

A Comparative Study of Approaches to Modulate the Pharmacotechnical Properties of BCS Class III Drug

Rakesh Rosan Kar, Lingaraj Mishra

Date of Submission: 01-01-2025

Date of Acceptance: 10-01-2025

I. 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 help full 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 maybe 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 VII 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, anti-haemophilic globulin and platelets. Factor VIIIa & factor IXa get together to form the factor IXa–factorVIIIa–phospholipidcomplex–akey activator of factor X. Factor Xa binds to negatively charged lipid 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 (Figure1.2).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-1 H- pyrazolo [3,4 c]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, cross carmellose sodium, sodium lauryl sulfate, and magnesium stearate. The components of film coating areas follows, lactose monohydrate, hypro mellose, titanium dioxide, triacetin, and yellow iron oxide (2.5mg tablets) or red iron oxide (5 mg tablets).

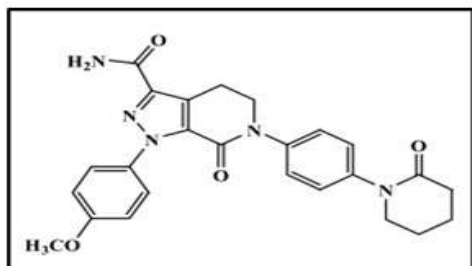


Fig.1.1 Structure of APX

1.3 Mechanism of Action(3)

The Factor X that is concerned within the

blood clotting process is hindered by APX and thus averts clot formation. It has an effect on each factor 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 inhibitor have predictable de-coagulant effects and don't need routine monitoring, not like another anticoagulants (Fig. 1.2).

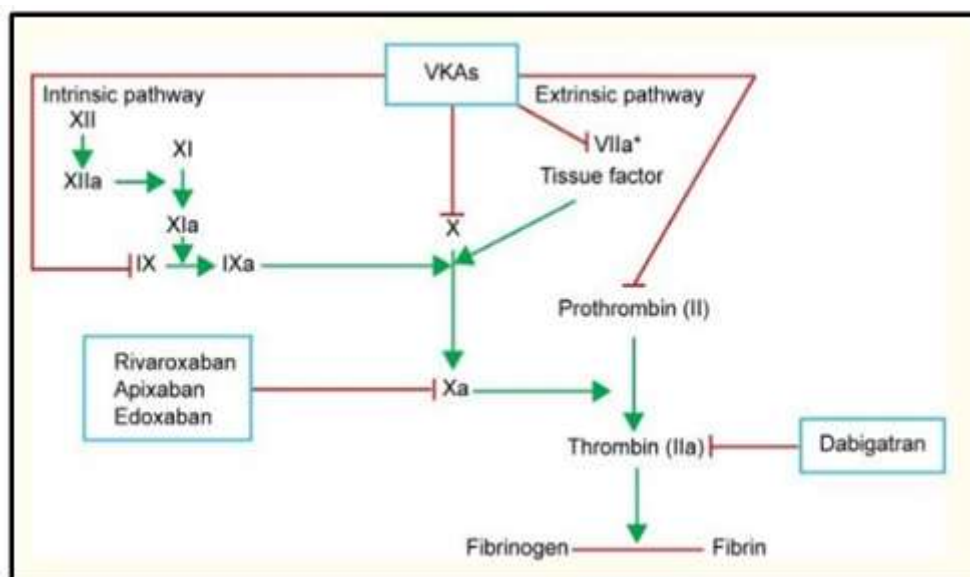


Fig.1.2: Mechanism of action of APX

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 lipo-philicity, 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's solubility and permeability through the intestinal cell layer. The classification is given in Table 1.1.

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 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 associated 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 with in 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 cephadrine, cefuroxime acetyl 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 grise of ulvinincitric 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.

1.6.1 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-absorptive metabolism by gut membrane-bound hemoprotein 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).

1.6.2 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).

1.6.3 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 there lease and enhance enteric permeability of the drug. The outcomes of in-vitro permeability unconcealed that drug-phosphatidyl choline 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 there to 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 nano pores 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 silicabased upon pore dimension

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

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 USFDA(32). In practice, the porous silicon dioxide and silicon materials diverge in their formulation techniques; porous silica materials are synthesized 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 like, comprising of siloxane groups(–SiO– 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 techniques 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 pores 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 alongside 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.

Super critical 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 its 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 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 porous 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 nano particles, the researcher have formulated 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. Zhanget. 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 adjacent 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 porous structure. Jianget

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 lipophilic 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 Songet al by altering the mesopores with a rotaxane moiety that was gated with an adamantane component conjugated

through an esterlink(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 polyvinylcaprolactam–polyvinylacetate–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 ap-gp efflux inhibitor in enhances the permeability of p-gp substrate drugs as well (40–42).

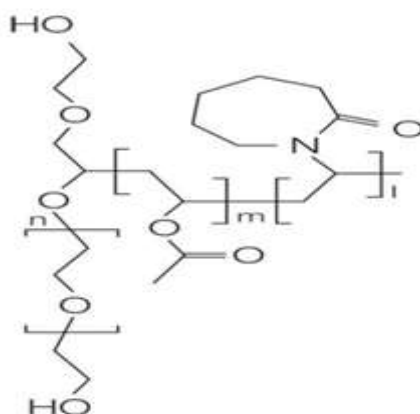


Fig1.3 Structure of Soluplus®

II. 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 pulmonary 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).

Frostet.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 Itraconazole 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 unlash

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 Nursing 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).

Nelet.al.(US2016/0008283A1)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)

Mekaet.al.(2018)studied the loading of BCS category IV drug Vorino stat 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%)comparedtoSyloidXDPthirty50thatshowedhighestandvirtuallycompletereleaseatintervals30min (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 anti tumour 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).

Sumitet.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)

