IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Formulation Strategy For Dissolution Enhancement Of Atovaquone

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1. INTRODUCTION

1.1 Oral Solid Dosage Forms[1]

The oral route is considered as the most promising route of drug delivery. The high level of patient compliance in oral drug delivery systems is due to the ease of administration and handling of these systems. An Oral Dosage Form is the physical form of a dose of a chemical compound used as a drug or medication intended for administration or consumption by oral route. Common oral dosage forms are tablets or capsules. Tablets are solid preparations each containing a single dose of one or more active substances with or without excipients usually obtained by compressing uniform volumes of particles. Tablets are intended for oral administration. Some are swallowed whole, some after being chewed, some are dissolved or dispersed in water before being administered and some are retained in the mouth where the active substance is liberated. The excipients can include binders, glidants and lubricants to ensure efficient tabletting and disintegration to promote tablet break-up in the digestive tract; sweeteners or flavours to enhance taste; and pigments to make the tablets visually attractive. These are included in the formulations to facilitate easy handling, enhance the physical appearance, and improve stability and aid in the delivery of the drug to the blood stream after administration. A polymer coating is often applied to make the tablet smoother and easier to swallow, to control the release rate of the active ingredient, to make it more resistant to the environment (extending its shelf life), or to enhance the tablet's appearance.

Solid dosage forms include; powders, granules, tablets, capsules.

1.1.1 Advantages of Solid Dosage Forms

1. More stable than liquids, with longer expiration dates.
2. Easy shipping and handling.
3. Less needed shelf space.
4. No preservation requirements.
5. Accurate dosage (single dose).
6. Suitable for sustained release preparation.

1.1.2 Disadvantages of Solid Dosage Forms

1. Their preparation needs complicated and expensive machines.

1.2 Tablet

1.2.1 Introduction to Tablet[2,3,4]

Tablets are solid unit dosage form, flat or biconvex in shape, prepared by compressing a drug or a mixture of drugs with or without suitable excipients.

Tablets may be swallowed whole or being chewed. Some are dissolved or dispersed in water before administration. Implants or pesseries may also be presented in the form of tablet. Tablet may vary in shape and differ greatly in size and weight depending on the amount of medicinal substance and the intended mode of administration.

1.2.2 Advantages

- They are unit dosage form, and they offer the capabilities of all oral dosage forms for the dose precision and the least content variability during dosing.
- Accuracy and uniformity of drug content
- Optimal drug dissolution and hence, availability from the dosage form for absorption consistent with intended use (i.e., immediate or extended release).
- Usually taken orally, but can be administered sublingually, rectally or intravaginally.
- Their cost is lowest of all oral dosage forms
- They are the most compact of all oral dosage forms
- They are in general the easier and cheaper to package and ship as compare to other oral dosage forms
- Product identification is simple and cheap, requiring no additional processing steps when employing an embossed or monogrammed punch face
- They are ease to administer, does not require a specialist
- They are better suited to large-scale production than other unit oral forms
- They have the better properties of chemical, mechanical and microbiological stability

1.2.3 Disadvantages

- Some drugs resist compression, due to their amorphous nature or low-density
- Drugs having bitter taste, objectionable odour or drugs that are sensitive to oxygen may require encapsulation or coating of tablet ➢ Bioavailability problems.
- Chance of GI irritation caused by locally high concentrations medicament.
- Difficulty in swallowing tablets in a small proportion of people and so size and shape become important considerations.
- Slow onset of action as compared to parenterals and solutions.

1.2.4 Type and classes of Tablet[3]

A. Oral Tablets for Ingestion

- Compressed tablets
- Multiple compressed tablets
- Layered tablets
- Compression-coated tablets
- Repeat-action tablets
- Delayed-action and enteric-coated tablets
- Sugar and chocolate-coated tablets
- Film coated tablets
- Chewable tablets

B. Tablets Used in the Oral Cavity

- Buccal tablets
- Sublingual tablets
- Troches and lozenges
- Dental cones

C. Tablets Administered by Other Routes

- Implantation tablets
- Vaginal tablets

D. Tablets Used to Prepare Solutions

- Effervescent tablets
- Dispensing tablets
- Hypodermic tablets
- Tablet triturates

1.2.5 Excipient Used In Tablet Dosage Form[5]

Tablet generally consists of mixture of active pharmaceutical ingredient and excipient. Excipient means any component other than the active pharmaceutical ingredient(s). While selecting excipients for any formulation following things should be considered wherever possible.

- Keep the excipients to a minimum in number
- Minimize the quantity of each excipients and
- Multifunctional excipients may be given preference over unifunctional excipients.

Excipients are chosen in tablet formulation to perform varieties of functions like

- For providing essential manufacturing technology functions (binders, glidants, lubricants may be added)
- For enhancing patient acceptance (flavours, colorants may be added)
- For providing aid in product identification (colorants may be added)

- For Optimizing or modifying drug release (disintegrants, hydrophilic polymers, wetting agents, biodegradable polymers may be added)
- For enhancing stability (antioxidant, UV absorbers may be added)

1.2.6 Ideal Characteristics of Excipients

- They must be non toxic with no pharmacological activity and acceptable to the regulatory agencies in the countries where the product is to be marketed
- They must be commercially available in an acceptable grade in countries where the product is to be manufacture
- Cost effective
- They must be physiologically inert
- They must be physically and chemically stable by themselves and in combination with other drugs and tablet components
- They must not have an adverse effect on the bioavailability of the products.

Excipient	Function	Example
Diluents	Used as filler designed to make up the required bulk of the tablet.	Lactose,starch, mannitol, sucrose, sorbitol etc.
Binders and Adhesives	These are used to produce cohesive compact, either in dry or wet form.	Acacia, starch, povidone, cellulose derivative etc.
Disintegrants	Used to facilitate a breakup of the tablet.	Starch, clays, cellulose, alginate, povidone etc.
Lubricants	Used to reduce the friction during tablet ejection between the walls of die cavity.	Stearic acid, stearic acid salts, polyethylene glycol, talc, waxes etc.
Antiadherants	Used to reduce sticking or adhesion of any tablet granules or powder to the faces of punches or die wall.	Talc, polyethylene glycol, hydrogenated castor oil, glyceryl behenate etc.
Glidants or flow promoters	Used to promote flow of the tablet granules or powder material by reducing friction within particles.	Silica derivatives, talc, cornstarch etc.

Table 1: Excipients used in tablet formulation

1.2.7 Tablet Manufacturing[6,7]

The manufacturing of oral solid dosage forms such as tablets is a complex multi-stage process under which the starting materials change their physical characteristics a number of times before the final dosage form is produced. Traditionally, tablets have been made by granulation. Both wet granulation and dry granulation or direct compression is used.

1.2.7.1 Operation Involved in Tablet Manufacturing[7]

The manufacture of oral solid dosage forms such as tablets is a complex multi-stage process.

Numerous unit processes involved in making tablets are as follow,

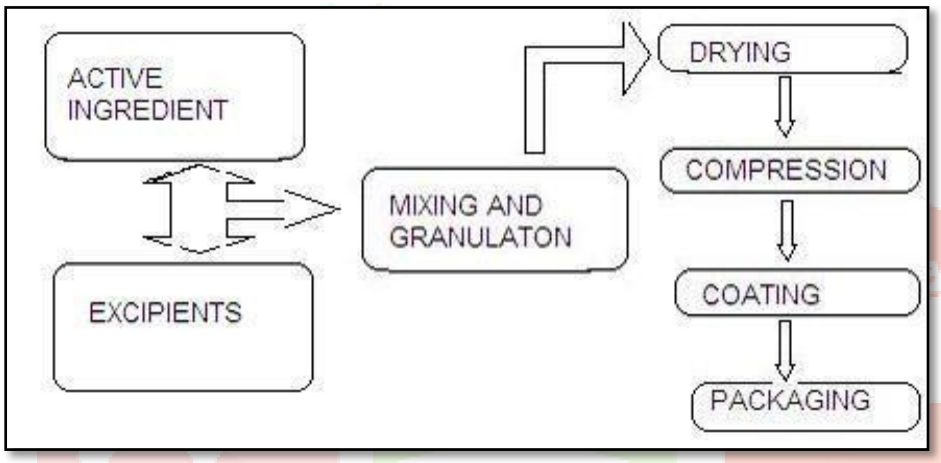


Figure 1: Flow chart for tablet manufacturing process

In general, the choice of method for the manufacture of tablets is dependent on a number of factors like:-

a. The physical and chemical stability of the therapeutic agent during the manufacturing process.
b. The availability of the necessary processing equipment.
c. The cost of the manufacturing process and
d. The excipients used to formulate the product.

1.2.8 Fate of Tablet with in GIT[8]

After the administration of tablet series of event occur until its absorption in to systemic circulation as depicted in following figure 2.

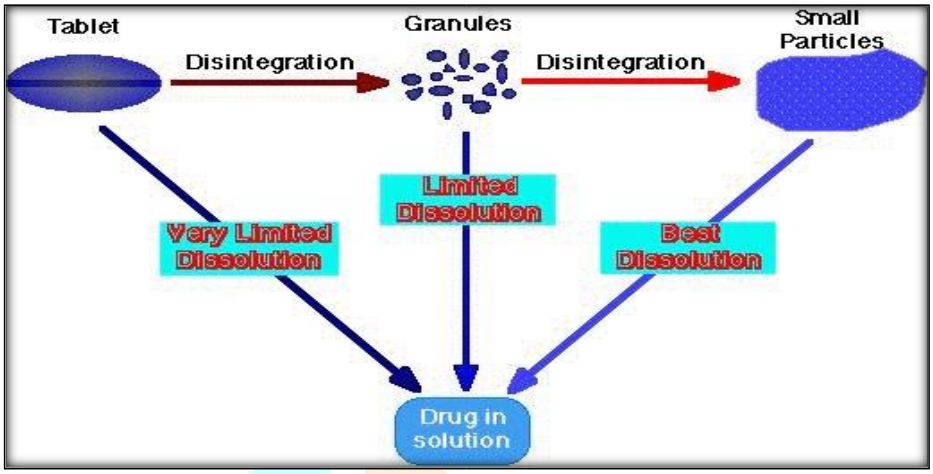


Figure 2: Event of tablet after administration

The process consists of four steps:

1. Disintegration of tablet in granules
2. Disintegration of granules into small particles
3. Dissolution of the drug in aqueous fluids at absorption site
4. Movement of dissolved drug through the GI membrane into the systemic circulation

1.2.9 Problems in Tablet Manufacturing[9],[10]

Following are the defects that are found during tablet manufacturing

- Capping
- Lamination / Laminating
- Chipping
- Cracking
- Sticking / Filming
- Picking
- Binding
- Mottling
- Double impression

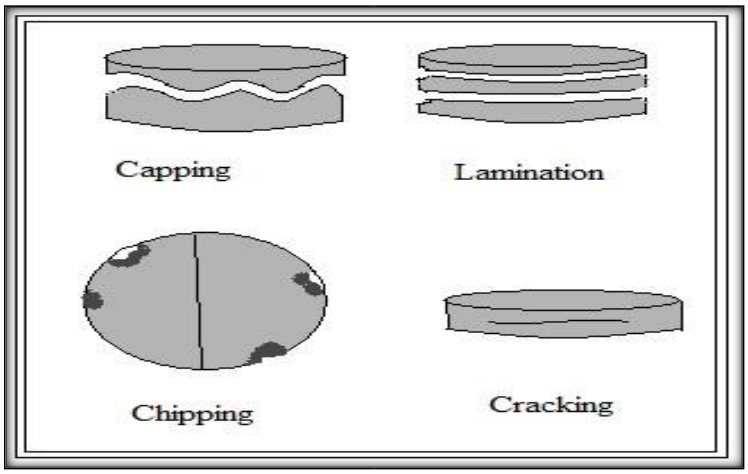


Figure 3: Tablet Defects

1.2.10 Evaluation Parameter for Tablet

To design tablet and later monitor tablet production quality, quantitative evaluation and assessment of tablets chemical, physical and bioavailability properties needs to be evaluated by using following parameters.[11]

- General appearance
- Unique identification marking
- Organoleptic properties
- Hardness and friability
- Weight variation
- Drug content
- Disintegration time
- Dissolution profile
- Impurity profile

1.3 Immediate Release Tablet[12,13]

The need for new oral drug delivery system continues, due to poor patient acceptance for invasive methods, need for exploration of new market for drugs and coupled with high cost of disease management. Developing new drug delivery techniques and utilizing them in product development is critical for Parma companies to survive this century.

Immediate release drug delivery systems are designed to provide immediate drug levels in short period of time. Immediate release drug delivery is desirable for drugs having long biological half life, high bioavailability, lower clearance and lower elimination half life. But main criterion for immediate release dosage form is poor solubility of the drug and the need of immediate action of drug to treat unwanted defect or disease. An immediate release dosage form allows a manufacturer to extend market exclusivity, while offering patient convenient dosage form or dosage regimen. Immediate Release Tablet is those tablets which are designed to disintegrate and release their medication with no special rate controlling feature. Such as special coating and other techniques. Recently immediate release tablet have started gaining popularity and acceptance as a drug delivery system, mainly because they are easy to administer, has quick onset of action

is economical and lead to better patient compliance. They are also a tool for expanding market, extending product life cycles and generating opportunities.

1.3.1Advantages of Immediate Release Tablets[14] ➢ Economical and cost effective.

- Quick onset of action.
- Suitable for industrial production.
- Improved stability and bioavailability.
- Provides some advantages of liquid dosage forms.
- Adaptable and amendable to existing processing and packaging machinery.
- Unique product differentiation.
- Allows high drug loading.
- Improved solubility of the pharmaceutical composition
- Decreased disintegration and dissolution times for immediate release oral dosage forms.
- Adaptable and amenable to existing processing and packaging machinery.

1.3.2 Disadvantages of Immediate Release Tablets

- Rapid drug therapy intervention is not possible.
- Sometimes may require more frequency of administration.
- Dose dumping may occur.
- Reduced potential for accurate dose adjustment.

1.3.3 Desired Criteria for Immediate Release Drug Delivery System[15,16,17]

- In the case of solid dosage it should dissolve or disintegrate in the stomach within a short period.
- In the case of liquid dosage form it should be compatible with taste masking.
- Be portable without fragility concern.
- Have a pleasing mouth feel.
- It should not leave minimal or no residue in the mouth after oral administration.
- Exhibit low sensitivity to environmental condition as humidity and temperature.
- Be manufactured using conventional processing and packaging equipment at low cost.
- Rapid dissolution and absorption of drug, which may produce rapid onset of action.

1.3.4 Biopharmaceutical Consideration[18]

When new drug delivery system put on, it is must that to consider Biopharmaceutical factor like metabolism and excretion.

