar and pestle to obtain a uniform sized powder. The prepared sample was stored in a plastic container under room temperature.

Solvent immersion

A previously reported traditional method of drug loading was used in this technique(30). Briefly, APX was dissolved in DMF and poured into a beaker and weighed amount of Syloid® XDP 3150 was added and stirred overnight. The powder was scrapped and kept in vacuum oven for 2hr to remove residual solvent. The prepared sample was stored in a plastic container under room temperature and was used for further evaluation.

Incipient wetness impregnation

This method is a modification of solvent immersion method[30], where the concentrated solution of drug in solvent was added drop-wise to the mesoporous silica. The prepared sample was dried and stored in a plastic container under room temperature and was used for further evaluation.

Physical mixture

In this method, APX and Syloid® XDP 3150 were weighed and mixed physically using mortar and pestle for an hour.

8.3.2 Preparation of APX: Soluplus® complex

APX being a p-gp substrate, has low oral BA which could be improved by incorporating p-gp inhibitors along with APX. Based on previously reported literature Soluplus® was chosen as it improves the solubility of drugs as well as effectively inhibits p-gp efflux of drug and there improves bioavailability of actives.[41]

In this method, APX and Soluplus were weighed and mixed physically using mortar and pestle for an hour in three different ratios.

All the prepared samples were kept in a desiccator. Prepared complexes were then characterized for physicochemical properties such as Fourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), X-ray Diffractometry (XRD) and solubility analysis in order to screen the suitable and best method of preparation for APX formulation with improved pharmaco-technical properties.

8.4 Solid State Characterization of APX:MSN & APX: Soluplus® Complexes

8.4.1 Saturation solubility

The solubility of pure APX and formulated preparations was determined in water. Briefly, excess amount of Apixaban powder was added to beakers containing 50 mL of the water. The

beakers were placed in a shaking water bath rotating at 50 rpm and maintained at 37 ± 1°C. After 24 hours, the dispersion was filtered through a 22µ syringe filter and analyzed using UV-Spectrophotometer.

8.4.2 X-ray powder diffraction (XRPD)

XRPD was performed using a Rigaku miniflex D600 diffractometer with Cu Kα radiation 1.54 angstrom (0.154 nm). An acceleration voltage of 40 kV was used. Samples were scanned between 10° to 80° 2θ with a step size of 0.01° and a step time of 10° per minute.

8.4.3 Fourier-transformation infrared spectroscopy (FTIR)

The FTIR spectra was recorded using Jasco FT/IR 4000, Japan. Potassium bromide (KBr) was allowed for activation in hot air oven for 15-20 minutes. Drug samples were mixed with KBr in the ratio 1:9 and filled in the cavity of apparatus. A blank KBr spectrum was run to minimize the interference of peaks due to functional groups present in KBr. Sample spectrum was run in the range of 4000 to 400 cm-1 using Fourier transform Infra- red spectrophotometer. Each sample was measured in triplicate.

8.4.4 Differential scanning calorimetry

The thermal properties of the drug, silica and the complex were analyzed by subjecting them to DSC studies. DSC studies were performed using Mettler DSC 822e (Mettler, Toledo, and Giessen, Germany). The thermogram of pure APX was compared with APX: SYL matrix prepared using different methods. 4mg of the samples were weighed in an aluminium pan and an empty aluminium pan was kept as reference. The scans were performed with a heating rate of 10ºC/min from 30ºC to 300ºC. During the measurement, inert nitrogen gas was purged into the system.

8.5 Scanning electron microscope (SEM)

The SEM study was performed to analyze the morphological characters of the prepared complex. The pure API, Syloid® XDP 3150 silica and the prepared complex were outsourced to University of Pune for SEM analysis.

8.6 In-vitro drug release study

The dissolution profiles of pure APX, marketed formulation of APX (Eliquis), APX:SYL complex & APX:SOL physical mixture were generated for a comparative analysis of the release profiles. The dissolution profiles over a period of 45 minutes were performed for the

pure APX, APX-SOL physical mixture & marketed product wherein the dissolution profile for the prepared APX: SYLOID XDP 3150 complex (containing 5 mg equivalent of drug) was performed for 3 hours. The dissolution studies were performed using an USP type II apparatus with a paddle rotating speed of 75 RPM & 900 ml of dissolution media at 37±1°C. The dissolution media used was 0.05 M Sodium Phosphate Buffer with 0.05% SLS, pH 6.8 which is a reported dissolution media in the FDA database.[60] 5 ml samples were withdrawn and immediately replaced with fresh 5 ml phosphate buffer pH 6.8 to maintain the sink condition. The collected samples were filtered through a 22µ syringe filter and analyzed under UV-Spectrophotometer. The dissolution profiles were performed in triplicates.

8.7 Ex-vivo permeation study

The permeation study was performed using a non-everted intestinal gut sac method.[61] The goat ileum was used for checking the permeability behavior. The ileum was washed and cleaned thoroughly with clean water and Ringers solution prior to use. The cleaned ileum was cut into pieces of 5 cm, tied with a thread at 1 cm from one end. The sample was dispersed in 1 ml of distilled water and filled into the sac tied at one end using a micropipette and another end of the sac was tied using a thread at 1 cm from the end. The permeation study was performed using a USP Dissolution type II apparatus with a paddle rotating speed of 100 RPM & 250 ml of Ringers solution at 37 ± 1 °C. 5 ml samples were withdrawn and immediately replaced with fresh 5 ml Ringers solution to maintain the sink condition. The collected samples were filtered through a 22 µ syringe filter and analyzed under UV-Spectrophotometer. The permeability profiles were performed in triplicates.

