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COLLEGE OF HEALTH SCIENCES
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FORMULATION, OPTIMIZATION AND *IN-VITRO* EVALUATION OF AQUEOUS-BASED ENTERIC COATED DOXYCYCLINE HYCLATE TABLETS

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FORMULATION, OPTIMIZATION AND *IN-VITRO* EVALUATION OF AQUEOUS-BASED ENTERIC COATED DOXYCYCLINE HYCLATE TABLETS

A thesis submitted to the Department of Pharmaceutics and Social Pharmacy, School of Pharmacy, College of Health Sciences, Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Science in Pharmaceutics

Seife Shimels (B.Pharm)

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This is to certify that the thesis investigated by Seife Shimels, entitled: "*Formulation, Optimization and In-Vitro Evaluation of Aqueous Based Enteric Coated Doxycycline Hyclate Tablets*" and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Pharmaceutics complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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ABSTRACT

Doxycycline Hyclate (DXYH) is a tetracycline-class antimicrobial used to treat infections caused by susceptible Gram-positive and Gram-negative organisms. Due to doxycycline's antibacterial effects on a wide range of pathogens, it is currently one of the most commonly prescribed antibiotics worldwide for treating infectious diseases. There are some disadvantages associated with the standard formulation of doxycycline such as gastrointestinal upset, anorexia, vomiting, and nausea, limiting its acceptability by patients. Enteric coating is recommended to overcome these problems.

In the present study, different formulations of DXYH core tablets were developed using micro crystalline cellulose as a dry binder and different disintegrants in different proportions. From those formulations, formulation with average Concentration of crospovidone (FCP^2) was selected as optimized core tablet for enteric coating formulation as it was mechanically stronger with least friability and disintegrating within 3.50±0.12 min. Further, optimized formulation was coated by varying the concentration of enteric coating polymers i.e. Eudragit-L30-D55® and Kollicot® MAE 100 P with aqueous based enteric coating systems.

A central composite design was chosen by considering factors as polymer percentage, 1,2-propylene glycol percentage, coating level, and dependent variables as disintegration in 0.1 N HCl, dissolution in 0.06 N HCl and dissolution in phthalate buffer of pH 5.5. Linear and curvature regression models were developed for response variables. The model was used to achieve an optimized response characteristic of disintegration in 0.1 N HCl, dissolution in 0.06 N HCl and dissolution in phthalate buffer of pH 5.5 with predicted input variables of polymer percentage, 1,2-propylene glycol percentage, and coating level. With the optimized process parameters, tablets were coated and the suitability of the model determined.

The results revealed that the prepared enteric coated tablets exhibited cumulative release 99.3± 1.89 % of DXYH from those tablets coated with Eudragit-L-30-D-55® and 98.5± 2.58 % of DXYH from Kollicot® MAE 100 P within 30 min at factors set to polymer percentage, 1,2-propylene glycol percentage, coating level as (97 %, 8.5 % and 5.52 %) for FCP^2E-14 and (91.7 %, 8.5 % and 12.5 %) for FCP^2K-18), respectively. Further, low percentage of DXYH release were observed in acidic media 9± 0.71 % in 0.06 N for FCP^2E-14 and 9.5+ 1.58 % for FCP^2K-18 over a period of 20 min of dissolution. Furthermore, the results of accelerated stability studies for these optimized batches

showed no significant changes in the physical parameters of the tablets, drug content and *in-vitro* dissolution data until the end of 3 months from the initial values.

Thus the study fulfilled the objective of developing efficient Doxycycline Hyclate aqueous-based enteric coted tablets.

Key Words: Aqueous-based enteric coating, Eudragit-L-30-D-55®, Kollicot® MAE 100 P, Doxycycline Hyclate, Optimization