1.3.4.1 Pharmacokinetics

In this consideration, study has done on absorption, distribution, metabolism and excretion. After absorption, drug attains therapeutic level and therefore elicits pharmacological effect, so both rate and extend of absorption is important. In conventional dosage form there is delay in disintegration and therefore dissolution is fast. Drug distribution depends on many factors like tissue permeability, perfusion rate, binding of drug to tissue, disease state, drug interaction etc. Duration and intensity of action depends upon rate of drug removal from the body or site of action

i.e. biotransformation. Decrease in liver volume, regional blood flow to liver reduces the biotransformation of drug through oxidation, reduction and hydrolysis. Excretion by renal clearance is slowed, thus half-life of renal excreted drugs increase.

1.3.4.2 Pharmacodynamic

Drug reception interaction impaired in elderly as well as in young adult due to undue development of organ. Decreased ability of the body to respond reflexive stimuli, cardiac output, and orthostatic hypotension may see in taking antihypertensive like prazosin. Decreased sensitivity of adrenergic agonist and antagonist. Immunity is less and taken into consideration while administered antibiotics. Altered response to drug therapy-elderly show diminished bronchodilator effect of theophylline shows increased sensitivity to barbiturates. Concomitant illnesses are often present in elderly, which is also taken into consideration, while multiple drug therapy prescribed.

1.3.5 Drug-Plasma Level after Oral Administration of a Drug from an Immediate-Release Dosage Form[19]

Immediate release drug delivery system employed plays a vital role in controlling the pharmacological effect of the drug as it can influence the pharmacokinetic profile of the drug, the rate of drug release, the site and duration of drug action and subsequently the side-effect profile. An optimal drug delivery system measures that the active drug is available at the site of action for the correct time and duration. The drug concentration at the appropriate site should be above the minimal effective concentration (MEC) and below the minimal toxic concentration (MTC). This concentration interval is known as the therapeutic range and the concept is illustrated in Figure 1.1, showing the drug plasma levels after oral administration of a drug from an immediate release dosage form. Achieving the desired concentration of a drug is dependent on the frequency of dosing, the drug clearance rates, the route of administration and the drug delivery system employed.

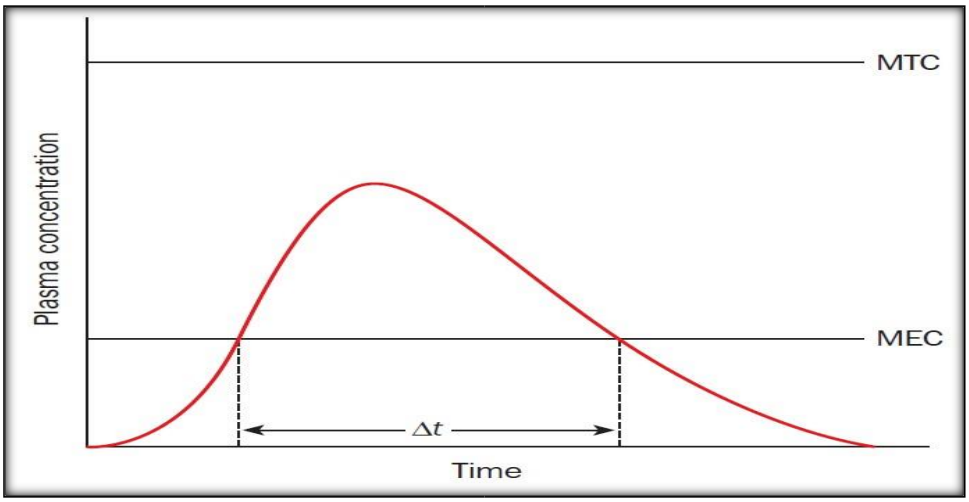


Figure 4: Drug plasma level after oral administration of a drug from an immediate-release dosage form

1.3.6 Techniques Used In Manufacturing of Immediate Release Tablet Immediate release tablet are manufactured by following method,

- Direct compression
- Wet granulation
- Dry granulation

1.3.6.1 Direct Compression[20,21]

The term direct compression is used to define the process by which tablets are compressed directly from powder blends of the active ingredient and suitable excipients which will flow uniformly into a die cavity and form into a firm compact. No pre-treatment of the powder blends by wet or dry granulation procedures is necessary. The manufacture of tablets using wet granulation or dry granulation methods is both time-consuming and potentially costly. The mechanisms of particleparticle interactions in tablets produced by direct compression are similar to those operative in tablets produced by dry granulation. The advent of direct compression was made possible by the commercial availability of directly compressible tablet vehicles that possess both fluidity and compressibility.

The simplicity of the direct-compression process is obvious. But direct compression should not be conceived as a simplified modification of the granulation process for making tablets. It requires a new and critical approach to the selection of raw materials, flow properties of powder blends and effects of formulation variables on compressibility. During the wet granulation process the original properties of the raw materials are, to a great extent, completely modified. As a result, a new raw material is what is finally subjected to compression. Many inadequacies in the raw materials are covered up during the granulation step. This is not true in direct compression and therefore the properties of each and every raw material and the process by which these materials are blended become extremely critical. Direct compression is often preferred because of its simplicity and relatively low cost, but may not always be technically feasible. It has been estimated that less than 20 percent of pharmaceutical materials can be compressed directly into tablets. The rest of the materials lack flow, cohesion or lubricating properties necessary for the production of tablets by direct compression.

Procedure for Direct compression

Step 1: The active ingredient and excipients were weighed and mixed Step 2: Pre-milling of formulation components.

Step 3: Mixing of the therapeutic agent with the powdered excipients (including the lubricant).

Step 4: Compression of the mixed powders into tablets.

Advantages

Direct compression is more suitable for moisture and heat sensitive drugs, since it eliminates wetting and drying steps and increases the stability of active ingredients by reducing detrimental effects.

- Changes in dissolution profiles are less likely to occur in tablets made by direct compression on storage than in those made from granulations.
- It is suitable for low dose drugs.
- Saving equipment, space and personnel.
- Fewer formulation excipients.
- Better physical and chemical stability.
- Superior tablet disintegration.
- Shorter "time to market".
- The high compaction pressure involved in the production of tablets by slugging or roller compaction can be avoided.
- The chances of wear and tear of punches and dies are less.
- Materials are "in process" for a shorter period of time, resulting in less chance for contamination or cross contamination, and making it easier to meet the requirement of current good manufacturing practices .
- Due to fewer unit operations, the validation and documentation requirements are reduce.
- Due to the absence of water in granulation, chance of microbial growth is minimal in tablets.

Disadvantages

- Direct compression is more prone to segregation due to the difference in density of the API and excipients.
- The dry state of the material during mixing may induce static charge and lead to segregation.
- Most of the directly compressible materials can accommodate only 30-40 % of the poorly compressible active ingredients like acetaminophen.
- API that has poor flow properties and/or low bulk density is difficult to process by direct compression.
- There is a need for greater quality control in purchasing of raw material to assure batch uniformity.

1.3.6.2. Wet Granulation[22,23]

Wet granulation is a process of using a liquid binder to lightly agglomerate the powder mixture. The amount of liquid has to be properly controlled, as over-wetting will cause the granules to be too hard and under-wetting will cause them to be too soft and friable. Aqueous solutions have the advantage of being safer to deal with than solvent-based systems.

Procedure for wet granulation

Step 1: The active ingredient and excipients are weighed and mixed.
Step 2: A wet granulate is prepared by adding the liquid binder to the powder blend and mixing thoroughly.
Step 3: Screening the damp mass through a mesh to form pellets or granules.
Step 4: Drying the granulation.
Step 5: After the granules are dried, they are passed through a screen of smaller size than the one used earlier.
Step 6: After granulation a final lubrication step is used to ensure that the tableting blend does not stick to the equipment during the tableting process. **Advantages:-**

- Reduced segregation of formulation components during storage and/or processing, leading to reduced intra- and inter batch variability.
- Useful technique for the manufacture of tablets containing low concentrations of therapeutic agent.
- Employs conventional excipients and therefore is not dependent on the inclusion of special grades of excipients.
- Most manufacturing plants are built around wet granulation tablet manufacture.
- Tablets produced by wet granulation are amendable to post processing unit operations, e.g. tablet-coating techniques.
- A wide variety of powders can be processed together in a single batch and in so doing their individual physical characteristics are altered to facilitate tableting.
- Bulky and dusty powders can be handled without producing a great deal of dust and airborne contamination. **Disadvantage**
- Because of the large number of processing steps, it requires a large area with temperature and humidity control.
- It requires a number of pieces of expensive equipment.
- It is time consuming, especially the wetting and drying steps.
- There is a possibility of material loss during processing due to the transfer of material from one unit operation to another.
- There is a greater possibility of cross-contamination than with the direct- compression method.
- It presents material transfer problems involving the processing of sticky masses.
- It can slow the dissolution of drugs from inside granules after tablet disintegration if not properly formulated and processed

1.3.6.3 Dry Granulation[24,25,26]

Dry granulation processes create granules by light compaction of the powder blend under low pressures. The compacts so-formed are broken up gently to produce granules (agglomerates). This process is often used when the product to be granulated is sensitive to moisture and heat. Dry granulation can be conducted on a tablet press using slugging tooling or on a roll press called a roller compactor. Dry granulation equipment offers a wide range of pressures to attain proper densification and granule formation. It is simpler than wet granulation, therefore the cost is reduced. However, this method often produces a higher percentage of fine granules, which can compromise the quality or create yield problems for the tablet. Dry granulation requires

drugs or excipients with cohesive properties, and a 'dry binder' may need to be added to the formulation to facilitate the formation of granules. At last powdered lubricants are added. **Advantages**

- Both roller compaction and slugging require conventional grades of excipients.
- These methods are not generally associated with alterations in drug morphology during processing.
- No heat or solvents are required. **Disadvantages**
- Because of the large number of processing steps, it requires a large area with temperature and humidity control.
- It requires a number of pieces of expensive equipment.
- It is time consuming, especially the wetting and drying steps.
- There is a possibility of material loss during processing due to the transfer of material from one unit operation to another.
- There is a greater possibility of cross-contamination than with the direct- compression method.
- It presents material transfer problems involving the processing of sticky masses.
- It can slow the dissolution of drugs from inside granules.

1.4 Malaria[27]

Malaria is a mosquito-borne infectious disease of humans caused by eukaryotic protists of the genus Plasmodium. It is widespread in tropical and subtropical regions, including much of Sub-Saharan Africa, Asia and the Americas. The disease results from the multiplication of malaria parasites within red blood cells, causing symptoms that typically include fever and headache, in severe cases progressing to coma, and death. Four species of Plasmodium can infect and be transmitted by humans. Severe disease is largely caused by Plasmodium falciparum. Malaria caused by Plasmodium vivax, Plasmodium oval and Plasmodium malaria is generally a milder disease that is rarely fatal. A fifth species, Plasmodium knowlesi, is a zoonosis that causes malaria in macaques but can also infect humans.

1.4.1 Malarial Life Cycle[28]

Plasmodium life cycle

The life cycle (Figure 5) is almost the same for all the five species that infect humans and follows three stages:

(i) Infection of a human with sporozoites

(ii)Asexual reproduction

(iii) Sexual reproduction

The two first stages take place exclusively into the human body, while the third one starts in the human body and is completed into the mosquito organism.

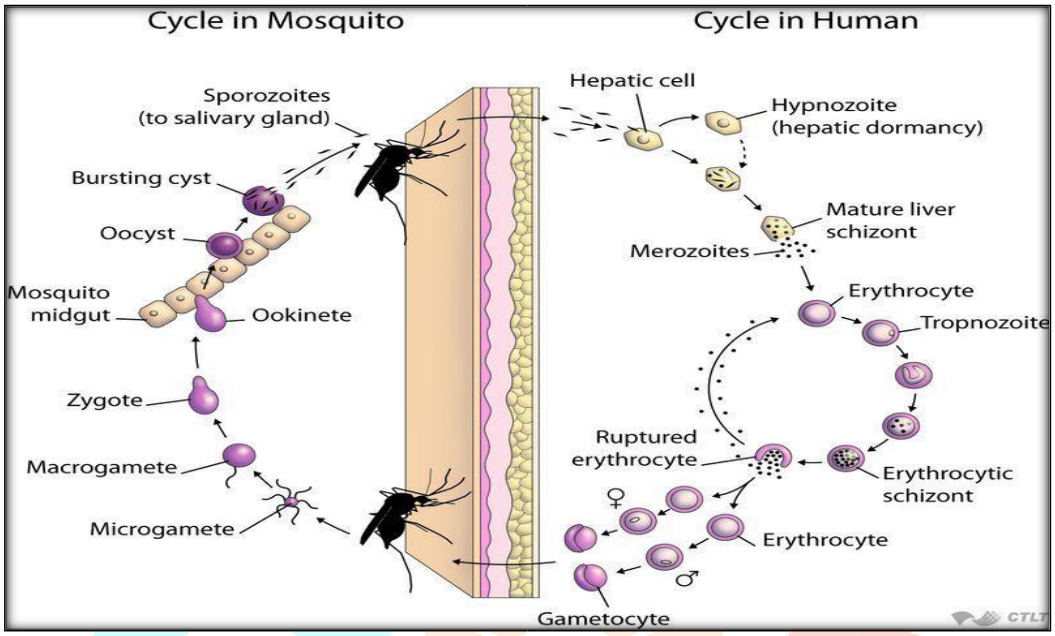


Figure 5: Plasmodium life cycle

1.4.2 Sings and symptoms

- Headache
- Fever
- Chill
- Sweating
- Dry cough
- Fatigue
- Pain
- Spleen enlargement
- Nausea
- Vomiting

1.5 Antimalarials[29]

Antimalerials are designed to prevent or cure malaria. Such drugs may be used for some or all of the following

- Treatment of malaria in individuals with suspected or confirmed infection
- Prevention of infection in individuals visiting a malaria-endemic region who have no immunity (Malaria prophylaxis)
- Routine intermittent treatment of certain groups in endemic regions (Intermittent preventive therapy)

Example of Antimalerial Drug:

- Artemither
- Atovaquone
- Lumifantrine
- Proguanil

Among the various antimalerial drugs Atovaquone was selected for present research work. Since Atovaquone is BCS II Class drug it is practically insoluble in water**.**

The aim of present work was "Formulation strategy for Dissolution Enhancement of Atovaquone".

Atovaquone is a hydroxy-1,4-naphthoquinone, used for the prevention and treatment of Pneumocystis jevorici (formerly carinii) pneumonia and prevention and treatment of P. falciparum malaria. The main aim of this study was to enhance the dissolution rate of a poorly water-soluble antimalarial drug, Atovaquone , by „"

Formulation Development and Evaluation of Immediate Release Tablet".