8.8 Bioanalytical Method

8.8.1 Instrument and liquid chromatography conditions

The Jasco HPLC system was equipped with PU-1580 Intelligent HPLC pump, UV-1575 intelligent UV/VIS detector. Data was integrated using Jasco Borwin version 1.5, LC-Net II/ADC system.[62,63] The mobile phase was filtered through 0.45µ membrane filter. The chromatographic separation was performed at ambient temperature utilizing Hypersil GOLD C18 column (250 mm × 4.6 mm, 5 µm) coupled with guard column (RP-18, 33 mm Kromasil®, 5 µm).

8.8.2 Method development

Combinations of solvents and their ratios were explored to find the suitable mobile phase for efficient elution of APX. Isocratic elution was carried out using mobile phase ratio (50:50 v/v)

of acetonitrile and water maintained at a flow rate of 0.8 mL/min. The UV/VIS detector was model operated at wavelength of 276 nm. The sample injection volume was 20 µl. Extracting solvent (ES) plays a crucial role in bioanalytical estimation as it precipitates the proteins in plasma leaving behind the plasma-drug solution. Solvents *viz.* methanol, chloroform, ethylacetate, dichloromethane, dimethyl formamide, acetonitrile and perchloric acid (10 %v/v) were investigated for their extraction efficiency. Further, the optimum quantity of ES was determined by altering its volume in sample preparation.

8.8.3 Preparation of standard solution, calibration and quality control samples using plasma

The standard solution of 1000 µg/ml was prepared by weighing 10mg APX and diluting it upto 10ml with mobile phase. APX's working samples contained concentrations viz. 0.01, 0.1, 1, 10, 100 ppm. They were prepared by accurately withdrawing respective volumes from standard stock solution into eppendorf tubes using micropipette and diluting it to respective concentration with mobile phase.

The plasma working samples were prepared in eppendorf centrifuge tubes by externally spiking Apixaban using volume as follows:

a) 150µl of plasma
b) 150µl of drug solution
c) 300µl of extracting solvent

These samples in eppendorf tubes were vortexed for 3min on Tarson's Spinix Vortex Shaker and then centrifuged on Centrifuge 5424R at 4˚C and 15000 rpm for 15 minutes. After centrifugation, the supernatant was taken and filtered through 0.22µ PVDF filter.

8.9 In-vivo pharmacokinetic studies

The oral bioavailability of APX, optimized batches and marketed tablet were assessed in male albino Wistar rats (250 ± 20 g). Before the beginning of the study, animals were deprived of food 12 h, with free access to water. Male wistar rats were separated into three groups of six animals per group and dose was administered via oral gavage with the following samples: APX, optimized batches and marketed tablet, suspended in water at a dose of 2.0 mg/kg. Blood samples were obtained from the retro-orbital puncture (ROP) into a heparinized tube at 0.5,1.0, 2.0, 3.0, 4.0, 8.0 and 12.0 h post-treatment. The plasma fraction was prepared by centrifugation at 15,000 rpm for 20 min at 4°C and was preserved at -20°C until analysis. To 100 µL aliquots of plasma, 300 µL of acetonitrile (ACN) was added. The samples were vortexed for 3 min and

centrifuged at 15,000 rpm for 20 min at 4°C. The whole supernatant was collected from above step and filtered through 0.22µ nylon syringe filter and injected directly into the HPLC. Animal experiments were approved by Institutional Animal Ethics Committee and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (CPCSEA/PCP/PCT10/2020) and conducted in accordance with the CPCSEA guidelines. Pharmacokinetic parameters were quantified using PK Solver (version 2.0). The maximum plasma concentration (Cmax) and the time to reach Cmax (Tmax) were determined. The area under the plasma concentration-time curve (AUC) was computed by the linear trapezoidal method.

8.10 Stability study[64]

The stability study was carried out on optimized formulation at room temperature for three months as per ICH guidelines. The tablets were evaluated by dissolution study.

9. RESULTS AND DISCUSSION

9.1 Pre-formulation studies

9.1.1 Drug analysis

9.1.1.1. Description

APX was white to off white crystalline powder.

9.1.1.2 FTIR analysis

The infrared spectroscopy was used to analyze the purity of APX and probable structure modification of drug sample if any. The sample was analyzed in the region of 4000 to 400cm-1. Major peaks were observed in the FTIR spectrum at the range corresponding to functional group as given in **Table 9.1**. Appearance of major peaks in spectrum confirmed that the tested sample was APX **(Fig. 9.1).**

Table 9.1: FT-IR interpretation of APX.

Functional Groups	Wavenumber (cm-1)	Functional Groups
NH-stretch	3482.81	NH-stretch
Aromatic ring	3313.11	Aromatic ring
Amide	1688.25	Amide

