"Lipid-Based Nano-Delivery for Oral Administration of Atazanavir : Design , Optimization and Characterization "

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BY

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UNDER THE GUIDANCE OF

DR. Shital Butani (Guide)

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CERTIFICATE

*This is to certify that the dissertation work entitled "**Lipid-Based Nano-Delivery for Oral Administration of Atazanavir : Design ,Optimization and Characterization**" submitted by Ms. **SEVAK KAJOL DIPAK** with Regn. No. (18mph105) in partial fulfillment for the award of Master of Pharmacy in "Pharmaceutics" is a bonafide research work carried out by the candidate at the Department of Pharmaceutics, Institute of Pharmacy, Nirma University under my/our guidance. This work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.*

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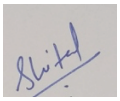

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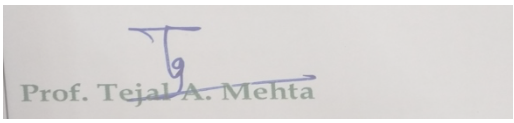

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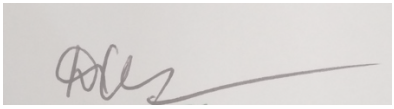

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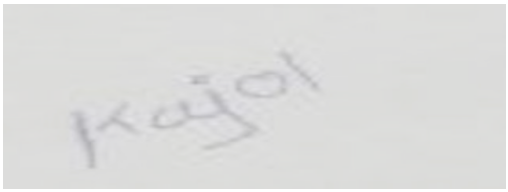
DECLARATION

I hereby declare that the dissertation entitled "*Lipid-Based Nano-Delivery for Oral Administration of Atazanavir : Design ,Optimization and Characterization*" *is based on the original work carried out by me under the guidance of Dr. shital Bhutani , Associate Professor, under the Department of Pharmaceutics, Institute of Pharmacy, Nirma University. I also affirm that this work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.*



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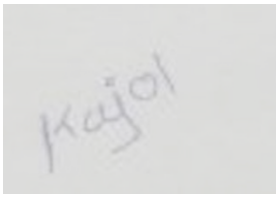
In providing concrete shape of my thesis. There are numerous people who have taken part. I take this opportunity to express my deep sense of gratitude and hearty thanks to my guide Dr. Shital Bhutani madam for her valuable guidance and support for the project. I am heartily thankful to her for providing me valuable suggestions and remarks to complete this project. Their extensive knowledge of subject and the way, they imparted the same to me has enabled me to develop the thesis in cohesive manner.

At last but not least, I would like to express my heartiest thanks to my family and friends to extend their help as and when required.



SEVAK KAJOL DIPAK.

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Lipid-Based Nano-Delivery for Oral Administration of Atazanavir : Design ,Optimization and Characterization

Abstract: -

In the world there are millions of people suffering from the HIV positive. There are various medications are available for the HIV, which includes the various antiretroviral treatment having the safety. Antiretroviral are given orally so also there is possibility of the drug interaction. Lipid carries used to solubilize the very poorly soluble drug. Lipids with the short chain, long chain medium chain were used as oil phase. The Atazavair is the class four drug having the very solubility so the purpose of this study is to check the solubility of the drug Atazanavir sulfate with the various lipids. This lipid carrier was formulated an evaluated with the particle size, zeta potential, drug loading. Materials and methods: - Various types of oils and surfactant, co-surfactant was used for the solubility study like capmul, transcutol, labrazol. The phase diagram was used for the study.

[1] Aim/Objective of the Work

Now a day, Humans are getting infectious by the various known and unknown disease. Acquired immune deficiency syndrome(AIDS) which is caused by the Human Immunodeficiency virus. HIV is the virus which demolish the immune system of the body and damage and weakens the other functions of the human body. In the HIV infection AIDS is the most critical level in the body. This infection can be spread through the various body fluids like blood donation, semen, breast milk etc.

The records from the world Health Organization established that the HIV is the most dangerous disease in the world, in 2014 the world wide around 1.2 million people were died due to cause of AIDS infectious illness and at that same time around 37 million people were suffering from the HIV disease as per world health organization data.

It's is challenging to control the increasing the infection of the HIV disease day by day. There are various drug are available in the market but it's not much as effective although the surgery cannot be done. So, the increasing the effectiveness of the drug is the wide option now a day to control the infection of the HIV in the body which helps to improve the immune system strong than the destroying it.

The various marketed formulations are available which are in the form of powder, tablets, capsules, sachets but there is no Nano emulsion are available in the officially market formulation.

So, our goal of this study is to develop the Lipid based Nano-emulsion having the good stability and to give it by the oral system.

[2] Introduction

Human Immune Deficiency virus, which affects and destroys the immune system of the human body. This Virus is also known as the "Lentiviruses". This Lenti Viruses comes from another virus category called "Retroviruses" This Retroviruses are different from the other viruses because of their gene structure comprises of RNA. The meaning of the Lentivirus is "slow virus" so, the adverse effect of this virus comes after very such a long time to the human body. Generally, the Human Immune Deficiency virus is classified into two types which are HIV-1and HIV-2. HIV -2 virus is not easily sprayed and duration of the early infection and symptoms long lasting in this case. So, the worldwide superior virus is HIV -1, with this it's refer as the HIV without any specification type of it. HIV-2 is rarely found in the region of the west Africa or somewhere else.[1]

[2.1] HIV

1) HIV-1

Detection or the symptoms of the HIV virus was first found out in 1981in the United states by the scientist in the young man later on it was found that the infected HIV pregnant woman infect the infant. HIV is the disease which demolishes the immune system of the body and it majorly affects the CD4+ T cells. The amount of the CD4+ T cells gets decreases by the infection in the body. Earlier in the 1980 the symptoms were found more in the Africa and also in other parts of the Europe countries. At that time the HIV Virus was known as the "Lentivirus".

In 1984 the other viruses were recognized by the scientist "JAY LAVY". In the lente virus and AIDS there were some same properties and symptoms which was correlated with the Retroviruses. So, there were three types of prototypical viruses lente virus, Human T cell leukemia virus and retroviruses. These three viruses were belonging from the family "Lentivirinae". These group of viruses is called as "HIV" by the International Committee on Taxonomy of Viruses. In the west Africa another virus was found and named HIV-1 and the earlier was named as HIV-2.[2]

So, there are two types of viruses are there: HIV-1 AND HIV-2.

2) HIV-2

In “Cape Verde” which is located in the west Africa where people were found to have the symptoms having the AIDS ritonavir’s which was of HIV-2 virus. Also, in the “Senegal” the more patients were having the aids indications, more than 55% of the patients were isolated having the HIV-2 virus.

The HIV-1 and HIV-2 viruses are very similar but there is small difference in their genome structure. Genome which contains DNA which is made up of too many of proteins in their structure. The dissimilarity in both HIV is, in HIV-2 virus there is the presence of protein Vpx and there is an absence of the protein called Vpu in its structure.

The antibodies of the human body get reacts with these viruses in the body. Generally, the antibodies cross responds to the HIV-2 virus proteins Gag and Pol but not identifies the HIV-1 virus in the body. Though the HIV-2 virus covers up the glycoproteins of it and try to respond it with the SIV. SIV is the simian immunodeficiency virus, which is small group of viruses, from the lentiviruses. So, we can say that HIV-2 virus was also originated from the simian immunodeficiency viruses.[2]

[2.2] HIV STRUCTURE

Similar to the other viruses the HIV virus having the proteins in its structure, the diameter of the virus is in the range of 100-200nm. From the cell membrane buds of the virus particle got out side and forms the circle with the lipid bilayer having the Glycoprotein 120 and glycoprotein 41 covering the other glycoproteins of it. The viral membrane and the nucleoside are covered by the missile under which the Gag matrix and other proteins like MA, p17 gets formed.

The interface takes place between the RNA and the protein like Gag. In the structure of the RNA there are two strands in which one is RNA-dependent and another one id DNA polymerase. There are so many other proteins are present in the HIV structure like 1) Vif 2) Nef 3) Vpr 4) Vpx. These proteins are present in the virions and also the these all are responsible for the infection in the human body. Here are other proteins are also present in the virus structure which are as 1) Actin 2) Ezrin 3) Emerin 4) Moesin 5) Coflin.

As discussed earlier the structure of the HIV-1 and HIV-2 is mostly the same, but LTR which is long terminal repeats are same in the structure. N-terminal in the RNA structure which alters the Gag protein which was integrated on the cytosolic ribosomes. Alteration done by the N-terminal which is responsible for the more interface with the membrane. The Long terminal repeats are surrounded by the transcriptase sequences, RNA signals and also the integrating and packaging sites.[2]

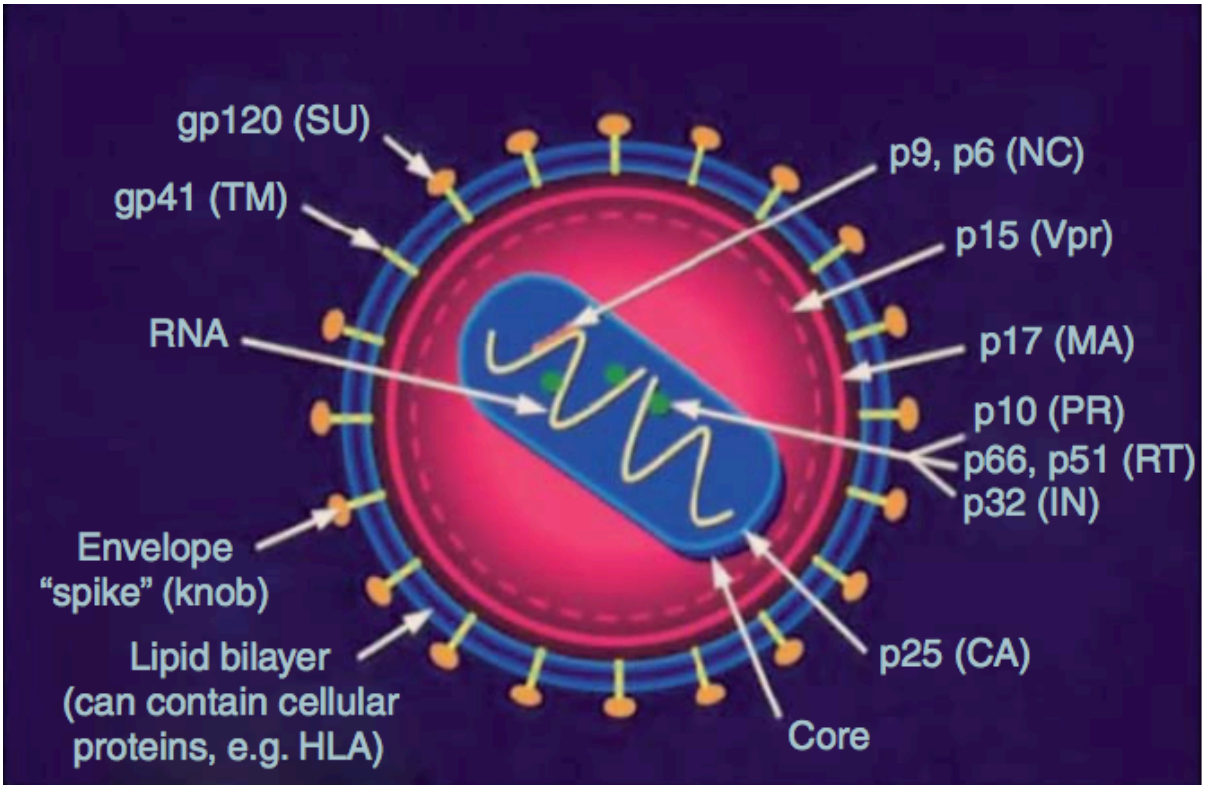


Figure 1

This figure shows the HIV virus structure with its identified proteins.

[2.3] HIV Life Cycle: -

The HIV life cycle is divided into main three phase. [3]

1) Entry and Integration

2) Transcription and Translation

3) Budding

[2.3.1] Entry and Integration

In body there are various T cells presents, T cells get infected by HIV infection, so infection spread in all over the body. Interface done between the both the protein and the CD4+ T cells which provide the infection to the body. The interacting protein is gp120. Sometime the interface not only the way for the entry of the cells of HIV. In the virus there are various other proteins are presents which represent as the co-receptors and will bind to the chemotic peptide, which is called as the "CHEMOKINES".

The capacity of the protein gp120 is different, based on the capacity of the protein gp120 it will bind to the different chemokine receptors of the body. The complex formed is divided based on its binding to the chemo receptors, so it is classified into the two groups as below:

1) Series of the Monocytes cells are having the chemokine receptors named CCR5, which generally gets bind to the gp120 protein.

2) Lymphocytes cell line contains the CXCR4 chemokine receptors, these receptors also bind with the HIV virus proteins.

The mammalian immune system is having the antigen presenting cells, which are known as the dendritic cells. These cells bind upper surface has the lectin and that bind to the virus surface and form the cluster of the virus particle, which get the other cells infectious.

The CCR5 and CXCR4 both chemokine receptors are the primary receptors. The Monocytes and Lymphocytes both the cell line are very important in the understanding of the pathogenesis. The inconsistency of the virus protein lead to involve it in many other ways.

The transmembrane protein gets exposed when virus with the CD4+T cells and chemokines receptors gets attached with each other and change there is conformational change in the structure get happened. The transmembrane molecules are having the various colloidal chain structure in it, with hydrophobic and hydrophilic part, but on the hydrophobic part which allows permeation into the target cell membrane. This approach leads the virus to cover the cell

membrane and then binding happens. After binding the cell cytoplasm contains the virus plasmid into it. Alteration of the diploid RNA into the dsDNA started by the capsid of the virus by the reverse transcriptase process. Also, at that time the genome is carried to the nucleus. Without dividing the nucleus cell and get into the nucleus, to desegregate into it successfully can only done by the lentiviruses. Whereas the other viruses require the dissolution of the nucleus membrane which allow them to get into nucleus by dividing the nucleus. Entry into the nucleus achieved by the transport signal which are 1) p17 matrix protein 2) integrase enzyme 3) Vpr protein.