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Seife Shimels

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ACRONYMS

1,2-PG:	1,2-Propylene Glycol
ANOVA:	Analysis of Variance
Avg.:	Average
CCD:	Central Composite Design
CCNa:	Crosscarmelose Sodium
Conc:	Concentration
CP:	Crospovidone
DS:	Dried Starch
DXYH:	Doxycycline Hyclate
EPHARM SC:	Ethiopian Pharmaceuticals Manufacturing Share Company
$FCCNa^1$:	Formulation with Higher Concentration of Crosscarmelose Sodium
$FCCNa^2$:	Formulation with Average Concentration of Crosscarmelose Sodium
$FCCNa^3$:	Formulation with Lower Concentration of Crosscarmelose Sodium
FCP^1:	Formulation with Higher Concentration of Crospovidone
FCP^2:	Formulation with Average Concentration of Crospovidone
FCP^2E:	Formulation of Eudragit-L30-D55® coated tablets for optimization batches
FCP^2K:	Formulation of Kollicot® MAE 100P coated tablets for optimization batches
FCP^3:	Formulation with Lower Concentration of Crospovidone
FDS^1:	Formulation with Higher Concentration of Dried Starch
FDS^2:	Formulation with Average Concentration of Dried Starch

FDS^3:	Formulation with Lower Concentration of Dried Starch
FMHACA:	Food, Medicine and Healthcare Administration and Control Authority
$FSSG^1$:	Formulation with Higher Concentration of Sodium Starch Glycolate
$FSSG^2$:	Formulation with Average Concentration of Sodium Starch Glycolate
$FSSG^3$:	Formulation with Lower Concentration of Sodium Starch Glycolate
FT-IR:	Fourier transformed infrared spectroscopy
MAE:	Methacrylic Acid and Ethyl acrylate
MCC	Micro Crystalline Cellulose
Mg Stearate:	Magnesium Stearate
NDDS:	Novel Drug Delivery System
NF:	National Formulary
PVC/PVDC:	Polyvinyl chloride/Polyvinyl dichloride
rpm:	Revolution per Minute
RSM:	Response Surface Methodology
SD:	Standard Deviation
SSG:	Sodium Starch Glycolate
T_g	Glass Transition Temperature
USP:	United States Pharmacopoeia
UV/Vis:	Ultra Violet Visible
W/W:	Weight by Weight

1. INTRODUCTION

The oral route is the most commonly preferred means of drug administration and acceptable bioavailability of the active ingredient with the utmost importance to ensure a therapeutically successful treatment outcome (Alderborn and Aulton, 2002; Liu *et al.*, 2009; Desai *et al.*, 2012). Among them, tablet is the most popular conventional solid dosage form because of its convenience in terms of self-administration, compactness, accurate dosage and ease in manufacturing of all ethical pharmaceutical preparations produced (Naazia *et al.*, 2014; Sandeep *et al.*, 2015; Nasir *et al.*, 2017). Recent development in novel drug delivery system (NDDS) aims to improve safety by formulating convenient dosage forms for administration to achieve better patient compliance (Bansal *et al.*, 2014; Kaushik, 2016).

Tablet size, shape and coating are pharmaceutical parameters which can be controlled to minimize esophageal contact of a dosage form with esophageal tissue. For instance, enteric coating of some tablets is desirable for preventing stomach upset or irritation in patients taking daily doses (Bushra *et al.*, 2010; Kishore *et al.*, 2016). Thus, pharmaceutical technology has played a fundamental role in the investigation of systems that efficiently deliver a drug to its site of action. The use of modified release dosage form allows a drug to be delivered at a rate dictated by the needs of the body over the period of treatment and also avoids or directs the drug to specific sites, as in delayed release (Rafati *et al.*, 2006; Souza *et al.*, 2013).

1.1 Type of Tablet Coating Techniques

Coating is a process by which an essentially dry, outer layer of coating material is applied to the surface of the dosage form to achieve specific benefits. Coating may be applied to a wide range of oral dosage forms. When coating composition is applied to a batch of tablets in a coating pan, a tablet surface becomes covered, with a tacky polymeric film. Before the tablet surface dries the applied coating changes from a sticky liquid to a tacky semisolid and eventually to a non-sticky dry surface pans. Generally, three traditional methods are used for tablet coatings, as sugar coating, film coating and enteric coating (Cole, 1995; Conway, 2008).

1.1.1 Sugar Coating

Sugar-coating tablets are such type of compressed tablets containing sugar coating. Such coating may be colored and beneficial to covering up drug substances having unpleasant taste or odor and in protecting material sensitive to oxidation. Tablet coating developed originally from the use of sugar to mask the taste and provide an attractive appearance to the core. The process of tablet coating consists of several steps, namely, sealing, sub-coating, syrup coating, coloring and polishing (Cole, 1995).