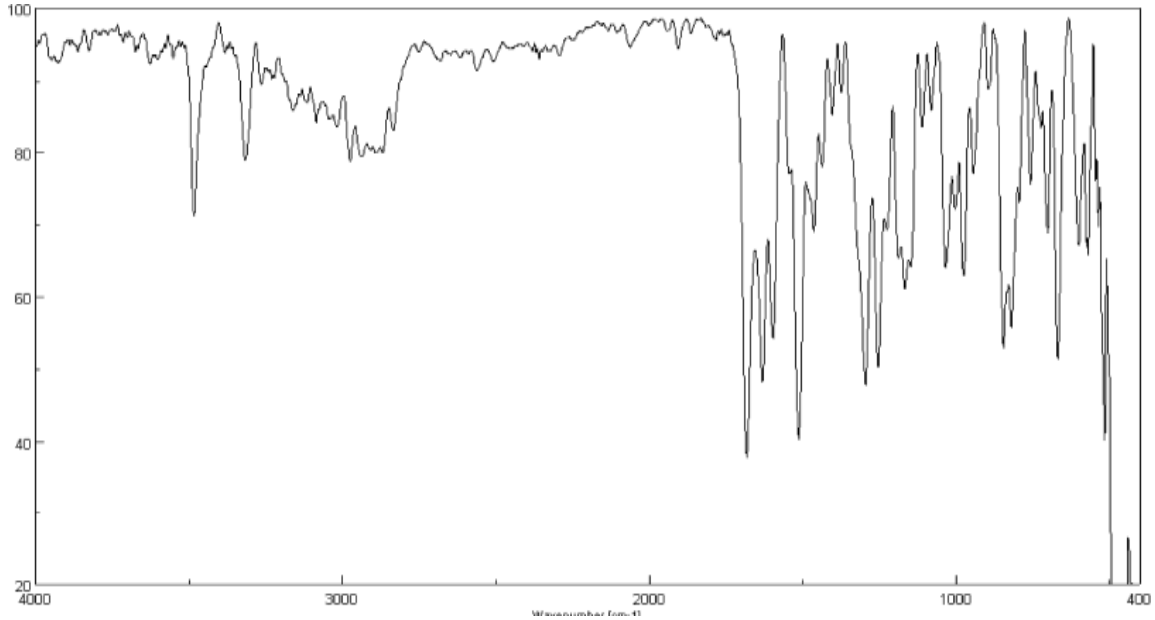


Fig. 9.1: FTIR spectra of pure APX.

9.1.1.3 Determination of wavelength

UV spectra of the solution of 10 µg/ml was recorded in the range of 400 – 200 nm. The maximum absorbance was found at 276 nm **(Fig. 9.2)**

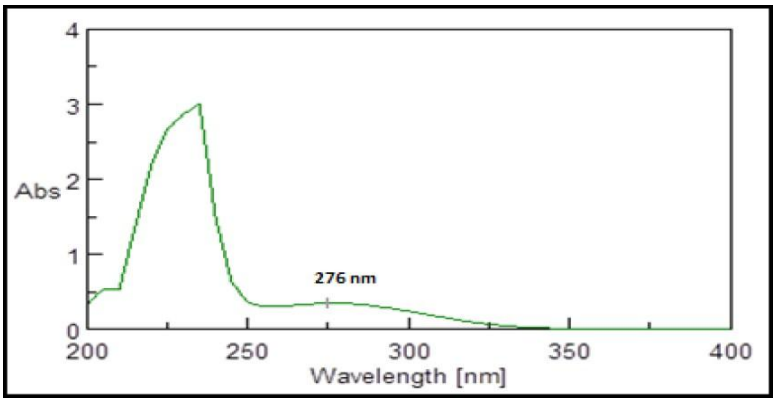


Fig. 9.2: Determination of λmax of APX.

9.1.1.4. Standard curve of APX

A standard curve of APX was obtained by calculating absorbance values at different concentrations using UV spectrophotometer and plotting them against each other. Graph of absorbance vs. concentration demonstrated in **Fig.9.3** and **Fig 9.4** and were linear in the concentration range of 2-27 µg/ml in distilled water and 2- 32µg/ml in 0.05M Sodium phosphate buffer pH 6.8 with 0.05% SLS respectively at 276 nm obeying Beer-Lambert's law.

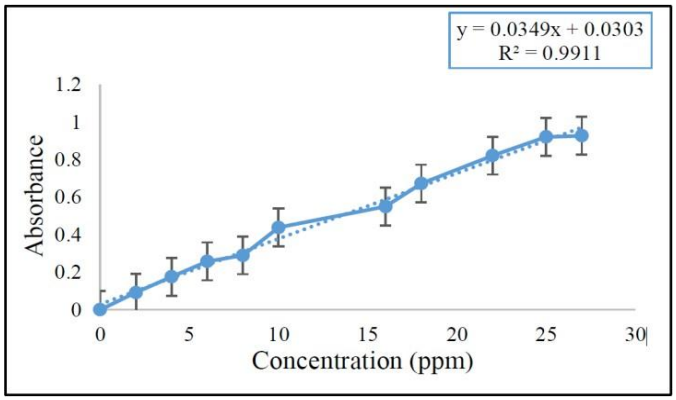


Fig. 9.3: Calibration curve in distilled water.

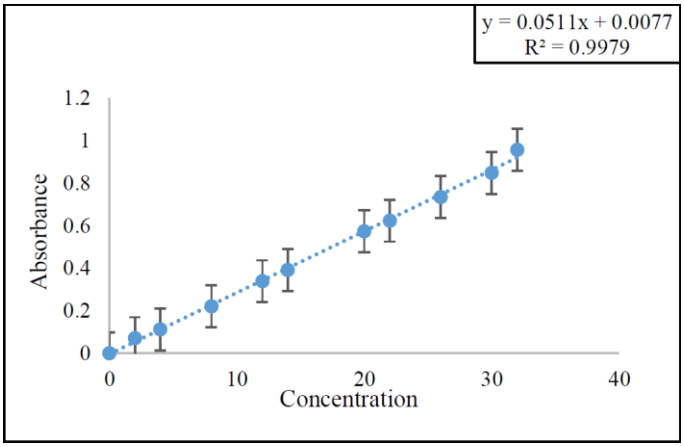


Fig. 9.4: Calibration curve in in 0.05 M Phosphate buffer pH 6.8 with 0.05% SLS.

9.1.1.5 Drug excipient compatibility studies

No change was observed in the physical appearance of the drug with all the excipients in dry form as represented in Table 9.4. Also, the FTIR graphs **(Fig. 9.5)** illustrates that there were no chemical interaction and all the principal peaks of API were present. This confirms the drug and excipient are compatible with each other.