Atovaquone is a unique naphthoquinone with broad-spectrum antiprotozoal activity. It is effective for the treatment and prevention of Pneumocystis carinii pneumonia (PCP), Malaria and Babesiosis .In spite of this wide spectrum of pharmacological activity, its use in pharmaceutical field is limited because it suffers from low aqueous solubility (less than 0.0002mg/ml at 25˚C) and belongs to class II of the biopharmaceutical classification system (BCS). As a result it exhibits poor dissolution and insufficient oral bioavailability. Thus, an efficient oral formulation of Atovaquone with an enhanced dissolution rate and hence, an improved bioavailability is highly desired. Reducing the particle size was an alternative to improve the oral bioavailability of Atovaquone, but was associated with some major limitations. The use of conventional jet milling method was incapable of reducing the particle size below 6 µm without causing fracture of the crystal structure. Complexity of the method resulting in longer processing time coupled with high cost of the equipment and its maintenance were the drawbacks associated with the micro fluidization technique. As a result, there is a need for alternative methods to increase aqueous solubility of Atovaquone. Thus to use immediate release tablet formulation by direct compression method employed to improve the solubility and bioavailability of poorly water soluble drug.

2. REVIEW OF LITERATURE

Casstell D.,(2001)[30] Immediate-release omeprazole (Zegerid, Santarus) was the first immediate-release oral proton pump inhibitor to reach the market. As a powder formulation for oral suspension, it is indicated for the treatment of gastroesophageal reflux disease, erosive oesophagitis, duodenal ulcer and gastric ulcer, and is the only proton pump inhibitor approved for the reduction of risk of upper gastrointestinal bleeding in critically ill patients. Administration of immediate-release omeprazole at bedtime results in a rapid and sustained elevation of gastric pH, and seems to provide better night time control of gastric acidity than that observed with conventional morning dosing of delayed-release proton pump inhibitors. The immediate-release formulation may provide a good treatment option for patients who require flexible dosing, quick onset of action and nocturnal gastric acid control.

Patel Jignesh *et al.*,(2005*),* has been described a method for simultaneous estimation of Candesartan Cilexetil and Hydrochlorothiazide in tablet dosage form. The method is based on UV-Spectrophotometric determination using Q-absorbance method. It involves, formation of Q-absorbance equation at 258.14nm (isoabsorptive point) and

271 nm λ max of Hydrochlorothiazide in methanol. Linearity was obtained in the range 2-24µg/ml for Candesartan and 2-24 µg/ml for Hydrochlorothiazide. The method allows rapid analysis of binary pharmaceutical formulation with accuracy. The % recovery lies in the range of 101.2 – 102.1 for CAN and 99.2 – 99.7 for HCTZ. Result was validated statistically and was found satisfactory.

Palaparthi *et al*.,(2005)[31]formulated and in vitro evaluated the immediate release tablet comprising telmisartan and hydrochlorthiazide with Inactive ingredients are sodium hydroxide, meglumine, povidone, sorbitol, magnesium stearate, lactose monohydrate, microcrystalline cellulose, maize starch, sodium starch glycolate, and a colouring agent that is either ferric oxide red or ferric oxide to yellow. Similar products are sold outside the U.S. as MICARDIS PLUS®.

Monika Bakshi *et al*.,(2007)[32] this write-up provides a review on the development of validated stability-indicating assay methods (SIAMs) for drug substances and products. The shortcomings of reported methods with respect to regulatory requirements are highlighted. A systematic approach for the development of stabilityindicating methods is discussed. Critical issues related to development of SIAMs, such as separation of all degradation products, establishment of mass balance, stress testing of formulations, development of SIAMs for combination products, etc. are also addressed. The applicability of pharmacopoeial methods for the analysis of stability samples is discussed. The requirements of SIAMs for stability study of biotechnological substances and products are also touched upon in the Development of validated stability-indicating assay methods.

Monica R P Rao *et al*.,(2008) [33] was study to develop and optimize fast disintegrating tablets of metoclopramide HCl with low friability and minimum DT, prepared by wet granulation technique for oral delivery. A computer aided optimization process11, 12 using a simplex centroid mixture design was employed to investigate the effect of three independent variable (factors) i.e.; amount of superdisintegrants: SSG, CCS and PGS. The DT and release after 15 min (rel15min) were taken as the response variables.

Parikh *et al*.,(2008)[34] They had developed solid oral formulations of Telmisartan which can be prepared. Preferably, the formulations should have immediate release characteristics and a dissolution showing no essential pH dependency within the physiological relevant pH interval of the gastrointestinal tract. Tablets were evaluated for various parameters like, weight variation, content uniformity, in-vitro dissolution studies were performed using United States Pharmacopeia (USP) apparatus type II. The effects of concentration of meglumine, povidone and different alkalizers on the release rate of Telmisartan were studied.

Ria *et al*.,(2009)[35] They had prepared Raloxifne hydrochloride immediate release tablet by wet granulation technique. In order to obtain the best optimized product, six different formulations were developed. Different filler, binder, disintegrant and lubricant were taken as variables. Weight variation, thickness, hardness, friability, disintegration time, in vitro release and pharmaceutical assay were studied as response variables. Steaking was observed when the formulation containing stearic acid and sodium stearl fumarate. However in remaining four formulations containing magnesium stearate no steaking was observed. The formulation NP061 was selected as an optimized product. The different physical properties and in vitro release profile showed best comparable with reference product.

Jain *et al*.,(2010)[36] Formulated and evaluated immediate release tablet of Nimesulide using agar, gum karaya and modified form of agar and gum karaya. From this study, they found that modified form of agar and gum karaya were more effective than agar and gum karaya.

Danielle Colussi *et.al*.,(2010)[37] This review highlights the Binding of artemether and lumefantrine to plasma proteins and erythrocytes. The serum/plasma protein binding and blood distribution of artemether and lumefantrine was studied in vitro. The techniques used were the erythrocyte partitioning and ultrafiltration methods with Clabelled compounds. Both artemether and lumefantrine were found to be highly bound to proteins in serum, 95–98% and .99%, respectively.

M. Gabriëls.,(2011)[38] this review highlights the Design of a dissolution system for the evaluation of the release rate characteristics of artemether and dihydroartemisinin from tablets. It consists of an organic solvent in the upper part and the aqueous phase, in which the dissolution test was executed. The main requirements for the selection of the solvent are: the density should be lower than 1; the analyte should dissolve in the organic part as much as required for „sink" conditions; if possible, the cut off should be near 200 nm, which allows direct HPLC measurement at 215 nm. The most suitable solvent for artemether is isooctane in a ratio of 100/150 ml aqueous phase. **Isabela Costa César *et al*.,(2012)** [39] this review highlights the Liquid chromatography–tandem mass spectrometry for the simultaneous quantitation of artemether and lumefantrine in human plasma: Application for a pharmacokinetic study A liquid chromatography–electrospray ionization tandem mass spectrometry (HPLC–ESI-MS/MS) method for the simultaneous quantitation of artemether and lumefantrine in human plasma was developed and validated. Artesunate was used as an internal standard (IS) and showed that no matrix effect was detected in the samples. The validated method was successfully applied to determine the plasma concentrations of artemether and lumefantrine in healthy volunteers, in a one-dose pharmacokinetic study, over the course of 11 days.

Paulo *et al*.,(2012)[40] discussed about the modeling and comparision of dissolution profiles. Whenever a new solid dosage form is developed or produced, it is necessary to ensure that drug dissolution occurs in an appropriate manner. The pharmaceutical industry and the registration authorities do focus, nowadays, on drug dissolution studies. The quantitative analysis of the values obtained in dissolution / release tests is easier when mathematical formulae express the dissolution results as a function of some of the dosage forms characteristics are used. In most of the cases the theoretical concept does not exist and some empirical equations have proved to be more appropriate. Drug dissolution from solid dosage forms has been described by kinetic models in which the dissolved amount of drug (Q) is a function of the test time, t or Q5f(t). Some analytical definitions of the Q(t) function are commonly used, such as zero order, first order, Hixson–Crowell, Weibull, Higuchi, Baker–Lonsdale, Korsmeyer–Peppas, and Hopfenberg models. Other release parameters, such as dissolution time (t), assay time (t), dissolution efficacy (ED), difference factor (f), x% x min 1 similarity factor (f) and Rescigno index (j and j) can be used to characterize drug dissolution / release profiles.

Giancarlo A Biagini.,(2012)[41] **t**his review highlights the Current drug development portfolio for antimalarial therapies . In response to the emergence of parasite drug resistance to currently deployed

antimalarials, the scientific community, in partnership with the pharmaceutical industry and public organizations, has fashioned an antimalarial drug development portfolio for the sustained development and registration of safe, effective and cheap antimalarial medicines. The management of this portfolio is being driven by MMV (Medicines for Malaria Venture), with a number of projects recently reaching the clinical end of this drug development pipeline.

Md. Elias-Al-Mamun *et.al*.,(2013)[42] this review highlights the study is to prepare a combination dosage form which can be used to treat both the diseases concomitantly, reducing pill burden and increasing patient compliance. Methodology Based on positive results of a feasibility study, including doctors‟ opinion and prescription survey, Gliclazide and Enalapril maleate were selected as the active ingredients for developing a FDC (Fixed Dose Combination) preparation. Immediate release combination tablet (Gliclazide and Enalapril maleate Tablet, GET), containing 80 mg of Gliclazide and 5 mg of Enalapril maleate, prepared by direct compression method. Their physical properties determined. The dissolution of Gliclazide and Enalapril maleate was 98% and 85% respectively at 45 minutes. During stability testing, slight discoloration of the tablets was observed at higher temperature although the assay results were found to be satisfactory.

Patel J A. *et al*.,(2013)[43] the purpose of this study was the task of developing immediate release tablet is accomplished by using a suitable diluents and superdisintegrants. Faster disintegration of the tablet administrated orally minimizes absorption time and improves its bioavailability in less time. Immediate Release tablet of Antibiotic drug is formulated using dry granulation using super disintegrant croscarmellose sodium. Azithromycin is Antibiotic drug is used to treat STDs due to Chlamydia and gonorrhea, community-acquired pneumonia, pelvic inflammatory disease, pediatric otitis media and pharyngitis, and Mycobacterium avium complex (MAC) in patients with advanced HIV disease. One of the important studies included in the present investigation is study on process parameter effect on performance of the Immediate Release tablets. The effect of selected process parameters on critical properties of immediate release (IR) tablets were studied, like effect of disintegration time, friability, dissolution profile.

Dr Guptal M M. *et al*.,(2013)[44] prepared Olmesartan medoxomile tablet by wet granulation method and also by direct compression. Effect of various fillers and disintegrants were also explored. Microcrystalline cellulose, lactose monohydrate, was used in wet granulation. In order to obtain acceptable product several trials were conducted. And ten different formulations were prepared.23

Sekar V. *et al*.,(2013)[45] have developed Immediate release tablets of telmisartan using superdisintegrant, in the study an attempt has been made to prepare immediate release tablets of telmisartan by using Polyplasdone XL-10 (Crosspovidone) at intragranular, extra granular and partly intra and extra granular level of addition to increase the rate of drug release from dosage form to increase the dissolution rate and hence its bioavailability.17

Debjit B. *et al*.,(2013)[46] gave a review on Fast Dissolving Tablet: An Overview, the disintegrants used and the properties in mouth dissolving tablet. The mechanism of disintegration with the examples was particularly illustration

3. OBJECTIVE

The basic aim of the present investigation is the dissolution enhancement of poorly water soluble ant-malarial drug Atovaquone by formulating and evaluating immediate release tablet by using direct compression method.

The objective of present research would be:

- To enhance the Dissolution rate of Atovaquone by developing stable and efficacious immediate release tablet.
- To study the preformulation parameter of drug and excipient.
- To carry out the characterization of drug and compatibility studies of drug and excipients with each other.
- To study the effect of different concentration of Solubilising agent and surfactant on release pattern of Atovaquone.
- To optimize the formulation by using 3^2 full factorial design.
- To evaluate the prepared immediate release dosage form for different official and unofficial tests.
- To perform the Accelerated stability studies on optimized formulation batch as per ICH guidelines.

4. JUSTIFICATION

Tablet is the most popular among all dosage forms existing today because of its convenience of self administration, compactness and easy manufacturing; however in many cases immediate onset of action is required than conventional therapy. To overcome these drawbacks, immediate release pharmaceutical dosage form has emerged as alternative oral dosage forms. There are novel types of dosage forms that act very quickly after. The development of immediate release therapy also provides an opportunity for a line extension in the marketplace, The basic approach used in development of tablets is the use of disintegrants, surfactant which provide instantaneous disintegration of tablet after administration.

The aim of present work was "Formulation strategy for Dissolution Enhancement of Atovaquone". Atovaquone is a hydroxy-1,4-naphthoquinone, used for the prevention and treatment of Pneumocystis jevorici (formerly carinii) pneumonia and prevention and treatment of P. falciparum malaria. Atovaquone is poorly water soluble drug having aqueous solubility (less than 0.0002mg/ml at 25˚C) and belongs to class II of the biopharmaceutical classification system (BCS). As a result it exhibits poor dissolution and insufficient oral bioavailability. Thus, an efficient oral formulation of Atovaquone with an enhanced dissolution rate and hence, an improved bioavailability is highly desired.

Atovaquone interferes with the mitochondrial electron transport and thereby ATP and pyrimidine biosynthesis and in Plasmodia, it is found to target cytochrome bc1 complex and disrupt the membrane potential.

- Atovaquone is poorly water soluble drug.
- The half life of Atovaquone is 2–3 days, that"s why immediate release dosage form is prepared.
- Monotherapy of Atovaquone is not available in the market.