1.1.2 Film Coating

As the sugar coating process is very time consuming and is dependent on the skills of the coating operator, this technique has been replaced by film coating technology. The process involves spraying of a solution of polymer, pigments and plasticizer onto a rotating tablet bed to form a thin, uniform film on the tablet surface. Film coating is deposition of a thin film of polymer surrounding the tablet core. Conventional pan equipment may be used, but nowadays more sophisticated equipment is employed to have a high degree of automation and coating time. The polymer is solubilized into solvent. Other additives like plasticizers and pigments are added. Resulting solution is sprayed onto a rotating tablet bed. The drying conditions cause removal of the solvent, giving thin deposition of coating material around each tablet core (Cole, 1995; Bagade *et al.*, 2014).

1.1.3 Enteric Coating

An enteric coating is a coating that remains intact in the stomach, but that dissolves and releases the contents once it reaches the upper small intestine. The prime intention is to delay the release of drugs, which are inactivated by the stomach contents or those that may cause nausea or bleeding by irritating the gastric mucosa. Cracking of the film either during application or on storage will result in a loss of enteric properties. Therefore, consideration must be given to the mechanical properties of the applied film. Enteric coatings are usually formulated with synthetic and/or natural polymers that contain ionizable functional groups that render the polymer water solubility at a higher pH value. The choice of the polymer and the thickness of the coated layer are critical to control the pH solubility profile of the enteric coated dosage form (Cole, 1995; Sakuma *et al.*, 2009; Das *et al.*, 2013).

1.1.4 Film forming mechanisms

The film formation mechanisms essentially depend on the type of coating formulation i.e. aqueous versus organic.

1.1.4.1 Non-aqueous based enteric coating

The non-aqueous solutions generally contain various materials to provide the desired coating to the tablets. A film former should be able to produce smooth thin films and be applicable to a variety of tablet shapes. An alloying substance provides water-solubility or permeability to the film to ensure penetration by body fluids and therapeutic availability. During the coating process the solvent evaporates and a highly viscous gel is formed around the core tablet as shown in Fig. 1.1. Upon complete solvent evaporation, a continuous polymeric film is formed. A ***plasticizer*** produces the flexibility and elasticity of the coating. This may enhance durability. ***Surfactants*** enhance spread-ability of the film during application. ***Opaquants*** and ***colorants*** make the appearance of the coated tablets attractive and distinctive. ***Sweeteners***, ***flavors***, and ***aromas*** enhance the acceptability of the tablet to the patient. A ***glossant*** provides luster to the tablets without a separate polishing operation. Volatile solvents allow spreading of the other components over the tablets while allowing rapid evaporation to permit an effective yet speedy operation. Owing to the high cost of using volatile solvents and the problem of releasing these potentially toxic agents into the atmosphere, organic solvents are not widely used anymore (Muschert, 2008; Grodowska and Parczewski, 2010; Nollenberger and Albers, 2013).

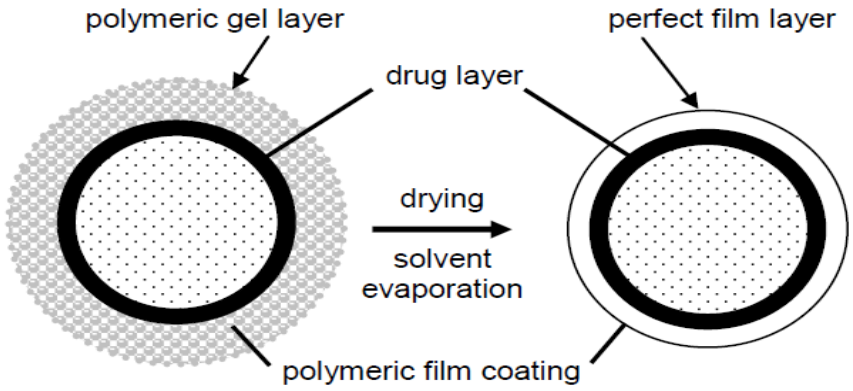


Fig. 1.1: Schematic presentation of the film forming mechanism from organic polymer Solutions (Muschert, 2008).