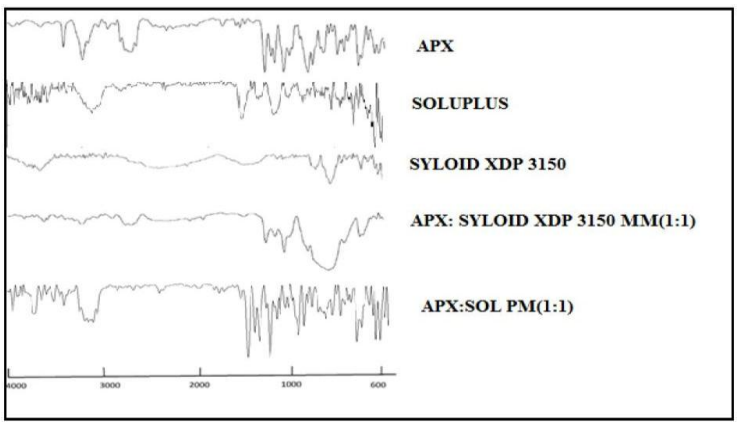


Fig. 9.5: FTIR graph for compatibility study.

Table 9.2: Drug and excipient compatibility study.

Sr. No	Sample	Initial Observation	After 400C / 75% RH (4 weeks)
1	APX	White to off white powder	White to off white powder
2	APX:Soluplus® (1:1)	White to off white powder	White to off white powder
3	APX:XDP 3150 (1:1)	White to off white powder	White to off white powder

9.2 Solubility studies

The solubility of APX in various buffer systems and distilled water was evaluated **(Table 9.3)**. The solubility of APX was found to be higher in pH 6.8 buffer with 0.05% SLS as compared to water and other pH buffers. This was due to the solubilizing effect of SLS, which acts as a surfactant. A surfactant or surface-active agent is a substance that, when present at low concentration in a system, has the property of adsorbing onto the surfaces or interfaces of the system and of altering the surface or interfacial free energies of those surfaces to a marked degree. As, APX remains unionized throughout the gastrointestinal tract, it neither gets protonated nor deprotonated. Due to this reason, it possesses pH independent solubility across the physiological pH.[65]

Table 9.3 Solubility data of APX in various media.

Sr. No.	**Media**	**Solubility (µg/ml ± SD)**
1	Water	24.6±0.63
2	0.1N HCl (pH1.2)	24.15±.59
3	pH4.5 Acetate Buffer	25.68±0.43
4	pH 6.8 Sodium Phosphate buffer	25.14±0.37
5	pH 7.4 Phosphate buffer	24.8±0.67
6	pH 6.8 Sodium Phosphate buffer with 0.05% SLS(OGD media)	**42.78±.94**

9.3 Characterization of APX: MSN & APX: Soluplus® Complexes

9.3.1. Saturation Solubility

The APX-SYL complex prepared in 1:1 ratio by melt method showed highest improvement in solubility of 0.1002±0.10 mg/ml (4.056 folds) as compared to solubility of pure APX (0.0247± 0.10 mg ml) wherein the APX:SOL complex prepared with 1:1 ratio showed maximum increase in solubility i.e. 0.1890± 0.12 mg/ml (7.6518 folds).

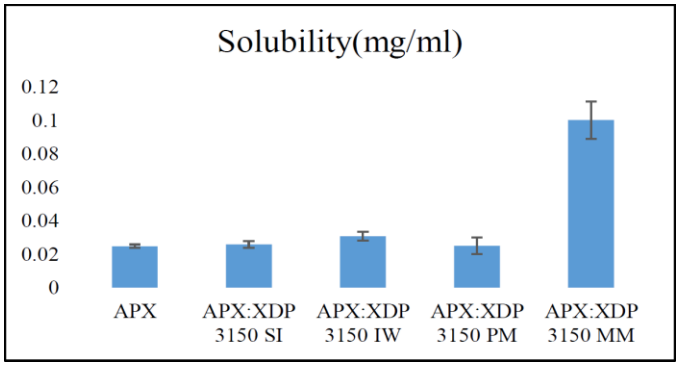


Fig. 9.6: Solubility enhancement using different drug loading methods.

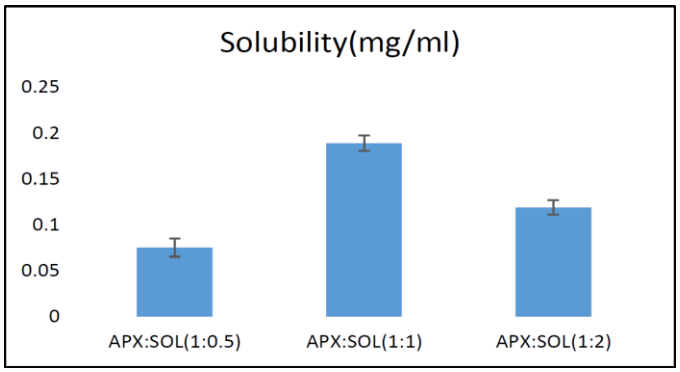


Fig. 9.7: Solubility enhancement using different ratios of drug & Soluplus®.