5. PLAN OF WORK

In order to achieve the objective mentioned earlier the plan of work was planned as follows

I.Phase I

1. Problem selection
2. Literature survey **II. Phase II**

1. Selection of drug candidate and excipients
2. Procurement of drug candidate and excipients

III. Phase III: PREFORMULATION

1. Identification and characterization of drug
    - Melting Point
    - UV Spectrophotometry
    - FT-Infrared spectrophotometry (FTIR)
2. Differential scanning calorimetry (DSC)
3. Bulk characterization of drug Bulk density
    - Tapped density
    - Angle of repose
    - Carr"s index

- Hausner‟s ratio

4. Excipient compatibility study
    - FT-Infrared spectrophotometry (FTIR)
    - Differential scanning calorimetry (DSC)
    - Bulk characterisation of Powder blend(Drug+Excipient)

IV. Phase IV: EXPERIMENTAL

1. Analytical Method Development
    - UV Spectra of Atovaquone
    - Calibration Curve of Atovaquone
2. Analytical Method Validation
3. Formulation development
    - Preliminary batches
    - Development and Optimisation of Immediate release tablet formulation using 3^2 full factorial design
    - Evaluation of optimized Immediate release tablet formulation
    - Anova study
    - Drug release kinetic study
    - Accelerated Stability Study by ICH guideline

V. Phase V

1. Summery and Conclusion

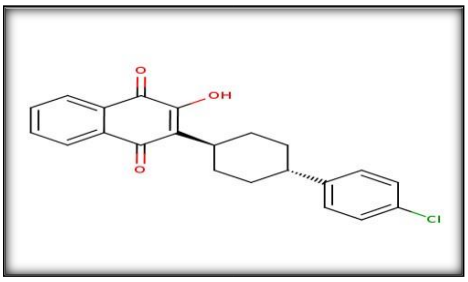
VI. Phase VI

1. Dissertation writing and submission

6. DRUG AND EXCIPIENT PROFILE:

6.1 Drug Profile:[47,48]

Structure of Atovaquone



Category: Antimalarial

Dose: 5mg, 250mg

Description: Yellow powder

Chemical Name: 2-(Trans-4-(P-Chlorophenyl) cyclohexyl)-3-hydroxy-1, 4-Naphthoquinone

Molecular formula: $C_{22}H_{19}ClO_3$

Molecular weight: 366.84 g/mol

Melting point: 214 - 219°C

Solubility: practically insoluble in water, Freely soluble in N- methyl-2-pyrrolidone; soluble in chloroform; sparingly soluble in acetone, in PEG 400; slightly soluble in alcohol, in 1, 3-butanedi, in PEG 200; very slightly soluble in 0.1N sodium hydroxide.

Half life: 2 to 3 days

Partition Coefficient: n-octanol/water**:** 5.31 @ pH7

Pka: 8.23

USP Limit: Atovaquone contains not less than 97.5% and more than 1014.5% of $C_{22}H_{19}CLO_3$, Calculated on the anhydrous and organic solution basis.

Wavelength: 251 nm **Pharmacology:**

This compound belongs to the naphthoquinones. These are compounds containing a naphthohydroquinone moiety, which consists of a benzene ring fused to a bezene-1, 4-dione (quinone).

Pharmacodynamics:

Atovaquone is a highly lipophilic drug that closely resembles the structure ubiquinone. Its inhibitory effect being comparable to ubiquinone, in sensitive parasites atovaquone can act by selectively affecting mitochondrial electron transport and parallel processes such as ATP and pyrimidine biosynthesis. For illustration, cytochrome bc1 complex (complex III) seems to serve as a highly discriminating molecular target for atovaquone in Plasmodia atovaquone has the advantage of not causing myelosuppression, which is an important issue in patients who have undergone bone marrow transplantation.

Mechanism of Action:

Atovaquone is a hydroxy- 1, 4- naphthoquinone, an analog of ubiquinone, with antipneumocystis activity. The mechanism of action against *Pneumocystis carinii* has not been fully elucidated. In Plasmodium species,

the site of action appears to be the cytochrome bc1 complex (Complex III). Several metabolic enzymes are linked to the mitochondrial electron transport chain via ubiquinone. Inhibition of electron transport by atovaquone will result in indirect inhibition of these enzymes. The ultimate metabolic effects of such blockade may include inhibition of nucleic acid and ATP synthesis. Atovaquone also has been shown to have good *in vitro* activity against *Toxoplasma gondii.*

Pharmacokinetics:

Absorption: The bioavailability of atovaquone is low and variable and is highly dependent on formulation and diet. Bioavailability of the suspension increases twofold when administered with meals. When administered with food, bioavailability is approximately 47%. Without food, the bioavailability is 23%.

Volume of Distribution: 0.60 ± 0.17 L/kg

Metabolism: Some evidence suggests limited metabolism (although no metabolites have been identified).

Route of Elimination: The half-life of atovaquone is long due to presumed enter hepatic cycling and eventual fecal elimination. There was little or no excretion of atovaquone in the urine (less than 0.6%).

Protein Binding: Atovaquone is extensively bound to plasma proteins (99.9%) over the concentration range of 1 to 90 µg/ml.

Food Interactions:

- Fatty foods increase absorption
- Take with food, bioavailability is increased 2 to 3 fold **Drug Interaction:**
- **Rifabutin:** Rifabutin decreases the effect of atovaquone
- **Rifampicin:** Rifampin may decrease the effect of atovaquone
- **Tetracycline:** Tetracycline may decrease the effect of atovaquone.
- **Zidovudine:** Atovaquone increases the effect and toxicity of zidovudine.

Contraindication:

- you are allergic to any ingredient in atovaquone
- you have severe kidney problems and are using atovaquone to prevent malaria
- you are taking rifampicin or rifabutin

Therapeutic Use:

- For pneumocystis pneumonia although it is not approved for treatment of severe cases
- For toxoplasmosis: the medication has ant parasitic and therapeutic
- for malaria, along with proguanil
- for babesia, it is often used in conjugation with azithromycin
- used in PCP when patient is allergic to sulphonamide **medication such as TMP-SMX**

6.2 Excipient profile:[49,]

For present research work various excipients such as superdisintegrants, disintegrant, binder, bulking agent, sweetener, lubricant and glidant was used. The excipients profile is as follows.

1. Disintegrant

For present research work disintegrant selected was Microcrystalline Cellulose

MCC33 (Microcrystalline Cellulose) Synonym: Avicel PH 102 or 101, Celex.

Chemical name: Cellulose.

Functional category**:** Adsorbent, suspending agent, tablet and capsule diluents, and tablet disintegrant.

Pharmaceutical applications**:** It finds wide range of applications in pharmaceutical industry. In tablet preparation it is used as binder in both wet granulation as well as in direct compression. It also has lubricant and disintegrant property. As a disintegrant it is used in the concentration range of 5-15%.

2. Solubilising agent

Starch

Synonym: Maize starch

Chemical formula**:** $(C_6H_{10}O_5)_n$

Functional category: Compressible aid, tablet and capsule disintegrante, binder.

Pharmaceutical applications: In the pharmaceutical industry, starch is also used as an excipient, as tablet disintegrant or as binder. In tablet preparation it is used as binder in both wet granulation and dry granulation. When 5% starch is used in formulation it acts as a binder for tablet formulations where as when it is used in dry form it can perform the function of a disintegrant.

3. Surfactant:

Sodium laurel sulphate:

Synonym: Sodium dodecyl sulfate; SODIUM LAURYL SULFATE; Irium;

Anticerumen; Neutrazyme.

Molecular wt.:288.379269 g/mol

Chemical formula**:** $C_{12}H_{25}NaO_4S$

Functional category**:** Surface active agent, solubilising agent.

Mechanism of action**:** Like other surfactants, SLS is amphiphilic. It thus migrates to the surface of liquids, where its alignment and aggregation with other SLS molecules lowers the surface tension. This allows for easier spreading and mixing of the liquid. SLS has potent protein denaturing activity and inhibits the infectivity of viruses by solubilising the viral envelope and/or by denaturing envelope and/or capsid proteins.

Pharmaceutical applications: It is used as surfactant. In tablet formulation it is used as solubilising agent (20%), it possess the advantage of improving the dissolution rate of poorly soluble drug at low carrier concentration.

4. Glidant:

Talc:

Synonym: Magnesium silicate

Chemical formula: $Mg_3Si_4O_{10}(OH)_2$

Functional category: Talc is used as an anti-sticking agent, an anti-caking agent, a lubricant, a carrier, a thickener, strengthening filler, smooth filler, and an adsorbent

Pharmaceutical application**:** Because of talc‟s crystalline platy structure and softness, talc is used as a lubricant or glidant in the manufacturing of pharmaceutical tablets. It is also commonly used as an in ingredient in enteric (time release) tablet coating formulations. Talc has been shown to improve direct compression of tablet formulation disintegration properties and can be used in combination with magnesium stearate to restore disintegration and dissolution properties caused by the addition of magnesium stearate as a lubricant. Smaller particle size talc have also been shown to improve lubricant efficiency. It is used to improve powder flow in tablet compression.

5. Lubricant:

Magnesium sterate:

Synonym: octadecanoic acid,

Chemical formula: $Mg(C18H35O2)_2$

Functional category: Anti-adherent, lubricant.

Pharmaceutical application: Magnesium stearate is often used as an anti-adherent in the manufacture of medical tablets, capsules and powders. In this regard, the substance is also useful, because it has lubricating properties, preventing ingredients from sticking to manufacturing equipment during the compression of chemical powders into solid tablets; magnesium stearate is the most commonly used lubricant for tablets.

7. EXPERIMENTAL WORK:

7.1 Selection and procurement of API and excipients.

API and excipients were selected based on the literature survey

7.1.1 API

Active pharmaceutical ingredients were selected by considering different drug candidate properties for preparing immediate dosage form. Active pharmaceutical selected were of antimalerial drug category. Illustrative examples of antimalerial category drugs are Artemither, Lumifantrine, Proguinil, Atovaquone etc. among these Atovaquone was selected as drug candidate for present research work.

7.1.2 Disintegrant

For present research work microcrystalline cellulose is selected as disintigrante.

7.1.3 Solubilising agent

Starch is selected as solubilising agent for present research work.

7.1.4 Surfactant

For present research work sodium laurel sulphate is selected as surfactant.

7.1.5 Lubricants

The selected lubricant for present research work was Magnesium stearate because of its good lubricant efficacy.

7.1.6 Glidants

Glidants mostly used includes colloidal silicon dioxide and talc. Talc was selected for present research work.

7.2 Identification and characterisation of drug

7.2.1 Melting point determination

The melting point of the given drug sample was carried out using Method I specified in the Indian Pharmacopoeia 2007 (2.4.21) using the Liquid paraffin as a bath liquid by Digital Melting Point apparatus. The melting point was noted and the readings were taken in triplicate.

7.2.2 Differential scanning calorimetry

Thermogram of Atovaquone was recorded on a TA-60 WS Thermal Analyzer
(Shimadzu). The samples were hermetically sealed in aluminium pans and heated at a constant rate of 20°C/min over temperature range of 50 to 200°C. Inert atmosphere was maintained by purging nitrogen gas at flow rate of 50 ml/min.

7.2.3 FT-IR spectrum

The infrared absorption spectrum Atovaquone was recorded with a KBr disc over the wave number 4000 to 400 cm-1 on an IR 200 Thermoelectron.

7.3 Preformulation study[50]

Preformulation study relates to pharmaceutical and analytical research work carried out proceeding and supporting formulation development efforts of the dosage form of the drug substance. Preformulation yields basic knowledge necessary to develop suitable formulation for the toxicological use. It gives information

needed to define the nature of the drug substance and provide frame work for the drug combination with pharmaceutical excipients in the dosage form.

Preformulation testing is the first step in the rational development of dosage forms of a drug. It can be defined as a research work of physical and chemical properties of drug substance, alone and when combined with excipients. A complete evaluation of physicochemical properties may provide a rationale for designing formulation or support the need for molecular modification or merely confirm that there are no significant barriers to the compound development.

7.3.1 Organoleptic properties

Atovaquone was evaluated for its organoleptic properties like appearance, colour and odour and taste. **7.3.2 Solubility study**

Solubility determination was carried out in water, phosphate buffer pH 8, methanol, 40% IPA buffered with pH 8. For determining saturation solubility the excess quantity of the API was added in the media (5ml) in which solubility determination was to be determined. It was then rotary shaken and kept for equilibrium for 24 hrs. The equilibrated solutions on next day were filtered through Whatmann filter and were analyzed on UV-visible spectrophotometer for solubility determination. Samples were diluted as needed.

7.3.3 Powder characterisation Characterization of API and excipients[51,52,53] The powder blends evaluated for flow properties as given below:

All the ingredients were passed through mesh #60. Required quantity of each ingredient was taken for each specified formulation (depicted in the formula) and all the drug and excipients without lubricant were first mixed by pouch mixing for 30 minutes. After the addition of lubricants (# 30), the mixing procedure continued for 5 minutes.

a. Angle of Repose

The angle of repose is indicator for flow ability of the powder. Fixed base method was used to determine angle of repose. The lower tip of funnel was kept up at 2-4 cm from the surface of table. Excess of powder was poured from the funnel to form a pile. Then funnel was adjusted up to height of pile and a circle was drawn around the pile. The height of tip from the surface of table was measured as pile height (h) and diameter of pile (d) was measured taking average of three average diameters of circumference of the circle. The angle of repose was calculated by using the following equation

$$\theta = \tan^{-1} \times (h/r)$$

Where, θ is the angle of repose h is the height r is the radius

Angle of Repose	Flow
< 25	Excellent
25-30	Good
30-40	Passable
> 40	Very poor

Table 2: Flow properties according to angle of repose

b. Bulk density (Db)

It is the ratio of total mass of powder to the bulk volume of powder. Accurately weighed quantities of sample of powder were placed in a 100 ml measuring cylinder. The volume occupied by the powder was determined without disturbing the cylinder and bulk density was calculated using the equation. It was measured by **Db = m/Vb**

Where m is the mass of the powder

Vb is the bulk volume of the powder

c. Tapped density (Dt)

It is the ratio of total mass of the powder to the tapped volume of the powder. Accurately weighed quantities of sample of powder were placed in a 100 ml measuring cylinder. The measuring cylinder was tapped for fixed number of taps (~500) to obtain constant volume of powder bed. The final volume was noted and s the bulk volume of the powder. Tapped density was calculated by using the equation. It is expressed in g/ml and is given by

Dt = m/Vt

Where, M is the mass of powder Vt is the tapped volume of the powder.

d. Carr's Index

The simplest method of measurement of free flow of powder is compressibility, an indication of the ease with which material can be induced to flow is given by compressibility index (I) which is calculated as follows

$$I = \frac{Dt - Db \times 100}{Dt}$$

Where Dt is the tapped density of the powder

Db is the bulk density of the powder.

Carr's Index (%)	Flow Character
5-15	Excellent
12-16	Good
18-21	Fair- Passable
23-35	Poor
33-38	Very poor
< 40	Very, very poor

Table 3: Flow Properties According to Carr's Index

e. Hausner's Ratio

Hausner"s ratio is an indirect index of ease of powder flow. It is calculated by the following formula.

Hausner's ratio = Dt / Db Where Dt is the tapped density of the powder Db is the bulk density.

Lower Hausner"s ratio (<1.25) indicates better flow properties than higher ones.

7.4 Analytical Method Development[54]

UV spectrophotometer is widely employed for routine drug analysis. Therefore one of the objectives of the present study was to develop and validate an UV Spectrophotometric method for analysis of Atovaquone.