1.1.4.2 Aqueous based enteric coating

Aqueous film coating application is either in solution or dispersion, depending on the water solubility of the film former polymer. The process of film formation using aqueous polymer dispersions is usually divided in three phases. Phase I is the evaporation of water. The density of the dispersion increases until the colloidal particles come into contact with each other and, subsequently, form close-packed arrays. The particles then undergo deformation to polyhedra without inter-particle spaces in phase II, induced by an increase in temperature above the minimum film formation temperature (MFT), one of the most important parameters of film formation from aqueous dispersion based coatings. It is defined to be the minimum temperature at which a cast film becomes crack-less and clear. Below this temperature, the dried dispersion appears opaque and powdery. Increasing the temperature above the glass transition temperature (Tg) in phase III, the boundaries between the particles disappear through inter-diffusion of polymer chains developing a continuous film without distinguishable particles (Fig. 1.2) (Ghebre-Sellassie, 1997; Kablitz and Urbanetz, 2007; Yang *et al.*, 2010; Nollenberger and Albers, 2013).

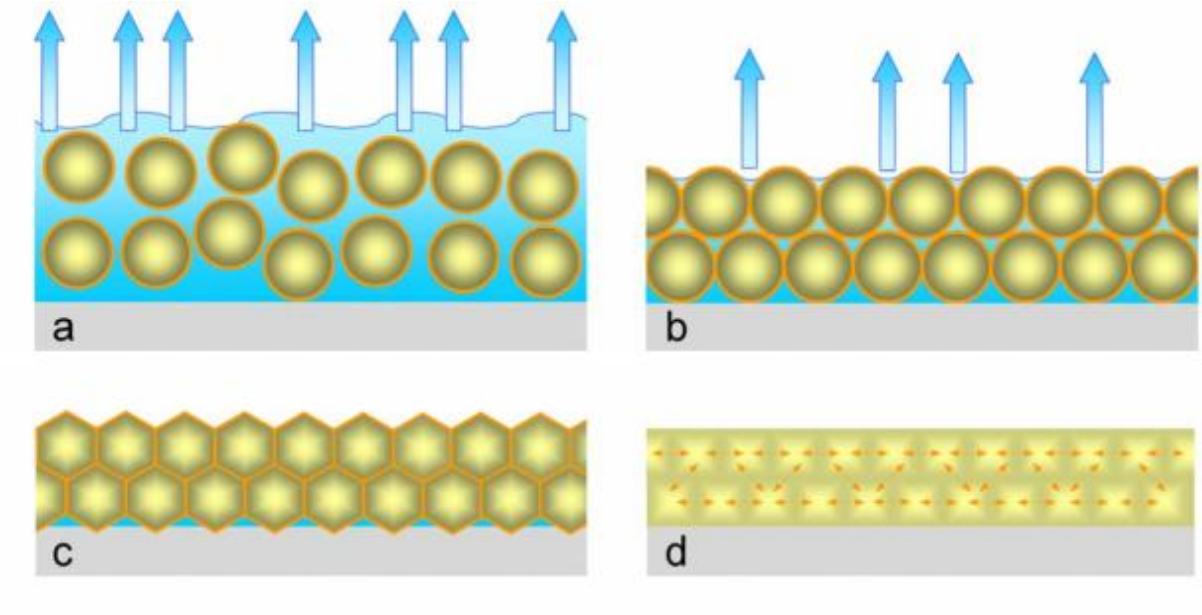


Fig. 1.2: Schematic presentation of the film forming mechanism from aqueous polymer dispersions: a) solvent evaporation; b) close-packed arrangement; c) deformation of latex particles d) coalescence of latex particles above the MFT (Ghebre-Sellassie, 1997).

Aqueous film coating is the quickest and least expensive method for enhancing tablet appearance and, unlike other methods, will not affect dissolution or disintegration profiles. It also offers customized color selection and color matching of immediate release tablet film coating products. Film coating has produced many enhanced polymer combinations resulting in new tablet coating options (Siepmann *et al.*, 2006).

