

Method Development of Oil Solubility Test of Aceclofenac

By

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A thesis submitted to the Department of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

Department of Pharmacy

Brac University
October 2019

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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis/project titled “Method Development of Oil Solubility Test of Aceclofenac” submitted by Joytee Ray (15346016) of Summer, 2015 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy on 10 October, 2019.

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Ethics Statement

This study does not involve any kind of animal or human trial.

Abstract

The aim of the study was to develop a method that would allow the solubility testing of Aceclofenac in different oils. The main target was to find a suitable method by using commercially available drugs like olive oil. The process involved screening of oil after which extraction was done in a separating funnel in order to differentiate the API using hexane and methanol. The absorbance was taken in UV spectrophotometer and wavelength scan of hexane layer was found out. Series of extraction was done to see up to how many extractions were needed until there was no more API peak in the hexane layer and all the Aceclofenac was dissolved in methanol layer. The results showed that, after 10 times extraction the absorbance of the methanol layer at 277 nm came out 0.78 which falls within the ideal range 0.5-1 and no peak was seen in the hexane layer within the wavelength range.

Keywords: Nanoemulsion, solubility, Aceclofenac, extraction of oil, hexane layer.

Dedication

Dedicated to my parents, and all my respected teachers.

Acknowledgement

Firstly, I would like to thank my almighty Lord for all her blessings that gave me courage and patience to complete the project. I would then like to express my gratitude for my supervisor Namara Mariam Chowdhury, Lecturer, Department of Pharmacy, Brac University, for giving me the opportunity to work with her. She gave me constant support, and motivated me to work hard & stay focused on my project. Her approach and encouragement towards this project not only allowed me to complete the project on time but also helped me in getting hands on experience in lab. Lastly, I would like to thank my parents for believing in me and giving me unconditional support, faith and support in achieving my goals.

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List of Acronyms

OA	Osteoarthritis
API	Active Pharmaceutical Ingredient
BCS	Biopharmaceutics Classification System
FDA	Food and Drug Administration
ALQ	Above Limit Quantitation

Chapter 1

Introduction

1.1 Biopharmaceutics Classification System (BCS)

The Biopharmaceutics Classification System was created to enable *in vitro* prediction of pharmacokinetic results of drug products from permeability (determined as the level of oral absorption) and solubility assessments. (Wu & Benet, 2005). It is divided into four classes. (Sachan, Bhattacharya, Pushkar, & Mishra, 2009)

Table 1: Biopharmaceutical Classification System

High Solubility High Permeability	Low Solubility Low Permeability
Class I Propranolol, Metoprolol etc.	Class III Acyclovir, Neomycin B etc.
Low Solubility High Permeability	High Solubility Low Permeability
Class II Ketoconazole, Aceclofenac etc.	Class IV Clorthiazide, Furosemid etc.

In 2000, the BCS framework, which is a science-based strategy was used by FDA to enable the waiver of *in vitro* bioavailability and bioequivalence screening of immediate release solid

dosage forms for high-solubility, high-permeability drugs in Class 1 where such drugs also show fast breakdown (FDA, 2002). The BCS at its root, is an experimental system with skills linked to pH and absorption, ideally adopting permeability and solubility. The BCS aims to anticipate the pharmacokinetic efficiency of drug products in vitro from permeability and solubility readings. When the high dose strength is soluble in 250 mL or less of aqueous media over a pH range of 1–7.5 at 37 ° C, a drug substance is considered as "highly soluble." A drug substance is regarded to be "extremely permeable" if the level of intake (parent drug in addition to metabolites) is found in an individual is $\geq 90\%$ after administration of dose, which depends on the mass balance determination or in comparison to an intravenous reference dose. (Wu & Benet, 2005)

1.2 Drug delivery through skin

Skin is one of the extensively used routes for the local and systemic drugs. In addition, it also acts as a pathway for the delivery of drugs as nanoparticles. Though skin provides a natural physical hindrance to particle transmission, therapeutic nanoparticles could be delivered, especially in diseased skin and hair follicle openings. (Prow et al., 2011) Due to the advantages of transdermal drug delivery system which includes, the option of systemic drug treatment, elimination of first-pass metabolism and minimization of side effects, the development of topical drug delivery systems for systemic effects in recent times has gained increasing attention. (Mei, Chen, Weng, Yang, & Yang, 2003) In spite of having wide opportunities in transdermal drug delivery, it suffers from low permeability of the skin, which makes the absorption of drugs quite small. (Edwards & Blankschein, 1995) To accomplish these objectives, many dermal vehicles contain chemical stimulants and solvents. (Rastogi & Yadav, 2012) However, particularly in chronic application, use of these chemical enhancers can be dangerous, as many of them are irritants. To promote drug permeation through the skin, it is therefore suitable to create a topical vehicle system, which does not involve the chemical

enhancers usage. Microemulsion or nanoemulsion are some prospective methods for improving transdermal permeation of drugs.(Faiyaz Shakeel et al., 2007) It has been shown that it is possible to rise the retention time of a drug in the body with the assistance of nanoemulsion as a delivery system, thus requiring a small number of drugs for therapeutic action.(Tiwari, Shenoy, & Amiji, 2006) Nanoemulsion was recognized as a prospective method of delivery for multiple drugs, including biopharmaceuticals. It is a heterogeneous arrangement consisting of one immiscible liquid that spreads into another liquid as droplets. The size of nanoemulsion droplets range from 20 to 500 nm and its droplet diameter along with surface characteristics play a significant part in the formulation's physiological behavior. The minute droplet size particles result in translucent emulsions so that an oil stage does not alter the appearance of the product. The word nanoemulsion also relates to a miniemulsion where the surfactant molecules are stabilized through interfacial film having a size from 20–600 nm that is fine oil / water or water / oil dispersion. Nanoemulsions are transparent due to tiny size. (Pagar & Darekar, 2019)The three types of nanoemulsion that can be formed are:

1. Oil in water nanoemulsion in which oil is dispersed in the continuous aqueous phase,
2. Water in oil nanoemulsion in which water droplets are dispersed in continuous oil phase
3. Bi-continuous nanoemulsion (F Shakeel et al., 2008)(Anton, Benoit, & Saulnier, 2008)

1.3 Importance of Solubility

Solubility depends on solute, which involves the characteristics of a solid, liquid, or gaseous chemical substance that dissolve in solvent that can be solid, liquid, or gaseous solvent in order to form a homogeneous solvent solution.A substance's solubility relies primarily on the

liquid used, heat and pressure. The magnitude of a substance's solubility in a particular solvent is assessed as the rate of saturation where the addition of more solvent does not elevate its quantity in the fluid. (Fox, 1970)

Due to its convenience of administration, strong patient compliance, cost effectiveness, lowest sterility limitations, and flexible in the layout of the dosage form, oral ingestion is the most useful and widely used method of drug delivery. As a consequence, many generic drug companies are more likely to create oral drug goods that are bioequivalent(Krishnaiah, 2010). However, the major challenge with the design of oral dosage forms lies with their poor bioavailability. The oral bioavailability depends on several factors including aqueous solubility, drug permeability, dissolution rate, first-pass metabolism, pre-systemic metabolism, and susceptibility to efflux mechanisms. The most frequent causes of low oral bioavailability are attributed to poor solubility and low permeability.(Savjani, Gajjar, & Savjani, 2012)