7.4.1 Selection of Wavelength and Preparation of Standard Calibration Curve a. Solvent Used

Atovaquone is practically insoluble in water, hence 5ml methanol was used to dissolve the drug then volume was made with water. **b. Preparation of Standard Stock Solution**

Accurately weighed 10 mg sample of Atovaquone was transferred to 100 ml volumetric flask and dissolved in 5ml methanol. The volume was made up to 100 ml with water to produce stock solution of 100µg/ml **c. Selection of Wavelength**

The aliquots of stock solution of drug were transferred (5ml) to 10 ml volumetric flask and volume was made up to 10 ml with water to get working standard solution of Atovaquone in concentration range 25µg/ml. These solutions were scanned from 200-400 nm.

d. Calibration curve procedure of Atovaquone:

The Standard solution of Atovaquone was subsequently diluted with water to obtain a series of solution containing 5, 10, 15, 20, 25µg/ml. The absorbance of solution was measured by using UV spectrophotometer (SHIMADZU 1800 UV) at 251 nm using water as blank. The absorbance so measured was plotted against concentration.

7.5 Analytical Method Validation[55]

a. Linearity

Aliquots of the stock solution of Atovaquone (1-5 ml of 100µg/ml) were transferred into 10 ml standard flasks and made volume using water. The absorbance of solutions of different concentrations were measured at 251 nm against water as blank **b. Precision**

The precision of the method was demonstrated by Repeatability, Intra-day and Inter- day variation studies. In repeatability studies pure drug solution (Within the working limits) was analyzed and being repeated six times. The relative standard deviation (%) was then calculated. In intra-day studies, three repeated measurements of standard and sample solutions were made in a day and percentage RSD were calculated. In inter- day studies, three repeated measurements of standard and sample solutions were made on three consecutive days and percentage RSD were calculated. **c. Accuracy & % recovery**

To ensure the accuracy, known amounts of pure drug were added to the solvent and these samples were reanalyzed by the proposed method and also % recovery was determined.

d. Detection Limit (DL)

It is the minimum limit that can be detected but not necessarily to be quantified. DL was calculated from the formula

$$DL = 3.3\sigma/S$$

Where, σ is standard deviation of the response and S is slope of calibration curve. **e. Quatitation Limit (QL)**

It is minimum limit that can be quantified

QL is calculated from formula

$$QL = 10\sigma/S$$

Where, σ is standard deviation of the response and S is slope of calibration curve.

7.6 Formulation Development of Atovaquone Immediate Release Tablet

Based on literature survey drug and excipients were selected. Next criteria were the selection of method for preparation. There are various methods for preparation, but from the feasibility point of view direct compression was selected. In this method directly compressible agents were utilized giving best results regarding DT and uniformity. Preliminary batches were performed to determine the disintegration efficacy of various disintegrants and other excipient at different concentrations. A generalized formula was developed to study preliminary batches.

7.6.1 Preliminary batch:

Weigh accurately all ingredients Atovaquone (250 mg), microcrystalline cellulose, Starch, Sodium lauryl sulphate, talc, Magnesium Stearte were passed through sieve no.18 individually. Then mix all ingredients together in mortar and pestal properly in one rotation. The mixed powder was passed through sieve no. 60. Tablets were prepared by direct compression method in single punch machine of 11mm punch.

Tablet weight was 400mg. Hardness of tablet was 3 to 3.2 mm.

Sr. No.	Ingredients	Amount(mg)/Tablet
1.	Active pharmaceutical agent	250
2.	MCC	35
3.	Starch	105
4.	Sod. Laurel Sulphate	6
5.	Magnesium stearate	2
6.	Talc	2
7.	Total	400

Table 4: Formula for Preliminary batch

7.6.2 Formulation batches using 3^2 full factorial design

Atovaquone is a poorly soluble drug. The solubility and the dissolution of atovaquone were very slow. But it was made soluble and brought into the dissolution first by the addition of solubilising agent such as Starch maize powder, surfactant SLS; and disintegrating agent MCC. Formulas for Factorial batches were shown in table 5 below.

Formulation code	Atovaquone (mg)	MCC (mg)	Starch (mg)	Sodium Lauryl Sulphate(mg)	Magnesium stearate (mg)	Talc (mg)
F1	250	25	115	6	2	2
F2	250	27	115	4	2	2
F3	250	29	115	2	2	2
F4	250	35	105	6	2	2
F5	250	37	105	4	2	2
F6	250	39	105	2	2	2
F7	250	45	95	6	2	2
F8	250	47	95	4	2	2
F9	250	49	95	2	2	2

Table 5: Factorial Batches (400mg/Tablet)

Optimization technique provides both a depth of understanding and an ability to explore ranges for formulation and processing factors. With a rational approach to the selection of the several excipients and manufacturing steps for a given product, one quantitatively selects a formulation. It is at this point that optimization can become a useful tool to quantify a formulation that has been qualitatively determined.

7.6.3 Coded values for 3^2 full factorial design

A randomized full factorial design was used in the present study. In this design 2 factors were evaluated each at 3 levels, and experimental trials were performed at all 9 possible combinations as shown in **Table 5**. The amount of solubilising agent Starch (X1) and the amount of surfactant SLS (X2) were selected as independent variables. *In vitro* dissolution time (DT) was selected as dependent variables presented below in **Table 6.**

Sr. No.	Formulation code	Variables X1	Level X2	Variable X1	Level X2
1.	F1	+1	+1	115	6
2.	F2	+1	0	115	4
3.	F3	+1	-1	115	2
4.	F4	0	+1	105	6
5.	F5	0	0	105	4
6.	F6	0	-1	105	2
7.	F7	-1	+1	95	6
8.	F8	-1	0	95	4
9.	F9	-1	-1	95	2

Table 6: Coded values for 3^2 Factorial designs

7.7 Evaluation of Factorial Batches of Immediate Release Tablet of Atovaquone[56]

Immediate release tablets were evaluated for official and unofficial tests as per IP as: **7.7.1 Appearance**

The control of general appearance of a tablet involves the measurement of number of attributes such as a tablets size, shape, and colour, odour and taste.

7.7.2 Weight Variation Test[56]

The weight variation testing was carried out as per the method described in the USP. Twenty tablets were weighed and the average weight was calculated. The individual weight was compared with the average weight. The tablets pass the test if not more than two tablets are outside the percentage limit and if no tablet differs by more than two times the percentage limit. The following percentage deviation in weight variation is allowed according to USP.

Average weight	Percentage deviation
130 mg or less	10
More than 130 mg and less than 324 mg	7.5
324 mg or more	5

Table 7: Percentage Deviation Vs Average Weight

In all the formulation the tablets weight is more than 324 mg, hence 5% maximum difference allowed.

7.7.3 Hardness

A significant strength of Fast dissolving tablet is difficult to achieve due to the specialized processes and ingredients used in the manufacturing. The limit of crushing strength for fast dissolving tablets is usually kept in a lower range to facilitate early disintegration in the mouth.

Tablet crushing strength (Fc) or hardness, the force required to break a tablet in a diametric compression, was measured using Monsanto tablet hardness tester.

7.7.4 Thickness

The crown thickness of individual tablets was measured using Vernier calliper, which permits accurate measurements and provides information of the variation between tablets. Tablets thickness should be controlled within ± 5% variation of a standard value.

7.5.5 Friability

Friction and shock are the forces that most often cause tablets to chip, cap or break. The friability test is closely related to tablet hardness and is designed to evaluate the ability of the tablet to withstand abrasion in packaging, handling and shipping. Friability of the tablets was determined using Veego Friabilator. Usually it should be below 1%, an indication of good mechanical resistance of tablets.

Pre-weighed sample of tablets was placed in the friabilator and subjected to 100 revolutions (25 rpm). The tablets were de-dusted using a soft muslin cloth and reweighed.

The friability (f) is given by the formula

$$F = \frac{(W\,o) - (W)}{(W\,o)} \times 100$$

Where, Wo is the weight of the tablets before the test and W is the weight of the tablets after the test.

7.7.6 Wetting time and water absorption ratio:

14 A piece of tissue paper folded twice was placed in a small petridish (i.d. = 6.5 cm) containing 6 ml of distilled water, a tablet was put on the paper, and the time required for complete wetting was measured. The wetted tablet was then weighed. Three trials for each batch were performed and standard deviation was also determined. Water absorption ratio, R, was determined using equation.

$$R = 100 \times (Wa - Wb) / Wb$$

Where,

Wb = weight of the tablet before water absorption

Wa = weight of the tablet after water absorption

7.7.7 Drug Content

The amount of active ingredient(s) is determined by the method described in assay and amount of active ingredient is calculated. New method was used for determination of drug content given below:

Twenty tablets were weighed and powdered. The blend equivalent to 400mg of Atovaquone was weighed and dissolved in sufficient quantity of 5% methanol. Then was filtered through Whatmann filter paper (no.41), suitably diluted with water and assayed at 251nm, using a UV spectrophotometer (SHIMADZU 1800 UV).

7.7.8 *In-Vitro* Disintegration Test

For a drug to be absorbed from a solid dosage form after oral administration, it must first be in solution, and the first important step toward this condition is usually the break-up of the tablet; a process known as disintegration. The *in vitro* disintegration time of a tablet was determined using USP disintegration test apparatus as per I.P. specifications without using disc. It is determined by using USP device which consist of 6 glass tubes that are 3 inches long, open at one end and held against 10 mesh screen at the bottom end of basket rack assembly.

To test for disintegration time, one tablet is placed in each tube and the basket arch is positioned in a 1 litre beaker of water at 37ºC ± 2ºC. A standard motor driven device is used to move the basket assembly up and down. To compliance with the USP standard, all tablets must disintegrate and all particles must pass through the 10 mesh in the time specified.

7.7.9 *In-Vitro* Dissolution Test

Dissolution is considered one of the most important quality control tests performed on pharmaceutical dosage forms and is now developing into a tool for predicting bioavailability and in some cases, replacing clinical studies to determine bioequivalence. Dissolution behaviour of drugs has a significant effect on their pharmacological activity. In fact, a direct relationship between *in vitro* dissolution rate of many drugs and their bioavailability has been demonstrated and is generally referred to as *in vitro-in vivo* correlation.

Atovaquone is a poorly soluble drug. The solubility and the dissolution of atovaquone were very slow. But it was made soluble and brought into the dissolution first by the addition of surfactant such as Starch maize powder, SLS; and carriers MCC. Drug release kinetics was done by using USP Type II paddle method using 40% Isopropyl alcohol (IPA) with pH 8 as dissolution medium at room temperature (37ºC) at 50 rpm speed. The sample was collected for 60 min. studies and percentage of drug release at different time interval was

calculated from the UV absorbance reading. The amount of drug was measured form the absorbance of UV spectrophotometer at 251nm. 5ml syringe was used to take 5ml sample from each sample basket and 5ml fresh distilled water was added after the sample was taken into each sample basket. Sample was filtered and percent (%) release of atovaquone was calculated from UV absorbance reading of sample.

7.7.10 Accelerated Stability Study as per ICH guideline

Stability studies were carried out according to ICH guidelines Q1A (R2). Formulation F7 (Optimized batch) was packed in marketed packs i.e. aluminium foils for stability study. Batches for stability were prepared in triplicate. Studies were carried out at 30ºC/65%RH, 40ºC/75% RH, and at room temperature for a 30 days and it was evaluated for *In-Vitro* dissolution and disintegration time, and % drug content. All three stability batches were kept as per ICH condition.

8. RESULT AND DISCUSSION

8.1 Identification and characterisation of Atovaquone

8.1.1 Melting point determination of Atovaquone

The melting point of Atovaquone was found between the range of **116ºC-119ºC**, indicating purity of drug sample.

8.1.2 Differential Scanning Calorimetric Analysis:

The DSC thermographs were shown below,

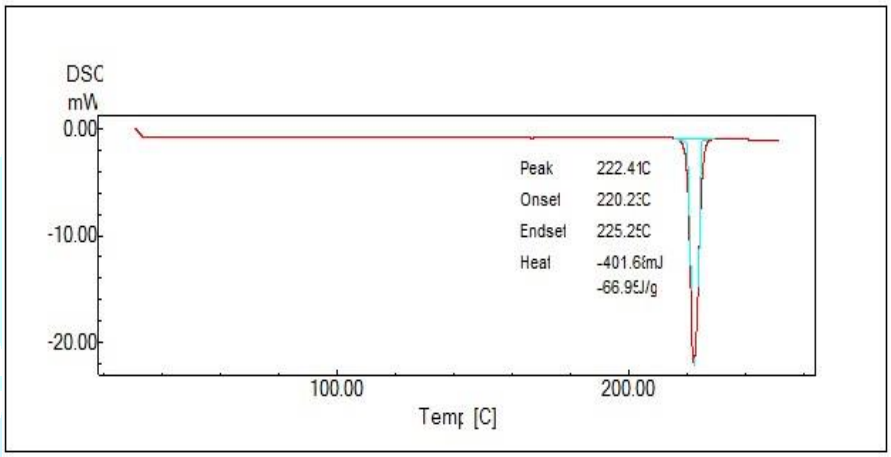


Figure 6: DSC Thermograph of API (Atovaquone)

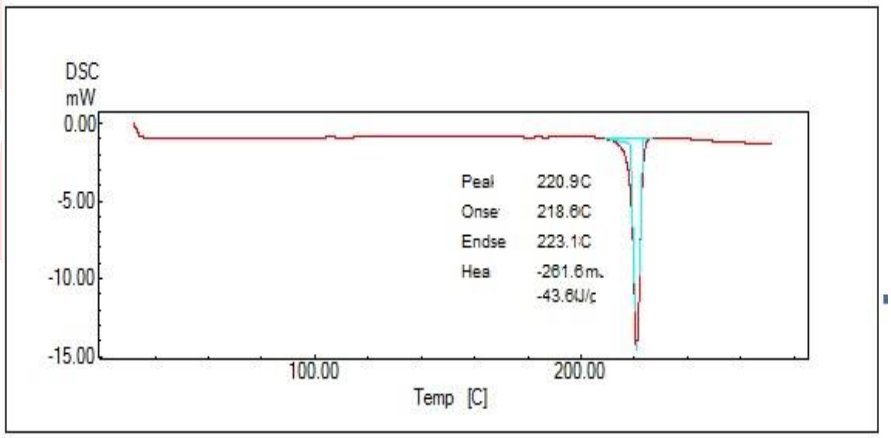


Figure 7: DSC Thermograph of Atovaquone + Excipient Discussion:

The sharp endothermic peak of Atovaquone was seen at 222.41ºC with onset of action at 220.23ºC. Sharp endothermic peak indicates the purity of drug sample. The DSC thermograph of Atovaquone final formulation was recorded in order to study the drug excipient compatibility. Study also shows the sharp peak at 220.91°C with onset at 218.66ºC indicating compatibility of drug with excipients. The DSC thermographs were shown in **figure 6** (Atovaquone) and **Figure7** (Formulation F7).