1.1.5 Enteric Coating Polymers

A polymer, natural or synthetic is a substance that is combined with a drug or other active agent to release drug in a pre-designed manner (Table 1.1). The development of NDDS has been made possible by the various compatible polymers to modify the release pattern of drug. Choice of polymers always suffers from the problems of non-biocompatible, non-biodegradable and cost and these can be solved with a polymer of different properties. The basic objective of controlled drug release is to achieve more effective therapies by eliminating the potential for both under and overdosing. Other advantages are the maintenance of drug concentration within a desired range, fewer administrations, optimal drug use and increased patient compliance (Rhodes and Porter, 1998; Sinha and Kumria, 2003; Zaid *et al.*, 2011; Kola and Kumar, 2013).

Type of the enteric polymer used and its threshold pH; enteric coating composition (polymer, plasticizer, anti-tacking agents, and pigments); core formulation, its swelling and disintegrant properties, and the nature of the drug in the dosage form; presence of imperfections in the coating, such as fissures that can result in loss of integrity of the coating; thickness of the film layers applied to the dosage form; *in vitro* testing conditions, such as the composition, pH ionic strength of dissolution media, and agitation intensity within the media; and fed and fasted gastric conditions are important factors that may influence the behavior of enteric coated dosage forms (Akhgari *et al.*, 2005; Shahrzad *et al.*, 2010; Kola and Kumar, 2013).

Table 1.1: pH-Sensitive polymers used in the production of delayed-release oral dosage forms (Bansal *et al.*, 2014)**.**

Polymer dissolution	Threshold pH	Dry powder	Aqueous dispersion
Cellulose Derivatives			
Cellulose acetate trimellitate	5.0	—	—
Hypromellose phthalate 50	5.0	HP50	—
Hypromellose acetate succinate L	5.5	—	Aqoat AS-L
Hypromellose acetate succinate M	6.0	—	Aqoat AS-M
Cellulose acetate phthalate	6.0	—	Aquacoat CPD
Acrylic Derivatives			
Poly (methacrylic acid, ethyl acrylate) 1:1	5.5	Eudragit-L30-D55®	Eudragit L100 55
	5.5	Kollicoat®MAE 100 P	Kollicoat® MAE 30 DP
	5.5		Acryl-eze
Poly (methacrylic acid, methyl methacrylate, methyl acrylate) 2.5:6.5:1	6.8	—	Eudragit FS 30D
Poly (methacrylic acid, methyl methacrylate) 1:2	7.0	Eudragit S100	—
Polyvinyl Derivatives			
Polyvinyl acetate phthalate	5.0	Phthalavin, Opaseal	Sureteric

Note: All polymers are available in powder/granule form for use in organic solutions and in some cases ready-to-use aqueous dispersion.

1.1.5.1 Kollicoat® MAE 100 P

Kollicoat® MAE 100 P is a non-dusting re-dispersible powder grade of Kollicoat® MAE 30 DP (methacrylic acid copolymer type C). Kollicoat® MAE 100 P is a copolymer consisting of methacrylic acid and ethyl acrylate in the ratio of 1:1 (Fig. 1.3). It is an anionic copolymer that can be neutralized by bases such as sodium hydroxide. The main application of Kollicoat® MAE 100 P is as a film former in enteric coatings for solid dosage forms such as enteric tablets and pellets. It is also used as an enteric matrix in hot-melt extrusion formulations. It dissolves in slightly alkaline, aqueous media but readily soluble in ethanol and methanol (Kolter *et al.*, 2010; USP36/NF31, 2013).

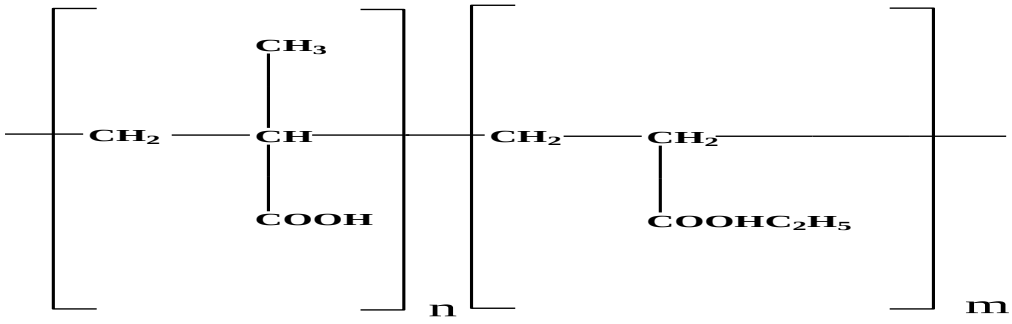


Fig. 1.3: Chemical structure of Kollicoat®MAE 100 P (Kolter *et al.*, 2010).