9.3.2 X-ray powder diffraction (XRPD) analysis

The X-ray diffractogram of APX shows sharp peaks at 13.40°, 13.91°, 17.03°, 18.46°, 21.56°, 21.93°, 24.74° and 27.03° suggesting the crystalline nature of APX. The principal peaks with lower intensity were observed in APX-SYL with SI, IW and PM wherein the APX-SYL prepared with MM & APX-SOL® detects absence of the principal crystalline peaks which proves that there is complete drug amorphization **(Fig. 9.8).**

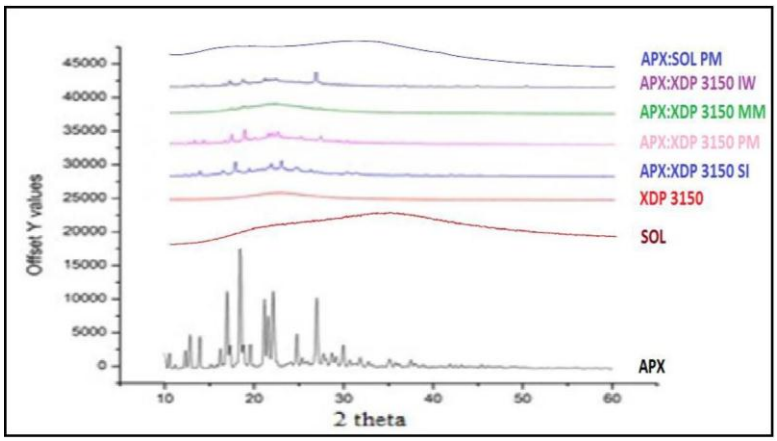


Fig 9.8 PXRD graphs of pure drug and formulated complexes.

9.3.3 Fourier-transformation infrared spectroscopy (FTIR)

The FTIR spectra of the pure drug detects all the characteristics peaks of APX. The FTIR spectra of APX: SYL & APX:SOL® shows that there is no significant change in the peaks which indicates that there are no drug excipient interactions.

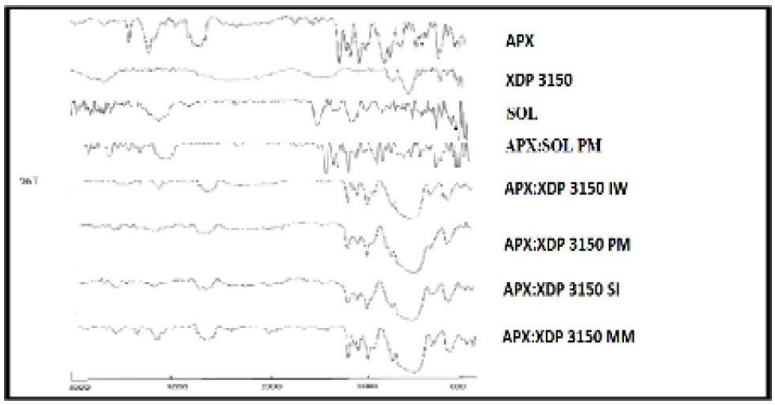


Fig. 9.9: FTIR spectra of pure drug and formulated complexes.

9.3.4 Differential scanning calorimetry

Fig. 9.10 illustrates the DSC thermograms of APX, Syloid XDP 3150, Soluplus®, APX-SYL and APX-SOL®. A sharp endothermic peak of APX at 240°C indicates crystalline nature of the drug. The thermogram of Syloid XDP 3150 & Soluplus® has not shown any distinct peaks. APX-SYL complex prepared by different methods except melt method displayed diminished endothermic peaks suggesting partial amorphization. Complex prepared with melt method and Soluplus® showed no obvious endothermic peak and confirmed complete amorphization of drug as well as a possible incorporation of drug within the porous network of Syloid. Based upon the results of solubility studies and solid state characterization of melt method for APX:SYL and physical mixture of APX:SOL® (1:1) were finalized for the further in-vitro drug release, ex-vivo permeation and pharmacokinetic studies.

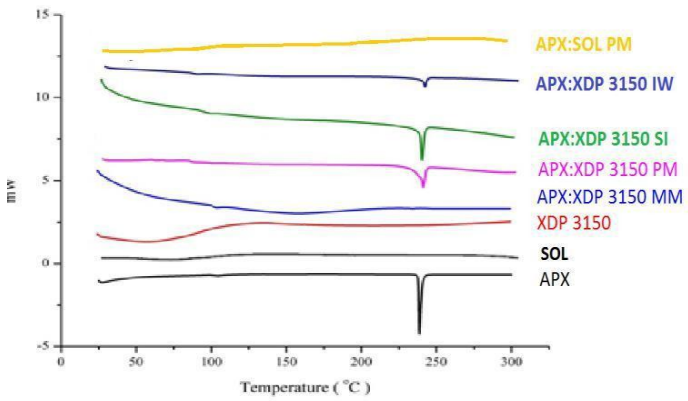


Fig 9.10 DSC thermograms of pure drug and formulated complexes.

9.4 Scanning electron microscope (SEM)

SEM images of the complex shows that there was no significant deposition of drug over the silica surface which indicates that the drug was incorporated into the pores of silica successfully.