Solubility also plays a major role for other dosage forms like parenteral formulations as well.(Kerns & Di, 2008) In order to obtain the desired pharmacological response, one of the important parameters for achieving required drug concentration in systemic circulation is solubility. (Vemula, Lagishetty, & Lingala, 2010)Drugs which have poor solubility often involve elevated amounts after daily oral administration to achieve therapy plasma levels. The main issue that is faced with the creation of formulations of new chemical entities along with generic development is having low aqueous solubility. In order to be absorbed at the site of absorption, a drug must need to be available aqueous solution. Water is the preferred solvent for formulation of liquid pharmaceuticals. Majority of drugs seem to be either poorly acidic or poorly basic with weak solubility in the aqueous solution.(Savjani et al., 2012)

The latest tendency towards improving the solubility/bioavailability of poorly soluble compounds is lipid-based structures such as nanoemulsions, microemulsions, solid

dispersions, strong lipid nanoparticles/nanosuspensions, nanosuspensions which are polymeric in nature, niosomes and liposomes, etc.(Faiyaz Shakeel & Ramadan, 2009)Lipids are analyzed as constitute of various oily liquids and dispersion hence it was seen from the point of view of the delivery of oral drugs which were aimed at elevating the solubility and bioavailability of drugs belonging to Class II & IV of the Biopharmaceutics Classification(V. P. Shah & Amidon, 2014)

Drug delivery system in case of nanoemulsion has become one of the prospective innovations and this is order to maximize the oral bioavailability of those drugs that are poorly soluble this technology being utilized.Nanoemulsion allows ultra-low tension between the interfaces and big interfaces. Nanoemulsions provides benefits over volatile dispersions as for example, emulsions and suspensions as they have greater solubilizing ability which is basic micellar alternatives and their thermodynamic stabilization and this is because they can be produced with little power consumption (thermal or blending) and have a lengthy storage existence.Nanosized particles resulting in huge interfacial fields connected with nanoemulsions will also affect the drug's transportation characteristics, a significant consideration in continuous and targeted drug delivery. (Welling, 2007)(Lawrence & Rees, 2012)in case of formulation of o/w nanoemulsion technologies much appeal resides in their capacity to integrate hydrophobic drugs into the oil stage and thus improve their solubility.(Lawrence & Rees, 2012)

1.4 Techniques for Solubility Enhancement

There are different techniques for improving the enhancement of solubility, which can be classified as physical modification, chemical modifications of the drug substance, and miscellaneous techniques. (Savjani et al., 2012)

- **Physical Modifications.** Reduction of particle size such as micronization and nanosuspension, alteration of crystal habit such as polymorphs, amorphous structure

and co-crystallization, dispersion of drugs in transports such as eutectic mixtures, solid dispersions, solid solutions and cryogenic methods.

- **Chemical Modifications** Modification of pH, buffer use, derivation, complexation and salt development
- **Miscellaneous Methods:** Supercritical method of fluid, adjuvants use such as surfactants, solubilizers, and co-solvency, hydrotrophy and special excipients.

1.5 Application of nanoemulsion

Nanoemulsion has already become a very appealing formulation for pharmaceuticals delivery. Nanoemulsion also has a strong cosmetic benefit. For the following reasons, the appeal of nanoemulsion formulations in pharmaceuticals and cosmetics. (Sharma N, Bansal M, Visht S, Sharma P K, 2010)

Due to its very tiny droplet, nanoemulsion never demonstrates creaming and sedimentation issues. With standard emulsion and even microemulsion, these issues are very prevalent. Generally, force of gravity impacts the emulsion droplet which is connected with both the issues. But the droplet size is very tiny for instance nanoemulsion, which minimized gravitational work.

- Again, tiny nanoemulsion droplet size avoids droplet coalescence. Droplets fall together in the coalescence method and create a big droplet with a larger volume that is liable for emulsion turbulence. But the tiny droplet volume of nanoemulsion prevents coalescence between them and prohibits distortion and fluctuation of the surface.
- The disintegration of nanoemulsion relative to microemulsion is quite high, this is because the tiny droplet volume blocks the droplet flocculation and this procedure causes the system to be dispersed in lieu of separation.

- The introduction of nanoemulsion offers a fast introduction of effective components through the hair owing to the big droplet layer. Even nanoemulsion is discovered to readily pass through tough skin at times. This nanoemulsion ownership minimizes the extra use of a unique surface enhancer accountable for product incompatibility.
- Compared to microemulsion, nanoemulsion composition needed a small quantity of surfactant. For example, about 20-25% surfactant is needed for microemulsion preparation, but in the case of nanoemulsion, 5-10% surfactant is sufficient. Again, it is possible to minimize the use of nanoemulsion surfactant.
- Due to the lack of any thickening agent and colloidal particles, nanoemulsion has a transparent and fluid structure that increases treatment patient compliance and is secure for administration.
- It is also noted that, nanoemulsion can be used specifically in cancer therapy for the targeting active ingredients delivery.
- For liposomes and vesicle types of transporting devices, nanoemulsion design can become a stable alternative.

1.6 Mechanism of Nanoemulsion

1. High pressure homogenization (HPH)
1. Hot homogenization
2. Cold homogenization
2. Ultrasonication or high-speed homogenization
3. Microemulsion
4. Phase inversion
5. Solvent Evaporation Technique
6. Double emulsion technique
7. Solvent emulsification-diffusion method

8. Spontaneous Emulsification

9. Microfluidization

10. Hydrogel Method

1.6.1 Highpressurehomogenization

Technique of high stress homogenization used to formulate NLCs. High stress homogenizers pass through a tight divide through a elevated stress fluid (100–2000 psi). The fluid accelerates to elevated speed (over 1000 km / h) at a tiny range. Very elevated cavitation and shear stress disrupts the solids down to the spectrum of submicrons. Usually 5-10% lipid material is used, but up to 40% lipid content is also studied. Two types of hot homogenization and dry homogenization are high stress homogenization. In this two, by dissolving or dispersing the drug in the lipid foam or liquid lipid, a design phase provides the drug integration into the total lipid. (Liu, Kurihara-Bergstrom, & Good, 1991) (Lippacher, Müller, & Mäder, 2000)

1.6.2 Hot homogenization

Homogenization happens in this technique at temperatures above the lipid melting point. Drug-loaded lipid foam is spread through the blending unit (Ultra-Turrax) in the warm aqueous surfactants (isothermal) stage and contributes to pre-emulsion creation. Because of the reduced viscosity at high temperatures, particle size becomes lesser mainly. There are three primary problems in Hot homogenization. The first is the drug's temperature-dependent degradation, the second is that the drug penetrates during homogenization into the aqueous stage and the final is the difficulty of the nanoemulsion's crystallization step causing to several changes and/or super-cooled melts. (Mehnert & Mader, 2001)

1.6.3 Cold homogenization

In the technique of hot homogenization, the drug is placed in the lipid bath and quickly dried by liquid nitrogen or dry ice. Milling results in the creation of 50-100 nm of nanoparticles that are dispersible in a cool surfactant stage that forms a pre-suspension. PHP is performed at room temperature, causing the nanoparticles to break into NLCs. The cold homogenization method was extended to solve warm homogenization issues (Loxley, 2009)(B. Mishra, Patel, & Tiwari, 2010)(Parhi & Suresh, 2010)(Naseri, Valizadeh, & Zakeri-Milani, 2015)