8.1.3 FTIR Spectroscopic Analysis:

Sr. NO.	Absorption peaks	Attributed to
1.	3652	O-H- stretching
2.	1646	-C=O- aldehyde stretching

3.	540	-C-Cl stretching
4.	3070	C-H aromatic strectching

Table 8: Interpretation by FTIR Spectroscopy

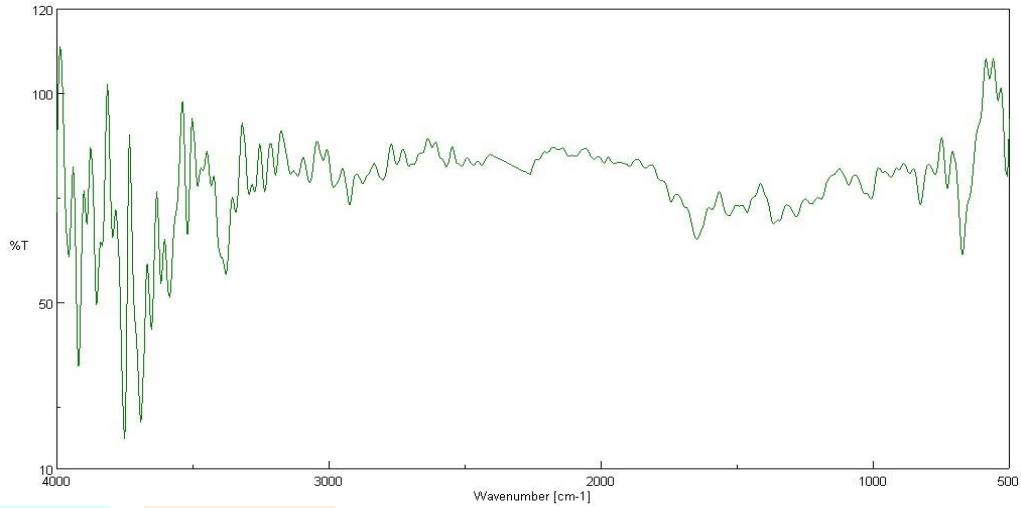


Figure 8: FTIR spectra of Atovaquone

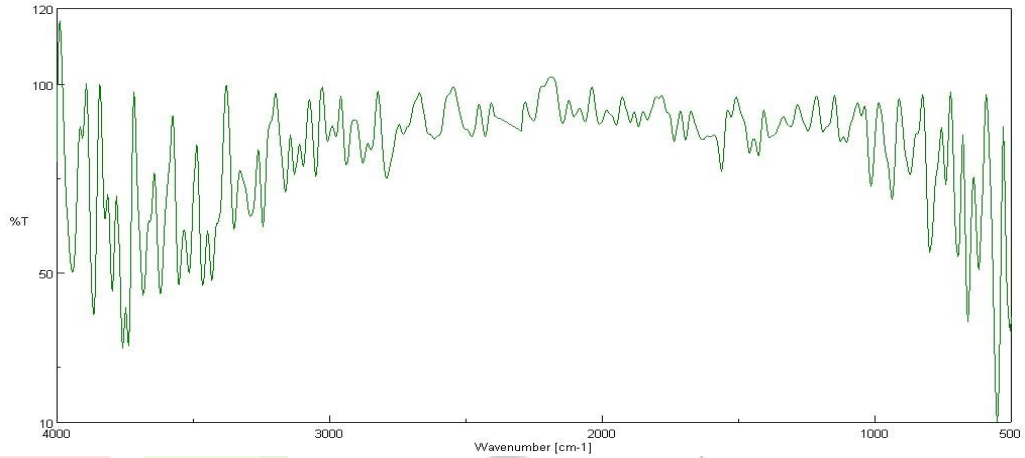


Figure 9: FTIR Spectra of Atovaquone + Excipient

Discussion:

The IR spectra of Atovaquone sample and drug+ excipient were recorded. The spectrum obtained was concordant with the reference as depicted in **Figure 10** and **11.** The IR structure of Atovaquone complies with the chemical structure 2-(Trans-4-(PChlorophenyl) cyclohexyl)-3-hydroxy-1, 4-naphthoquinone. Various groups present are given in **Table 11**.

On the basis of melting point, UV spectrum, FTIR spectrum and DSC thermogram, the procured sample of Atovaquone was found to be of Acceptable purity and quality.

The sample was taken for further studies.

8.2 Preformulation study

8.2.1 Organoleptic properties Atovaquone

Atovaquone was evaluated for its organoleptic properties such as odour, colour, and taste, and the observation were shown in **Table 8** below.

Sr. No.	Parameter	Result
1.	Colour	Yellow

2.	Odour	Odourless
3.	Taste	Tasteless
4.	Size	11mm
5.	Shape	Circular

Table 9 : Organoleptic properties of Atovaquone

8.2.2 Solubility study of Atovaquone

Solubility studies were carried out in Methanol,40% IPA, Phosphate buffer and water. All Medias have shown different solubility as shown in **Table 10**. The solubility was found in 40% IPA as compared to Methanol, phosphate buffer of pH 8 and water. As saturation solubility was found maximum in 40% IPA.

Sr. no.	Media	Solubility (mg/ml)
1.	40%IPA	1.8634
2.	Methanol	1.308
3.	Water	Insoluble
4.	Phosphate buffer pH 8	Insoluble

Table 10: Solubility study of Atovaquone in different media

8.2.3 Characterization powder of API and excipients

For preliminary batch, blends of API and excipients were prepared and evaluated for various parameters as explained in **table 11** shows the flow property of each API and Excipients. From these results it was found that the API and formulation excipients have good flow properties.

Sr. No.	Flow Properties	Preliminary batch	API	Description
1.	Bulk density(g/ml)	0.41	0.44	Good
2.	Tapped Density(g/ml)	0.47	0.50	Good
3.	Angle of Repose	25.74	31	Good
4.	Carr‟s Index (%)	14.20	14	Good
5	Hausner‟s Ratio	1.17	1.15	Good

Table 11: Flow properties of API and preliminary batch

8.3 Analytical Method Development:

8.3.1 UV spectra of Atovaquone

The pure drug Atovaquone was scanned over a range of 200-400 nm to determine its λmax. The maximum absorption was found at 251 nm in 5% methanol corresponds to the literature review. Spectra of Atovaquone were shown in **figure10** below.

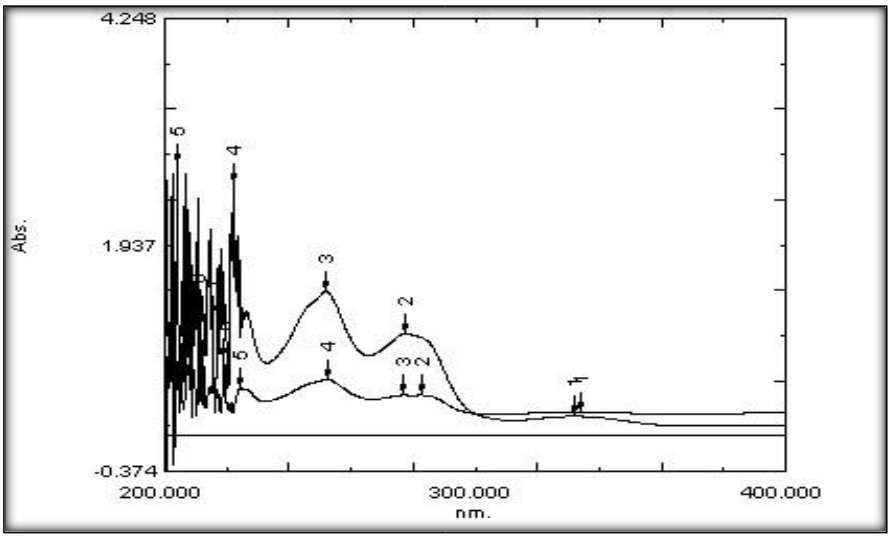


Figure 10: Spectra of Atovaquone

8.3.2 Calibration Curve of Atovaquone

The standard curve of Atovaquone was obtained by plotting the graph of concentration vs. Absorbance. The **Table 12** given below shows the values of absorbance‟s of Atovaquone. The standard curve as shown in figure12 shows the slope of 0.01260, and regression coefficient of **$(R)^2$ 0.999**, and the equation of line was **y = 0.023x+0.167**, the curve was found to be linear in the range of 5-25µg/ml obeying Beer‟s law at 251 nm.

Sr. No.	Concentration(µg/ml)	Absorbance
1.	5	0.281
2.	10	0.401
3.	15	0.515
4.	20	0.631
5.	25	0.745

Table 12: Calibration curve of Atovaquone

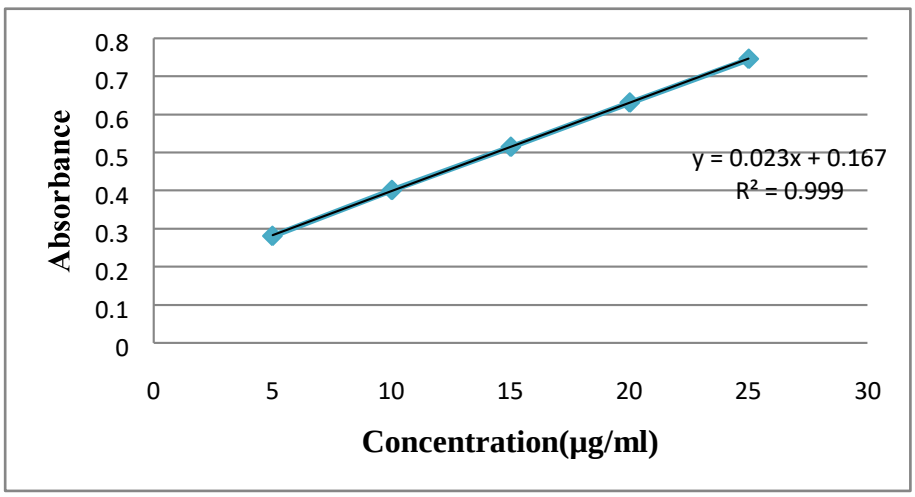


Figure 11: Calibration curve of Atovaquone in methanol + water

8.4 Analytical Method Validation

The parameters studied for method validation were linearity and range, intraday and interday precision, accuracy study by % recovery, LOD (Detection Limit) and LOQ (Quantitation Limit). The results were listed in **Table13** below.

Sr. No.	Parameter	Result	Limit
1.	Accuracy	99.92%	98-102%
2.	Interday precision	0.05158%RSD	%RSD<2
3.	Intraday precision	0.04434%RSD	%RSD<2
4.	LOD	3.3µg/ml	-
5.	LOQ	10µg/ml	-
6.	Range	20-30µg/ml	-
7.	Linearity	0.999	R^2>0.9997

Table 13: Method Validation

8.5 Flow properties of Factorial Batches

Factorial batches were evaluated for their flow properties. Prior to compression, the powder blends of various batches were evaluated for their bulk and tapped densities and from these values compressibility index and hausner‟s ratio were calculated. While the flow properties of powder blend were accessed from angle of repose. Compression was excellent for this minimal formulation with a wide range of compression forces producing acceptable tablets. The results were shown in **Table 14** and it was found that all factorial batches shows good flow property.

Formulation code	Bulk Density (gm/cm3)	Tapped Density (gm/cm3)	Carr's Index (%)	Hausner s ratio	Angle of repose (θ)	Flow
F1	0 .41	0.47	14.18	1.16	25.78	Good
F2	0.42	0.49	13.25	1.15	25.45	Good
F3	0.44	0.51	13.25	1.15	29.37	Good
F4	0.44	0.50	13.41	1.15	31.10	Good
F5	0.45	0.51	11.76	1.13	28.32	Good
F6	0.43	0.51	15.29	1.18	28.18	Good
F7	0.43	0.52	16.66	1.20	26.19	Good
F8	0.43	0.51	15.47	1.18	27.38	Good
F9	0.44	0.51	13.25	1.15	28.18	Good

Table 14: Evaluation Flow Property of Factorial Batches

8.6 Results for Evaluation of Atovaquone Immediate Release Tablets:

8.6.1 Evaluation of Preliminary Batch Tablets:

Effect of Starch and SLS was considerably seen in showing its solubilising and good friability property, as there was increase in the concentration of SLS, and decreased concentration of Starch it showed good friability and also increased the disintegration time. The use of MCC as a conventional disintegrant agent showed a key role in disintegration. The rapid swelling of these tablets upon wetting may partly be attributed to the recovery of deformation and disintegrates in the form of fine particles. Depending upon the disintegration time, Dissolution rate and friability Primary batch was selected.

The Primary batch was evaluated for Hardness, Friability, Thickness, Disintegration time, Dissolution and Drug content. The results were shown in **Table 15** below.

Sr. No	Evaluation Parameters	Result
1.	Hardness	3.5kg/cm2
2.	Friability	0.87%
3.	Thickness	3.55mm
4.	Disintegration time	42sec
5.	% Drug content	99.24%
6.	% Drug release	97.76%

Table 15: Evaluation of preliminary batch

8.6.2 Evaluation of Factorial Batches

All the tablet formulations were subjected for organoleptic, physical and chemical evaluations as shape, thickness, hardness, friability, weight variation, *in vitro* disintegration time, wetting time, drug content, *and in vitro* dissolution studies**.**

a. Weight Variation Test

The percentage weight variation for all the formulation is tabulated in **Table 17**. All the tablets passed weight variation test as the % weight variation was within the pharmacopoeial limits of ≤ 375 ±5%. The weight of all the tablets was found to be uniform. Uniform weight due to uniform die fill with acceptable variation as per USP standards were obtained since blend of material was free-flowing.

b. Hardness

Tablet crushing strength, the critical parameter was controlled as the resistance of tablets to capping, abrasion or breakage under conditions of storage, transportation and handling before usage depends on its hardness. Hence, hardness for all Trial batches and Factorial batches were between **2.8-4 kg/cm^2**. The results were shown in **Table17**. These results were observed due to constant tablet press setting across all batches design irrespective of weight variation.

d. Thickness

The thickness of the tablets was measured by using Digital Vernier Calliper by picking the tablets randomly. The values were shown in **Table 17**. The values were almost uniform in all formulations. Thickness for all formulation batches was found to be between **3.55 – 3.72 mm** due to constant tablet press setting across all batches design irrespective of weight variation with constant diameter 11mm. **e. Friability**

To achieve percent friability within limits for a fast dissolving tablet was challenge to the formulator since all methods of manufacturing of Fast dissolving tablets was responsible for increasing the % friability values. The % friability values for all formulation batches were found to be between **0.74 - 0.95%,** due to constant tablet press setting across all batches of design irrespective of weight variation. The results of friability were shown in **Table17**

f. Wetting time and water absorption ratio:

Wetting time is used as an indicator from the ease of the tablet disintegration in stomach. It was observed that wetting time of tablets was in the range of **24 to 40** seconds result were shown in **table 17** and type of the Disintegrant affects the wetting of the tablets.