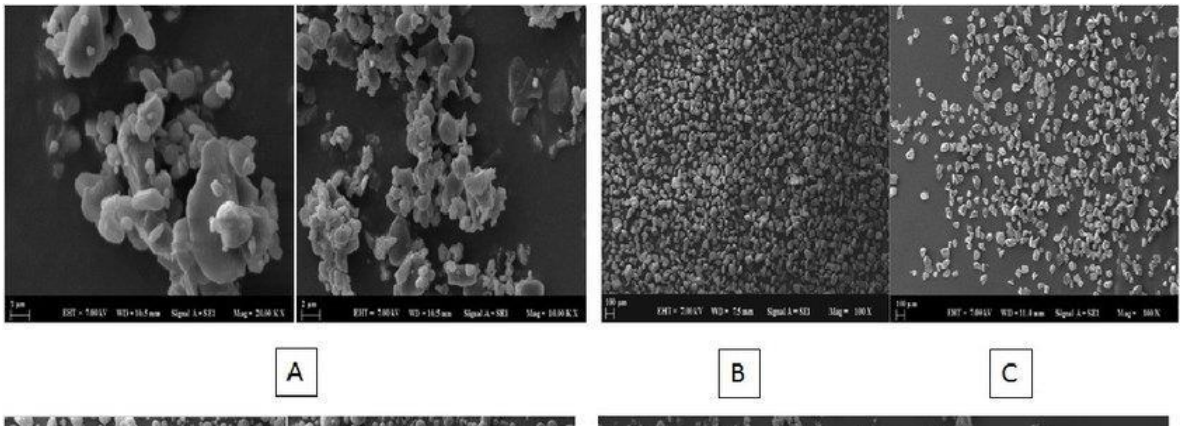


Fig. 9.11 SEM pictures of (A) APX; (B) Syloid® XDP 3150; (C) APX: Syloid® XDP 3150 (1:1).

9.5 In-vitro drug release study

The dissolution profiles of pure drug, marketed formulation and APX:SOL® physical mixture showed more than 85% drug release in 30 minutes indicating the immediate release profile wherein the drug release from the APX: SYL was found to be modified as 48% drug release was observed in 30 minutes and the complete drug release was found at the end of 300 minutes.

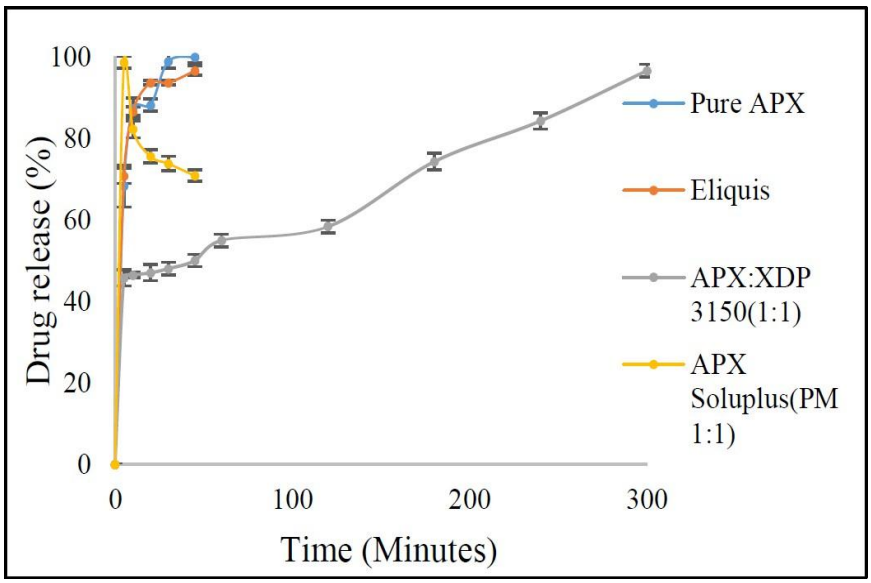


Fig. 9.12: In-vitro drug release profiles for pure drug, marketed formulation and prepared formulations.

9.6 Ex-vivo permeation study

Some of the previously reported literatures suggested that MSN might improve the permeability of APX and enhance the in-vivo pharmacokinetics by reducing the p-gp efflux(66). Though the permeability profiles of pure drug and prepared formulation predict that the MSN as such have no impact on the p-gp efflux transporter and permeability of the drug. In case of Soluplus there is marked improvement in the permeability of drug which indicates the p-gp efflux inhibitory properties of Soluplus. The permeability profile of Eliquis (marketed formulation) depicts increased permeability as compared to pure drug which could be subject to presence of SLS as an excipient which may act as a permeation enhancer (4).

Table 9.4: Flux values of pure drug, marketed formulation (Eliquis) & prepared formulations.

Sr.No.	Formulation	Flux (µg/cm2 / hr)	Fold improvement
1	Pure APX	2.9262	-
2	APX:SYL complex	2.9175	0.9970
3	APX:SOL® PM	4.1737	1.4262
4	Eliquis	5.0317	1.7195

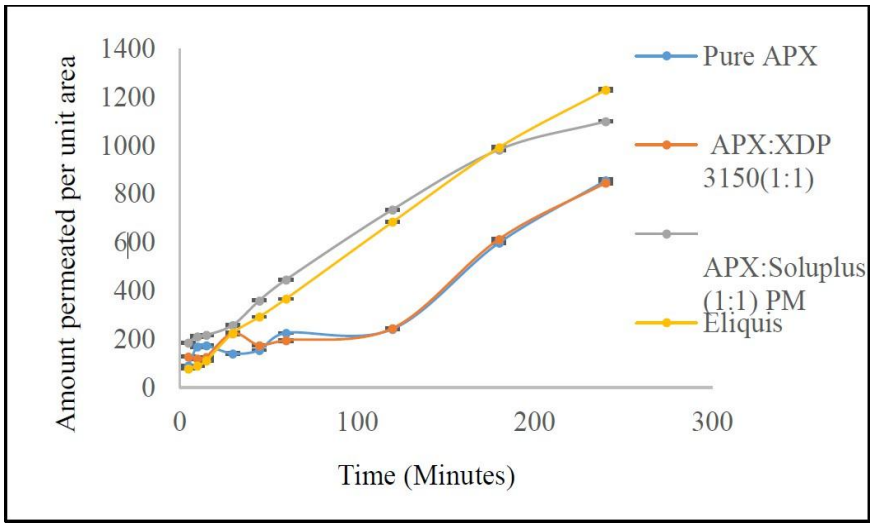


Fig. 9.13: Ex-vivo drug permeability profiles for pure drug, marketed formulation and prepared formulations.