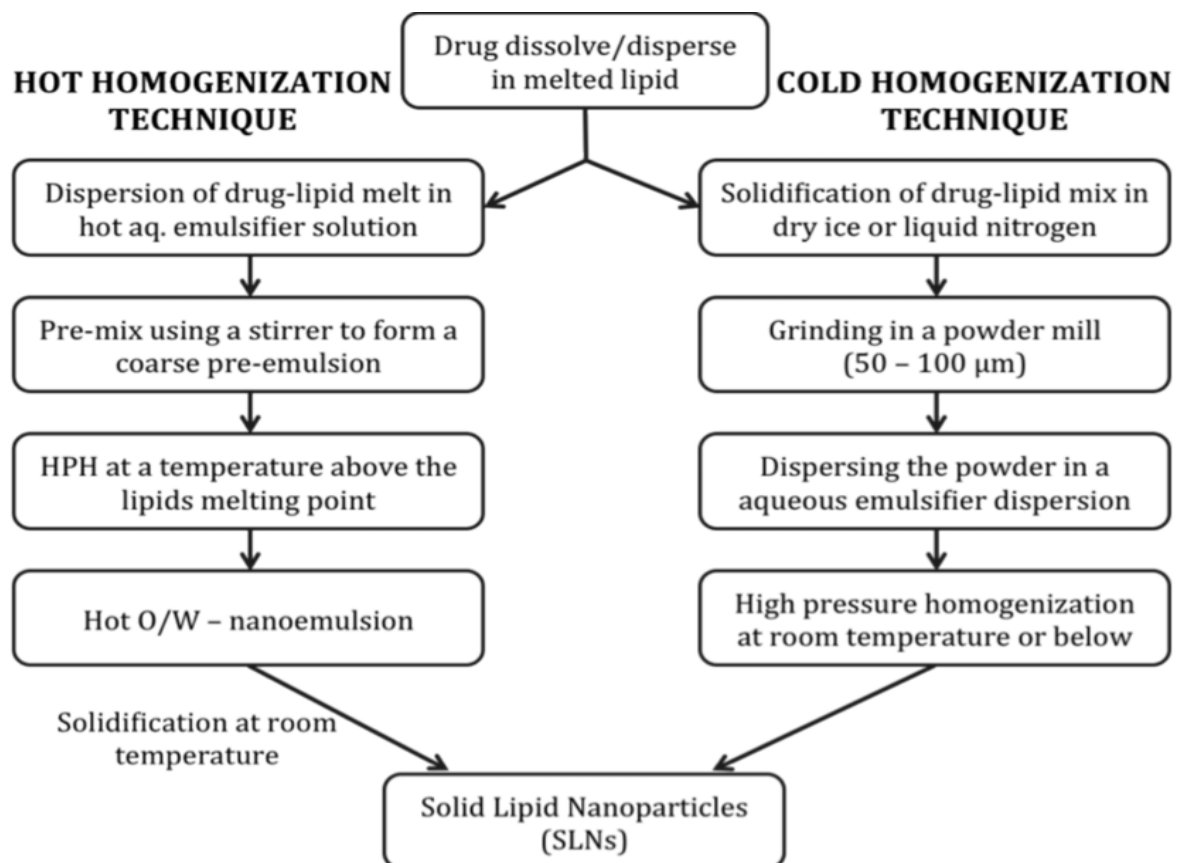


Figure 1: Hot homogenization and cold homogenization method(Khatak & Dureja, 2015)

1.6.4 Ultrasonication or high-speed homogenization

NLCs have also been created by stirring or sonication at elevated velocity. A major advantage is that any machinery used here is very prevalent in any laboratory. The issue with

this technique is a wider distribution of particle size varying from micrometer to micrometer. This physical disruption result prefers the development of particles after processing. Also, a large issue in this technique is potential metal contamination owing to ultrasonics. Therefore, for creating a stable version, different study organizations have carried out trials that combine and perform elevated velocity mixing and ultrasonics at elevated temperatures. (Eldem, Speiser, & Hincal, 1991)(Puglia et al., 2008)



Figure 2: Ultrasonication (Gajanan, Milind, & Adhikrao, 2017)

1.6.5 Microemulsion

This method is based on the dilution of microemulsions. As micro-emulsions are two-phase systems composed of an inner and outer phase (e.g. o/w microemulsions). They are made by stirring an optically transparent mixture at 65-70°C, which typically composed of a low melting fatty acid (e.g. stearic acid), an emulsifier (e.g. polysorbate 20), co-emulsifiers (e.g. butanol) and water. The hot microemulsion is dispersed in cold water (2-3°C) under stirring. NLCs dispersion can be used as granulation fluid for transferring in to solid product (tablets, pellets) by granulation process, but in case of low particle content too much of water needs to be removed. High-temperature gradients facilitate rapid lipid crystallization and prevent aggregation.(Priano et al., 2007)(Gharge & Pawar, 2017)

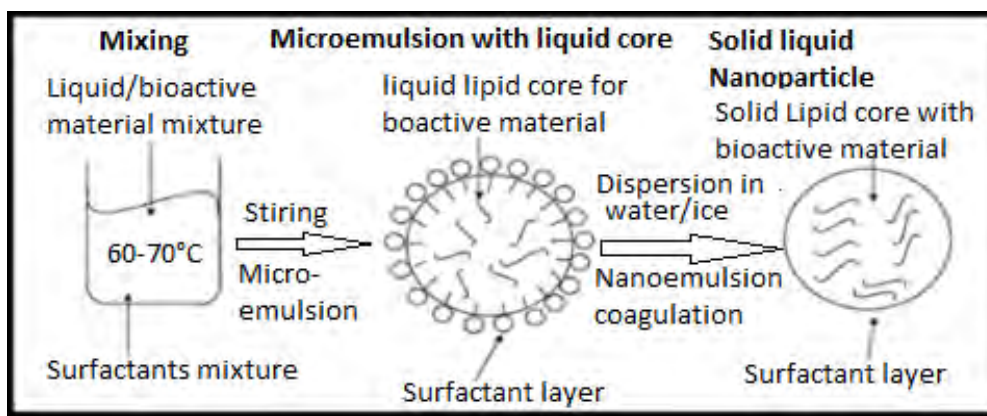


Figure 3: Microemulsion Method (Gajanan et al., 2017)

1.6.6 Phase inversion method

In this technique, the chemical energy arising from phase transitions generated by the emulsification process is acquired through good dispersion. The phase transition is achieved by changing the emulsion structure and maintaining a steady temperature or vice versa. Shinoda et al first performed the phase inversion temperature. It was found that by degradation of the polymer chain with the temperature, the rise in temperature outcomes in chemical changes of polyoxyethelene surfactants. (Baid & Nadu, 2011)

1.6.7 Solvent Evaporation Technique

This is a technique similar to NLCs solvent evaporation by precipitation in o / w emulsions. The lipid is dissolved in solvent emulsification-evaporation in a water-immiscible organic solvent (e.g. toluene, chloroform) which is then emulsified in an aqueous phase before the solvent is evaporated under reduced pressure conditions. The lipid precipitates upon evaporation of the solvent thus forming nanoparticles. When the solvent is evaporated, the lipid precipitates thus forming nanoparticles. First, an organic stage comprising the lipid content embedded in a water-immiscible organic solvent has been generated and then the drug is boiled or spread in the fluid. Mechanical stirring in an o/w surfactant that contains

aqueous stage emulsifies this organic stage. Following rapid expulsion of the solvent by evaporation from the acquired o/w emulsion under mechanical stirring or decreased nanoparticulate stress dispersion, lipid precipitation in the aqueous environment is created. To prevent aggregation of particles, the solvent evaporation phase must be swift. This technique is appropriate for incorporating extremely thermolabile medicines owing to heat prevention during preparing, but the existence of solvent particles in initial dispersion may cause issues owing to legislative concerns. Limited solubility of lipids in organic materials generally leads to dilute dispersions and need to concentrate by means of another process such as ultra-filtration, evaporation or lyophilization. On the other hand, small particle size around 100 nm with narrow size distribution can be achieved by this method. Limited lipid solubility in organic products usually results in diluted dispersions and need to be concentrated through other processes such as ultra-filtration, evaporation, or lyophilization. On the other side, this technique can achieve tiny particle size of around 100 nm with a tight volume allocation. (Shahgaldian, Da Silva, Coleman, Rather, & Zaworotko, 2003) (Wissing, Kayser, & Müller, 2004)