Into the DNA staggered cut made up by the cellular chromosome to implant virus DNA of the integrase virus.

[2.3.2] Transcription and translation

After the incorporation of the virus to the nucleus the, it behaves as the common cellular gene. mRNA is produced by the RNA polymerase II. After getting integrated the virus as not active, it is in the inactive state of it, silently the transcription of the virus is done and in the latent stage the viruses get activate. While the transcription instigates, there are wide size of RNA gets produces. During the RNA transcription there are many variable sequences got formed, which has the short message to the proteins Tat and Rev, which are of the RNA encoding in all RNA infection. After the translation get completed the mRNA moved to the cytoplasm and the proteins moves back to the nucleus. [4]

Tat-Tat proteins are the widely responsive proteins which leads to the interaction and increase the viral RNA production. Rev proteins gathers and cooperate with the other rev proteins which and transferred to the cytoplasm. In the cytoplasm this translated mRNA to construct other structural and the enzymatic proteins which are necessary to produce the other viral particles in the body. Tran scripted Rev proteins cooperate with the nucleus and its proteins to produce the other transport RNA in the cytoplasm.

[2.3.3] Budding

Before glycoproteins are transferred to the cell surface, the glycoproteins interpreted on the endoplasmic reticulum and then administered by the Golgi contraption. After getting on the cell surface these Glycoproteins combat the other gag protein which had been transformed upon the unrestricted ribosomes in the cytoplasm. Same way the other capsid of the virus incorporates into the nucleus and then encompass the RNA molecules, these transcript viruses

and the covered glycoproteins of the surface interact and then produce the other Gag proteins, replicative enzymes and other auxiliary viral proteins.

[2.4] Antiretroviral therapy for HIV

The person with the HIV should receive for viral symptom suppression, this antiretroviral therapy is the most effective and important treatment. Antiretroviral therapy is recommended to the patients having the following symptoms: [5]

1) opportunistic infection 2) immune reconstitution inflammatory syndrome 3) drug interaction

US department of the "Health and Human services" this all treatment for the patient with the high CD4 amount in body. There is very less side effect in the 1st line treatment with the antiretroviral therapy, which consist 1 or 2 pills. The patients having the history with treatment of the HIV should reviewed genotype and phenotype results of it and then select antiretroviral drugs. There is some limitation while giving the antiretroviral dug to patients because patients with having less amount of the CD4 + T cells counts in body cannot give some specific drugs. The amount of the CD+T cells in the body should not be less than 200 cells/mm.

In this type of cased of having less CD+4 T cell count drugs like Rilpivirine and also the combination of the drug like 1) Darunavir + Raltegavir 2) Lamivudine + Dolutegravir cannot be given. Some time there are many failures seen with the other combinations given to the patients given 1) abacavir + lamivudine which also the coalesced with the other combination 2) efaviren + boosted atazanavir. But drug-drug interaction takes place with the combination treatment in the patients and produce the toxicities in the patients. It not only affects the drug interaction but also the other disease will also have affected with the antiretroviral treatment.

[2.4.1] Goals and the Principals of the Antiretroviral Therapy: - [5]

1) Convalesce and increase the immunity level of the immune system of the body by growing the CD4+T cells in the body

2) Elongate Persistence

3) decrease the HIV related illness

4) Decrease the Transmission of the HIV

[2.5] History of Advantages of Antiretroviral therapy since 1987-2014

1) NRTI Monotherapy (1987-1993)[6]

In this therapy the patients with the HIV had better-quality of survival and decrease the infection in the body, but no increase in the amount of CD4+Tcells.

2) Dual NRTI Therapy (1993-1996)

This therapy had the more decrease in the viral infection but also increase the toxicities to the body.3

3) PMTCT (1994)

This therapy was given with drug zidovudine in different patients and different route. 1) it was given in the pregnant woman by oral route 2) it was given by the IV routes to the labor 3) to the children by the oral route.

4) 2-NRTI treatment

This is also known as the highly active antiretroviral therapy and also HAART. This therapy decreased the amount of the HIV RNA in the body and increase the CG4+ T cells, which increases prolong the survival.

5) Ritonavir active pharmaceutical ingredient (1990- early 2000)

Ritonavir is the widely used drug which has the potency to increase the bioavailability of the protease kinase inhibitors so it is use with the blocking agent to the protease kinase inhibitors.

6) Second generation antiretroviral drug (2003-2008)

This second-generation antiretroviral drug includes 1) Tenofovir 2) Tipranavir 3) Darunavir 4) Etravirine. This all second-generation was found to increase the potency of the earlier drug efficiency.

7) First fusion inhibitors (2003)

Enfuvirtide as the fusion inhibitor was approved for the multi drug resistance in the HIV. This drug was given in combination with the T-20 to check the background treatment, which results into the decreasing the amount of the HIV RNA in the patients. This combination was given by the IV route so it had some limitation of it.

8) Atripla (2006)

As its name this treatment was given in the three-drug combination was 1) Efavirens 2) Tenofovir 3) Emtricitabine. These three drugs were approved. Combination of these drugs reduce the frequency of the daily dose of the drug to the patients.

9) CCR5 Antagonist (2007)

The CCR5 antagonist approved was the maraviroc. First it was given based on the testing and need to the people and then given to the naïve patients.

10) Raltegravir (2007)

This drug belongs to the first INSTI approved for the HIV for multi drug resistance. It decrease the amount of the HIV RNA in the body but had some resistance so after that it was given to the naïve patients.

11) Antiretroviral used as the prevention of transmission to the uninfected persons (2011)

In this therapy, study was done based on the controlled and uncontrolled group of the patients of the HIV, based on that conclusion was that the antiretroviral therapy decreases the transmission of the infection to the other person.

12) Pre-exposure of the prophylaxis with tenofovir plus emtricitabine

Tenefovir was approved by the US FDA but after the constant exposure of this drug leads to the high risk to the patients with the HIV negative so the earlier antiretroviral therapy was used.

[2.5.1] Targets of the anti-retroviral therapy approved by the US FDA

1) CCR5 Antagonist [7]

Drug: - Maraviroc

2) Fusion Inhibitors

Drug: - Enfuvirtide

3) NRTI

Drugs: - Zidovudine, Didanosine, Zalcitabine, Stavudine, Lamivudine, Abacavir, Tenofovir, Emtricitabine

4) NNRTI

Drugs: - Nevirapine, Delavirdine, Efavirenz, Etravirine, Rilpivirine

5) INSTI

Drug: - Ralteegravir, Elvitegarvir, Dolutegravir

6) Protease kinase Inhibitors

Drugs: - Saquinavir, Indinavir, Ritonavir, Nelfinavir, Amprenavir, Lopinavir, Fosaamprenavir , Atazanavir , Tipranavir, Darunavir

[2.6] Nucleoside Reverse Transcriptase Inhibitors

This nucleoside inverse Tran scripted inhibitors are the widely used and oldest therapy used in the antiretroviral therapy. In this treatment the phosphorylation takes place which stimulates the both the diphosphate and triphosphate which metabolites and gives the deoxyribonucleoside triphosphate. This deoxyribonucleoside triphosphate get amalgamated into the DNA structure by the reverse transcriptase, which is not having the 3-OH group in its structure so there will no chain formation between the other formed deoxyribonucleoside triphosphate. Human immune deficiency virus has developed the resistance against the nucleoside reverse transcriptase inhibitors by differentiating the NRTI similarity and some time by removing the phosphorylation done into the DNA structure. This process is known as the thymidine similarity mutation. Cytochrome P450 enzymes cannot metabolized the NRTIS so drug- drug interaction takes place between the other drugs in the body, which develops the side effects in body like 1) Lactic acidosis 2) Hepatic Steaosis. These side effects are common in all the NRTIs.[8]

1) Abacavir

Abacavir is the similar to the guanine. Abacavir is completely get absorbed and metabolized with the regular dosage of 300mg two times a day. The combination given with the lamivudine and dolutegravir. As there are side effects of the Abacavir like hypersensitivity, myocardial infraction.

2) Lamivudine and Emtricitabine

Since there are many similarities in the structure of the Lamivudine, Emtricitabine so they are similar to the cytosine. But the Emtricitabine structure contains the fluorine in its structure. Generally, the Emtricitabine is given in the combination with the Tenofovir and Disoproxil. Addition of the fluorine to the structure can be done and also be removed. In the Hepatitis B disease, the both the drug used. The regular dose of the lamivudine is 25 mg but it can be used up to 150 mg dose.

3) Tenofovir

Tenofovir is similar to the adenine. Different 2 prodrug are available of tenofovir which are Tenofovir disoproxil and tenofovir alafenamide and tablets of tenofovir with this combination are available in market in the single tablet form. Toxicity of the tenefovir disoproxil is the nephrotoxicity beacusse of the high amount of TDF in the (Tenofovir disoproxil) in the blood.

4) Zidovudine

Zidovudine is similar to the thymidine. The dosage form of the Zidovudine is Intravenous Formulation, tablets and also the suspension form is available in the market. There are too many side effects of the Zidovudine like Headache, Nausea, Fatigue and also the risk of the bone marrow suppression so it's no widely used drug. This drug is given in the combination with the Lamivudine. [9]

[2.7] NNRTI (Nonnucleoside Reverse transcriptase inhibitors)

To the reverse transcriptase the NNRTIS get impasse to the HIV virus and attach to the dNTPS. There is various new second generation drug established to maintain the activity to the increased level of mutation to NRTIS. Between Doravirine and Rilpivirine cross resistance occurred. Rashes is the most common side effect of the all the NNRTIS. Also, the Nevirapine having the most dangerous side effect called "Johnson syndrome".[8]

1) Efavirenz

Dosage form of the Efavirenz available in the market is capsule and tablet with the regular dose of the 600 mg. Metabolism of Efavirenz is done by CYP3A4 and CYP2B6. Taking this drug with the milk it increases the absorption of drug through the intestine, but there are major side effect are produced so it is given on empty stomach before the meal.

2) Rilpivirine

This drug comes from second generation of NNRTIS. The dosage form of Rilpivirine is tablet and regular dose is 25 mg per tablet. This tablet is given in combination with 1) Emtricitabine 2) TDF 3) TAF and 4) dolutegravir. The drug is metabolized by the CYP3A4 enzymes in the intestine. If it's given with the food which increases its bioavailability to the body.

3) Doravirine

The newest 2nd generation NNRTIS drug is Doravirine. Generally, it is given in the oral dosage form by the tablet of the daily dose of 100 mg per day. It is given with the combination of lamivudine and Tenofovir Disoproxil. This Doravirine gets metabolized by the CYP3A4 enzyme. There is no change in bioavailability if it is given with food or without food. [10]

[2.8] Integrase Inhibitors: -

These integrase strand transfer inhibitors get attach to the magnesium moiety which are present on the integrase enzyme, which counter act with the DNA and prevent the entry of the DNA into the cellular DNA. As we know Magnesium is the very good chelating agent and get chelated by the cations calcium, aluminum, Zinc and iron. Chelating decreases the absorption of the integrase inhibitors and this problem solved by decreasing the chelation of the magnesium ion by taking drug with the food into the body. The weight gain occurs by taking the integrase inhibitors.[8]

1) Raltegravir

This is the 1st drug approved by the FDA for INSTI. It is given in oral dosage form tablet with daily dose of 400 mg a day and also available in the 600 mg dose. Raltegravir is not digested by the enzymes called CYP but it goes for the glucuronidation by uridine diphosphate glucuronosyltransferase, which increases the amount of creatine kinase. Raltegravir has the less resistance but need to take the drug three times a day which is little disadvantage. As per the guidelines of "US Department of Health and Human Services" (DHHS) it is the 1st line of drug for the pregnant woman.

2) Dolutegravir

It is 2nd generation INSTI and given by oral dosage form tablet with the dose of 50mg, 10mg, 25mg tablets are available in the market. It is given with the combination of 1) abacavir 2) lamivudine 3) rilpivirine. Dolutegravir completely absorbed by glucuronidation and sometime moderately metabolized by CYP3A4 enzyme. It is the blocking agent of organic cation transporter2.

3) Bictegravir

It is also the 2nd generation INSTI and given in oral doage form tablets. It is given in combination with emtricitabine and tenofovir alafenamide. same as the dolutegravir, absorbed by UGT1A1 and moderately by CYP3A4 enzymes. It has very less drug-drug interaction so its chosen as the 1st line therapy for the treatment.

[2.9] Protease Inhibitors: -

Protease inhibitors get attached to the protease and avoid the splitting of the GAG-Pol Proteins of the infected cells. This process stops the growing of the viral particles in the body. Protease inhibitors are widely used antiretroviral agents than other because of their less side effects into the body and also have additional metabolic effect. [11]

1) Atazanavir

Atazanavir is available in the sulphate form called Atazanavir Sulphate. It is available in the dosage form of tablets, capsules and also powder form are available in the market. There are wide range of the dose is available in the market are 100mg, 200mg, 300mg and 400mg, it is given in combination with the tenofovir disoproxil and tenofovir alfenamide. Atazanavir has side effects like nephrotoxicity because it gets crystalize.

2) Darunavir

Newest approved protease inhibitor is the darunavir. It is available in the oral dosage tablet form with daily dose of 800mg a day. It is given with ritonavir in combination with dose of 600 mg a tablet two times a day. It is also given with the combination of Combisistan, Emtricitabine, Tenofovir Alafenamide. there is sulfonamide moiety is present in Darunavir structure, so the patients having the sulfa hypersensitivities should not give Darunavir. It should be given with the food to increase the bioavailability and decrease the side effect in the GI.