9.7 APX calibration in plasma by HPLC

In the externally APX spiked plasma, calibration was performed in the concentration range of 0.1-100 µg/ml. The curve plotted has a regression coefficient of 0.9999. The **Fig.9.14** demonstrates the calibration curve in plasma.

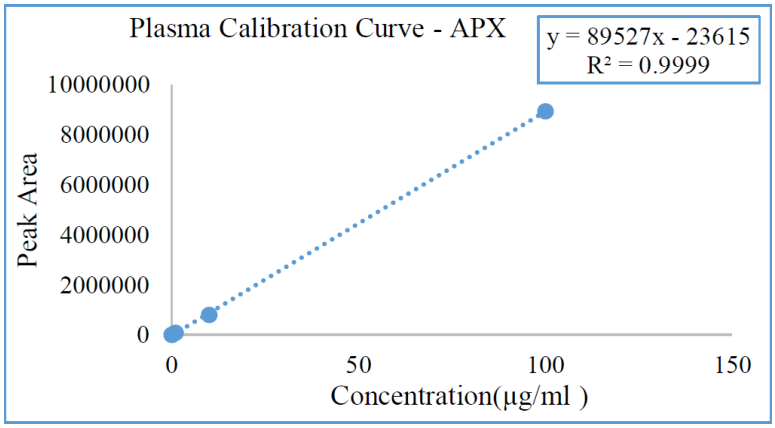


Fig. 9.14: APX calibration curve in plasma.

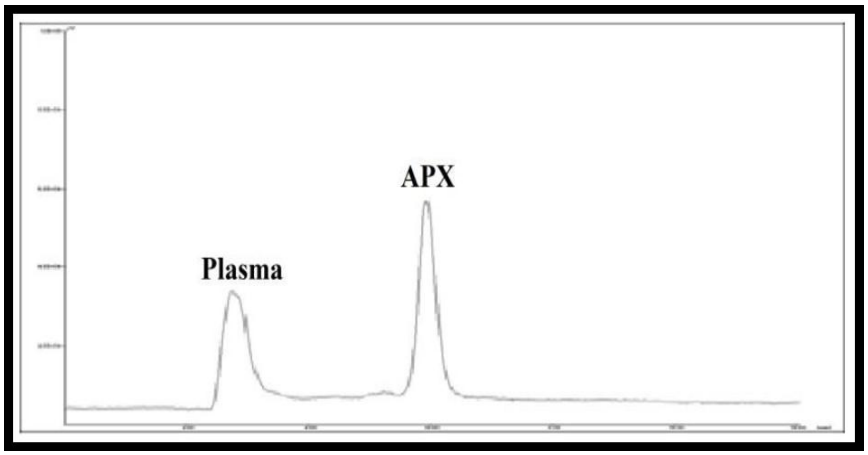


Fig. 9.15: Chromatogram obtained for 100ppm APX spiked plasma.

9.8 In-vivo pharmacokinetic study

Any formulation developed has to be evaluated for in-vivo pharmacokinetic behavior to understand how the formulation is going to behave in the body. Based on the results of solid characterization, dissolution study and permeability study, APX-SYL complex prepared by MM & physical mixture of APX-SOL® was chosen for an in-vivo study of bioavailability. **Figure 9.16** shows the mean plasma concentration-time profile of APX, optimized APX-SYL & APX-SOL® complex and marketed formulation in rats. The pharmacokinetic parameters were also determined and are summarized in **Table 9.5**. The Cmax, tmax and AUC0-24 was calculated using PK Solver software. The tmax was achieved in 4 hour for the APX control group, APX-SOL® complex & marketed formulation whereas it took 6 hours for the same in APX-SYL complex. The maximum concentration achieved in the plasma was 0.109 µg/ml in APX-SOL® physical mixture followed by 0.100 µg/ml in APX-SYL complex followed by 0.067 µg/ml in marketed formulation followed by 0.059 µg/ml in APX control group. 1.84 and 1.69 folds improvement in Cmax of APX:SOL® & APX:SYL complex was evident from this

study. The AUC0-24 was 3.55 and 29.15 times increased in APX-SOL® PM and APX-SYL complex respectively as compared to the pure APX. Wherein in comparison with marketed formulation the AUC0-24 of APX-SOL® PM was found to be 1.25 times higher. These results direct that the APX-SOL® complex shows improved BA as compared to pure drug which could be because of enhanced solubility of APX and successful inhibition of p-gp efflux & in case of APX-SYL complex the increase in BA could be attributed to enhanced solubility of the APX as well as modified release profile which must have increased the systemic exposure of the drug.