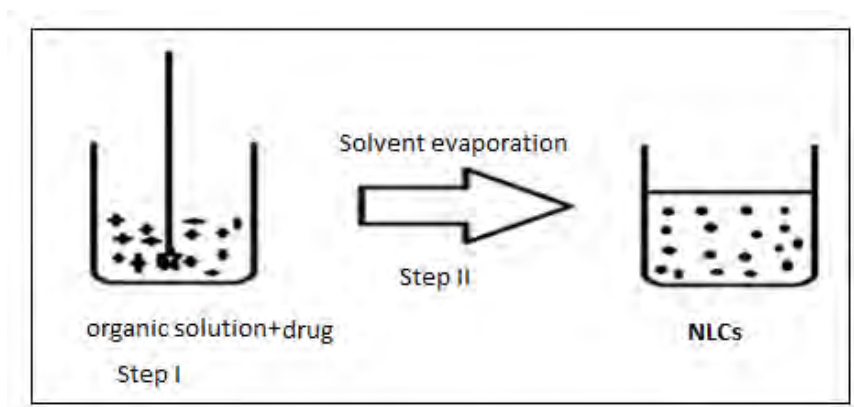


Figure 4: Solvent Evaporation Technique (Gajanan et al., 2017)

1.6.8 Double emulsion technique

To prepare NLCs, method of double emulsion is utilized. In this, the drug was dissolved in aqueous solution (mainly hydrophilic drugs) and then emulsified in melted lipid. By adding

stabilizer (e.g. gelatin, poloxamer-407), this main emulsion has been maintained. In an aqueous stage comprising hydrophilic emulsifier (e.g. PVA), this stable main emulsion was distributed. Double emulsion technique tries to avoid the need to melt the lipid in order to prepare peptide-loaded lipid nanoparticles and the exterior layer of the nanoparticles could be altered to stabilize them sterically by incorporating a lipid / PEG derivative. Sterical stability in the gastrointestinal liquids considerably enhanced the strength of these colloidal systems. This method is primarily used for hydrophilic (peptides) encapsulation.(Cortesi, Esposito, Luca, & Nastruzzi, 2002)

1.6.9 Solvent emulsification-diffusion method

The solvent emulsification-diffusion method can also produce NLCs. The average particle size relies on the quantity of lipids and the emulsifier used in the organic stage. This method can be used to obtain particles with median diameters of 30-100 nm. The most significant benefit of this method is the negligence of heat during the preparing. The lipid matrix is opened accompanied by emulsification in water-immiscible organic solvent, in an aqueous stage. Under decreased stress arising in dispersion of nanoparticles which is caused by lipid precipitation in aqueous fluid, the solvent is evaporated.(Trotta, Debernardi, & Caputo, 2003)

1.6.10 Spontaneous Emulsification

It involves three main steps:

- i. Preparation in water miscible solvent and hydrophilic surfactant of homogeneous organic fluid consisting of oil and lipophilic surfactant.
- ii. The organic stage was the placed under magnetic stirring in the aqueous stage, forming the o / w emulsion.

- iii. Under decreased stress, the water-miscible solvent was deleted by evaporation.(Mishra, Soni, & Mishra, 2014)

1.6.11 Microfluidization

Microfluidization is a mixing method that uses a microfluidizer machine. This unit utilizes a high-pressure positive displacement pump (500 to 20000psi) that pushes the item through the room of contact consisting of tiny tubes called "microchannels". Through the microchannels, the item runs into an impingement region arising in very good sub-micron distance droplets. To produce a rough emulsion in an upright homogenizer the two alternatives (aqueous stage and oily stage) are mixed and handled together. The dense emulsion is in a microfluidizer where a stable nanoemulsion is further handled. The crude emulsion is constantly moved through the microfluidizer of the contact room until the required particle size is achieved. The mass emulsion is then washed under nitrogen through a filter to extract big particles leading to a standardized nanoemulsion.(Hadgraft, 2001)

1.6.12 Hydrogel Method

It is comparable to the technique of solvents evaporation. The only distinction between the two techniques is that with the anti-solvent drug, the drug solvent is miscible. Higher shear force stops the development of crystals and the maturation of Ostwald.(R. K. Mishra, Soni, & Mishra, 2014b)

1.7 Importance of oil screening method

Before ensuring a good formulation of the nanoemulsion, it is necessary to consider some important issues like oil screening, co-surfactant and surfactant screening.

In o/w nanoemulsions, drugs which are lipophilic in nature are preferably solubilized, while w/o solutions appear to be a stronger option for hydrophilic drugs. In the growth of nanoemulsion structures for drugs that are poorly soluble, loading of drug per formulation is a

very critical layout variable, which depends on drug solubility in multiple parts of the formulation. This is particularly important in the case of oral formulation development, as the ability of nanoemulsion to maintain the drug in solubilized form is greatly influenced by the solubility of the drug in the oil phase. To deliver the drug's therapeutic dose in an embedded form, the volume of the formulation should be minimized as much as feasible. Drug solubility is an significant criteria for the choice of oils in the oil stage. (Azeem et al., 2009) If the surfactant or cosurfactant leads solubilization of drug, there may be possibility of precipitation, as nanoemulsion dilution in the gastrointestinal tract will reduce the surfactant or cosurfactant's solvent ability. (Narang, Delmarre, & Gao, 2007) (Lawrence & Rees, 2012) Thus, an awareness of variables affecting medication charging ability while preserving the solutions ability to experience monophasic fluid dilution and minimizing medication rainfall or crystallization trends in diluted structures is crucial for the development of sustainable and adequately low-volume nanoemulsion systems for applying the distribution of medication. Lately, novel semisynthetic that have medium-chain derivatives, which can be described as surfactant-defined amphiphilic compounds, are recommended. (Azeem et al., 2009)

From the literature, it was found that Aceclofenac is poorly soluble in water. For enhancement, elevating of the solubility of the API Aceclofenac oil screening study is important step for the identification of the suitable oils that will be most desirable for the drug. We have used olive oil and screened 10 times to see the results. It is noted that previous formulation of nanoemulsion of Aceclofenac was done by incorporating industrial graded oils. For this reason, another purpose was to see if it is feasible to formulate the nanoemulsion of Aceclofenac using commercially available oil like olive oil.