3) Ritonavir

Ritonavir is the widely used Protease inhibitors because it has ability to block the protease inhibitors. It is available in the oral dosage form tablet, capsule, powder and in combination is also widely used drug. Because ritonavir has the property to block many enzymes like CYP3A4, CYP2D6, CYP2C19, CYP1A2, CYP2B6, CYP2C9 AND UGT. The dose of ritonavir is very less 100mg and 200mg only. [11]

[2.10] Impact of Transporters on Protease Inhibitors: -

Protease kinase inhibitor therapy is used widely in the HIV infection. The protease inhibitors comes under the second generation inhibitors.[12] HIV Protease kinase inhibitor are the large molecules, so more in weight and mostly insoluble in the water. These inhibitors are widely absorbed by the enzymes called CYP3A4 throughout the intestinal region. so the molecules having the short half-life cannot be used as the protease inhibitors because of their metabolism to intestine, so molecules with the large half-life used as the protease inhibitors to achieve the therapeutic concentration in the body.[13] Ritonavir is the protease inhibitor which is widely used because it has property to block the CYP3A4 enzyme. Dose range of the ritonavir is 100 to 200mg per day. It is also known as the "boosting agent", but it is clinically approved that ritonavir has many side effect if it is use alone, which increases the side effect and toxicity profile of HAART regimen. [11]

Determination of the protease inhibitors done by the its pharmacological and other toxicological effect like 1) absorption 2) Distribution 3) Excretion. Various transporters are present in the intestine track which generally metabolise the inhibitors but the protease inhibitors very less metabolised by the these transporters and enzymes.[14]

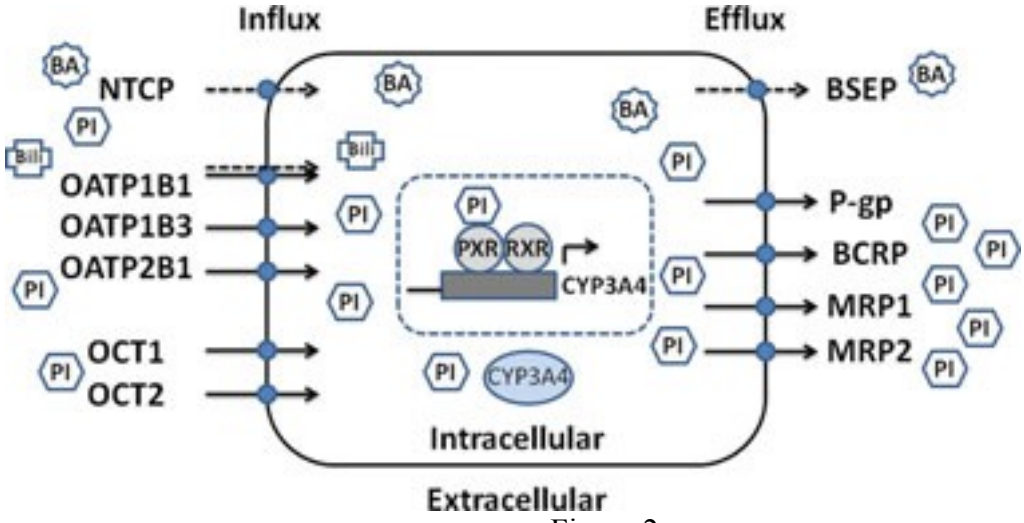


Figure 2

In this figure there are various transporters are present which belong to the SLC and ABC transporter family. OATPS and OCTS transporter belong to the SLC family and these transporter are helping the protease inhibitors to the cells and efflux of the protease inhibitors done by the ABC family. BRCP and MRP transporters comes under the ABC family. So here the influx of the protease inhibitors done by the SLC family and efflux done by the ABC family which shown in this image.[15]

After entry of the PI into the cell the PI bind to the PXR and RXR, which are nuclear receptor of the cells and induction of CYP3A4 done. Before that Bili rubin and Bile Acid were inhibited by the PI and get attached to the nuclear receptors.[16]

[2.11] Emulsion

Emulsion is widely used formulation in the pharmaceutical industry now a days. [17]Emulsion can be used as the internal and external application also. As internal application it is used as the solution and suspension, whereas externally it is used as the cream and lotion. Emulsion is well known as the biphasic system because of oil and water into it. [18]The one phase is known as dispersion phase and another phase is known as the continuous phase. The dispersion phase has the globules which are present in the continuous phase. Emulsion are generally unsteady thermodynamically, so to stabilise the emulsion surfactant and co-surfactant are added to the emulsion which forms the fine film around the globules. [19]The consistency of the disperse phase and continuous phase changes based on whether it is semi solid or sometime liquid also, so the viscosity of the emulsion get changes from high to low. Particle size also play the important role in the emulsion which has the range from the 0.1 to 100µm. [20]

[2.12] Types of Emulsion

Basically two type of emulsions are there: - 1) oil in water 2) water in oil. Other than this emulsion various complex emulsions are there like 1) multiple emulsion 2) Micro emulsion 3) Pickering Emulsion 4) Nano Emulsion.[17]

[2.12.1] Oil in Water Emulsion

In pharmaceutical industry emulsion are the combination of the aqueous phase and the oily phase. in oily phases various oil and wax can be used as an oil whereas the aqueous system water is used but sometime other water-soluble solvents used as the aqueous system. In this oil in water emulsion oil phase, oil is distributed as small droplets into the aqueous phase which is the continuous phase and oil becomes disperse phase. This emulsion is non-oily and can easily washed by the water from the skin. For the drugs which are soluble in aqueous system for that oil in water type of emulsion are used, so drug get easily solubilized.[21]

[2.12.2] Water in Oil Emulsion

This system is reverse system of the oil in water emulsion. In this emulsion the continuous phase is the oil phase whereas the disperse system is the water, water droplets are mixed in the oily phase. Drugs which are more soluble in the oil for these types of drugs water in oil emulsions are used. Because of continuous phase of oil the emulsion is more oil and not easily washable by water, and these type of emulsions are widely use as the cream, lotion for the cleaning of the skin.[22]

[2.12.3] Multiple Emulsion

This type of emulsion is having the complicated system. This emulsion is also known as the “emulsion of emulsion”. It has extensively use in the cosmetics pharma industry and also in the separation sciences. Multiple emulsion is made by the addition of the oil in water and water in oil emulsion to another aqueous phase or the oil phase system. This emulsion is also uses in taste masking, adjuvant vaccine, immobilization of enzymes and overdose treatment. Sustained release of drug can also be achieved by this emulsion system. Multiple emulsions are widely used for moisturizer formulation in the cosmetics, but there is drawback of multiple emulsion is that thermodynamically it is unstable.[23]

[2.12.4] Micro emulsion

Micro emulsion can be also O/W and W/O system. Micro emulsions are isotropic and thermodynamically more stable than other system. For stability of the emulsion surfactants are added to the emulsion, which is based the hydrophilic and lipophilic system of the emulsion. In emulsion co-surfactant are added which gives the gel types of system to emulsion and then further added to the aqueous or oil and end into the good stable emulsion. [21]

[2.12.5] Nano emulsion

Nano emulsion are also same as the micro emulsion but the particle size of the emulsion is in the Nano range. Nano emulsion are widely use for the increasing the bioavailability of the drug to the body because of the decrease size. [24]

[2.12.6] Pickering emulsion

As the emulsion stabilizer solid particles can be use in the colloidal size. This type of solid particles is known as the "Pickering Emulsion". Pickering emulsions are widely use now days in many pharma industries like 1) Cosmetics 2) Food 3) Oil and Water treatment plants.[25]

[2.12.7] Solid in O/W Emulsion

There are various multiple emulsions are formed for the oral administration. From one of them Insulin S/O/W emulsion was prepared by the "Tooriaka". In this emulsion 1st the insulin was mixed with the surfactant so coating around the surfactant get done after that it is mixed with another solution hexane lipophilic surfactant and then it is mixed with the soybean oil by ultra-sonication method. This forms the S/O emulsion. This S/O emulsion was then mixed with the aqueous solution by mixing it with rotor-stator homogenizer, which give S/O/W emulsion.

Vitamin B12 S/O/W emulsion was prepared by the "Kikizaki" by the membrane emulsification method.[25]

[2.13] Nano Emulsion: -

From past few days Nano Drug Delivery widely used system for the delivery of the Bio-active constituents or active pharmaceutical ingredient to body. This drug delivery system help to target the drug to its specific cell or the receptors of the body. The particle size of this Nano emulsion is in Nano range which increases the solubility and bioavailability of the API. Nano emulsions are based on the Nano encapsulation. The particle size of the Nano emulsion is in the range of between 20 – 200 nm. Also it is homogeneous , stable thermodynamically and isotropic system for oil, water and Smix phase. Nano emulsion improves the stability of the emulsion. It has capacity to increase the release of drug from the formulation. Encapsulation of Nano emulsion increases the bioavailability of the drug.[26]

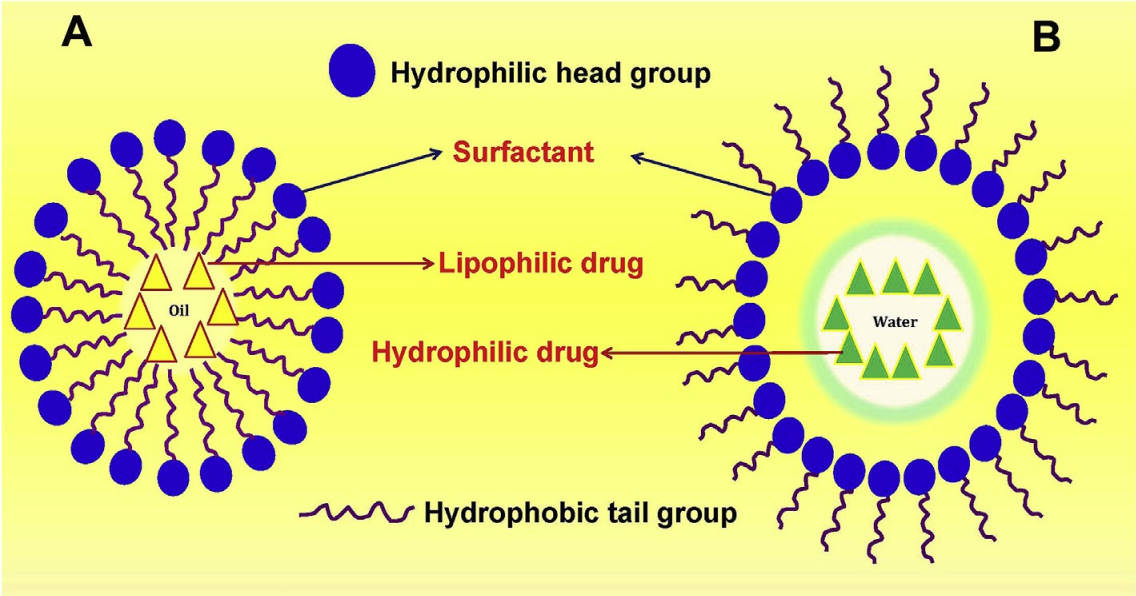


Figure 3

This figure shows the structure of the Nano emulsion/emulsion.

In figure A part shows the O/W emulsion whereas B part shows the W/O emulsion.

[2.13.1] Nano Emulsion Advantages: -

1) It has many advantages, it increases the solubility of drug, absorption, increases the bio-membrane infusion and also increases the Bioavailability of the class IV drugs.

2) Nano emulsion increases the stability of herbal bioactive by encapsulating it into the emulsion.

3) Nano emulsion gives the target specific and sustained drug delivery system.

4) Nano emulsion can also give topically also given by the IV route so avoid the first pass metabolism which increases the blood plasma concentration.

5) It promote the patient compliance.

6) it also reduces drug dosing

7) increases the pharmacokinetic drug profile[27]

[2.14] Method of Preparation Of Nano Emulsion

Selection of excipients, it's concentration ,pattern of addition of all the excipients , mixing time and rotation per minute these all points are very important while formulating the emulsion. Sometime change in the methods creates the problem to the Nano emulsion and which also affects the particle size of the nanoparticles. There are various approaches/ techniques used for the formulation of the Nano emulsion.[28]

Generally , two methods are widely used for Nano emulsion formulation which are as below:

1) High energy emulsification method

2) Low energy emulsification method

[2.14.1] High energy emulsification method: -

In this method high energy force is applied which break down the intermolecular forces between the molecules, which can be hydrogen bond, Vander Waals force present between the very high and low surface tension of liquid. High energy is applied by shear or ultrasonic waves which forms the droplets of the small Nano size. Tremendous heat gets produced during this process. High force mixer is used extreme speed motor.[29]

High pressure homogenization creates turbulence and shear between oil and water. The ultrasonic waves produce cavitation bubble that breakdown and release the energy in system which creates the small droplets in the emulsion. Though, more energy produces free radicals of the water molecules like H+ and OH- and produce breakdown of surfactant on the surface. Turbulence creates small size globules having a size fewer than 100nm.[30]

Micro fluidization system is also used for the formulation of the multiple emulsion, in this system emulsion is formulated by conflict of two immiscible liquid under high pressure. The stability of this emulsion dependents on the micro channels of the micro fluidizer.[31]