8.6.2. % Drug Content for factorial batches

Drug content for all formulation batches was found to be in the range of **94% to 100.01%**. The results were shown in **Table16** and **figure12.** The results indicated that in all the formulations the drug content was uniform.

Sr. No.	Formulation	% Drug content
1.	F1	94
2.	F2	95
3.	F3	96
4.	F4	98
5.	F5	97
6.	F6	95
7.	**F7**	**100.01**
8.	F8	94
9.	F9	97

Table 16: % Drug content for Factorial Batches

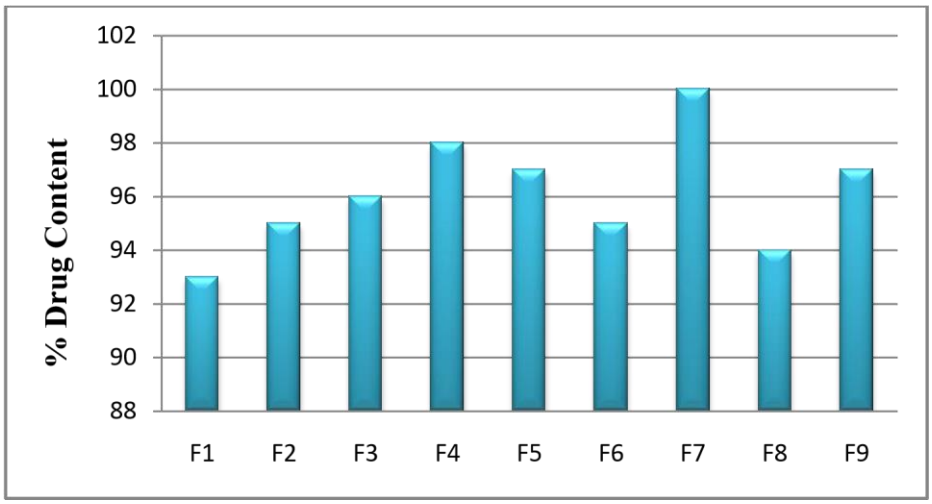


Figure 12: % Drug content for Factorial batches

Formulation code	Weight Variation mg±%SD	Hardness (Kg/cm) ±SD	Friability (%)	Thickness (mm) ± SD	Wetting time
F1	400±0.55	3.3±0.05	1.25	3.5±0.012	24±0.003
F2	400±0.45	2.9±0.1	0.94	3.54±0.01	27±0.05
F3	400±1.28	3.5±0.30	1.00	3.65±0.05	26±0.04
F4	400±0.19	3.3±0.11	0.79	3.60±0.02	27±0.03
F5	400±0.56	3.4±0.05	0.79	3.57±0.05	29±0.06
F6	400±0.85	3.1±0.17	0.75	3.67±0.0	30±0.03
F7	**400±1.42**	**3.6±0.05**	**0.17**	**3.71±0.02**	**26±0.03**
F8	400±1.41	4.2±0.12	0.74	3.69±0.06	32±0.06
F9	400±1.11	4.1±0.15	0.94	3.72±0.06	40±0.03

Table 17 Evaluation of Factorial Batches

8.6.3. *In-Vitro* Disintegration Time

All tablets disintegrated rapidly without disc in test especially when used at optimum concentrations of selected Solubilising agent and surfactant. The results of disintegration time were shown in **Table 18** and **Figure14**. *In vitro* disintegration time for factorial batch F7 containing SLS 1.5% and 23% Starch 23% gives best results among the factorial batches.

Sr No.	Formulation code	DT (sec)
1.	F1	51
2.	F2	50
3.	F3	61
4.	F4	59
5.	F5	46
6.	F6	48
7.	**F7**	**40**
8.	F8	49
9.	F9	58

Table 18: Disintegration Time Profile of Factorial Batches

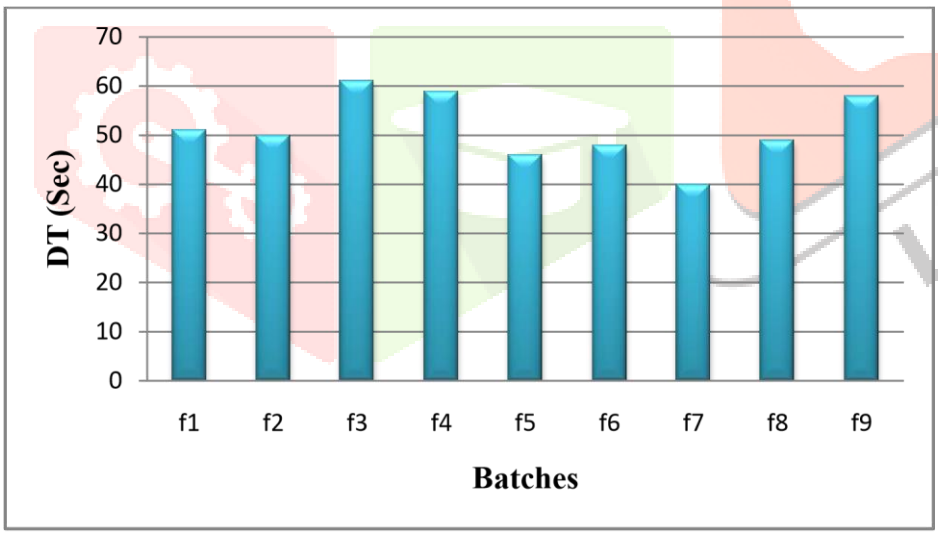


Figure 13:DT of factorial batches

8.6.4 *In-Vitro* Dissolution Study

As discussed above, differences in the particle size generated in the disintegrated tablets could affect drug dissolution. Since breaking tablets into finer fragments may promote drug dissolution by providing larger total surface areas for drug dissolution. Different concentration of MCC, Starch and SLS shows different drug release. The concentration of MCC at 11% & Starch at 24% and SLS at 1.5% shows the increased % drug release. The complete dissolution profile of factorial batches were shown in **Table 19.**

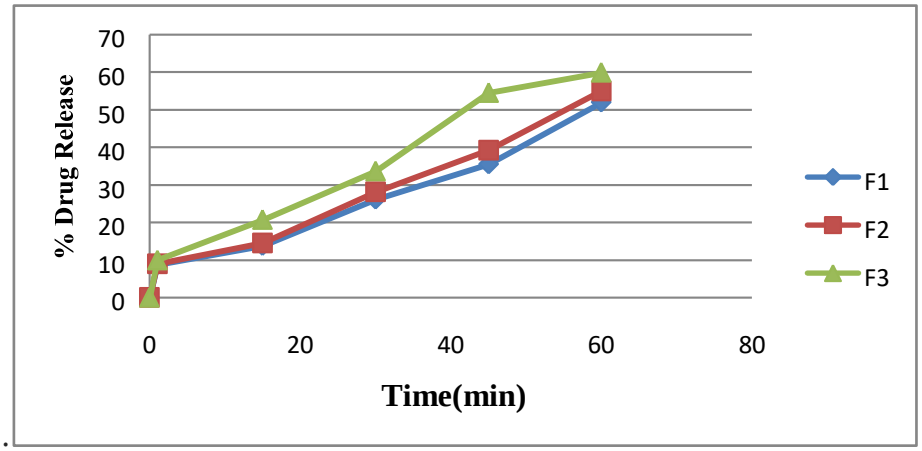


Figure 14:*In vitro* dissolution profile for F1, F2, F3 batches

The F1,F2 &F3 contain 6-7% MCC and 28% starch and SLS 1.5%, 1%, 0.5% respectively. Different concentration of MCC, Starch, SLS shows different drug release. The Percent Drug release at 60 min. was found to be 52%, 55%, 60% respectively and the dissolution behaviour were shown in **figure14.**

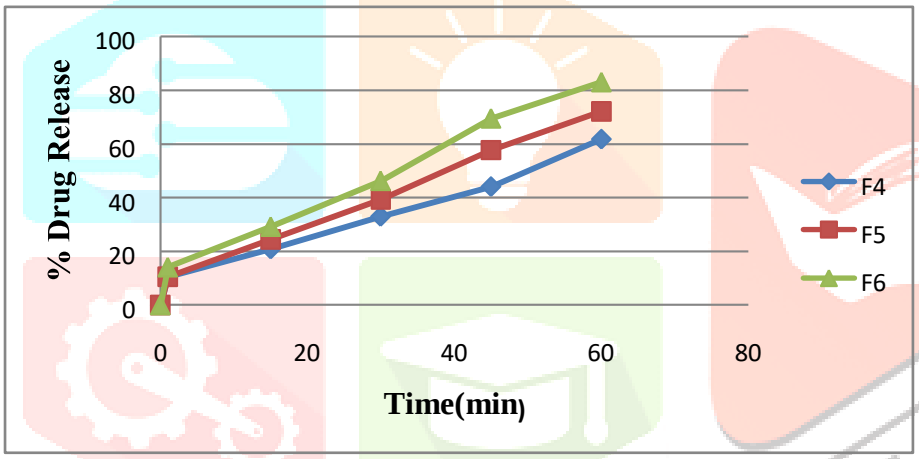


Figure 15: *In vitro* dissolution profile for F4, F5, F6 batches

F4, F5, F6 contain 8-9% MCC and 26% starch and SLS 1.5%, 1%, 0.5% respectively. The Factorial batches F4, F5% F7 shows 62%,72%,and 83% drug release, and the release behaviour were shown in **figure 15**.

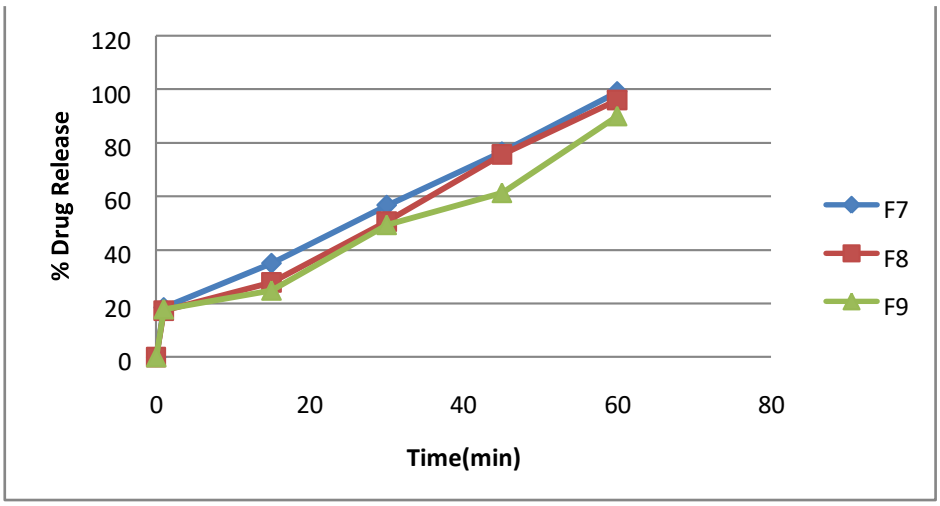


Figure 16: *In vitro* dissolution profile for F7, F8, F9 batches

F7,F8,F9 contain 11-12% MCC,23% starch and SLS 1.5% .The release of factorial batches were found to be 99.18%,96% and 90%, and result were shown in **Figure 16**.. Factorial batch **F7** shows better drug release,

among all factorial batches. As the concentration of MCC, Starch decreases and concentration of SLS increases, percent drug release was found to be increases. The complete dissolution profile was shown in **Table 19**

Time (min)	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	8.76 ±1.25	9.01 ±1.35	10.02 ±1.29	10.47 ±1.56	10.52 ±1.48	14.13 ±1.14	**18.60 ±1.31**	17.29 ±1.88	17.84 ±2.0
15	13.84 ±1.58	14.54 ±1.88	20.70 ±1.55	20.90 ±1.4	24.43 ±1.44	29.24 ±1.5	**35.11 ±1.44**	27.90 ±1.75	24.81 ±2.4
30	26.13 ±1.41	28.09 ±1.65	33.65 ±2.07	33.00 ±1.34	39.28 ±2.21	46.19 ±0.15	**56.69 ±1.28**	50.66 ±0.46	49.40 ±1.74
45	35.57 ±1.46	39.24 ±1.46	54.54 ±1.82	44.02 ±1.19	57.61 ±2.5	69.50 ±1.24	**76.82 ±1.4**	75.64 ±1.42	61.36 ±1.48
60	52.00 ±1.4	55.04 ±1.54	59.94 ±1.9	62.00 ±1.67	72.03 ±2.45	83.04 ±1.76	**99.18 ±1.31**	96.02 ±1.45	90.00 ±1.98

Table 19: Complete Dissolution Profile of Factorial Batch

8.4.6 Drug release kinetic studies

In vitro dissolution has been recognized as an important element in drug development. Under certain conditions it can be used as a surrogate for the assessment of bioequivalence. There are several models to represent the drug dissolution profiles where ft is a function of t (time) related to the amount of the drug dissolved from the pharmaceutical dosage system. The kind of drug its polymorphic form, crystalline, particle size, solubility and amount in that pharmaceutical dosage form can influence the release kinetics. A water soluble drug incorporated in a matrix is mainly released by diffusion while for low water soluble drug the self erosion of the matrix will be the principle release mechanism. The Matrix model relates drug release exponentially to time. The Korsmeyer-Peppas model relates drug release exponentially to time. It is described by the following equation,

$$\mathbf{Mt / Minf = atn}$$ Where, Mt / Minf = fractional release of drug

a= constant depending on structural and geometric characteristics of the drug dosage form n= release exponent.

This model is used to analyze the release from polymeric dosage forms, when the release mechanism is not well known or when there is a possibility of more than one type of release phenomenon being involved. In the present study, the drug release was analyzed by PCP Disso Version 3 software to study the kinetics of drug release mechanism. The results showed that F7 batch followed Korsmeyer-Peppas model. The R values of Korsmeyer-Peppas model are shown in table. n values were found to be less than 0.5 indicating that the mechanism is diffusion controlled or fickian type.

Formulation	Models	R	n	K
F7	Zero Order	0.967		
	First Order	0.843		
	Matrix	0.976		
	Korsmeyer Peppas	**0.952**	**0.3917**	**16.6964**
	Hixon Crowell	0.939		

Table 20: Drug release kinetic study of Tablet formulation

8.4.7 ANOVA Study

Optimization was performed using State ease design of experiment software version 9.0.4.1 suite-480 and predicted vs. actual, contour plot, surface response plot were plotted. ANOVA study for Formulations was performed by Starch (A) and Solubilising Agent (B) formulation variables and for percent drug release at 60 Min. as response. ANOVA results were as summarized in **Table 22**. And predicted vs. actual, contour plot, surface response plot were given in **Figure 20**, **Figure 19** and **Figure 21** respectively.