1.8 Reason behind choosing Aceclofenac

Osteoarthritis (OA) prevalence increases with age, affecting 60% of men and 70% of women after 65 years of age. (Pareek et al., 2009) In elderly people it is affected particularly in the knee and hip joints. (Litwic, Edwards, Dennison, & Cooper, 2013) Predominant indications are pain in the joints, rigidity, restricted motion, and reduced performance of lives. Disease progression can contribute to pain and disability joint loss. (Litwic et al., 2013) Osteoarthritis creates harm and reduction of articular cartilage in the synovial joints, reduces the density of the subchondral layer and contributes to subarticular bone remodeling, osteophyte development, ligament inflexibility, periarticular muscle shrinking, synovial inflammation along with formation of cyst in the subchondral bone. (Litwic et al., 2013) (Mukherjee, Rachita, Aisen, & Pasinetti, 2001) Cytokines are produced by synovial tissue neurons and subchondral osteoblasts. In the catabolic process of cartilage degradation, IL-1 beta and tumor necrosis factor (TNF)-alpha are important cytokines. (Mukherjee et al., 2001) (Berenbaum, 2011)

OA is one of the most prevalent types of diseases among arthritis, which is also ascribed to as degenerative joint disease, or diabetes of “wear and tear”. (Arthritis Foundation, 2008) It is generally linked with aging and most probably affects joints that are constantly strained over the years, including ankles, hips, toes and lower spine area. (“WHO | Chronic rheumatic conditions,” 2016) Osteoarthritis is spread worldwide that comprises about nearly 9.6% of males and 18% female who are at the age of over 60 years old. (“WHO | Chronic rheumatic conditions,” 2016) It is referred as India’s top five chronic diseases that affects about 4-6 percent of the adult population. (“Arthritis-India”, 2014) Though some treatment attempts to slow the progression of the disease, the ultimate objective of osteoarthritis treatment is to reduce pain and improve function, as there is no disease cure. (“Diseases and Conditions Osteoarthritis”, 2019). Most often, physical interventions, drug therapy and sometimes

surgery are included in the mixture of therapies. (“Diseases and Conditions Osteoarthritis”,2019) OA (osteoarthritis) pharmacotherapy concentrates primarily on pain alleviation, performance of lives retention, and cognitive independence conservation. Currently, there has been found no pharmacological drugs that can retard development or prevent OA. (“Osteoarthritis Treatment Information”,2011). Oral acetaminophen, cannabis and topical non-steroidal anti-inflammatory drugs (NSAIDs), oral specific cyclooxygenase-2 (COX-2) agents, opiates, topical capsaicin, intraarticular steroidal injections and hyalronans are pharmacological products for the therapy of OA.(Steinmeyer & Kontinen, 2006)

To decrease pain and inflammation, the most frequently used drugs are non-steroidal anti-inflammatory drugs (NSAIDs).(Alvarez-Figueroa & Blanco-Méndez, 2001)In case of the therapy of the rheumatoid arthritis and osteoarthritis, suggestion for oral drug is Aceclofenac, which is a NSAID.(Attwood, Mallon, Ktistis, & Taylor, 1992)(Baboota et al., 2007) It also has functions that are anti-inflammatory, antipyretic, & analgesic.(Baboota, Shakeel, & Kohli, 2006) Gastrointestinal ulcers and gastrointestinal bleeding with acute use are caused by oral administration of aceclofenac. It also creates anemia due to gastrointestinal bleeding.(Attwood et al., 1992). Anemia is also created due to gastrointestinal bleeding. Using the transdermal pathway, it eliminates these side effects, improves patient compliance, prevents metabolism in the first step and retains the amount of plasma drugs for a longer span of moment (Shah, Magdum, Patil, & Niakwad,2010)

It is noted that, there is much advantages for Aceclofenac in improving knee activity in people with osteoarthritis in this meta-analysis. No important distinctions were found in pain frequency relief between Aeclofenac and regulate analgesics.A significant restriction of long-term use of NSAID in osteoarthritis is gastrointestinal adverse events. It is seen that, there are less gastrointestinal danger adverse events with Aceclofenac.(Patel & Patel, 2017)

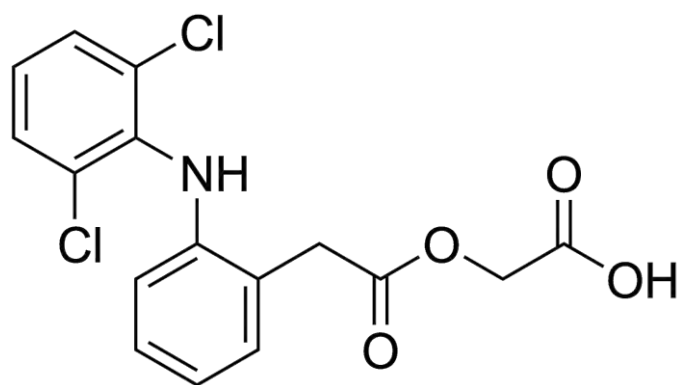


Figure 5: Structure of Aceclofenac (Malik, Ahmad, Minhas, & Munir, 2014)

After oral administration, it is processed with first-pass hepatic metabolism.(Mohammed S & Sheikh Shafiq, 2009) Due to its low aqueous solubility, which presents a dissolution-related issue of absorption, attempts have been produced to enhance its solubility and dissolution with various oils(Malik et al., 2014) For this reason, Aceclofenac has been used as API in this experiment.

1.9 Aims and Objectives

The aim of this experiment is to develop a method that would permit the quantification of the amount of API that was dissolved in olive oil. The objective of the study was to find out up to how many extractions there will be no more API peak present in the hexane layer and all the Aceclofenac will be dissolved in the methanol layer.

Chapter 2

Materials

For conducting the study, the Aceclofenac was chosen as an API. The API was sent by Quality pharmaceuticals as a gift. The solvents that were chosen include Methanol (Merck, Germany) and n-Hexane (DaeJung, Korea). The study involved the oils which is specifically Olive oil (Span Olive, Spain). The other reagents that was used for the purpose of this study ensured analytical grade and also were prepared or procured from the Brac University labs. UV spectrometer (Shimadzu UV Spectrometer, Model no: UV 1800) was used to conduct the analysis of those oils.

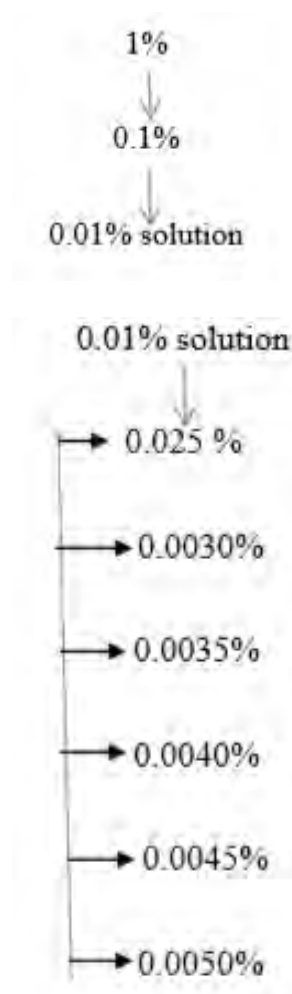
Chapter 3

Method

3.1 Preparation of Standard Curve

For keeping the absorbance within 0.5 to 1 dilution was conducted.

Calculation:



Then 6 different solutions were made with that solution. Absorbance was checked for each.

The standard curve was made using MS Excel.

3.2 Extraction of oil

After the standard curve was prepared, screening was conducted. The oil that was chosen for the purpose of the study was olive oil. In a 5.67mL of oil an excess amount of 0.03 gm of API was added to make the desired solution. Then the solution was kept in Digital Shaking Incubator for about 72 hours with 37°C at 150rpm. The solution was then kept in the centrifuge machine at 400rpm for about 15 minutes. With the help of cotton the undissolved API was filtered out. It was essential to extract the dissolved API first from the oil as it creates hindrance in UV readings. Hence, 5mL of methanol and 5mL of hexane was placed in the separating funnel for the purpose of filtrating out the solution. The oil has the tendency to dissolve into the hydrophobic hexane more whereas Aceclofenac or the API has more tendency to dissolve into hydrophilic methanol. It was kept for some time to get finely mixed from where the upper along with the lower layer was separated from the separating funnel. At the end, for the purpose of taking the readings of the absorbance, UV spectrometer was used where the absorbance of Aceclofenac was taken 277nm. Then wavelength scan of the hexane layer was taken through UV taking the range from 200 to 400 nm, to check for any remaining API. The whole procedure was repeated until there was no more API peak in the hexane layer.