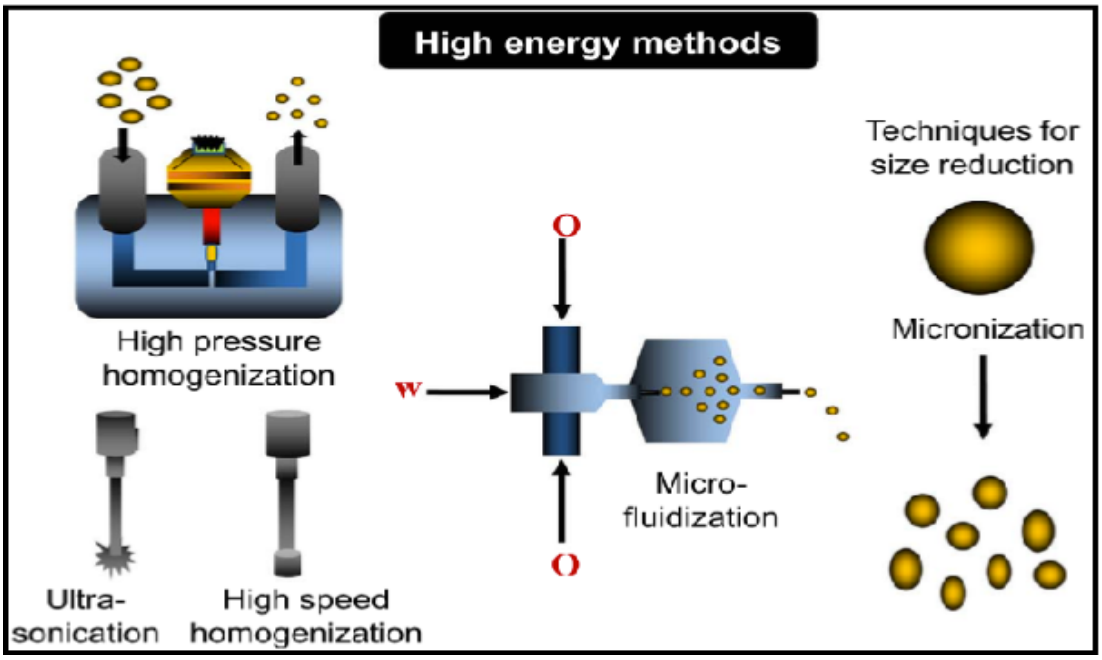


Figure 4

This figure shows the different tech used in the high energy method. Here is 1) High pressure homogenization 2) ultra-sonication 3) High speed homogenization 4) Micro Fluidization

[2.14.2] Low Energy Emulsification method: -

This technique also generates more stable thermodynamic emulsion. It consumes the energy stored in the system and produce small droplets. The approaches for emulsion taken by different considerations like temperature which directly affect HLB system of emulsion.[32]

Phase inversion temperature system is used for the temperature effective emulsions. For example, emulsion having molecules which are derivation of ethoxylated molecules which gets affected by the temperature, at low temperature hydrophilic form. The main difficulty of this method is "Ostwald Ripening". Sometime change in the excipients interrupts HLB system and leads to phase reversal at constant temperature, which fluctuates the surfactant mono layer.[33]

So, Phase inversion can also have done by the other technique like 1) addition of electrolytes 2) Surfactant 3) Alcohol to the formulation.

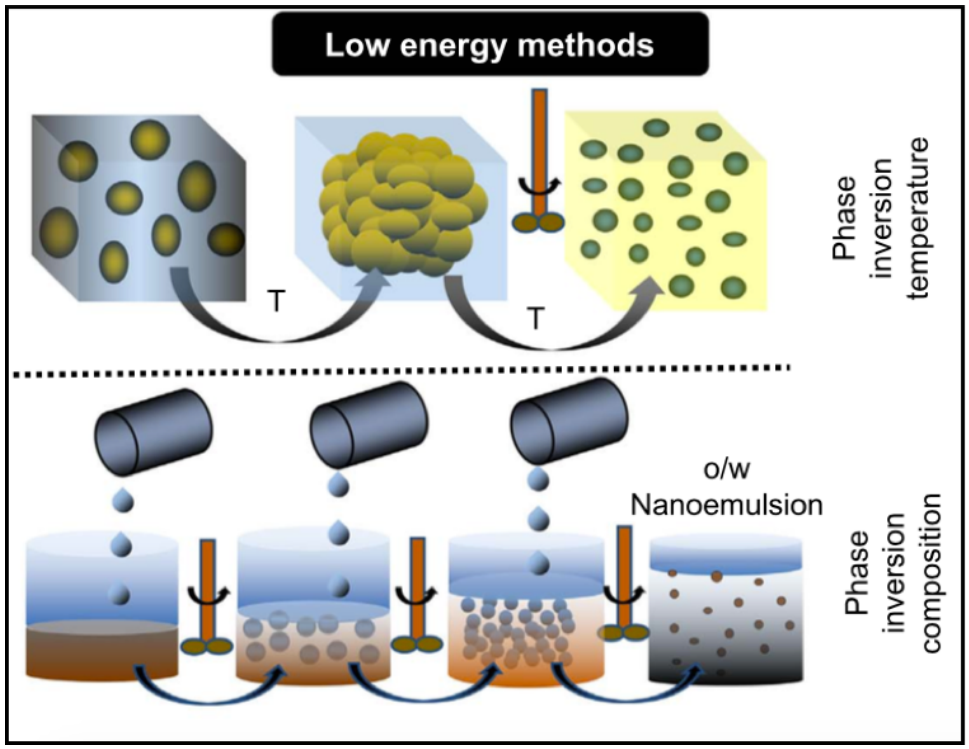


Figure 5

This figure shows the different technique used in low energy method which are 1) Phase Inversion Composition 2) Phase inversion Temperature

[2.14.3] Spontaneous Emulsification method

In this method after mixing if the two immiscible liquids and then at high speed homogenization or mixing done at high rotation per minute. [34]

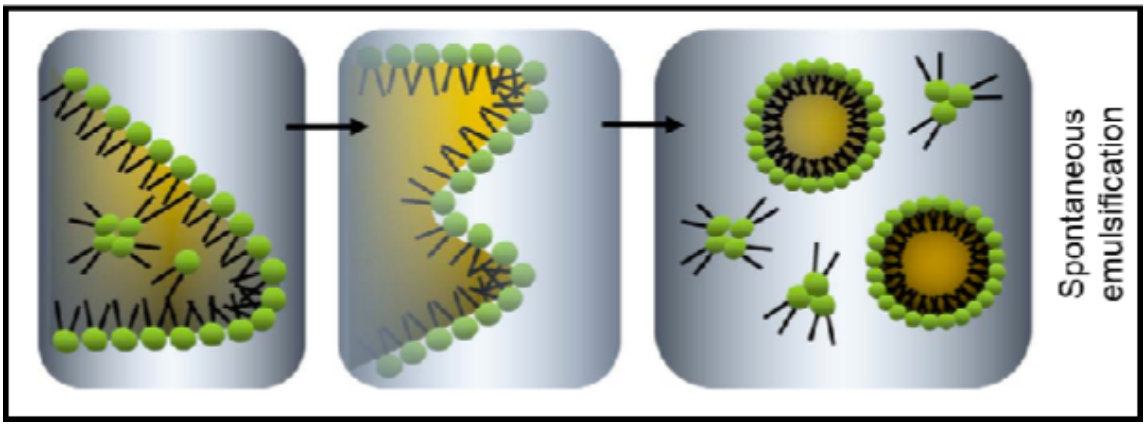


Figure 6

In this the process of spontaneous emulsification is shown.

[2.15] Drug Profile review: - ATAZANAVIR SULFATE

Atazanavir sulphate is an antiretroviral drug of the protease inhibitor (PI) class. Like other antiretroviral, it is used to treat infection of human immunodeficiency virus (HIV). Atazanavir is distinguished from other PIs in that it can be given once-daily (rather than requiring multiple doses per day) and has lesser effects on the patient's lipid profile (the amounts of cholesterol and other fatty substances in the blood). Like other protease inhibitors, it is used only in combination with other HIV medications.[35]

It is also used in combination with other antiretroviral agents as part of an expanded post-exposure prophylaxis regimen to prevent HIV transmission for those exposed to materials associated with a high risk for HIV transmission.

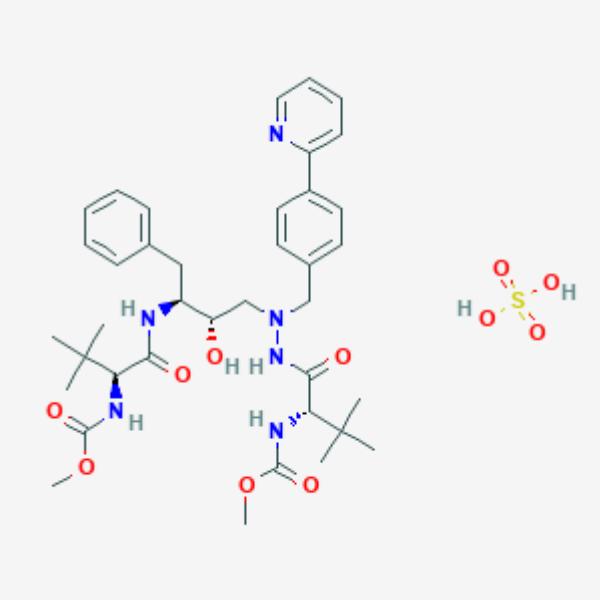


Figure 7

Structure of Atazanavir sulphate

Table 1

: Drug Profile – Atazanavir sulphate

Drug	Atazanavir sulphate
Chemical Formula	$C_{38}H_{54}N_6O_{11}S$ methyl *N*-[(2*S*)-1-[2-[(2*S*,3*S*)-2-hydroxy-3-[[(2*S*)-2-(methoxycarbonylamino)-3,3-dimethylbutanoyl]amino]-4-phenylbutyl]-2-[(4-pyridin-2-ylphenyl)methyl]hydrazinyl]-3,3-dimethyl-1-oxobutan-2-yl]carbamate;sulfuric acid
Description	White to pale yellow powder
Drug category	Protease inhibitor
Indication	In combination treatment of HIV infection (AIDS)
Mechanism of action	Atazanavir (ATV) is an azapeptide HIV-1 protease inhibitor (PI). The compound selectively inhibits the virus-specific processing of viral Gag and Gag-Pol polyproteins in HIV-1 infected cells, thus preventing formation of mature virus.
BCS Class	II (Low soluble, High permeable)
Molecular Weight	802.9 (sulfuric acid salt). The free base molecular weight is 704.9
Melting point	Reported between 195- 200°C
Water solubility	9.2 µg/mL (pH 8.7) at 25°C
Partition coefficient	3.23 (at pH 9) 3.47 (at pH 5)
Bioavailability	60-68 %
Protein binding	86 %
Half life	6.5 hrs
Dose	Capsules: 150 mg, 200 mg, 300 mg. (3, 16) Oral powder: 50 mg packet.
Marketed Products	REYATAZ

[3] Literature Review: -

1) Melinda J, Reese, Paul M. Savina, (2012)

Excipients: - Dolutegravir, cryo preserved hypatocytosis, cimetidine, digoxin, HPLC reagents, cell culture

Work done: -

They had described the use of dolutegravir with the other drug with use of human hypatocytosis. Dolutegravir is PI inhibitor but has drug interaction with other drug and but also increase the blocking of many enzymes excluding CYP1A2, CYP2B6, CYP3A4.

2) Mark berlin, Aaron Ruff, Flippos Kesisglou (2015)

Excipients: - Atazanavir sulfate capsule, glyceryl mono-oleate, sodium taurocholate, sodium oleate, Maleic acid, ortho phosphoric acid, Sodium hydroxide

Work done: - They resulted that there are factors are affecting the absorption of drug, they had compared the factors which are 1) pre-absorptive 2) absorptive 3) post absorptive and done PBPK modeling with in silico model and which is helpful for the potential formulation development study.

3) Lukas cerveny, zuzana ptakova, Marketa Durisova (2018)

Excipients: - Radiolabeled Atazanavir sulfate, Ritonavir, cell line, Indomethacin, HPLC reagents, rat placenta

Work Done: -

They had shown the effect of the combination of Atazanavir and ritonavir on ABC family transporter. They had resulted the Atazanavir get affected by the ABC family transporter which is ABCB1 and leads to the drug-drug interaction.

4) E. Gue, M. since, S. Ropars, R. Herbinet, L. Le Pluart (2016)

Excipients used: - Amiodarone HCL, Ciprofloxacin, Ibuprofen, Paracetamol, tween 80, Labrasol, Kolliphor HS15, Methanol, Acetonitrile, HPLC grade water.

Work Done: -

They had characterized the Nano emulsion with different six API, with having dissimilar physio chemical property. Nano emulsion was prepared at room temperature with the

phosphate buffer by admixture of triglyceride and surfactants. They resulted that except ciprofloxacin all the other API easily solubilize in the emulsion containing labrasol and emulsion was well characterized and compared with each other. They had done characterization by DSC, zeta potential, pH. At the end they concluded that comparing various Nano emulsion gives formulation approach.

5) Teofilo Vasconelos, Francisca Araujo, Carlos lopes (2019)

Excipients: - Labrasol, Gelurice, Capryol, Softigen 767, softigen 701, Miglyol 810, Transcutol, Labrafac, Labrafil, tween 80, Resveratrol

Work Done: -

They had prepared the self-emulsifying drug delivery system of API Resveratrol with two different composition excipients. 1st composition of Lauroglycol, Labrasol, Capryol and 2nd is tween 80, Transcutol, Imwitor. Caco-2 cell line study was done toc check free drug in both formulation, in the 2nd formulation they got more amount of free drug in the 2nd formulation than the 1st formulation based on the AUC and Cmax studies of formulation.

6) Mehdi Nouraei, Egar J. Ascota (2017)

Excipients: - Soybean lecithin as surfactant, glycerol mono oleate, polyglycerol caprylate , ethyl caprate as oil

Work done: -

Mehdi nouraei showed that use of ternary phase diagram in the formulation of emulsion is very useful for the solubilize feature, they prepared self-emulsifying drug delivery system in which no water is used, emulsion get diluted by water. They used the ternary phase diagram without using water and predicted the hydrophilic lipophilic difference which is very useful.

7) Claudia Menzel, Thomas Holzeisen, Flavia Laffuer

Excipients: - Sodium docusate, Cremophore, Labrafil, Capmul-PG 8, Propylene glycol, exenatide.