III. 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®.

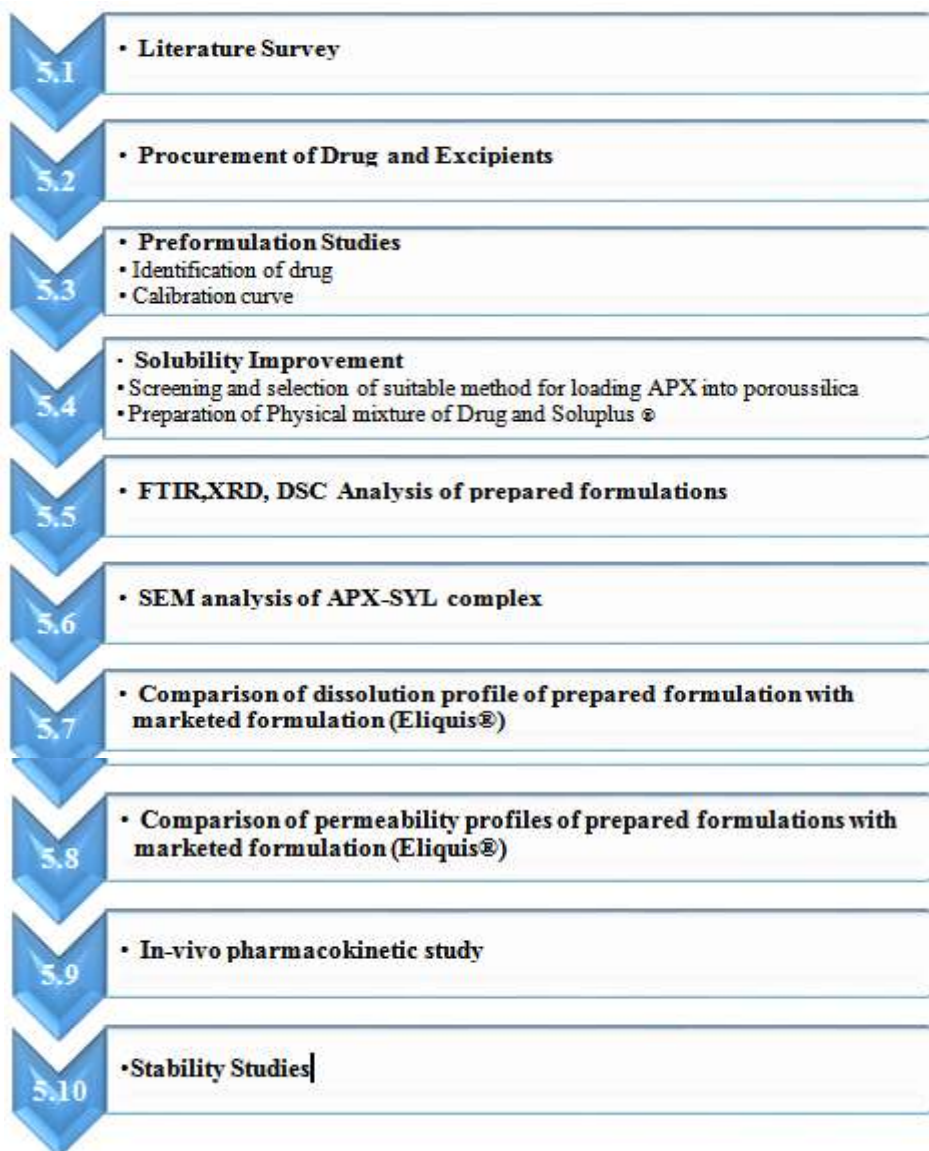
IV. AIMAND OBJECTIVES

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.

V. PLAN OF WORK



VI. DRUG AND EXCIPIENTS

PROFILE

6.1 Drug profile Apixaban(APX) Description

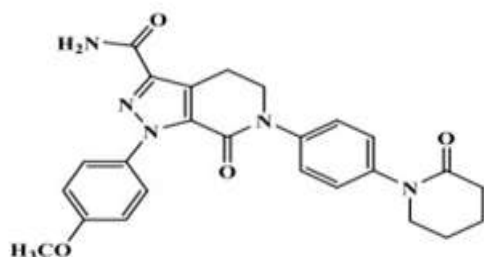
APX is an anticoagulant drug indicated to reduce the risk of stroke and systemic embolism in patients with nonvalvular atrial fibrillation. APX is an oral, direct, and highly selective factor Xa (FXa) inhibitor of both free and bound FXa, as well as prothrombinase, independent of antithrombin III for 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)
- **LogP:** 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.
- **t_{1/2}:** 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 5mg)
- **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: 1.7 cc/g.

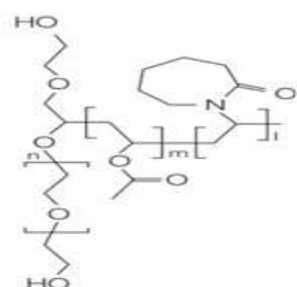
Regulatory status: Available in FDAs Inactive Ingredient list. Maximum strength permitted per

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 copolymer (PCL- PVAc-PEG)
- **Molecular weight:**1,18,000 g/mol
- **Glass transition temperature(Tg):**~70°C
- **Density:**1.082g/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.

VII. MATERIALS AND INSTRUMENTS

The following materials were used for the study.

Table7.1 Materials used in the study

Sr.No.	Material	Manufacturer
1.	Apixaban	Natco Pharma,India.
2.	Syloid®XDP3150	Grace Ltd, India
3.	Soluplus®	BASF India
4.	Micro crystalline cellulose	FMC Ltd.,India
5.	Lactose monohydrate	DFE Pharma,India.
6.	Croscar mellose sodium	Dupont, Germany.
7.	Magnesium stearate	Peter Greven,India.
8.	Sodium lauryl sulfate	Merck,India.
9.	Sodium dihydrogen phosphate	Lobachem PvtLtd, India
10.	Disodium hydrogen phosphate	Lobachem Pv tLtd, India
11.	Isopropyl alcohol	Merck, India
12.	N,N-Dimethylformamide	Merck ,India
Sr.No.	Material	Manufacturer
13.	Acetonitrile(HPLC grade)	Merck,India
14.	Milli porewater	In-house

The following instruments were used for the study

Table7.2Instruments used for the study

Sr.No.	Instrument	Manufacturer
1.	Tablet Compression	Karnavati, India
3.	Electronic Balance	Shimadzu,Japan

4.	Tap Density Tester(USP)	Electrolab, India
6.	Friabilator	Electrolab,India.
7.	Tablet Hardness Tester	3in 1tester,Incorp.
8.	Dissolution Test Apparatus USP type II	Electrolab, India.
9.	UV-Visible Spectrophotometer	Jasco,Japan
Sr.No.	Instrument	Manufacturer
10.	FTIR	Jasco,Japan
11.	Differential Scanning Calorimetry	TA Instruments,India
13.	X-rayDiffraction	Rigaku,India
14.	Melting apparatus	Labindia, India
15.	pH Meter	Thermo scientific,USA fisher
16.	HPLC	JascoJapan
17.	Centrifuge	Eppendorf,Germany
18.	Vortex mixer	Spinix

Apixaban COA


Natco Pharma Limited
Regd. O/E : NATCO PHARM, Road No. 2, Banjara Hills, Hyderabad-500 034.
 Telangana, INDIA. Tel : +91 40 23547552, Fax : +91 40 23548243
 CIN : L24230TG1981PL030301, www.natcopharma.com



CERTIFICATE OF ANALYSIS

Name of the Product : APIXABAN			
Grade : In-house		A/R. No. : GAP1230/16	
Batch No : ASN / 0030516		Date of Analysis : 12.05.2016	
Batch Size : 7.870 KG		Retest date : 03.11.2016	
Date of Manufacture : 04.05.2016		Page : 1 of 2	
Inspection Lot No : 049100181200			
S.No	TESTS	RESULTS	SPECIFICATIONS
1.0	Description	White crystalline powder	White to off-white powder.
2.0	Solubility	Complies	Sparsely soluble in dimethylformamide, insoluble in water.
3.0	Identification		
	3.1) IR Absorption spectrum	Complies	The sample IR spectrum should be concordant with that of reference standard IR spectrum.
	3.2) HPLC	Complies	The sample HPLC retention time should be concordant with that of reference standard HPLC retention time as obtained in test for Assay(HPLC).
	3.3) Polymorphism (by PXRD)	Complies	The sample XRD diffractogram should match with that of reference standard (Form N-1) XRD and should exhibit characteristic 2 theta values.
4.0	Loss on drying (At 105°C for 2 hours)	0.06 %w/w	Not more than 0.5 %w/w
5.0	Residue on ignition	0.02 %w/w	Not more than 0.1 %w/w
6.0	Heavy metals	< 10 ppm	Not more than 10 ppm
7.0	Related substances (HPLC)		
	a) Impurity-A	Not detected	Not more than 0.15 %
	b) Impurity-B	Not detected	Not more than 0.15 %
	c) Impurity-C	0.02 %	Not more than 0.15 %
	d) Impurity-D	Not detected	Not more than 0.15 %
	e) Impurity-E	Not detected	Not more than 0.15 %
	f) Unspecified Impurity	0.01 %	Not more than 0.10 %
	g) Total Impurities	0.05 %	Not more than 0.5 %