Chapter 4

Results

4.1 Preparation of Standard Curve:

Table 2: Table for standard curve of Aceclofenac

Concentration (% w/v)	Absorbance
0.0025	0.539
0.0030	0.746
0.0035	0.826
0.0040	0.919
0.0045	1.108
0.0050	1.167

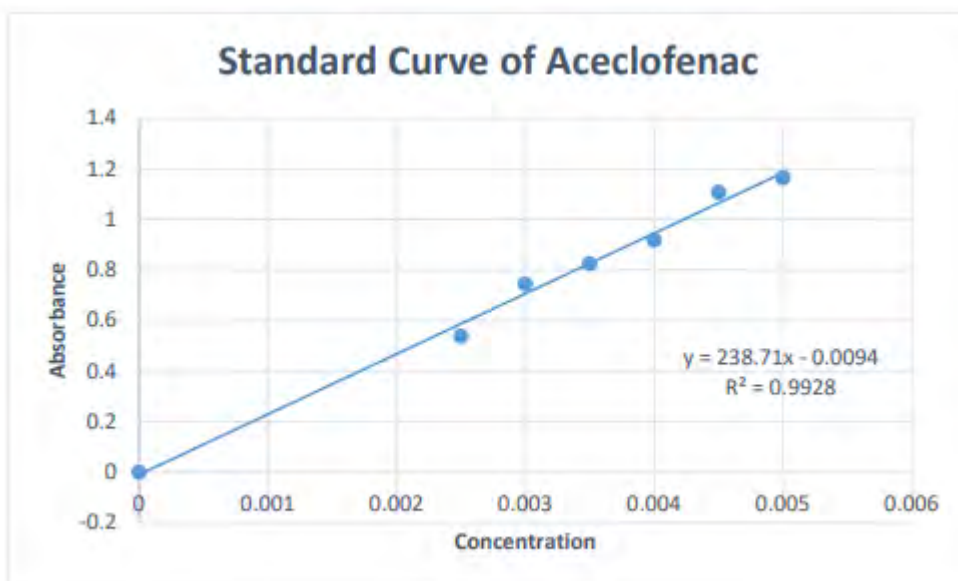


Figure 6: Standard curve of Aceclofenac

After putting the value on the equation, that was found from the standard curve $y=238.71x-0.0094$.

The R square value was found to be 0.9928. This equation was used in order to calculate the concentration of the API Aceclofenac extracted from the olive oil.

4.2 Three times extraction of Olive oil:

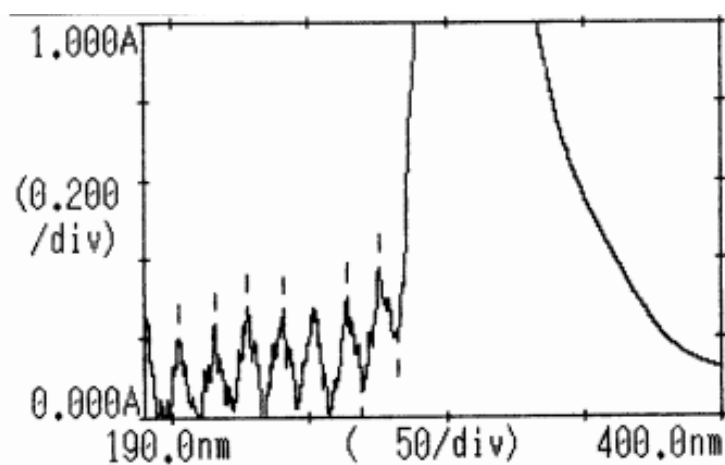


Figure 7: Wavelength of Hexane layer after 3 times extraction

Table 3: Absorbance vs Peak after 3 times extraction

No.	Wavelength	Absorbance
1	307.4	3.261
2	275.4	0.378
3	263.6	0.305
4	240.4	0.273
5	227.4	0.279
6	215.4	0.234
7	202.8	0.202

After 3times extraction, the absorbance of methanol layer was 0.155 so further extraction were needed as hexane layer still showed API peak.

4.3 Five times extraction of olive oil:

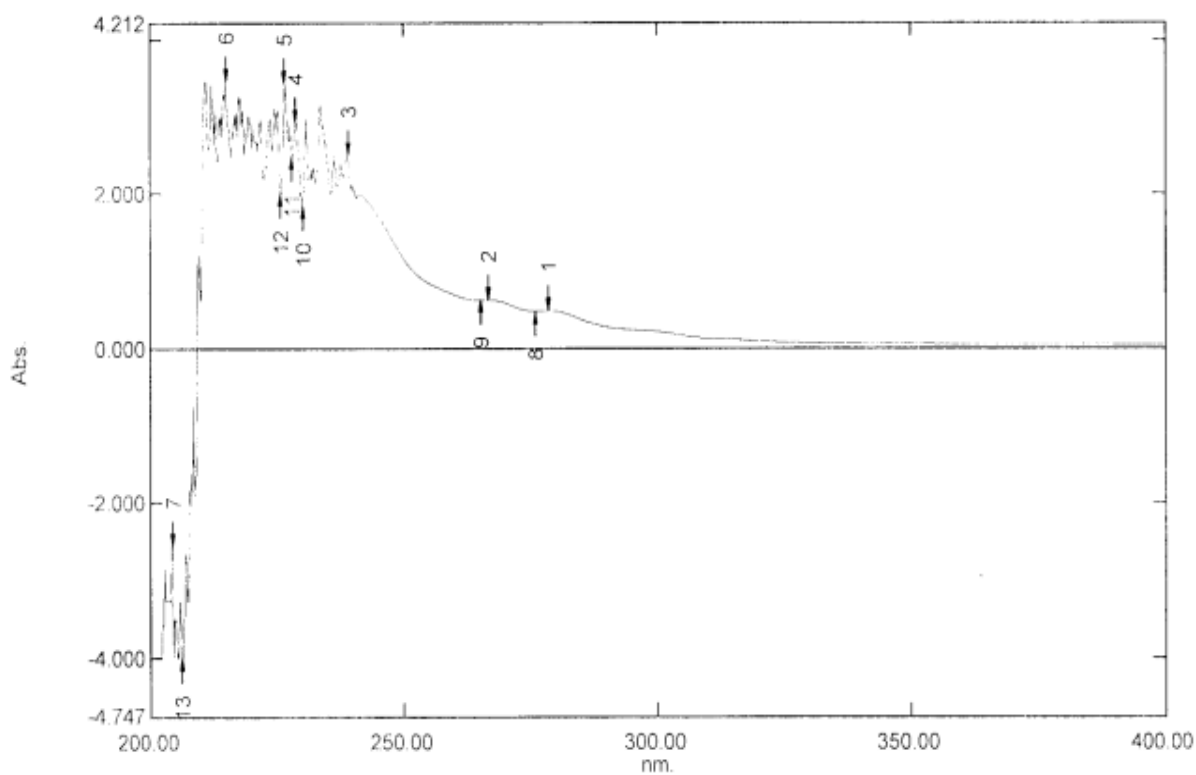


Figure 8: Wavelength of Hexane layer after 5 times extraction

Table 4: Absorbance vs Peak after 5 times extraction

No.	Wavelength	Absorbance
1	278.40	0.491
2	266.60	0.628
3	239.20	2.529
4	229.00	2.949

After 5times extraction, the absorbance of methanol layer was 0.418 so further extraction were needed as hexane layer still showed API peak.