Work done: -

The SEDDS of exenatide was formulated and then evaluation done by the IN VIVO method, and formulated the SEDDS by using mentioned above excipients, and characterization was done and got the results that molar ratio 1:4 of exenatide: sodium docusate gives more effective

log P and increase in the bioavailability. So, they concluded that use of hydrophobic ion pairing give result in oral delivery.

8) Pavan Puligujja, Shantanu Balkundi, Lindsey Kendrick

Excipients: - Atazanavir sulfate, Ritonavir, human serum, cell growth medium.

Work Done: -

They had formulated the Nano formulation of combination of Atazanavir Sulfate with the Ritonavir and its pharmacodynamics on the folic acid receptors. They formulated the long acting Nano crystals of Ritonavir and Atazanavir given to the lymphocytes system in rat and evaluation was done, they found that it increases the bioavailability in the rat and also accelerate drug carriage also antiretroviral reactions.

9) Agnieszka snela, Barbara Jadach, Anna Froelich (2019)

Excipients: - Atorvastatin calcium trihydrate, Aerosil, Tween 80, Span 80, Ethyl oleate, Neusilin US2, Transcutol.

Work Done: -

They had formulated SEDDS of Atorvastatin which was absorbed on the solid carrier and then IN VITRO study was done. With the use of pseudo ternary diagram, they had prepared the ternary diagram and formulated SEDDS and resulted that interaction happened with the carrier and the API which and more interaction of drug was with the aerosol, so solid carrier has no straight relation with the release of the drug.

10) Wanxu Wang, Hongtu Wei, Zhiping Du

Excipients: - Oleic Acid, Methyl Laurate, Propanol, Glycerol, amyl alcohol,

Work Done: -

They formulated fully dilutable emulsion and done the characterization of emulsion with the help of pseudo ternary phase diagram, from that selected different range and formulation prepared but had problem with the crystallization of the particle.

11) K. Geetha Bhavani, K. Bala Murali.

Excipients: - Atazanavir Sulphate, HPLC reagents, Tert butyl hydrazine carboxylate

Work Done: - They had genotoxicity of the Atazanavir Sulphate drug. They had use various methods from which determination of genotoxic impurities. But LC-MS methods give the better genotoxic impurities of atazanavir.

12) Olena Kis, Sharon L. Walmsley, Rein Benayan

Excipients: - Cac0-2 cell line, Cell line Growth medium, pH Buffer (pH 4.5- 8.5).

Work Done: -

They had worked on the Caco-2 cell line study, with difference pH buffer range of 4.5 to 8.5in the fasted and fed state, which shows the bioavailability of the drug in the intestine. In lumen pH absorbance of Atazanavir drug is increasing with acid reducing substances.

[4] Materials and Methods: -

[4.1] Chemicals and Materials

Table 2

Sr. No	Chemical Name	Manufacturer
1	Capmul MCM	Gattefosse sas, India
2	Capryol 90	Gattefosse sas, India
3	Labrafil M2125	Gattefosse sas, India
4	Maisine	Gattefosse sas, India
5	Labrasol	Gattefosse sas, India
6	Plurol	Gattefosse sas, India
7	PEG	
8	Transcutol	Gattefosse sas, India
9	Spam 80 (Sorbitan Monooleate)	SRL Lab, India
10	Span 20 (Sorbitan Mono-laurate)	SRL Lab, India
11	Tween 20 LR	Rasayan Lab, India
12	Plurol	
13	Lecithin	Dr. Reddy's Lab, India

[4.2] List of Equipment: -

Table 3

SR. NO	INSTRUMENTS	VENDOR'S NAME
1	Digital Balance	Citiweight-Tejas exports, India
2	Electronic Weighing Balance	Shimadzu Corporation Ltd.
3	Mechanical Stirrer	Remi motors Ltd. India
4	Vortex shaker	Remi motors Ltd. India
5	Ultraviolet Spectrophotometer	Shimadzu UV 1800 Corporation
6	Refrigerated Micro Centrifuge	Rajendra Electrical Industries Ltd.
7	Ultrasonicator	Trans-o-sonic D-Compact, India
8	pH meter	Ana lab Scientific instruments, India
9	Brookfield Viscometer	Brookfield Engineering Laboratories.

[4.3] Methods

[4.3.1] Physical Characterization and identification of Atazanavir Sulfate

The sample of Atazanavir sulfate was characterized on the basis of it's some properties like color, water solubility and also the form of the powder. Melting Point determination, DSC, FTIR were also carried out and compared with the literature.

[A] Organoleptic Properties

Atazanavir Sulfate was characterized for its physio chemical properties.

[B] Melting Point Identification

Melting Point of Atazanavir Sulfate was done by using Capillary melting point method. Very small quantity of drug added to the capillary tube. Then capillary was hang on paraffin bath and melting point was taken with standardized thermometer and result was reported.

[C] Differential Scanning calorimeter

The sample of Atazanavir sulfate around 4-5 mg was taken on the small pan od DSC and sealing pf pan was done, then placed into the DSC. After that range was selected of melting point and then DSC was run in the presence nitrogen gas. The temperature of the sample was recorded.

[D] Fourier Transform Infrared Spectroscopy

FTIR of sample Atazanavir sulfate was done by KBr pellet method. Very small quantity of sample and KBr was taken in range of 0.2 to 0.1%. The range of wavelength was taken in the range of 200 to 4000 cm-1 and spectra was recorded.

[4.3.2] Analytical method or Drug substance characterization: -

Spectrophotometric Method for Determination of Atazanavir-Sulphate

Preparation of 0.1 N HCL :-

A) Preparation of stock solution in HCL and Preparation of calibration curves in HCL

Accurately weighted API Atazanavir-Sulphate 100 mg and transfer it to the 100ml of the volumetric flask and dilute it with 0.1N HCL, which gives the 1000 ppm solution. From this stock solution take 1 ml of it and bring it to the 10 ml volumetric glass flask, which gives 100-ppm stock solution. From 100 ppm solution take 1 ml of it and dilute to 10 ml. which gives 1 ppm. The 1ppm, 2ppm, 3ppm ,4ppm, 5ppm, 10 ppm, 15 ppm ,20 ppm, 25 ppm, 30 ppm. The Absorbance was taken in Shimadzu double beam spectrophotometer taking HCL as blank. The graph of Absorbance vs Concentration of the drug was plotted and standard curve obtained. The Wavelength of the UV was between 200-800 nm.

B) Preparation of stock solution in Methanol and Preparation of calibration curves in Methanol

Accurately weighted API Atazanavir-Sulphate 100 mg and transfer it to the 100ml of the volumetric flask and dilute it with Methanol which gives the 1000 ppm solution. From this stock solution take 1 ml of it and transfer it to the 10 ml volumetric flask, which gives the 100-ppm stock solution. From 100 ppm solution take 1 ml of it and dilute to 10 ml. which gives 1 ppm. The 1ppm, 2ppm, 3ppm ,4ppm ,5ppm, 10 ppm, 15 ppm ,20 ppm, 25 ppm, 30 ppm. The Absorbance was taken, in Shimadzu double beam spectrophotometer taking Methanol as blank. The graph of Absorbance vs Concentration of the drug was plotted and standard curve obtained. The selected range of the wavelength was in between 200-800nm.

[4.3.3] Pre-formulation study for Emulsion

The aim behind the pre-formulation study was to check the solubility of the drug in the different oil solution and idea about how much drug was getting solubilized in the different chemicals, from this we could understand that the stability of the drug in oil, surfactant, co-surfactant and co-solvent.

1) Screening of Oil, Surfactant, Co-Surfactant and Co-Solvents

The solubility of the drug in the different oils, surfactant and solvents was done. Around 20mg of drug was weighed and added to the excipients like oil, surfactant and solvents. The visual inspection was done whether drug was visually getting solubilized or not. Based on the visual inspection solubility study the further super saturation study was done.

2) Preparation for the Super-saturation solubility study: -

The super-saturation solubility study was done by addition of the excess amount of the drug into the exact amount of oil, surfactant, surfactant, co-solvents. Which gives the exact amount of drug getting dissolved into that solution. Here we had taken 2ml of the Eppendorf tube, in which addition of drug done by the dissolution of that drug. The vortex was done for the solidarization of the drug into the solution, after achieving the super saturated solution the absorbance was taken in the UV spectrophotometer.

(a) Preparation of Transcutol with drug super saturation solution

The drug was added gradually slowly added to the Eppendorf tube of 2 ml of transcutol and vortex was done for around 15-20 minute to dissolve the drug into the solvent, after dissolving the centrifugation was done which settled down the undissolved drug in the solvent.

(b) Preparation of Capmul MCM with drug super saturation solution

The drug was added continuously to the capmul in the Eppendorf tube of 2ml and then vortex was done for fifteen to twenty minutes for dissolving of the drug, after solubilizing the drug the vortex was done and then centrifugation was done.

(c) Preparation of Labrasol with drug super saturation solution

The drug was added slowly and continuously to the labrasol and the same procedure was followed as above.

(d) Preparation of Labrafil M2125 with drug super saturation solution

The drug was added continuously to the labrafil solvent and the same procedure was followed as above.

(e) Preparation of Plurol with drug super saturation solution

The drug was added continuously to the Plurol and then same procedure was followed as above.

(f) Preparation of Capryol with drug super saturation solution

The 34.6mg drug was dissolved in the solvent and then same procedure was followed.

(g) Preparation of Span 20 with drug super saturation solution

The 27mg of drug was added to the solvent and then same procedure followed.

(h) Preparation of Tween 20 with drug super saturation solution

The 25.9 mg drug was added to the Tween20 surfactant and the same procedure was followed.

(i) Preparation of Tween 80 with drug super saturation solution

The drug was added gradually in small amount to the Tween80 solution and then same procedure was followed.

[4.3.4] Development of Pseudo-Ternary Phase Diagram

This study indicates thein which ratio of surfactant and co-solvents gives the more stabilized Nano-emulsion. Also, the range of the surfactant and co-solvents can be optimized by the ternary phase diagram. Based on the above screening study and the super saturation study the selected oil, surfactant and co-solvents was mixed in specific ration of it. The surfactant and co-solvents were mixed with ration of 1:1, 1:2, 2:1. The ratio of Oil and Smix was 9:1,8:2,7:3,6:4,5:5,4:6,3:7,2:8 and1:9. So in the three Smix ratio the oil and Smix ration was performed with the water titration method. In the water titration method, the water was added by the increasing 5% to the solution of oil and Smix, which shows the stability of the emulsion at the room temperature also at the higher and lower temperature.

[4.3.5] Selection of mixture ratio of components for the formulation of the Nano-Emulsion by

After developing the pseudo ternary phase diagram, the concentration of oil, surfactant and water was done.

[4.3.6] Optimization by Mixture design of Atazanavir sulfate loaded Nano Emulsion

After selection of the range of each excipient, the optimization done by mixture design. The higher and lower limit of the excipients selected and put into the mixture design which gives the idea of the optimized batch of the Nano emulsion and preferred result will be obtained. The selected mathematical model will give the precise statistical parameters.

[4.3.7] In-Vitro study

In vitro study of Nano emulsion will be done by Dialysis Membrane method. In this method membrane was taken, put it in distilled water for 24 hrs. and one dose of Nano emulsion will be put in the membrane and for 24 to 48 hrs. at specific time interval sample was taken UV spectra will be taken, which shows the drug passed through the cell membrane.

[4.3.8] Ex-Vivo Study

Ex-vivo study will be done Caco-2 cell line which shows the how much amount of drug is passing through the intestinal cell line. The Caco-2 cell line is the intestinal cell line, in which

there are various transporters are present. This transporter will allow or block the formulated Atazanavir Nano emulsion.

Cell Line Growth: -

Caco-2 cell line will have maintained by the Hank's salt solution which contains the growth medium KH_2PO_4 ,Na_2HPO_4, $Cacl_2$. This solution will be added to the buffer system and maintained the pH of solution at 7.4. After Preparing the solution Caco-2 cell line was incubated with this buffer system and cell growth maintained.

[4.4] Evaluation of Nano-Emulsion

[4.4.1] Viscosity

Viscosity will be measured by the brooked field viscometer, at specific shear rate and specific temperature which gives the idea about the viscosity of an emulsion.

[4.4.2] Globule size

Globule size of Nano emulsion measured by the different technique like light scattering method, Transmission Electron microscopy.

[4.4.3] pH

pH of Nano emulsion will be measured by pH meter.

[4.4.4] Dilution Test

Dilution test of the Nano emulsion will be done check the stability of the emulsion in diluted state. So, Nano emulsion one dose will be diluted to 100 ml distilled water or 0.1 N HCL and shows the stability of Nano emulsion.

[4.4.5] Polydispersity Index

Polydispersity index is the most important evaluation of Nano emulsion. PI of Nano emulsion done by Malvern Particle size analyzer. Higher the polydispersity index, less will be the uniform droplets size in Nano emulsion. So, decrease in polydispersity index shows the good emulsion.

[4.4.6] Dye test

Dye test will be done to check the which type of emulsion is formed which show it is O/W emulsion or W/O emulsion. In water soluble dye is used and the emulsion is get dye color which means emulsion is O/W and disperse phase remains colorless.

[4.4.7] Zeta Potential

Zeta Potential will be measured by the Malvern Zeta analyzer. This zeta analyzer shows the stability of the emulsion by the charges carried by the surfactant and drug molecules.

[4.4.8] Fluorescence Test

Fluorescence test is also done to check the which type of emulsion is formed, it is O/w or W/O emulsion.

[4.4.9] Percentage transmittance

Percentage transmittance of emulsion is done to check the clarity of emulsion, the greater percentage transmittance more clear emulsion will be. The percentage transmittance of emulsion is done by the UV spectrophotometer.