PREPARED BY Name: <i>P. Suresh</i> Date: 14-05-16	CHECKED BY Name: <i>P. Raju</i> Date: 14-05-16	APPROVED BY Name: <i>MCV REDDY</i> Gen. Manager-QC Date: 14-05-16
---	--	--

Manufacturing Site : Chemical Division (ISO 14001 Certified)
 Mekaguda (Village), Kothur (Mandal), Mahabubnagar Dist., Telangana, India, Pin : 509
 Tel : +91 8548 249493, 249496, Fax : +91 8548 249491

FOR INFORMATION ONLY



CERTIFICATE OF ANALYSIS

Name of the Product : APIXABAN			
Grade : In-house		A.R. No. : CAP9235/16	
Batch No : ABN / 0030516		Date of Analysis : 12.05.2016	
Batch Size : 7.870 KG		Retest date : 03.11.2016	
Date of Manufacture : 04.05.2016		Page : 2 of 2	
Inspection Lot No : 04000163300			
S.No	TESTS	RESULTS	SPECIFICATIONS
8.0	Assay (HPLC)	99.6 %w/w	Between 98.0%w/w and 102.0%w/w on dried basis
9.0	Residual solvents 9.1) Method-I (by HS-GC) a) Methanol b) Ethanol c) Acetone d) Isopropyl alcohol e) Methylene chloride f) Toluene g) Benzene 9.2) Method-II (by HPLC) a) Dimethylformamide	40 ppm Not detected Not detected Not detected Not detected Not detected Not detected Not detected	Not more than 2000 ppm Not more than 5000 ppm Not more than 5000 ppm Not more than 5000 ppm Not more than 600 ppm Not more than 890 ppm Not more than 2 ppm
Additional Test(s)			
10.0	Particle size d (0.5)	13.1 µm	Not more than 25 µm
SPECIFICATION REF NO: NPCD/QA/FP/SPIU135/NATCO(K)			
Remarks: The product conforms / Does not conform to the above specifications.			
PREPARED BY: <i>P. Srinivasulu</i>		CHECKED BY: <i>C. R.</i>	
Name: <i>P. Srinivasulu</i>		Name: <i>P. Srinivasulu</i>	
Date: 14.05.16		Date: 14.05.16	
		APPROVED BY: <i>McV Reddy</i>	
		Name: MCV REDDY Gen. Manager-QC	
		Date: 14.05.16	

VIII. 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. according to the specifications given in the certificate of analysis (COA).

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 ratio 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 1ml 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 (R^2 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:-

Arrange of final concentration of 2,4,6,8,10,12,14,16, 18,20,25 and 27 $\mu\text{g/ml}$ 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 2.7 ml solution

From the standard stock solution respectively and diluting them to 10 ml with distilled water. The graph of concentration ($\mu\text{g/ml}$) 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 $37 \pm 1^\circ\text{C}$. After 24 hours, the dispersion was filtered through a 22 μ 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 2 hr 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 ap-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 $37 \pm 1^\circ\text{C}$. 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 mini flex D600 diffractometer with $\text{CuK}\alpha$ radiation 1.54Å (0.154 nm). An acceleration voltage of 40 kV was used. Samples were scanned between 10° to $80^\circ 2\theta$ 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 DSC822e (Mettler, Toledo, and Giessen, Germany). The thermogram of pure APX was compared with APX: SYL matrix prepared using different methods. 4 mg 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 $10^\circ\text{C}/\text{min}$ from 30°C to 300°C . 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 where in 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 $37 \pm 1^\circ\text{C}$. 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 in1ml of distilled water And filled into the sactieda tone endusing a micropipette and another end of the sac was tie dusing a thread at1cm from the end. The permeation study was performed using a USP Dissolution type II apparatus with a paddle rotating speed of 100 RPM&250ml of Ringerssolutionat37±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 Bio analytical 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 integrate dusing 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×4.6mm,5µm)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 .Isocrati celution was carried out using mobile phase ratio(50:50v/v)of acetonitrile and water maintain data flow rate of 0.8mL/min. The UV/VIS detector was model operated at wavelength of 276 nm. The sample injection volume was20µl.Extractingsolvent(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:

- 150µlof plasma
- 150µlofdrugsolution
- 300µlofextractingsolvent

Thesesamplesineppendorftubeswerevortex edfor3min 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 of2.0mg/kg. Blood samples were obtained from there tro- 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 15,000rpm 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 onAnimals(CPCSEA)

(CPCSEA/PCP/PCT10/2020) and conducted in accordance with the CPCSEA guidelines. Pharmacokinetic parameters were quantified using PK Solver (version2.0).The maximum plasma concentration (C_{max})and the time to reach C_{max} (T_{max})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.

IX. 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 complying with COA.

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	Wave number(cm^{-1})
NH-stretch	3482.81
Aromatic ring	3313.11
Amide	1688.25

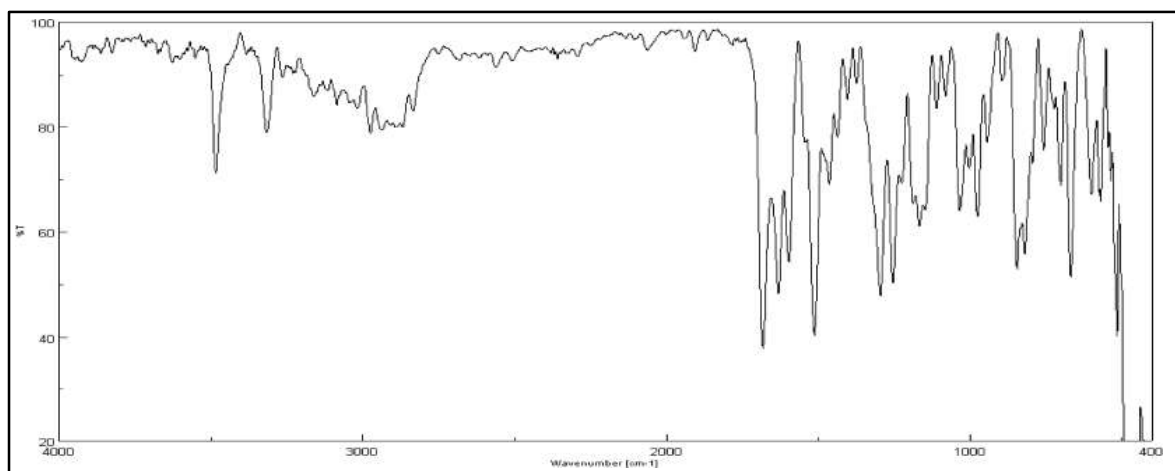


Fig.9.1 FTIR spectra of pure APX

9.1.1.3 Determination of wavelength

UV spectra of the solution of $10\text{ }\mu\text{g/ml}$ was recorded in the range of 400 – 200nm .The

maximum absorbance was found at 276 nm (**Fig. 9.2**).