4.4 Seven times extraction of Olive oil:

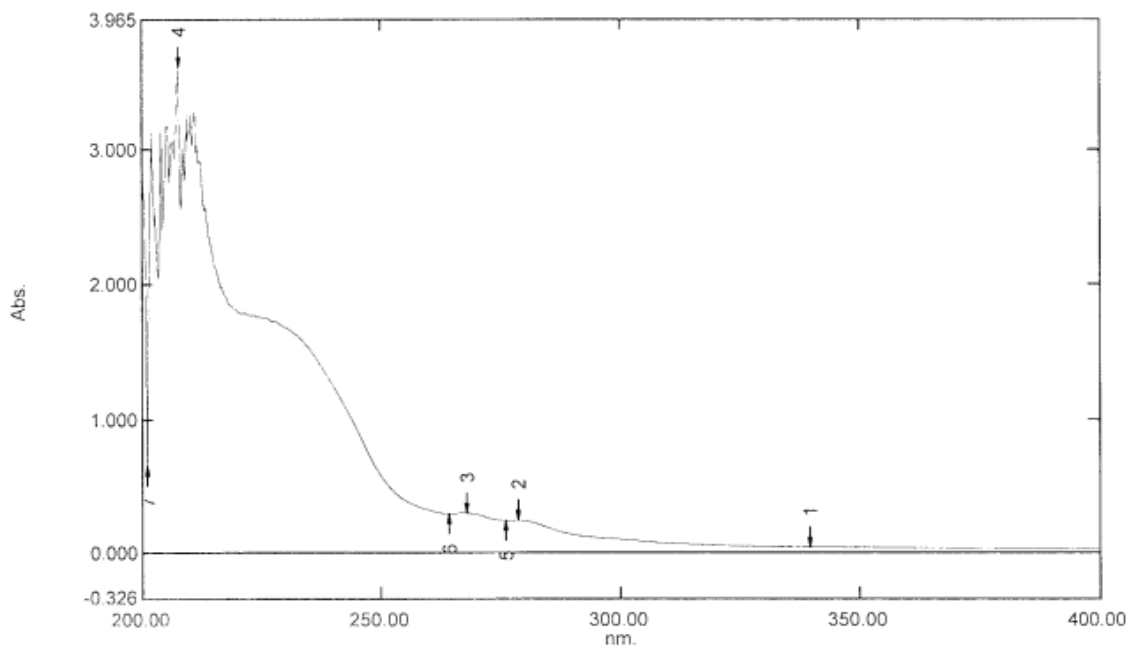


Figure 9: Wavelength of Hexane layer after 7 times extraction

Table 5: Absorbance vs Peak after 7 times extraction

No.	Wavelength	Absorbance
1	339.60	0.048
2	278.600	0.246
3	267.60	0.301

After 7times extraction, the absorbance of methanol layer was 0.564 so further extraction were needed as hexane layer still showed API peak.

4.5 Nine times extraction of Olive oil:

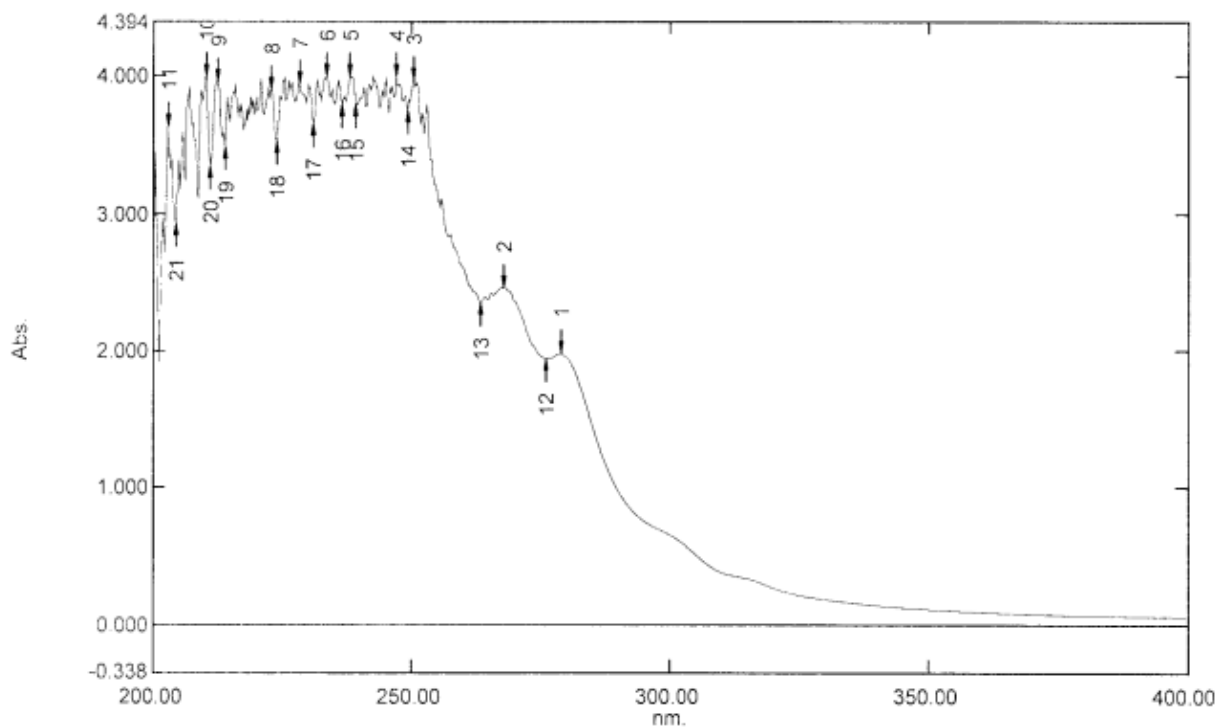


Figure 10: Wavelength of Hexane layer after 9 times extraction

Table 6: Absorbance vs Peak after 9 times extraction

No.	Wavelength	Absorbance
1	278.80	1.982
2	267.80	2.463
3	250.40	3.975

After 9times extraction, the absorbance of methanol layer was 0.764 so further extraction were needed as hexane layer still showed API peak.