1.1.5.2 Eudragit®-L-30-D-55

Eudragit-L-30-D-55® is the aqueous dispersion of an anionic copolymer based on methacrylic acid and ethyl acrylate. The ratio of the free carboxyl groups to the ester groups is approx. 1:1 at 30% solids (Fig 1.4). This film solubilizes at a pH above 5.5. The dispersion is miscible with water in any proportion, the milky-white appearance being retained. A clear or slightly cloudy, viscous solution is obtained by mixing 1 part Eudragit-L-30-D-55® with 5 parts acetone. The same results are obtained by mixing with ethanol or isopropyl alcohol. Initially, the polymer is precipitated, but then dissolves again in the excess organic solvent. A clear or slightly cloudy liquid is obtained by mixing 1 part Eudragit-L-30-D-55® with 2 parts 1 N sodium hydroxide. Eudragit-L-30-D-55® can also be obtained by re-dispersing the solid Eudragit-L-30-D-55® in water (Rahman and Ali, 2008; Thakral *et al.*, 2013).

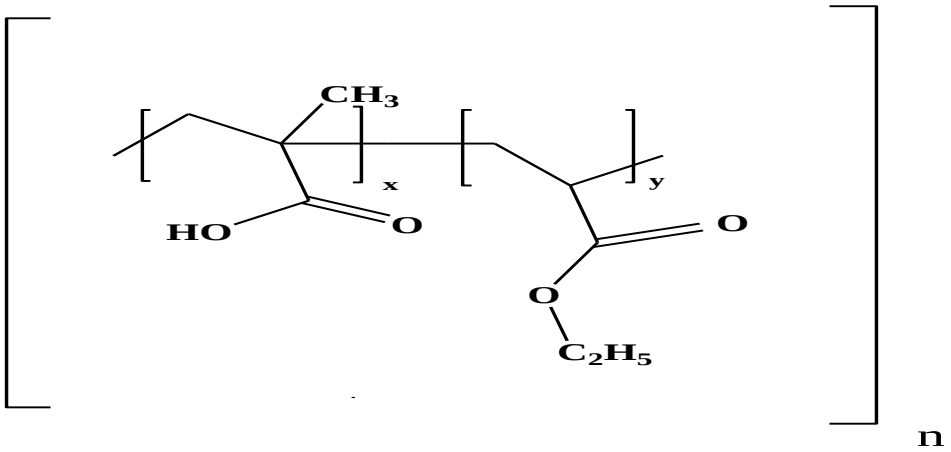


Fig. 1.4: Chemical structure of Eudragit-L-30-D-55® (Thakral *et al.*, 2013) .

1.1.5.3 Other coating composition

i) Plasticizers

The success of enteric coating efficiency mostly relies on the addition of plasticizers. Plasticizers are a group of auxiliary components that improve elasticity of the polymeric film (Suyatma *et al.*, 2005). Among them, 1,2-propylene glycol (1,2-PG) is an effective plasticizer as other traditional compounds such as triethyl citrate, but its adverse effect on drug release is less. This is particularly important with tablet coatings that dissolve in gastric juice. Furthermore, 1,2-PG has no negative influence on the tackiness of the coating. In concentrations of 10–15 % as a proportion of the film-forming agent, 1,2-PG considerably reduces the minimum film-forming temperature (Zhu *et al.*, 2002; Bühler, 2008; Galgatte *et al.*, 2013).

ii) Glidants/anti-tacking agents

Glidants are added to coating formulation to prevent tackiness and agglomeration during coating, drying, or storage, as these would otherwise cause film damage. Commonly used glidants are talc, glycerol monostearate, magnesium stearate and precipitated silica. Talc is the most effective glidant for enteric poly(meth)acrylate coatings, as it does not influence the gastro resistance of the coatings (Nollenberger and Albers, 2013). Talc is traditionally used as an anti-tacking agent in coating formulations. Generally, the addition of higher amounts of talc is seldom used because the high solids content could alter drug release from the dosage form (Kucera *et al.*, 2013).