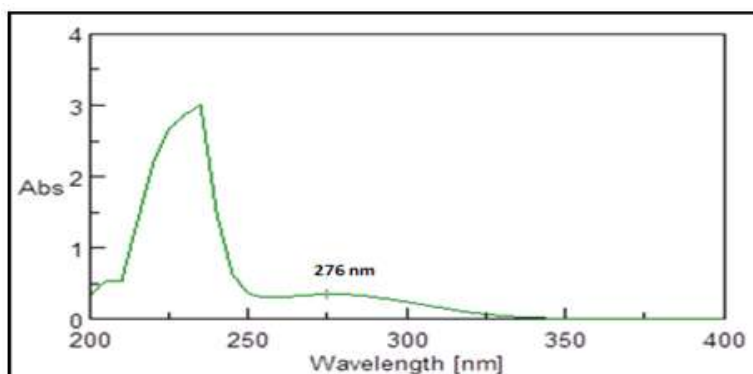


Fig.9.2Determination of $\lambda_{\max}$ of APX

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 $\mu\text{g/ml}$ in distilled water and 2- 32 $\mu\text{g/ml}$ in 0.05M Sodium phosphate buffer pH 6.8 with 0.05% SLS respectively at 276 nm obeying Beer-Lambert's law.

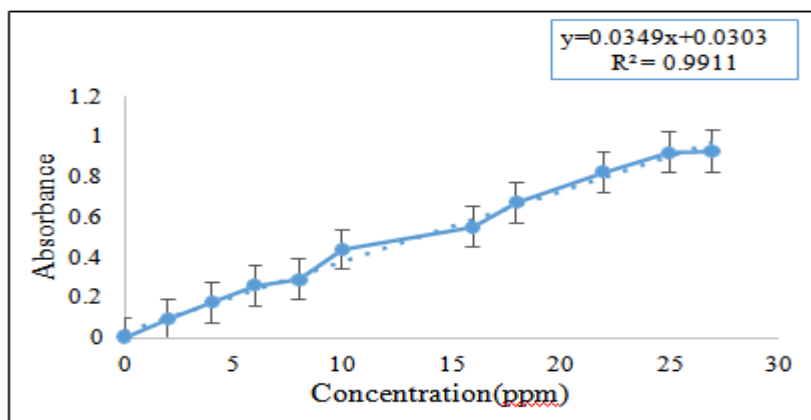


Fig.9.3Calibration curve in distilled water

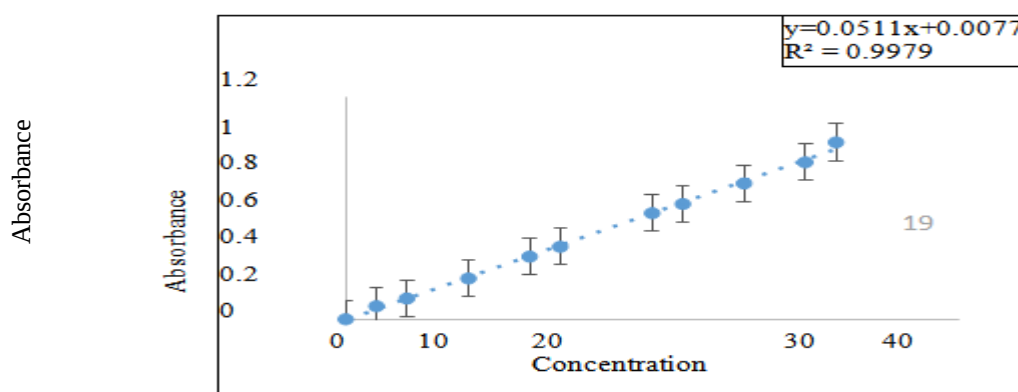


Fig9.4Calibration curve in 0.05M Phosphate buffer pH 6.8 with 0.05% SLS

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.

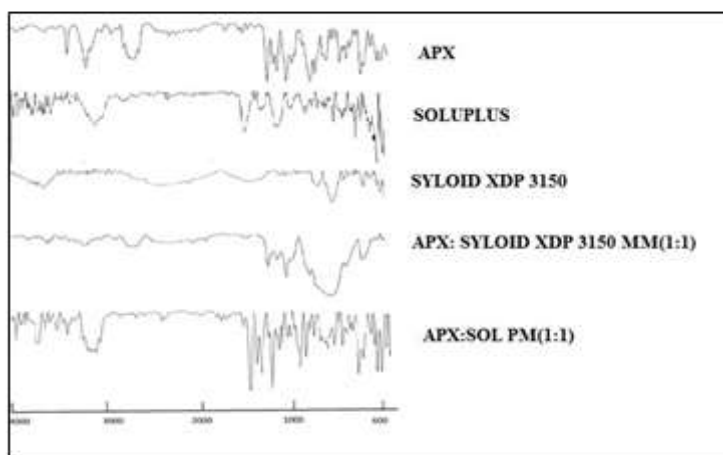


Fig 9.5 FTIR graph for compatibility study

Table 9.2 Drug and excipient compatibility study

Sr. No	Sample	Initial Observation	After 40°C/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: XDP3150(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 (6.5).

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 (pH 1.2)	24.15 ± 0.59
3	pH 4.5 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 ± 0.94

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) where in 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).