4.6 Ten times extraction of Olive oil:

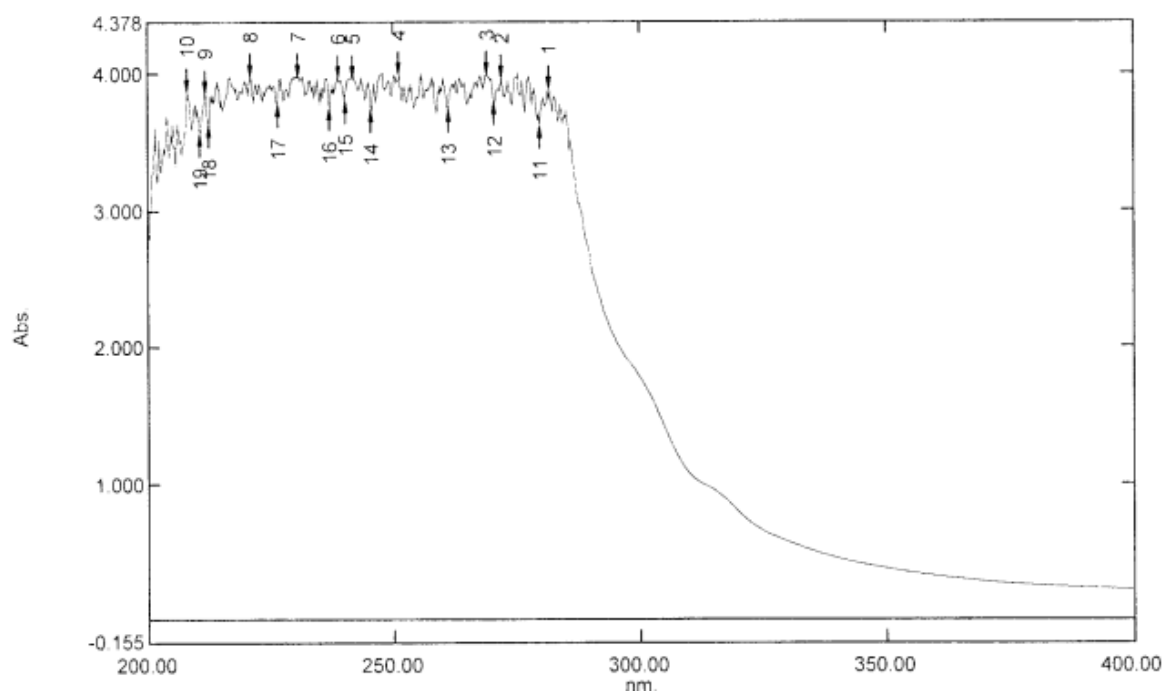


Figure 11: Wavelength of Hexane layer after 10 times extraction

Table 7: Absorbance vs Peak after 10 times extraction

No.	Wavelength	Absorbance
1	281.60	3.888
2	271.80	3.970
3	269.00	3.996
4	251.20	4.000
5	242.00	3.976
6	238.80	3.982
7	230.60	3.985
8	221.20	3.994
9	211.80	3.868

After 10times extraction, the absorbance of methanol layer was 0.78 and there was no API peak in the hexane layer for which no more extraction was needed.

The absorbance of the methanol, was then put into the standard curve equation and the concentration of API that was dissolved in the oil was 0.00331 % w/v or 0.0331 mg/mL

Chapter 5

Discussion

From the literature, it was known that, the process of solubility test of Aceclofenac in different oils in previous studies involved a lengthy procedure where at first the solubility in methanol, ethanol, isopropyl alcohol and n-butanol were found out to build their calibration equations in the multiple solvents since Aceclofenac is easily soluble in these solvents. For each of those solvents, Aceclofenac stock solutions had been prepared and serial dilutions were produced from stock solutions respectively. Secondly, through UV spectrophotometer at wavelength 276 nm, the solubility to Aceclofenac in different suspensions including water was measured and further in all sample matrices, an excess of Aceclofenac (5mL of each fluid) was put in triplicate in 20 mL stopped glass vials in order to achieve balance, these stopped glass vials were agitated at 25 ± 100 °C in a mechanical shaker steam bath for straight 72 hours (Malik et al., 2014)

In contrast to this procedure, our study involved less time as standard curve equation was made to calculate the Aceclofenac concentration in different oil. However, the API in olive oil was put in the shaking incubator for 72 hours too but the whole procedure was executed within less time than previous studies. The lambda max value of Aceclofenac is between 273 nm -276 nm but the best results came out at 277nm. (Dasgupta, Dey, Choudhury, & Mazumder, 2013) Extraction of olive oil was done at first done for three times but the absorbance found was 0.155 where ideal range is within 0.5-1. In the wavelength scan of hexane layer, it showed API peak for which the extraction process was repeated. After the 5 times of olive oil, there were still API peak in the hexane layer as a result; more dilution was needed for which the procedure was extracted for 7 times, 9 times and 10 times respectively. In the 9 times extraction, the absorbance was 0.764 which is within the ideal range but API

peak was seen between 270 nm and 280 nm and so finally the extraction was done for 10 times to see if any more peak still remains in the hexane layer or not.

In addition, it can be said that with increasing number of extractions, the amount of API in the hexane layer did decrease and more API was extracted into the methanol layer. This is because, with increasing number of extractions, the absorbance of the methanol layer, with the same dilution factor, also increased in value. Thus, indicating that more API was partitioning into the methanol layer.

After 10 times extraction, the absorbance of the methanol layer at 277 nm came out 0.78 and there was no API peak in the hexane layer. This shows that the API was able to eliminate from the olive oil or the hexane layer successfully and dissolved in methanol layer. For the preparation of nanoemulsion, it was found that almond oil and oleic acid were most used oils in order to test their solubility in Aceclofenac but no such comparison was seen between almond oil and olive oil for which our study focused on olive oil for analyzing the comparison. Therefore, this study should be repeated with almond oil and oleic acid to see the comparison for API that was extracted in literature and that of this prospective method of extraction.

Chapter 6

Conclusion

Solubility is one of the significant criteria for attaining required medication level in the systemic circulation in order to provoke pharmacological reaction. Drug effectiveness can be significantly restricted owing to bad water solubility and some drugs also have adverse effects owing to their poor solubility. (Chaudhary et al., 2012)

The aim of the study was to develop a method where solubility of Aceclofenac in different oil can be tested. The procedure started by preparing the standard curve for keeping the absorbance between 0.5-1. Afterwards in 5.67 mL of olive oil, API Aceclofenac was added until saturation. Then after keeping in shaking incubator for 72 hours, it was filtered out for extraction. Extraction was done to separate the API from the oil and it was done until Aceclofenac get fully dissolved in methanol layer and no API peak is seen in the hexane layer. In the 3 times extraction, no API peak was found in the hexane layer but the absorbance was too low for which consecutive extraction was done until the absorbance came between 0.5 to 1 and no API peak was found in hexane layer. After 10 times extraction, the absorbance was found 0.78 and no peak was seen which implies all the API was dissolved in the methanol layer successfully. The experiment was conducted up to 10 times with only a single oil. Therefore, this method must still be further conducted with other oils to check for reproducibility and reliability before making any definite conclusions about its possibility in oil screening.

It was found that, previous formulation of nanoemulsion of Aceclofenac was executed by incorporating industrial graded oils for which, another motive was to see if it is feasible to formulate the nanoemulsion of Aceclofenac using commercially available oil like olive oil. In this project, due to time constraint, only the test was done with olive oil but it needs to be done with other oil in order to see if this procedure works or not.

Chapter 7

Future Studies

For time limitation, the method was developed focusing only one commercially available oil which is olive oil. Future studies can involve using different other commercially available oils like almond oil, sunflower oil, oleic oil etc. for method validation. The outcomes of method verification can be used to assess the value, accuracy and accuracy of the analytical outcomes.

The validation results for parameters of validity are acquired during process validation. Selectivity, awareness, accuracy, precision, reproducibility and stabilization are the vital parameters needed by FDA Guidance. While these parameters are obtained, other parameters are also defined during verification (e.g., extraction efficiency, calibration spectrum and reaction feature [linear or discrete], positional variations within an analytical cycle, and quality of dilution for evaluating above quantity threshold [ALQ] specimens)(Bansal & DeStefano, 2007)

In addition to this, permeability test can be done along solubility test for ensure their effectiveness and improvement of solubility in the GI tract in order to improve the patient adherence to the drug Aceclofenac.

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