iii) Solvent

Film coating can be carried out using either an organic solvent or water. The liquid coating technology can obtain extremely uniform, smooth, gleaming coating surface. But, organic solvents are toxic and flammable. Organic solvents have limitations including: vapor of organic solvent causes environmental pollution and hazard to coating equipment operator, long processing time due to evaporation of solvent, vaporization of organic solvents is energy consumptive i.e. to clear residual, high cost, strict environmental regulation placed on use of organic solvent (Grodowska and Parczewski, 2010).The most superior films, showing the greatest combined strength of cohesiveness, have been reported when the coating solution solvation and polymer chain extension are at a maximum. Most coating formulations used in the pharmaceutical industry today are applied as aqueous-based systems. The use of water as the solvent is less expensive than organic materials, requires no solvent recovery system, and is environmental friendly. Moreover, the potential toxicities associated with residual solvents in the product are eliminated (Felton and Porter, 2013; Nollenberger and Albers, 2013).

iv) Pigments/Opacifier

Pigments commonly used in pharmaceutical systems include aluminum lakes of water-soluble dyes, opacifiers such as titanium dioxide, and various inorganic materials including the iron oxides. Pigments differ significantly in their physical properties, including density, particle shape, particle size, and morphology, and these differences contribute to the complex relationship with aqueous film coatings. In addition to affecting the mechanical properties of films, the incorporation of pigments into coating formulations has also been found to influence polymer adhesion (Felton and McGinity, 1999; Nyamweya *et al.*, 2001).

1.2 Coating process parameters and equipment

Aqueous film coatings of tablets is generally performed in different kinds of coating pans. The uniformity of tablet coating is very important as it affects the organoleptic quality of tablets and functionality of the coating, especially in the case of modified release formulations. Aqueous film coatings is a sensitive process in which a number of variables can affect the quality of the final product (Rege *et al.*, 2002). The variable inputs to a film coating process derived from differences

in equipment installations include but are not limited to inlet air volume, temperature and humidity, coating pan dimensions and rotating speed, numbers and distance between spray guns, distance between spray guns and tablet bed, atomizing air pressure, spray rate of the coating solution and nozzle orifice size. Therefore, these variables, relevant to the coating process, should carefully be controlled for acceptable quality of the final coating tablets. The coating formulation, however, should be selected carefully which requires extensive effort to apply, and is, therefore, time consuming (Sahni and Chaudhuri, 2011; Wang *et al.*, 2012; Just *et al.*, 2013). The coating process parameters and their effects on the formulation is tabulated in Table 1.2.

Table 1.2: Coating process parameters and associated defects (Bagade *et al.*, 2014).

Process parameter	Level	Possible defects
Atomization air pressure	Too high	Spray drying
	Too low	Picking and sticking, Roughness (orange peel texture), Log bridging and Twinning
Spray rate	Too high/fast	Picking and sticking, Roughness (orange peel texture), Log bridging and Twinning, Coating solution penetrating the core
	Too low/slow	Edge erosion, Surface erosion, Tablets too dry, subjects to abrasion, Tablet breakage
Bed/Product temperature	Too low	Log bridging, Picking and sticking (Over wetting)
Pan speed	Too low/slow	Colour variation, Picking and sticking (Over wetting), Twinning
	Too high/fast	Surface erosion, Edge wear and erosion, Tablet breakage
Spray Guns	Too few	Colour variation
	Too close together	Colour variation, Over wetting in over spray area
Air flow	Too high	Spray drying, Roughness (orange peel texture)
	Too low	Over wetting

In pharmaceutical industry, aqueous-based film coating of tablets is performed by using either an air-suspension (fluid-bed) coating apparatus or, today more often, by different kinds of perforated pans (Fig. 1.5). The side-vented perforated pan coating techniques has been designed for rapid and efficient production of aqueous film-coated tablets. In a side-vented pan coater the air current passes through a perforated pan to ensure continuous and consistent drying conditions. The construction of the rotating pan ensures complete mixing of the tablets. The aqueous coating liquid is commonly applied by pneumatic (air) spray systems, where the pressure of the spraying air disperses the coating liquid as appropriately sized droplets (Bagade *et al.*, 2014).