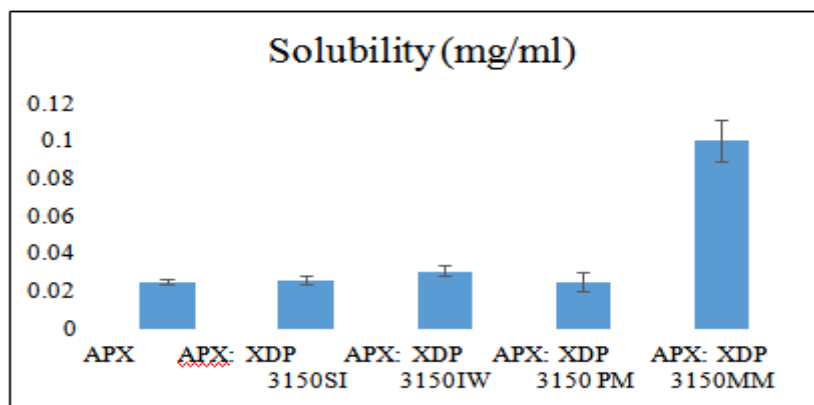


Fig 9.6 Solubility enhancement using different drug loading methods

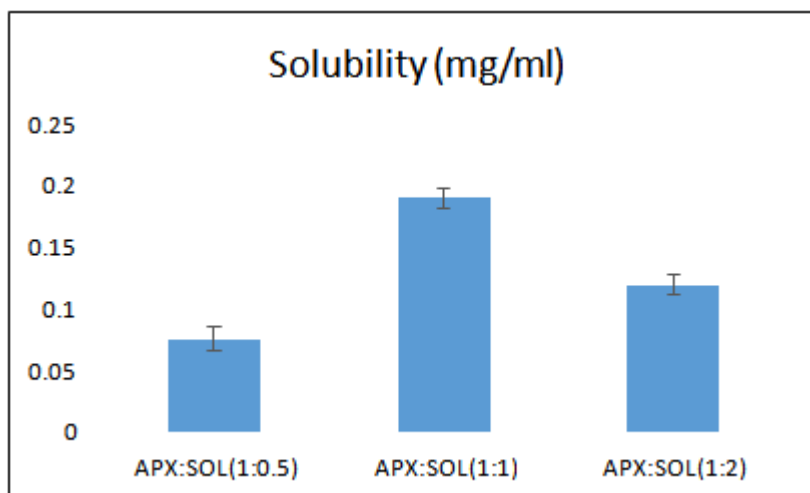


Fig 9.7 Solubility enhancement using different ratios of drug & Soluplus®

9.3.2 X-ray powder diffraction (XRPD) analysis

The X-ray diffract gram 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).

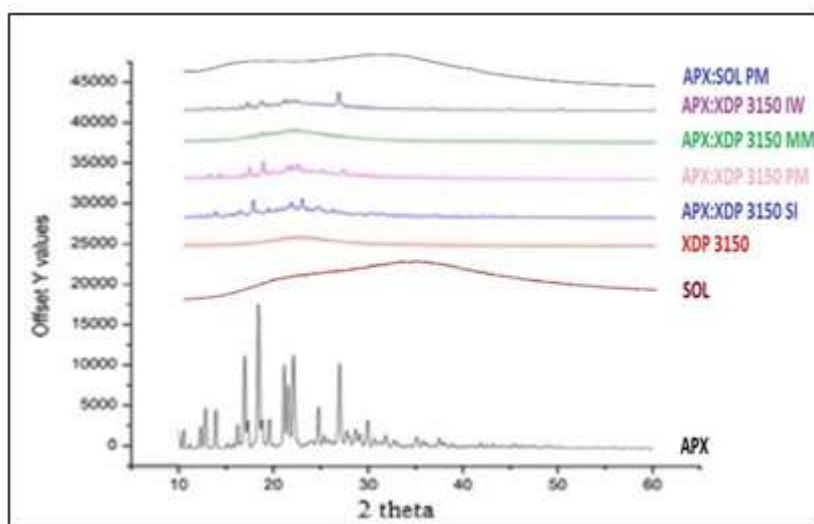


Fig9.8 PXRD graphs of pure drug and formulated complexes

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.

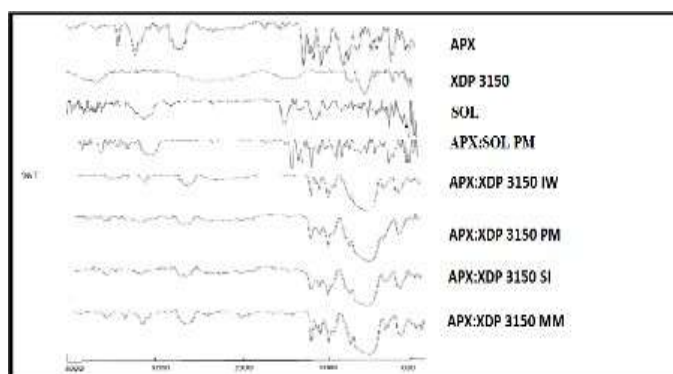


Fig9.9FTIR spectra of pure drug and formulated complexes

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 crystal in nature of the drug. The thermogram of Syloid XDP3150&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.

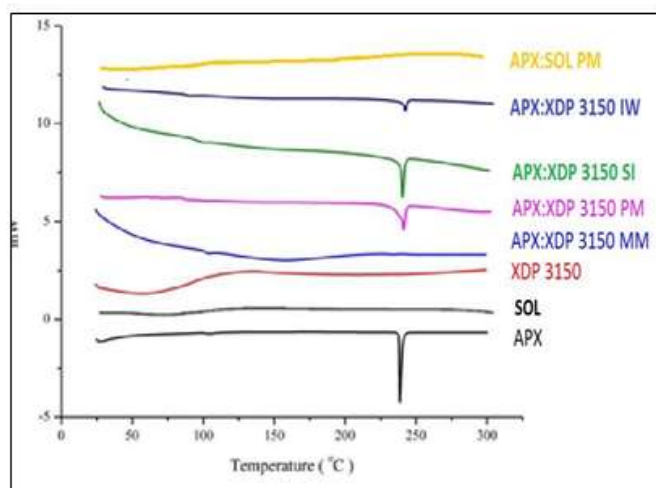


Fig 9.10 DSC thermograms of pure drug and formulated complexes

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.

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.

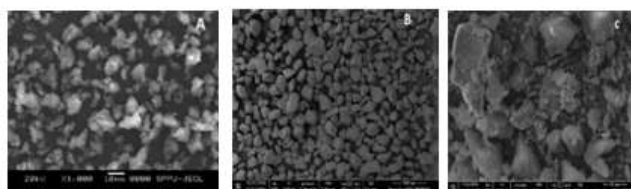


Fig.9.11 SEM pictures of (A) APX; (B) Syloid® XDP 3150; (C) APX: Syloid® XDP 3150 (1:1)

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 completed drug release was found at the end of 300 minutes.

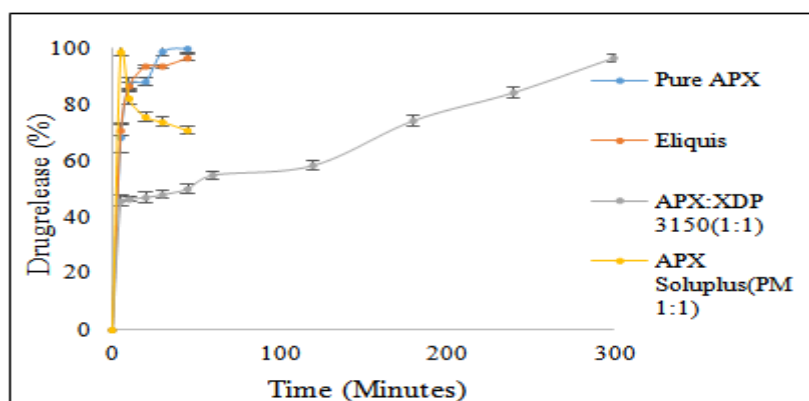


Fig9.12 In-vitro drug release profiles for pure drug, marketed formulation and prepared formulations

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($\mu\text{g}/\text{cm}^2/\text{hr}$)	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