[5] Results and Discussion

[5.1] Physical Characterization of Atazanavir Sulfate

[A] Organoleptic Properties:

Table 4

Sr. No	Parameters	Interpretation
1	Color	Yellowish white powder
2	Nature of powder	Fine crystalline powder
3	Odor	Odorless
4	Taste	Tasteless

[B] Melting Point:

The melting point was identified by capillary method. The melting point of Atazanavir sulfate was determined 196℃ and found melting point was between the range 195 -200℃.

[C] DSC (Differential scanning calorimeter)

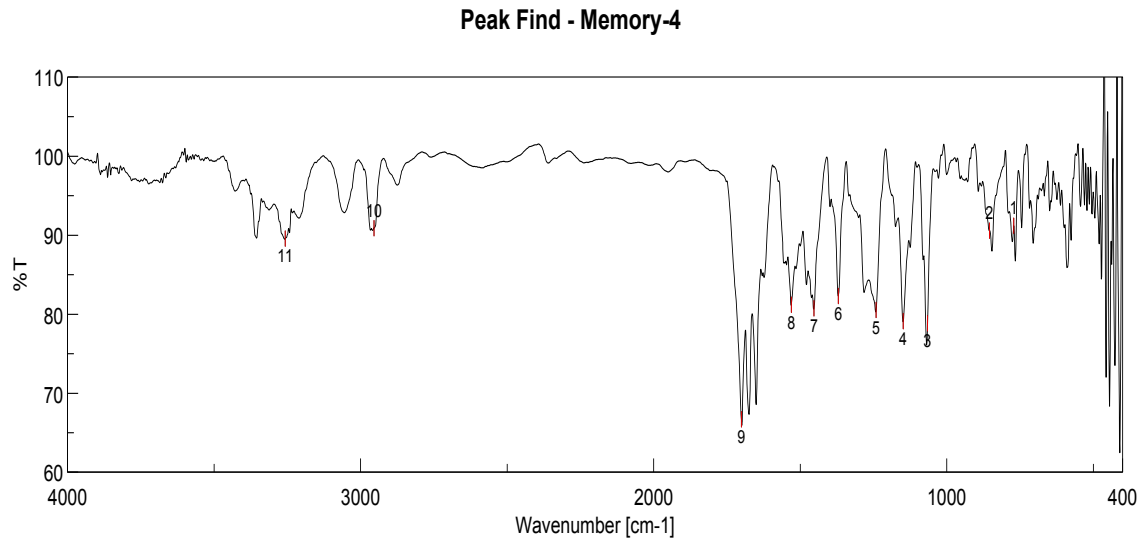
The endothermic peak of DSC was obtained at 198 ℃ and it was between the range of 195-200℃. So, it is concluded that the given sample was pure.

[D] Fourier Transform Infrared Spectroscopy

Observation: - FTIR spectra was taken by KBr pellet method and gives characteristic peaks at 771, 854, 1065, 1148, 1240, 1370, 1453, 1530, 1700, 2954, 3257 cm^{-1} which was recorded and compared with the original spectra of Atazanavir Sulfate, which gives the result that spectra found and authentic spectra were having the same peaks when compared.

Table 5

Functional Group	**Standard frequency (cm^{-1})**	**Observed frequency (cm^{-1})**
C=O Stretching	1680-1760	1697.36
Aliphatic 2º amine stretching (cm-1)	3360-3310	3312.40
-C-N stretching (cm-1)	1360-1180	1271.09, 1240.23
Secondary amine bending (cm-1)	1650-1550	1550.77



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	771.387	91.1867	2	854.311	90.5585	3	1065.48	78.8082
4	1148.4	79.0883	5	1240.97	80.5178	6	1370.18	82.2681
7	1453.1	80.7329	8	1530.24	81.1707	9	1700.91	66.6747
10	2954.41	90.8726	11	3257.18	89.5619			

Figure 8

This figure shows the FTIR recorded graph and its peaks with intensity.

[5.2] Analytical method or Drug Substance Characterization

[A] Preparation of calibration curve in 0.1N HCL

The UV spectra of sample Atazanavir was taken and recorded. The λmax of Atazanavir sulfate was obtained at 249 nm.

Calibration Curve: -

Table 6

Concentration (µg/ml)	λmax	Absorbance		Average Absorbance
		I	II	
0	0	0	0	0
4	249	0.19274	0.10933	0.1510
5	249	0.22048	0.16493	0.1927
10	249	0.36779	0.26977	0.3188
15	249	0.41082	0.36539	0.3881
20	249	0.57253	0.43341	0.5030
25	249	0.81295	0.54371	0.678

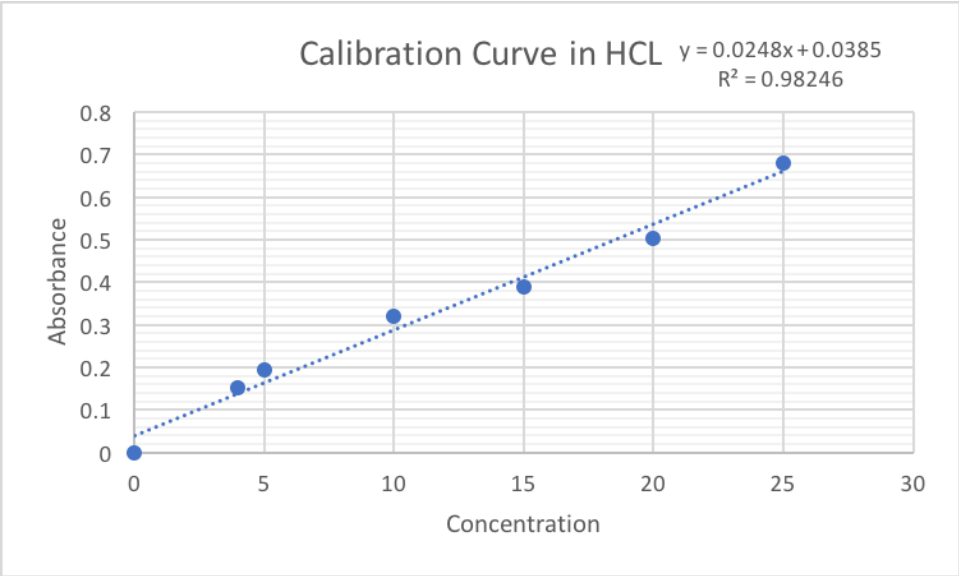


Figure 9

This figure shows the calibration curve in 0.1N HCL which gives the r square value 0.98246.

[B] Preparation of Calibration curve in Methanol

The UV spectra of sample Atazanavir was taken and recorded. The λmax of Atazanavir sulfate was obtained at 249 nm.

Table 7

Conc. (µg/ml)	Absorbance			Average
	I	II	III	
0	**0**	**0**	**0**	**0.000**
10	**0.374**	**0.3542**	**0.3443**	**0.358**
12	**0.413**	**0.418**	**0.423**	**0.418**
14	**0.476**	**0.49**	**0.509**	**0.492**
16	**0.574**	**0.5788**	**0.582**	**0.578**
18	**0.612**	**0.645**	**0.657**	**0.638**
20	**0.6963**	**0.718**	**0.722**	**0.712**
22	**0.766**	**0.808**	**0.795**	**0.790**
24	**0.853**	**0.87**	**0.9718**	**0.898**

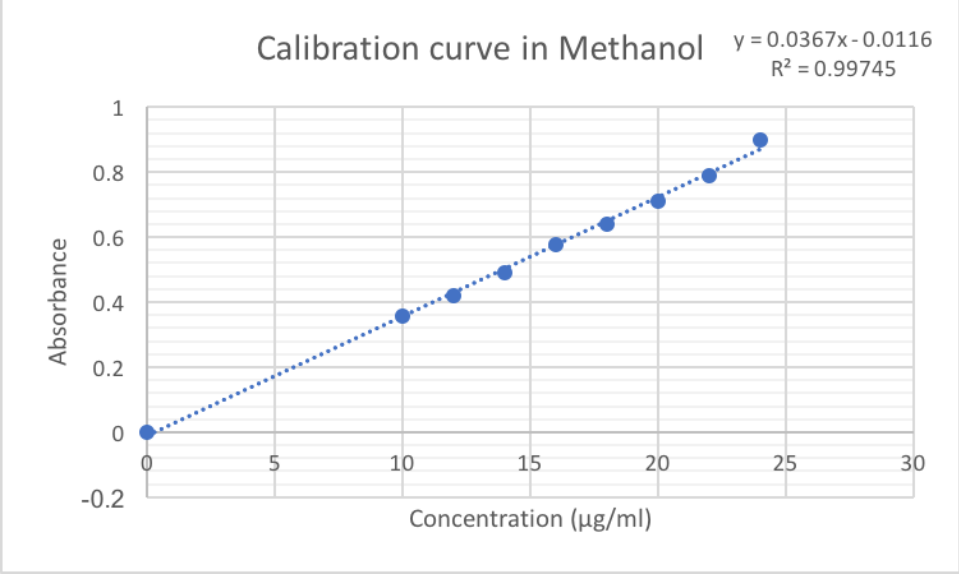


Figure 10

[5.2] Pre-formulation study

1) Screening of Oil, Surfactant, Co-Surfactant and Co-Solvents: -

The visual solubility study of drug is done, in this visual study the drug was solubilizing in the oil Capmul, Labrasol, Labrafil M2125, Capryol. The drug was also solubilized in the Co-solvent Transcutol. The drug was solubilized in the Surfactant which were PEG, Tween 80, Tween 20, Span 80

2) Super Saturation Solubility Study: -

The super saturation study shows the amount of drug getting dissolved in the selected excipients based on the screening of Oil, Surfactant, Solvents. After centrifugation it shows the undissolved got settled down to the Eppendorf tube and UV spectrophotometer shows how much drug got dissolved into the Oil.

a) Preparation of Transcutol with drug super saturation solution

In Transcutol around 140 mg got dissolved visually in 2ml of Eppendorf tube, the extra undissolved drug got settled down in the Eppendorf tube and then UV Spectra was taken, the absorbance was 1.09429 and peak was at 249 λmax.

(b) Preparation of Capmul MCM with drug super saturation solution

In Capmul MCM 129 mg drug got dissolved visually in 2ml of Eppendorf tube, after centrifugation undissolved drug settled down in the Eppendorf tube and absorbance was 0.1062 at λmax 249.

(c) Preparation of Labrasol with drug super saturation solution

In Labrasol 67 mg drug got dissolved visually in 2ml of Eppendorf tube, after centrifugation undissolved drug settled down in the Eppendorf tube and absorbance was 0.99359 at λmax 249.

(d) Preparation of Labrafil M2125 with drug super saturation solution

In Labrafil M2125 around 96.2 mg got dissolved visually in 2ml of Eppendorf tube, the extra undissolved drug got settled down in the Eppendorf tube and then UV Spectra was taken, the absorbance was 0.499102 and peak was at 249 λmax.

(e) Preparation of Plurol with drug super saturation solution

In Plurol 26 mg drug got dissolved visually in 2ml of Eppendorf tube, after centrifugation undissolved drug settled down in the Eppendorf tube and absorbance was 0.1973 at λmax 249.

(f) Preparation of Capryol with drug super saturation solution

In Capryol 34 mg drug got dissolved visually in 2ml of Eppendorf tube, after centrifugation undissolved drug settled down in the Eppendorf tube and absorbance was 0.13652 at λmax 249.

(g) Preparation of Span 20 with drug super saturation solution

In Span 20 surfactant 27 mg drug got dissolved visually in 2ml of Eppendorf tube, after centrifugation undissolved drug settled down in the Eppendorf tube and absorbance was 0.13155 at λmax 249.

(h) Preparation of Tween 20 with drug super saturation solution

In Tween 20 surfactant 25 mg drug got dissolved visually in 2ml of Eppendorf tube, after centrifugation undissolved drug settled down in the Eppendorf tube and absorbance was 0.11188 at λmax 249.

(i) Preparation of Tween 80 with drug super saturation solution

In Tween 80 surfactant 26 mg drug got dissolved visually in 2ml of Eppendorf tube, after centrifugation undissolved drug settled down in the Eppendorf tube and absorbance was 0.14589 at λmax 249.

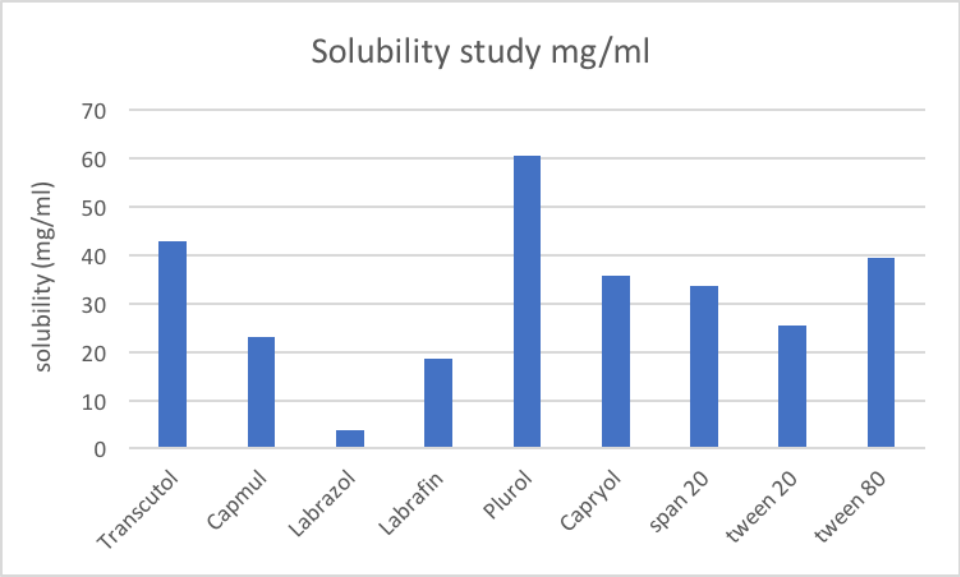


Figure 11

This figure shows the graph of solubility study of drug in the Oil, Surfactant and Solvents.