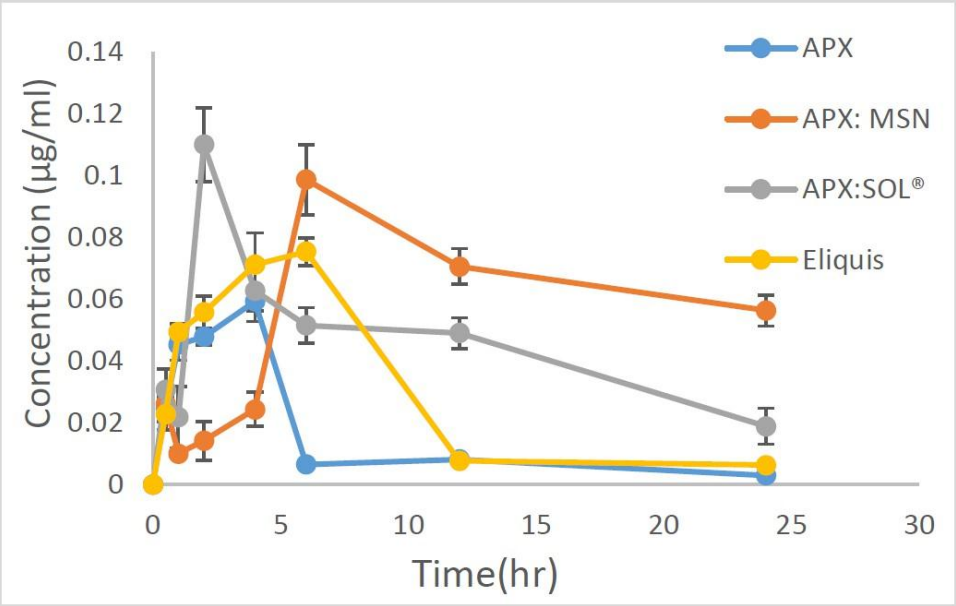


Fig. 9.16: APX plasma-concentration curve upon oral administration in rats (2 mg/kg).

Table 9.5: Pharmacokinetic Parameters of APX, prepared formulations and Eliquis® after oral administration to rats at 2 mg/Kg (Cmax: peak concentration; Tmax: time to reach peak concentration; AUC: area under the plasma concentration-time curve) Each value represents the mean ± standard deviation (n = 6). □ p < 0.05 compared withAPX powder.

Parameters	APX	Eliquis®	APX:Soluplus	APX:MSN
Cmax (µg/ml)	0.059 ± 0.74	0.067 ± 0.74	0.109 ± 0.74*	0.100 ± 0.74*
Tmax (h)	4.00 ± 1.00	4.00 ± 0.00	4.00 ± 0.11	6.00 ± 1.34*
AUC (h. µg/ml)	0.40 ± 0.16	1.13 ± 0.35	1.42 ± 0.68*	11.66 ± 0.88*
t½ (h)	12.52 ± 1.16	13.22 ± 1.23	11.69 ± 1.56	78.42 ± 1.65*
Kel(1/h)	0.055 ± 0.002	0.052 ± 0.003	0.059 ± 0.001	0.008 ± 0.001

9.6 Stability Studies

The optimized batch was subjected for stability storage condition (room temperature) for 3

month time period to study the impact of storage condition on dissolution profile. Drug release profiles were compared with the initial release. The stability study showed that there was no significant change in the release profile of formulation indicating no degradation of drug product.

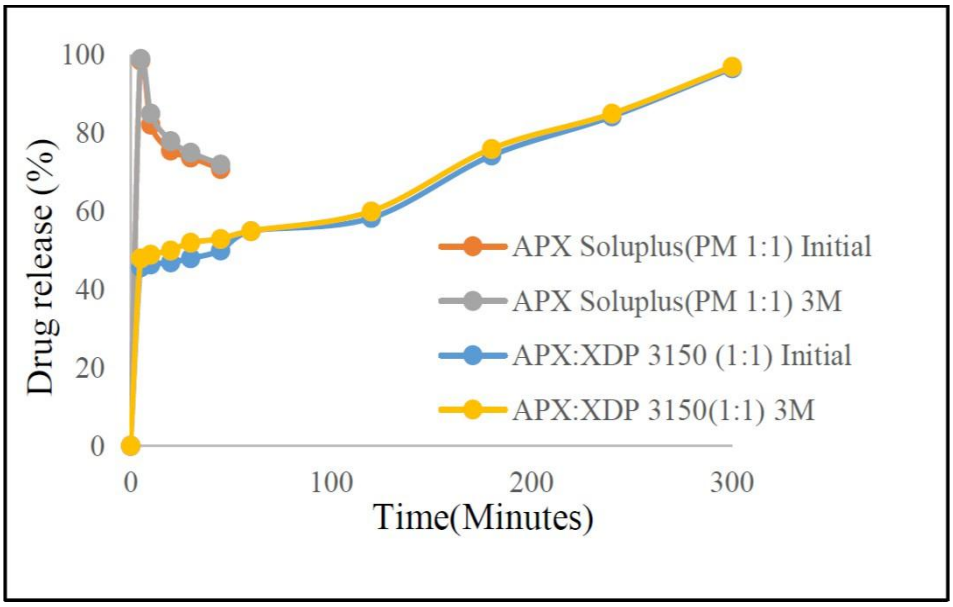


Fig. 9.17: Stability data of APX: Soluplus® and APX: XDP 3150- initial and after 3M.

10. SUMMARY AND CONCLUSION

- APX loaded Mesoporous silica complex were successfully prepared using melt method which improved the solubility of APX as compared to the pure drug.
- The physical mixture of APX and Soluplus® showed marked improvement in the solubility of APX as compared to the pure drug.
- From PXRD, DSC graphs, it is evident that complete amorphization was achieved in formulation prepared using melt method.
- The Dissolution profiles of APX (pure drug), Eliquis (Marketed formulation) and APX: SOL® physical mixture showed immediate release profile wherein the drug release from the APX: SYL complex was found to be modified.
- The permeability profiles of pure drug and prepared MSN complex indicates that the MSN as such have no impact on the p-gp efflux transporter and permeability of the drug. Wherein in case of Soluplus there is marked improvement in the permeability of drug which may be attributed the p-gp efflux inhibitory properties of Soluplus®.
- The observations of the pharmacokinetic studies reveal that though MSN have no impact on permeability of drug, there was marked improvement in the AUC of APX: SYL

complex because of the modified release of drug which increased the systemic exposure of drug for longer period of time. Also, in case of Soluplus® the AUC was improved as compared to the pure drug which may be attributed to improved solubility of drug and improved permeability because of p-gp inhibitory activity of the polymer.

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