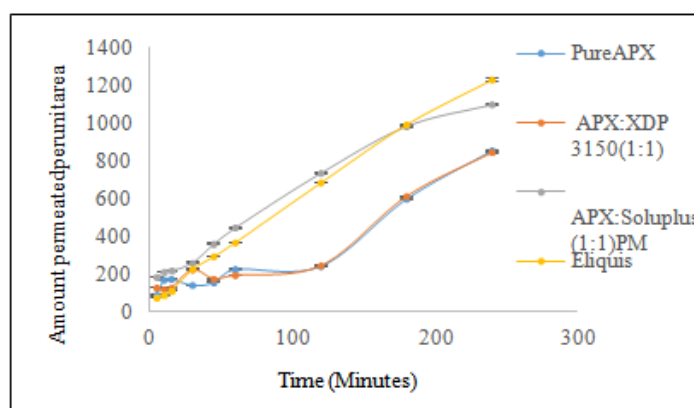


Fig 9.13 Ex-vivo drug permeability profiles for pure drug, marketed formulation and prepared formulations

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 $\mu\text{g}/\text{ml}$. The curve plotted has a regression coefficient of 0.9999. The **Fig.9.14** demonstrates the calibration curve in plasma.

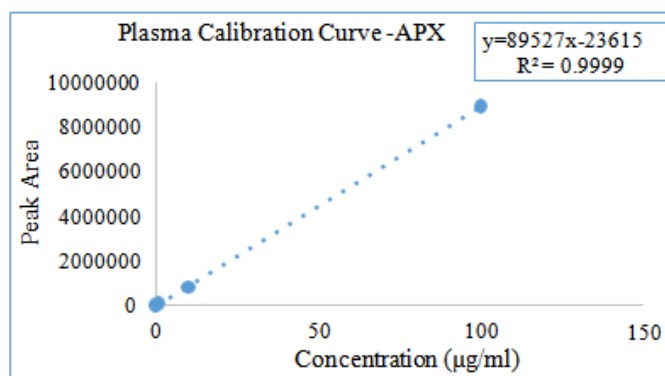


Fig.9.14APX calibration curve in plasma

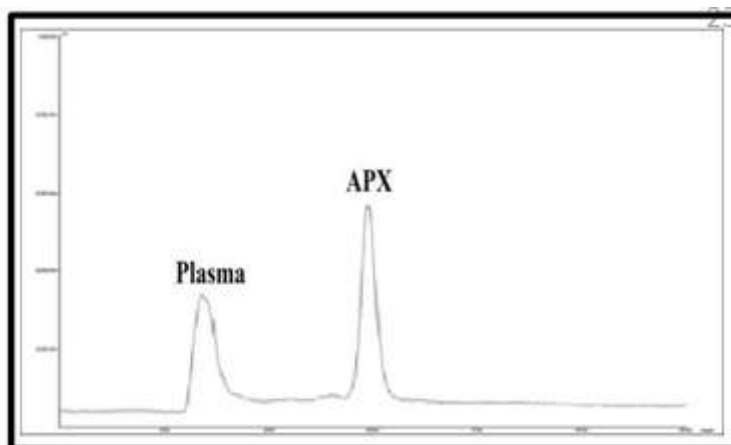


Fig.9.15 Chromatogram obtained for 100 ppm APX spiked plasma

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 C_{max} , t_{max} and AUC_{0-24} was calculated using PK Solver software. The t_{max} 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 $\mu\text{g/ml}$ in APX-

SOL® physical mixture followed by 0.100 $\mu\text{g/ml}$ in APX-SYL complex followed by 0.067 $\mu\text{g/ml}$ in marketed formulation followed by 0.059 $\mu\text{g/ml}$ in APX control group. 1.84 and 1.69 folds improvement in C_{max} of APX:SOL® & APX:SYL complex was evident from this study. The AUC_{0-24} 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 AUC_{0-24} 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.

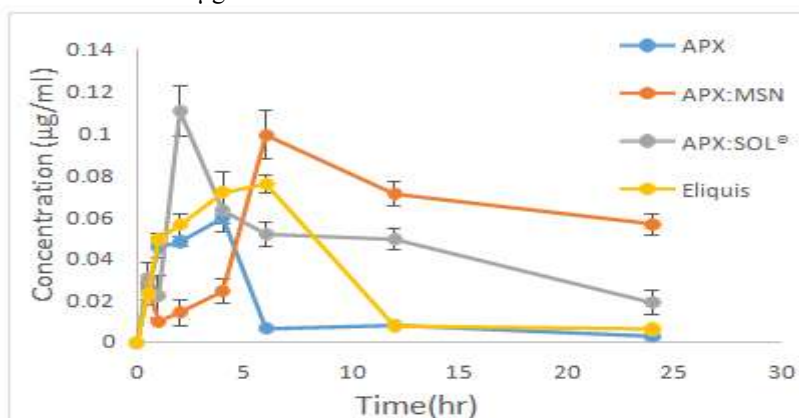


Fig.9.16: APX plasma-concentration curve upon oral administration in rats (2 mg/kg)

Table 9.5 Pharmacokinetic Parameters of APX, prepared formulations and Eliquis® after oral administration to rats at 2mg/Kg (C_{max}: peak concentration; T_{max}:time to reach peak concentration; AUC: area under the plasma concentration-time curve)

Parameters	APX	Eliquis®	APX: Soluplus	APX:MSN
C _{max} (µg/ml)	0.059± 0.74	0.067± 0.74	0.109± 0.74*	0.100± 0.74*
T _{max} (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*
t _{1/2} (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

Each value represents the mean± standard deviation(n=6). □ p<0.05comparedwithAPXpowder.

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 there lease profile of formulation indicating no degradation of drug product.