8.4.7.1 Response Surface Plot

The quadratic model obtained from the regression analysis used to build a 3-D graphs in which the responses were represented by curvature surface as a function of independent variables. The relationship between the response and independent variables can be directly visualized from the response surface plots.

The response surface plot for dependent variable i.e. % DR was generated using Design Expert software 9.0.4.1 suite-480 presented in Figure21 and. The effect of independent variables, X1 and X2 on the response was studied.

Graphical presentation of the data helped to show the relationship between the response and the independent variables. The information given by graph was similar to that of mathematical equations obtained from statistical analysis. Surface Response Plot and contour plots shows that drug release decreases with increasing concentration of independent variables Starch (A) and decreasing concentration of Surfactant (SLS) (B).

Factor	Name	Unit	Actual values		Coded values	
			Lowest	Highest	Lowest	Highest
A	Starch	%	23	28	-1	+1
B	SLS	%	0.5	1.5	-1	+1

Table 21: Summery of Statistical Design

The equation conveyed the basis to study of the effects of variables. The regression coefficient values are the estimates of the model fitting. The r2 was high indicating the adequate fitting of the quadratic model. The polynomial equations can also be used to draw conclusions considering the magnitude of co-efficient and the mathematical sign it carries; i.e. positive or negative. Where all p-values are less than 0.05%. We

can conclude that developed model is statistically valid. The model fitting equations are depicted in equation 1 and 2 for coded and actual values respectively.

Source	Sum of Squares	Degree of freedom	Mean Square	F Value	pvalue	Level of significance
Model	2395.79	2	1197.90	28.42	0.0009	Significant
Starch	2329.33	1	2329.33	52.2	0.0003	
SLS	66.47	1	66.47	1.58	0.2559	
Residual	252.94	6	42.16		-	-
Core	2648.7	8	-	-	-	-
Total	4					

Table 22: Analysis of variance

Interpretation for ANOVA:

we have selected 2 variables Starch and SLS, ANOVA study and null hypothesis states that the 2 variables have effect on each other, as F value calculated for starch was 52.2 which is greater than the F tabulated value, so it is concluded that the variables are significant.

Term	Coefficient	Standard Error	Low Confidence	High Confidence	VIF
Intercept	74.34	2.16	69.05	79.64	
A:Factor 1	-19.70	2.65	-26.19	-13.22	1.00
B:Factor 2	-3.33	2.65	-9.81	3.16	1.00

Table 23: Regression Analysis

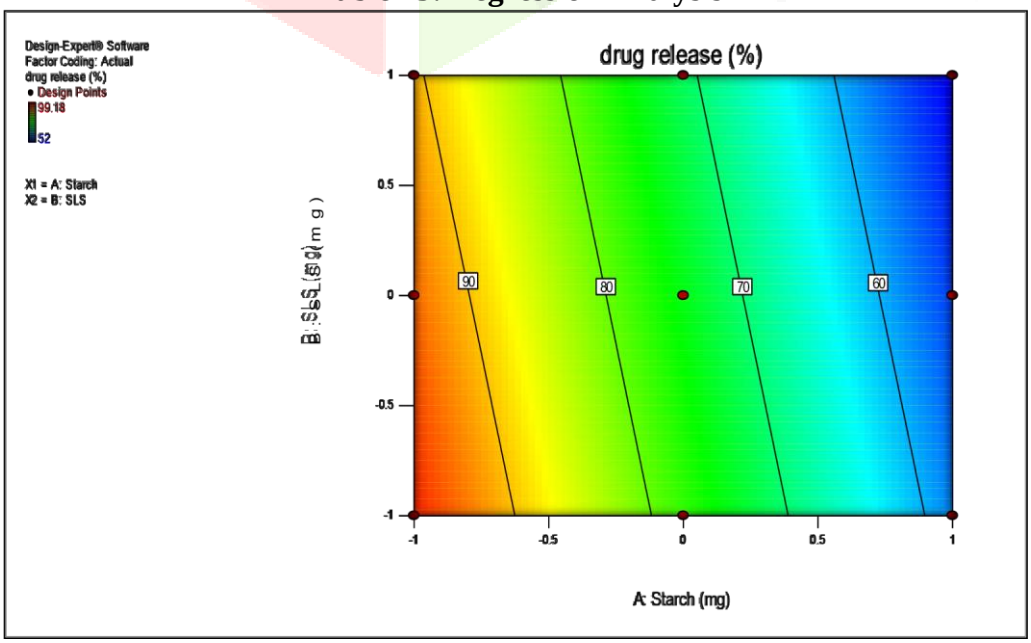


Figure 17: Contour Plot

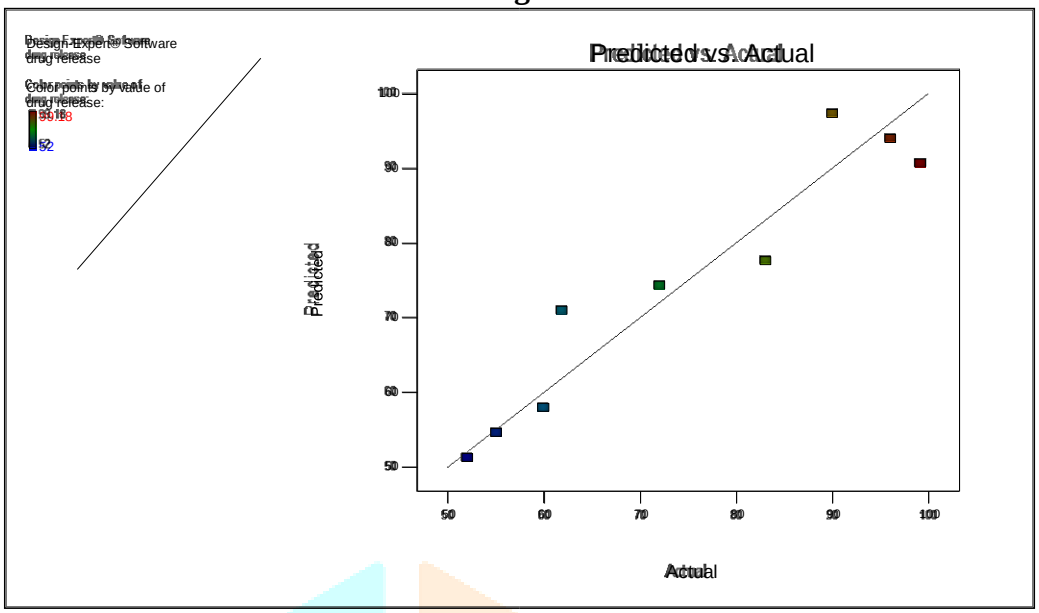


Figure 18: Predicted Vs Actual

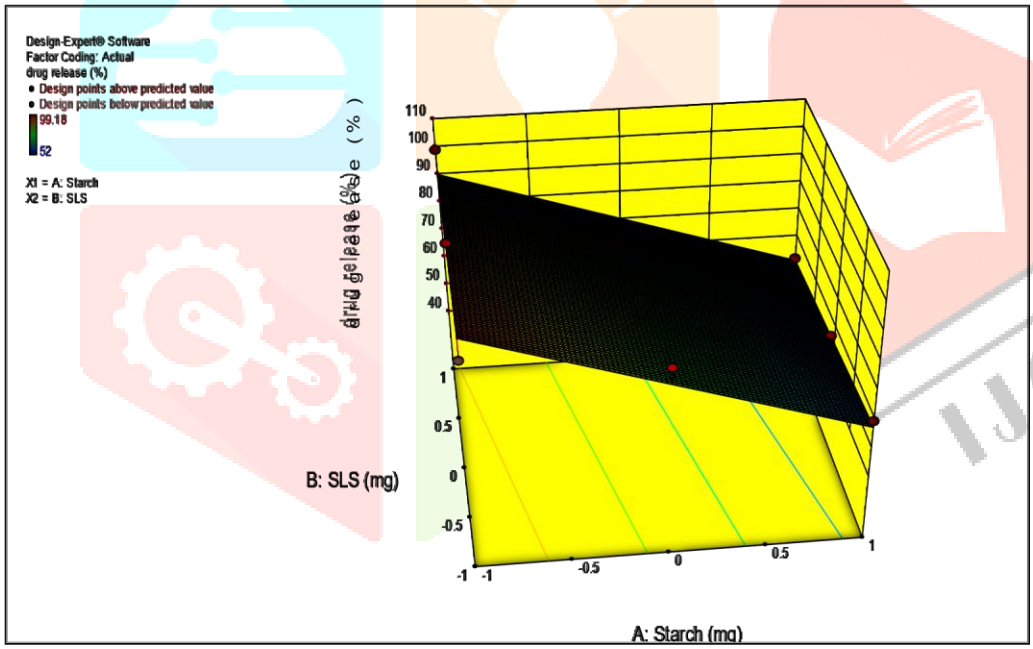


Figure 19: Surface Response Plot Equation 1: Coded Factors Final Equation in Terms of Coded Factors

% Drug Release=+74.34-19.70* Starch-3.33* SLS

R^2=0.9045

Equation 2: Actual Factor Final Equation in Terms of Actual Factors

Drug Release=+74.34222-19.70333* Starch-3.32833* SLS

8.4.8. Accelerated Stability study as per ICH guideline

Stability study was conducted as per ICH guidelines Q1A(R2). The optimized batch (F7) was studied for one month time period. Three different batches of F7 were kept for accelerated stability study.

Sr. no.	Formulation Parameter	F7A	F7B	F7C
1.	Colour	Yellow	Yellow	Yellow
2.	Odour	No	No	No
3.	*In-Vitro* DT(sec)	40	39	40
4.	*In- Vitro* DR	98.89	98.90	99.04
5.	Drug content	100.10	100.19	100.20

Table 24: One Month Stability Data at Room Temperature

Sr. no.	Formulation Parameter	F7A	F7B	F7C
1.	Colour	Yellow	Yellow	Yellow
2.	Odour	No	No	No
3.	*In- Vitro* DT(sec)	41	40	41
4.	*In- Vitro* DR	98.96	99.05	99.14
5.	Drug content	100.21	100.25	100.39

Table 25: One Month Stability Data at 40^0C ± 2ºC /75% RH ± 5% RH

Discussion

At room temperature the *In-Vitro* DT, was found to be 40, 39, 40, *In-Vitro* DR 98.89, 98.90, 99.04 and drug content 100.10, 100.19, 100.20 for F7A, F7B, F7C respectively. At 40^0C ± 2ºC /75% RH ± 5% RH *In-Vitro* DT was found to be 41, 40, 41, *In-Vitro* DR 98.96, 99.05, 99.14 and Drug content 100.21, 100.25, 100.39 respectively. From study it was concluded that there was no significant change in colour and odour and no significant variation in *In-vitro* disintegration time, dissolution time and drug content profiles after one month stability study for optimized formulation at different temperature after storage. The results were shown in **Table 24** and **Table 25**.

9. SUMMERY AND CONCLUSION

The main Aim of present research work is to evaluate and optimise the immediate release tablet of Antimalerial Drug Atovaquone to overcome the Dissolution problem. To minimize critical process parameters and since Atovaquone is moisture sensitive, direct compression method was selected for the formulation of immediate release tablets.

The conclusions drawn from the present investigation were given below

- Under the pre-formulation studies API characterization and drug- excipient compatibility studies were carried out. The API characterization showed compliance with the drug characteristics.
- Preformulation studies were carried out during the early stages of the work. The drug excipients compatibility studies were carried out to determine the interaction between API and the Excipient. DSC thermograms revealed that there was no interaction between the API and excipients indicating compatibility with the drug.
- UV spectrophotometric method was developed and validated for Atovaquone.
- The dintegrant and other excipients were selected based on the satisfying results produced during drug - excipient compatibility studies to develop the final formulation.
- The significant effects of interaction and polynomial variables on the investigated characteristics of immediate release tablets of Atovaquone were verified using 3^2 full factorial designs.
- This permitted the selection of a batch of tablets with desired disintegration time and improved dissolution rate after oral administration.
- The final suitable formulation (F7) was achieved fruitfully by the direct compression technique using MCC as superdisintegrant, Starch and SLS as solubilising agent.
- Which exhibited acceptable disintegration time (40 sec), percentage drug content per tablet (100.01%) and in vitro drug release (99.18%) ➢ Monotherapy of Atovaquone is not available in market.

SUMMERY AND CONCLUSION

- Drug release kinetic study of Optimized formulation(F7) were carried out by PCP Disso Version 3 software to study the kinetics of drug release mechanism
- One month Stability study results revealed that optimized formulation was stable i.e. Colour, Odor, Disintegration time, %DR; Assay does not change at Room temp.

From above discussion it was concluded that immediate release tablets of Atovaquone were prepared by the direct compression technique using MCC as disintegrant at lower possible concentration of 6%w/wt Starch 23%w/wt and SLS at higher concentration 1.5% w/wt which gives the disintegration time of 40 seconds and with improved dissolution rate and increased Aqueous solubility of Atovaquone.

10. APPENDIX

10.1 Chemicals and Equipments Used for Study 10.1.1 Chemicals

Sr. No	Materials	Supplier/Makes
1	Atovaquone	GSK Pharma. Pvt. Ltd Mumbai.
2	Microcrystalline cellulose(Avicel)	Lupin Pharma Ltd. Aurangabad.
4	Starch Maize powder LR	Research lab Fine Chem. Industries, Mumbai.
5	Sodium Lauryl Sulphate LR	Research lab Fine Chem. Industries, Mumbai.
6	Magnesium Sterate	Research lab Fine Chem. Industries, Mumbai.
7	Talc	Research lab Fine Chem. Industries, Mumbai.

Table 26: List of chemicals 10.1.2 Equipments

Sr. No.	Instrument	Make
1.	Electronic Balance	Citizen CS 65
2.	UV-Visible Spectrophotometer	SHIMANDZU 1800 UV
3.	IR Spectrophotometer	SHIMANDZU IR Prestige
4.	Differential scanning Colorimeter	Shimadzu Aurangabad (DSC 60)
5.	Tablet Compression Machine	Rotary Tablet Press
6.	Sieve	Alpine
7.	Tablet Hardness Tester	Monsanto Hardness tester
8.	Friability Test Apparatus	Veego
9.	Tablet Disintegration Tester	Veego
10.	Dissolution Test Apparatus	VDA-6DR USP Stds, Veego.
11.	Bath Sonicator	Chief Scientific Industries

Table 27: List of Equipment

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