Table 8

Calculation of Amount of drug added to the 1ml of each solution.

Sr. No.	Lipid	Absorbance	Concentration	Dilution	Actual (mg/ml)	Amount Added(mg)
1	Transcutol	1.094	42.979	1	42.97	140
2	Capmul	0.106	02.310	10	23.10	129
3	Labrazol	0.993	38.834	0.1	03.83	67
4	Labrafil	0.499	18.485	1	18.48	96
5	Plurol	0.197	06.065	10	60.65	26
6	Capryol	0.136	03.564	10	35.64	34
7	Span 20	0.135	03.360	10	33.60	27
8	Tween 20	0.111	02.550	10	25.50	25
9	Tween 80	0.145	03.950	10	39.50	26

[5.3] Development of Pseudo Ternary Phase Diagram

Based on the Super Saturation Solubility Study Oil, Surfactant and Solvent was selected. Capmul was selected as Oil, Tween 80 was selected as Surfactant and Transcutol as Co-Solvent. Different surfactant and co-solvents were mixed with ration of 1:1, 1:2, 2:1. The ratio of Oil and Smix was 9:1,8:2,7:3,6:4,5:5,4:6,3:7,2:8 and1:9. So in the three Smix ratio the oil and Smix ration was performed with the water titration method, which is shown in following tables. The based-on solubility of the formulation to the 100 ml water the selected range was plotted in the ternary phase diagram which shows the higher stable emulsion.

In Smix ration of 2:1 in that the ratio of Smix: Oil in 9: 1 ratio whole solution was clear but in the Smix: Oil ratio of 8:2 the solution was hazy at 90 % of addition of water which shows the instability of the water. In ration of Smix: Oil at 60% of water addition the solution was hazy more than before. Also, in the ratio of Smix: Oil at 45% of water addition the solution was hazy so the formulation was getting hazy more in further Smix ratio with Oil.

In Smix ration of 1:1 in that the ratio of Smix: Oil in 9: 1 ratio whole solution was clear but in the Smix: Oil ratio of 8:2 the solution was hazy at 60 % of addition of water which shows the instability of the water. In ration of Smix: Oil at 45% of water addition the solution was hazy more than before. Also, in the ratio of Smix: Oil at 30% of water addition the solution was hazy so the formulation was getting hazy more in further Smix ratio with Oil.

In Smix ration of 1:2 in that the ratio of Smix: Oil in 9: 1 ratio whole solution was clear but in the Smix: Oil ratio of 8:2 the solution was hazy at 90 % of addition of water which shows the instability of the water. In ration of Smix: Oil at 70% of water addition the solution was hazy more than before. Also, in the ratio of Smix: Oil at 65% of water addition the solution was hazy so the formulation was getting hazy more in further Smix ratio with Oil.

So, Smix ratio of 1:2 was selected for the formulation of Nano emulsion and further ternary phase diagram was plotted, which gives the range of the selection for formulation.

In 1:2 ratio of Smix there is more solubility of the Capmul oil and Surfactant Tween 80 in each other and also it gives the clearer solution in the 100ml water which is the dilution of solution so it's selected ratio for the formulation.

Also, in ternary phase diagram there is more area covered by 1:2 ratio of Smix ratio, which shows the more solublisation of Oil, Surfactant and Water with each other and clear solution obtained.

1) Smix Ratio (tween 80: Transcutol): - (2:1)

Table 9

1)Surfactant: Oil (9:1) (450:50) µl										
Trial	Surfactant (µl) Smix (300:150)	Oil µl	water		Total	% Surfactant	% Oil	% water	% Total	
			%	µl						
1	450	50	5	50	550	81	9.09	9.09	100	+++
2	450	50	10	100	600	75	8.33	16.66	100	+++
3	450	50	15	150	650	25	65.7	8.60	100	+++
4	450	50	20	200	700	64	7.14	28.57	100	+++
5	450	50	25	250	750	60	6.66	33.33	100	+++
6	450	50	30	300	800	56	6.25	37.5	100	+++
7	450	50	35	350	850	52	5.88	41.17	100	+++
8	450	50	40	400	900	50	5.55	44.44	100	+++
9	450	50	45	450	950	47	5.26	47.36	100	+++
10	450	50	50	500	1000	45	5.00	50	100	+++
11	450	50	55	550	1050	42	4.76	52.38	100	+++
12	450	50	60	600	1100	40	4.54	54.54	100	+++
13	450	50	65	650	1150	39	4.34	56.52	100	+++
14	450	50	70	700	1200	37	4.16	58.33	100	+++
15	450	50	75	750	1250	36	4.00	60	100	+++
16	450	50	80	800	1300	34	3.84	61.53	100	+++
17	450	50	85	850	1350	33	3.70	62.28	100	+++
18	450	50	90	900	1400	32	3.57	64.28	100	+++
19	450	50	95	950	1450	31	3.44	65.51	100	+++
20	450	50	100	1000	1500	30	3.33	66.66	100	++

+++ shows the whole clear solution in 100ml water

2) Smix Ratio (Tween 80: Transcutol): - (1:1)

Table 10

1)Surfactant: Oil (9:1) (450:50) µl										
Trial	Surfactant (µl) Smix (300:150)	Oil µl	water		Total	% Surfactant	% Oil	% water	% Total	
			%	µl						
1	450	50	5	50	550	81	9.09	9.09	100	+++
2	450	50	10	100	600	75	8.33	16.66	100	+++
3	450	50	15	150	650	25	65.7	8.60	100	+++
4	450	50	20	200	700	64	7.14	28.57	100	+++
5	450	50	25	250	750	60	6.66	33.33	100	+++
6	450	50	30	300	800	56	6.25	37.5	100	+++
7	450	50	35	350	850	52	5.88	41.17	100	+++
8	450	50	40	400	900	50	5.55	44.44	100	+++
9	450	50	45	450	950	47	5.26	47.36	100	+++
10	450	50	50	500	1000	45	5.00	50	100	+++
11	450	50	55	550	1050	42	4.76	52.38	100	+++
12	450	50	60	600	1100	40	4.54	54.54	100	+++
13	450	50	65	650	1150	39	4.34	56.52	100	+++
14	450	50	70	700	1200	37	4.16	58.33	100	+++
15	450	50	75	750	1250	36	4.00	60	100	+++
16	450	50	80	800	1300	34	3.84	61.53	100	+++
17	450	50	85	850	1350	33	3.70	62.28	100	+++
18	450	50	90	900	1400	32	3.57	64.28	100	+++
19	450	50	95	950	1450	31	3.44	65.51	100	+++
20	450	50	100	1000	1500	30	3.33	66.66	100	++

+++ shows the whole clear solution in 100ml water

3) Smix ratio (Tween 80: Transcutol): - (1:2)

Table 11

1)Surfactant: Oil (9:1) (450:50) µl										
Trial	Surfactant (µl) Smix (300:150)	Oil µl	water		Total	% Surfactant	% Oil	% water	% Total	
			%	µl						
1	450	50	5	50	550	81	9.09	9.09	100	+++
2	450	50	10	100	600	75	8.33	16.66	100	+++
3	450	50	15	150	650	25	65.7	8.60	100	+++
4	450	50	20	200	700	64	7.14	28.57	100	+++
5	450	50	25	250	750	60	6.66	33.33	100	+++
6	450	50	30	300	800	56	6.25	37.5	100	+++
7	450	50	35	350	850	52	5.88	41.17	100	+++
8	450	50	40	400	900	50	5.55	44.44	100	+++
9	450	50	45	450	950	47	5.26	47.36	100	+++
10	450	50	50	500	1000	45	5.00	50	100	+++
11	450	50	55	550	1050	42	4.76	52.38	100	+++
12	450	50	60	600	1100	40	4.54	54.54	100	+++
13	450	50	65	650	1150	39	4.34	56.52	100	+++
14	450	50	70	700	1200	37	4.16	58.33	100	+++
15	450	50	75	750	1250	36	4.00	60	100	+++
16	450	50	80	800	1300	34	3.84	61.53	100	+++
17	450	50	85	850	1350	33	3.70	62.28	100	+++
18	450	50	90	900	1400	32	3.57	64.28	100	+++
19	450	50	95	950	1450	31	3.44	65.51	100	+++
20	450	50	100	1000	1500	30	3.33	66.66	100	+++

+++ shows the whole clear solution in 100ml water

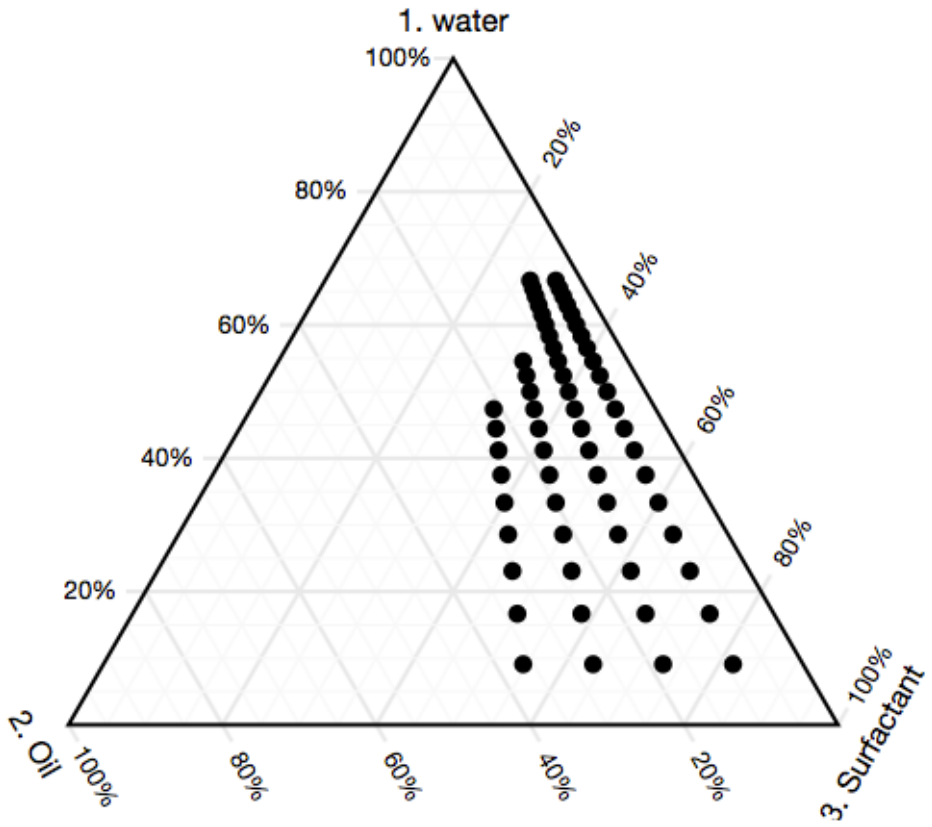


Figure 12

Smix ratio of Tween 80: Transcutol in 1: 2

This figure shows the ternary phase diagram of Smix ratio 1:2 and all the Oil and Smix ratio plotted which shows the very good solubility of emulsion in 100 ml water.

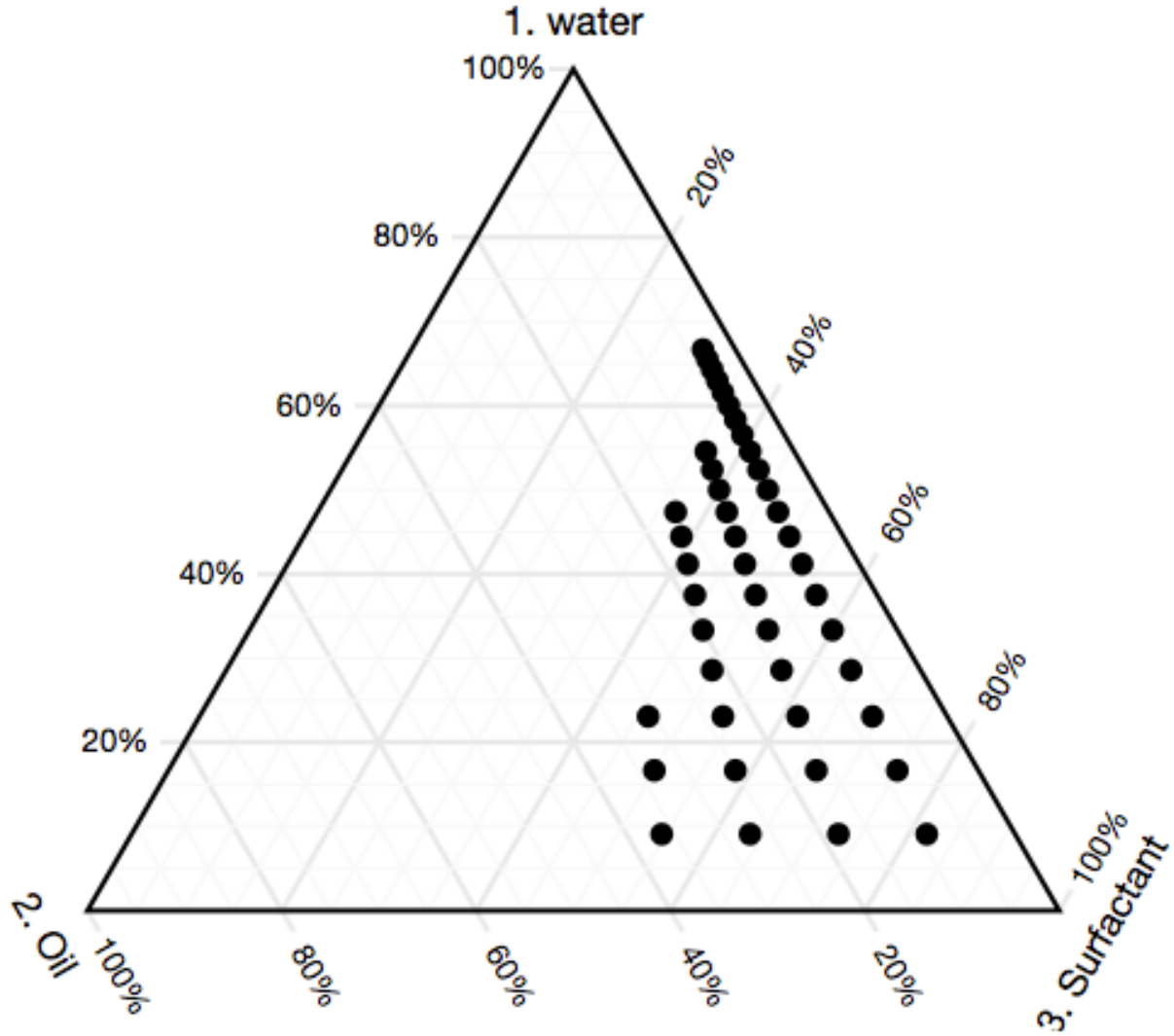


Figure 13

Smix Ratio of Tween 80: Transcutol 2: 1

This figure shows the ternary phase diagram of Smix ratio 2:1 and all the Oil and Smix ratio plotted which shows the very less solubility of emulsion in 100 ml water.