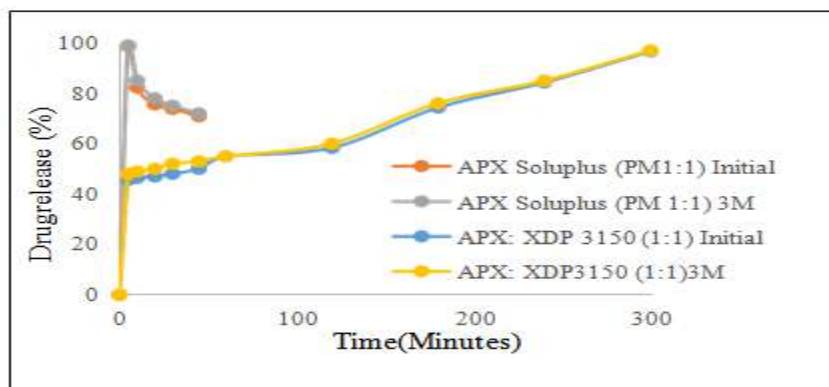


Fig.9.17Stability data of APX: Soluplus® and APX: XDP 3150- initial and after 3M

X. 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.

REFERENCES

- [1]. NameD.ListofFactorXainhibitors.Drugs.com[Internet].2021;1–2.Availablefrom:<https://www.drugs.com/drug-class/factor-xa-inhibitors.html>
- [2]. Apixaban |C25H25N5O4 - PubChem[Internet]. [cited 2021 Jul30].Availablefrom:<https://pubchem.ncbi.nlm.nih.gov/compound/Apixaban>
- [3]. Apixaban: a blood-thinning medicine to treat and prevent blood clots - NHS [Internet]. [cited 2021 Jul 30].Availablefrom: <https://www.nhs.uk/medicines/apixaban/>
- [4]. Alsenz J, Kansy M. High throughput solubility measurement in drug discovery and development ☆. 2007;59:546–67.
- [5]. Blagden N, Matas M De, Gavan PT, York P. Crystal engineering of active pharmaceutical ingredients to improve solubility and dissolution rates ☆. 2007;59:617–30.
- [6]. Papich MG, Martinez MN. Applying biopharmaceutical classification system (BCS) criteria to predict oral absorption of drugs in dogs: challenges and pit falls.The AAPS journal.2015Jul;17(4):948–64.
- [7]. Merisko-liversidge E, Liversidge GG, Cooper ER. N anosizing: a formulation approach for poorly-water- soluble compounds. 2003;18:113–20.
- [8]. Jain S, Patel N, Lin S. Solubility and dissolution enhancement strategies: Current understanding and recent trends. Drug Dev Ind Pharm. 2015;41(6):875– 87.
- [9]. Mosharraf M,Nyström C.The effect of particle size and shape on the surface specific dissolution rate of micro sized practically insoluble drugs. Int J Pharm. 1995;122(1–2):35–47.
- [10]. Keck CM, Müller RH. Drug nanocrystals of poorly soluble drugs produced by high pressure homogenisation. EurJPharm Biopharm.2006;62(1):3– 16.
- [11]. Nanoparticle Technology for Drug Delivery| Taylor & Francis Group[Internet].[cited2021Jul30].Available from:<https://www.taylorfrancis.com/books/edit/10.1201/9780849374555/nanoparticle-technology-drug-delivery-ram-gupta-uday-kompella>
- [12]. Zhang J, Shen Z, Zhong J, Hu T, Chen J, Ma Z, et al. Preparation of amorphous cefuroxime axetil nanoparticles by controlled nanoprecipitation method without surfactants. 2006;323:153–60.
- [13]. Rasenack N, Steckel H, Mu BW. Preparation of microcrystals by in situ micronization. 2004;144:291– 6.
- [14]. NII-ElectronicLibraryService.Chiou WL, Riegelman S.Preparation and dissolution characteristics of several fast-release solid dispersions ofgriseofulvin.JPharmSci.1969;58(12):150 5–10.
- [15]. Afifi SA, Hassan MA, Abdelhameed AS, Elkhodairy KA. Nanosuspension: An Emerging Trend for Bioavailability

- Enhancement of Etodolac. 2015;2015.
- [16]. Kedzierewicz F, Hoffman M, Maincent P. Comparison of tolbutamide / 3-cyclodextrin inclusion compounds and solid dispersions Physicochemical characteristics and dissolution studies. 1990;58:221–7.
- [17]. Remenar JF, Morissette SL, Peterson ML, Moulton B, Macphee JM, Guzman R. Crystal Engineering of Novel Cocrystals of a Triazole Drug with 1, 4-Dicarboxylic Acids. 2003;8456–7.
- [18]. Aakeroy CB. Crystal Engineering: Strategies and Architectures. 1997;1997:569–86.
- [19]. Agency EM. ICH guideline Q3C (R6) on impurities: guideline for residual solvents. 2019;31(June 2017).
- [20]. Chakraborty A, Bhattacharjee A, Dutta D, Mukhopadhyay G. A Review on Some Formulation Strategies to Improve the Bioavailability of Drugs with Low Permeability and High Solubility (BCS III). 2016;(February 2017).
- [21]. Rege BD, Kao JPY, Polli JE. Effects of nonionic surfactants on membrane transporters in Caco-2 cell monolayers. 2002;16:237–46.
- [22]. Moghassemi S, Hadjizadeh A. Nanoniosomes as nanoscale drug delivery systems: An illustrated review. J Control Release [Internet]. 2014;185:22–36. Available from: <http://dx.doi.org/10.1016/j.jconrel.2014.04.015>
- [23]. Attia IA, El-gizawy SA, Fouda MA, Donia AM. Influence of a Niosomal Formulation on the Oral Bioavailability of Acyclovir in Rabbits. 2007;8(4):1–7.
- [24]. Ara HH De, Sayer C. Solid lipid nanoparticles for encapsulation of hydrophilic drugs by an organic solvent free double emulsion technique. 2015;
- [25]. Mirchandani Y, Patravale VB, Brijesh S. Solid lipid nanoparticles for hydrophilic drugs. J Control Release. 2021 Jul 10;335:457–64.
- [26]. El-Leithy ES, Nasr M, El-Moneum RA. Development and characterization of solid lipid dispersion as a delivery system for hydrophilic antihypertensive drug atenolol. Int J Drug Deliv. 2012;4(2):219–28.
- [27]. Chen T, Yang J, Chen L, Qian X, Zheng Q, Fu T, et al. Use of ordered mesoporous silica-loaded phyto-phospholipid complex for BCS IV class plant drug to enhance oral bioavailability: a case report of tanshinone IIA. RSC Adv. 2016;6(116):115010–20.
- [28]. Pawar JN, Desai HR, Moravkar KK, Khanna DK, Amin PD. Exploring the potential of porous silica as a carrier system for dissolution rate enhancement of artemether. Asian J Pharm Sci [Internet]. 2016;11(6):760–70. Available from: <http://dx.doi.org/10.1016/j.ajps.2016.06.002>
- [29]. McCarthy CA, Ahern RJ, Dontireddy R, Ryan KB, Crean AM. Mesoporous silica formulation strategies for drug dissolution enhancement: A review. Expert Opin Drug Deliv. 2016;13(1):93–108.
- [30]. Arruebo M. Drug delivery from structured porous inorganic materials. Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology. 2012 Jan;4(1):16–30.
- [31]. Inactive Ingredient Search for Approved Drug Products.
- [32]. Patel VI, Dave RH. Evaluation of colloidal solid dispersions: Physicochemical considerations and in vitro release profile. AAPS Pharm Sci Tech. 2013;14(2):620–8.
- [33]. Bharti C, Nagaich U, Pal AK, Gulati N. Mesoporous silica nanoparticles in target drug delivery system: A review. International journal of pharmaceutical investigation. 2015 Jul;5(3):124..
- [34]. Barkat A, Beg S, Panda SK, S Alharbi K, Rahman M, Ahmed FJ. Functionalized mesoporous silica nanoparticles in anticancer therapeutics. Semin Cancer Biol [Internet]. 2021;69(August):365–75. Available from: <https://doi.org/10.1016/j.semcancer.2019.08.022>
- [35]. Keasberry NA, Yapp CW, Idris A. Mesoporous silica nanoparticles as a carrier platform for intracellular delivery of nucleic acids. Biochem. 2017;82(6):655–62.