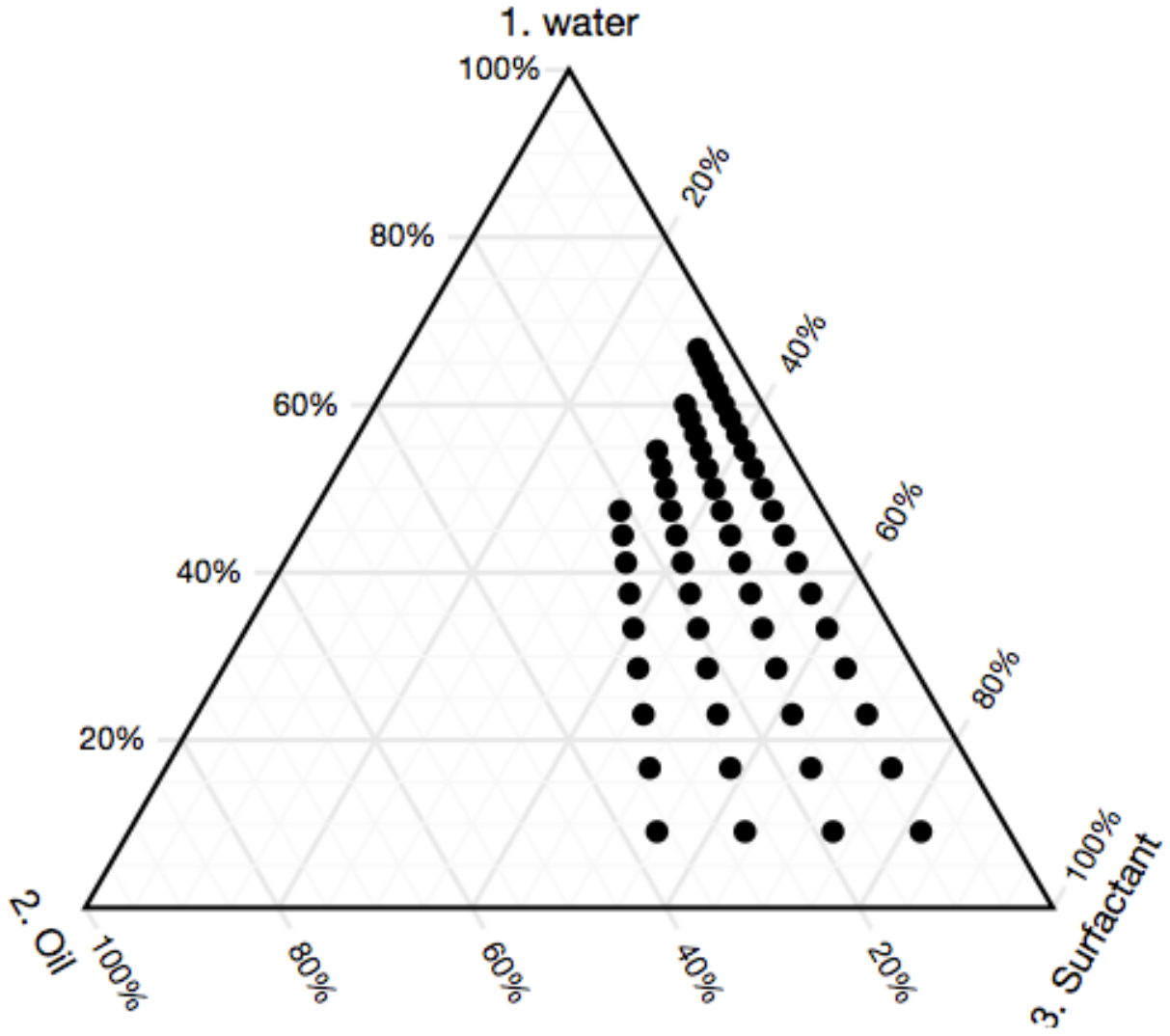


Figure 14

Smix Ratio of Tween 80: Transcutol 1:1

This figure shows the ternary phase diagram of Smix ratio 1:1 and all the Oil and Smix ratio plotted which shows the less solubility of emulsion in 100 ml water.

[5.4] Optimization by Mixture design of Atazanavir sulphate loaded Nano Emulsion

Based on the results of solubility data and pseudo ternary phase diagram the selected range was used for the development of Nano emulsion, the optimization of this emulsion done by applying the mixture design. The Oil Capmul MCM having lower range of 10% and higher limit 25% , Smix lower limit 25% and higher limit 40% and Water lower limit 50 % and higher limit 65%.

Oil was taken as Variable X1, Smix as Variable X2 and water taken as variable X3 as shown below table :-

Table 12

Variables taken in Mixture design

Independent Variables			Range (%)	
			Minimum	Maximum
X1	OIL	Capmul MCM	10	25
X2	Smix	Tween 80 + Transcutol	25	40
X3	Water	Water	50	65

Table 13

Sr. No	X1	X2	X3	Oil	Smix	Water
1	1	0	0	25	25	50
2	0	1	0	10	40	50
3	0	0	1	10	25	65
4	0.5	0.5	0	17.5	32.5	50
5	0.5	0	0.5	17.5	25	57.5
6	0	0.5	0.5	10	32.5	57.5
7	0.33	0.33	0.33	15	30	55

Batch A

Batch size : 4 ml

Table 14

B.No	Oil (ml)	Smix (ml)	Water (ml)	Drug	Observation	
A1	1	1	2	500	Clear Solution	+++
A2	0.4	1.6	2	500	Clear Solution	+++
A3	0.4	1	2.6	500	Clear Solution	+++
A4	0.7	1.3	2	500	Clear Solution	+++
A5	0.7	1	2.3	500	Clear Solution	+++
A6	0.4	1.3	2.3	500	Clear Solution	+++
A7	0.6	1.2	2.2	500	Clear Solution	+++

Particle Size Results of Batch A

Table 15

Sr. No	PI	Z -Average (nm)
1	0.849	473
2	0.348	3817.3
3	8.727	2545.8
4	4.948	2793.7
5	5.473	3733
6	4.355	1640
7	-	-

In this batch A From batch A1 to A6 we got the PI and Z-average but the soluctin was getting freeze so the batch was failed.

Conclusion: - After 5-6 hrs. drug was precipitate out/solidification so another batch B was prepared with the less amount of drug.

Batch B

Table 16

B.No	Oil (ml)	Smix (ml)	Water (ml)	Drug	Observation	
B1	1	1	2	300	Milky Appearance	++
B2	0.4	1.6	2	300	Solidification on standing	+
B3	0.4	1	2.6	300	1st milky then got freeze	+
B4	0.7	1.3	2	300	Solidification on standing	+
B5	0.7	1	2.3	300	Milky white appearance	++
B6	0.4	1.3	2.3	300	Clear Solution	+++
B7	0.6	1.2	2.2	300	1st clear then milky	+

Again there is solidification problems are occurring so further batches were taken.

Table 17

Sr. No	PI	Z -Average (nm)
1	12.287	3.6
2	-	-
3	9.204	4883.2
4	-	-
5	1.009	1498.8
6	0.577	893.6
7	-	-

Conclusion:

The Particle size and Z-average of the emulsion was not in the range and again emulsion was formulated again. Again there is solidification problems are occurring so further batches were taken.

Batch C

Batch Size 4 ml

Batch formulated using the 100 mg drug dissolved in the Varying concentration of oil, S mixture and Water . The drug stock table is shown in the Table.

Table 18

B.No	Oil (ml)	Smix (ml)	Water (ml)	Drug	Observation	
C1	1	1	2	100	Clear Solution	+++
C2	0.4	1.6	2	100	Clear Solution	+++
C3	0.4	1	2.6	100	Clear Solution	+++
C4	0.7	1.3	2	100	Clear Solution	+++
C5	0.7	1	2.3	100	Clear Solution	+++
C6	0.4	1.3	2.3	100	Clear Solution	+++
C7	0.6	1.2	2.2	100	Clear Solution	+++

Conclusion: -

Batch C Clear Solution was obtained at addition of 100 mg drug to Nano emulsion.

For better improvement in the emulsion and more batches were prepared with less change in excipients .

Batch D

Table 19

Sr. No	Excipients	Quantity
1	Drug	100mg
2	Capmul	1ml
3	Transcutol	1ml
4	Tween -80	0.8 ml
5	Water	1 ml

Table 20

Parameters	Result
Appearance	Hazy milky Solution

Batch E

Table 21

Sr. No	Excipients	Quantity
1	Drug	100mg
2	Capmul	1ml
3	Transcutol	1ml
4	Tween -80	0.8 ml
5	Span 80	0.5 ml
6	Water	1ml

In this batch combination of two surfactant was done and batch was analysed.

Table 22

Parameters	Result
Appearance	Milky Solution

Batch H

Table 23

Sr. No	Excipients	Quantity
1	Drug	100mg
2	Capmul	0.5ml
3	Oleic Acid	0.5 ml
4	Transcutol	1ml
5	Tween 80	0.5
6	Water	1 ml

Table 24

Parameters	Result
Appearance	Very clear solution

Batch I

Table 25

Sr. No	Excipients	Quantity
1	Drug	100mg
2	Capmul	1ml
3	Oleic Acid	0.5 ml
4	Transcutol	1ml
5	Tween 80	0.5
6	Water	1 ml

Table 26

Parameters	Result
Appearance	Hazy Solution

Batch J

Capmul and Oleic acid were taken in 1:1 ratio.

Table 27

B.No	Oil (ml) Capmul + Oleic acid	Smix (ml)	Water (ml)	Drug	Observation	
C1	1	1	2	100	Clear Solution	+++
C2	0.4	1.6	2	100	Clear Solution	+++
C3	0.4	1	2.6	100	Clear Solution	+++
C4	0.7	1.3	2	100	Clear Solution	+++
C5	0.7	1	2.3	100	Clear Solution	+++
C6	0.4	1.3	2.3	100	Clear Solution	+++
C7	0.6	1.2	2.2	100	Clear Solution	+++

Discussion:-

In batches D, E, I the formulation prepared having the problem with the solidification and also the formulation was getting hazy while formulating and upon dilution with the 100ml of water so , these batches got failed. Batch H has no hazy formulation problem and so solidification problem so batch H has good stability than other batches. Based on the batch H results as per mixture design batch J was prepared and had good results of solubility.

[6] Conclusion

From this study project we had investigated that Nano emulsion is the good drug delivery for Atazanavir sulphate for oral delivery. Compared to other formulation of Atazanavir Sulphate Nano emulsion is good and it can improve bioavailability of drug by this formulation.

Nano emulsion will get easily formulated and has more patient compliance than other formulation. This formulation has good dilution property of drug and can not precipitation of drug carried out.

Therefore, it is concluded that Atazanavir sulphate Nano emulsion might represent alternative treatment in HIV.

[7] Future Plan

1) In-vitro drug dissolution

As per OGD (office of generic drugs) Guideline for Dissolution testing to be done in medium as per FDA guidelines .

Drug Name	**Atazanavir Sulfate**	**Atazanavir Sulfate/ Cobicistat**
Dosage Form	Capsule	Tablet
USP Apparatus	II (Paddle)	II (Paddle)
Speed (RPMs)	50	75
Medium	0.025 N HCL	0.05 M Citrate Buffer (pH 2.8)
Volume (mL)	1000	1000
Recommended Sampling Times (minutes)	10, 20, 30 and 45	Atazanavir: 10, 15, 20, 30 and 45; Cobicistat: 5, 10, 15, 20 and 30

Same dissolution medium to per performed for the Nano emulsion For our Product.

2) Cytotoxicity Assay to be performed

Cytotoxicity assay can be performed on CACO-2 cell lines to determine the drug Cytotoxic Concentrations using with standard protocol .

- In briefly, different concentrations of drug Formulation varying from c (10µg/ml to 100µg/ml),) were mixed with virus and incubated for one hour at 37°C.
- The mixture was added to the CAC0-2 cell line in triplicates along with cell control, drug control and virus control and wait for one hour with irregular shaking.
- After one hour, incubate for 72 hours at 37°C under 5% CO2 and every day observed cells under light microscope.
- After 72 hours to estimate the cell viability by performing the Assay.

The effect will show a significant decrease in cell viability and an increase in cytotoxicity in a time- and dose-dependent study.

The Prospects are :-

1) Determination of In-vitro and Ex-vivo correlation of drug release study.
2) Determination of Pharmacokinetic of drug release.
3) Comparison of drug with marketed formulation.
4) Stability of Nano emulsion formulation.

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