- [36]. Wang M, Wang T, Wang D, Jiang W, Fu J. Acid and light stimuli-responsive mesoporous silica nanoparticles for controlled release. *J Mater Sci* [Internet]. 2019;54(8):6199–211. Available from: <https://doi.org/10.1007/s10853-019-03325-x>
- [37]. An X, Zhu A, Luo H, Ke H, Chen H, Zhao Y. Rational Design of Multi-Stimuli-Responsive Nanoparticles for Precise Cancer Therapy. *ACS Nano*. 2016;10(6):5947–58.
- [38]. Linn M, Collnot EM, Djuric D, Hempel K, Fabian E, Kolter K, et al. Soluplus® as an effective absorption enhancer of poorly soluble drugs in vitro and in vivo. *Eur J Pharm Sci* [Internet]. 2012;45(3):336–43. Available from: <http://dx.doi.org/10.1016/j.ejps.2011.11.025>
- [39]. Tanida S, Kurokawa T, Sato H, Kadota K, Tozuka Y. Evaluation of the micellization mechanism of an amphipathic graft copolymer with enhanced solubility of ipriflavone. *Chem Pharm Bull*. 2016;64(1):68–72.
- [40]. Metre S, Mukesh S, Samal SK, Chand M, Sangamwar AT. Enhanced Biopharmaceutical Performance of Rivaroxaban through Polymeric Amorphous Solid Dispersion. *Mol Pharm*. 2018;15(2):652–68.
- [41]. Bao S, Zhang Y, Ye J, Zhu Y, Li R, Xu X, et al. Self-assembled micelles enhance the oral delivery of curcumin for the management of alcohol-induced tissue injury. <https://doi.org/10.1080/10837450.2021.1950185> [Internet]. 2021 Jul 13 [cited 2021 Jul 22];1–10. Available from: <https://www.tandfonline.com/doi/abs/10.1080/10837450.2021.1950185>
- [42]. Richard G., Jansen M., Dosage forms of Apixaban. International Patent number WO 2010/147978 A1. Issued on 23 December 2010
- [43]. Frost C, Us PA, Jia J, Mead B, Us NJ, Vemavarapu C. (12) United States Patent. 2018;2(12).
- [44]. Kinnari P, Mäkilä E, Heikkilä T, Salonen J, Hirvonen J, Santos HA. Comparison of mesoporous silicon and non-ordered mesoporous silica materials as drug carriers for itraconazole. *Int J Pharm*. 2011;414(1–2):148–56.
- [45]. Uejo F, Limwikrant W, Moribe K, Yamamoto K. Dissolution improvement of fenofibrate by melting inclusion in mesoporous silica. *Asian J Pharm Sci* [Internet]. 2013;8(6):329–35. Available from: <http://dx.doi.org/10.1016/j.ajps.2013.11.001>
- [46]. Chen Y, Li L, Yao J, Chen J, Lu T. Improving the Solubility and Bioavailability of Apixaban via Apixaban – Oxalic Acid Cocrystal. 2016;1(Scheme 1).
- [47]. Santosh B, Neeraj R. Increasing the Oral Bioavailability of. 2016;2016(2):86–95.
- [48]. Data RUSA, Angeles L, Zink JJ, Angeles L, Meng H, Angeles L. (12) Patent Application Publication (10) Pub . No . : US 2016 / 0008283 A1. 2016;1(19).
- [49]. Meka - Enhanced Solubility, Permeability and Anticancer Activity of Vorinostat Using Tailored Mesoporous Silica Nanoparticles _ Enhanced Reader.pdf.
- [50]. Waters LJ, Hanrahan JP, Tobin JM, Finch CV, Parkes GMB, Ahmad SA, et al. Enhancing the dissolution of phenylbutazone using Syloid® based mesoporous silicas for oral equine applications. *J Pharm Anal* [Internet]. 2018;8(3):181–6. Available from: <http://dx.doi.org/10.1016/j.jpha.2018.01.004>
- [51]. Xi Z, Zhang W, Fei Y, Cui M, Xie L, Chen L, Xu L. Evaluation of the solid dispersion system engineered from mesoporous silica and polymers for the poorly water soluble drug indomethacin: In vitro and In vivo. *Pharmaceutics*. 2020 Feb;12(2):144.
- [52]. Liong, M.; Lu, J.; Tamanoi, F.; Zink, J.I.; Nel, A. Mesoporous Silica nanoparticles for biomedical applications. U.S. Patent 20100255103A1, Issued on 7 October 2010.
- [53]. Piao J, Lee J-Y, Weon JB, Ma CJ, Ko H-J, Kim D-D, et al. Angelica gigas Nakai and Soluplus-Based Solid Formulations Prepared by Hot-Melting Extrusion: Oral Absorption Enhancing and Memory Ameliorating Effects. Lu J, editor. *PLOS ONE*. Public Library of Science; 2015;10:e0124447.

- [54]. EMA. Assessment report for Eliquis. 2011;44(EMA/H/C/002148 Assessment):62. Available from:http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_Public_assessment_report/human/002148/WC500107726.pdf
- [55]. Inhibitors P, Howell D, Zaremski L, Fisher J, Ferrick K, Krumer A. Bleeding Risk with Apixaban when Using Cardiac. 2018;2(October):61–5.
- [56]. Sciences G D. Syloid Silica. 2014;1–8.
- [57]. Administration USF and D. Inactive Ingredient Guide database. 2020.
- [58]. Information T. Technical Information Soluplus®. 2019;(September 2015):1–14.
- [59]. Dissolution Methods [Internet]. [cited 2021 May 6]. Available from: https://www.accessdata.fda.gov/scripts/cder/dissolution/dsp_SearchResults.cfm
- [60]. Bothiraja C, Pawar AP, Dama GY, Joshi PP, Shaikh KS. Journal of Pharmacological and Toxicological Methods Novel solvent - free gelucire extract of Plumbago zeylanica using non-inverted rat intestinal sac method for improved therapeutic efficacy of plumbagin. J Pharmacol Toxicol Methods [Internet]. 2012;66(1):35–42. Available from: <http://dx.doi.org/10.1016/j.vascn.2012.05.007>
- [61]. Mandpe L, Kyadarkunte A, Varsha P. High-performance liquid chromatographic method for simultaneous determination of iloperidone and idebenone in spiked plasma. Int J Chem Anal Sci. 2013;4(1):19–23.
- [62]. Dhapte Vividha, Kadam Vivek PV. Determination of pyrimethamine in rat plasma by liquid chromatography and its application to pharmacokinetics. J Liq Chromatogr Relat Technol. 2013;36:304–14.
- [63]. Veszelszki Á. Q1F Who. Digilect [Internet]. 2017;(February 2003):309–52. Available from: https://database.ich.org/sites/default/files/Q1F_Stability_Guideline_WHO_2018.pdf
- [64]. Pharmacology C. CENTER FOR DRUG EVALUATION AND CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW (S).
- [65]. Zhang Y, Wang J, Bai X, Jiang T, Zhang Q, Wang S. Mesoporous silica nanoparticles for increasing the oral bioavailability and permeation of poorly water soluble drugs. Mol Pharm. 2012;9(3):505–13.