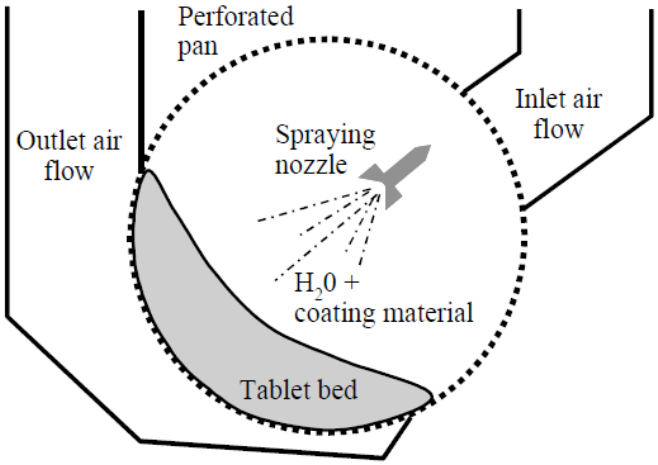


Fig. 1.5: Schematic of a modern pan coater (side-vented) and domain for the spray (Bagade *et al.*, 2014).

1.3 Doxycycline hyclate and its pharmacokinetic profile

The DXYH is a derivative of tetracycline consists of hydronapthacene nucleus four hexacyclic rings. It is a broad-spectrum antibiotic used in several countries to treat infectious diseases and as an additive in animal nutrition to facilitate growth (Shabeer *et al.*, 2013).

DXYH exists in hemi-hydrate and hemi-ethanolate forms. It is hygroscopic yellow crystalline powder, and should be stored in air tight containers and protected from light. It has the chemical name: 4-(dimethylamine)-1,4,4a,5.5a,6,11,12a-octahidro-3, 5,10,12,12a-pentahidroxi-6-methyl-1,11-dioxo-2-naphthacene-carboxamidemonohydrochloride monohydrate, combined with ethyl alcohol (USP36/NF31, 2013; Kogawa *et al.*, 2014). Its molecular formula is $(C_{22}H_{24}N_2O_8.\ HCl)_2$.

C_2H_6O. H_2O, CAS 24390-14-5 and it has a molecular weight of 1025.89 $g.mol^{-1}$ and PKa values: pKa1 3.02 ± 0.3; pKa2 7.97 ± 0.15; pKa3 9.15 ± 0.3 as indicated in Fig. 1.6 (Shariati *et al.*, 2009).

DXYH is a yellow crystalline soluble powder in water and in solutions of alkali hydroxides and carbonates. It has a high degree of lipid solubility and a low affinity for calcium binding. It is highly stable in normal human serum (Mistry and Menon, 2013).

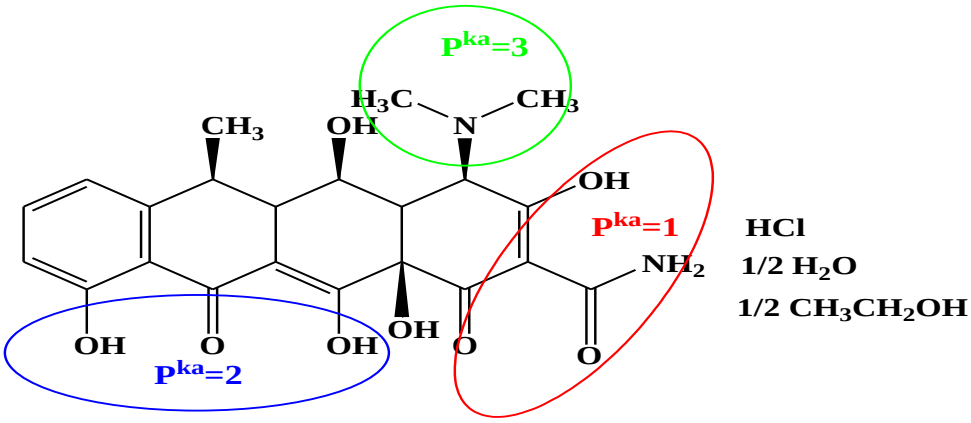


Fig. 1.6: Chemical structure of DXYH and its functional groups corresponding to the PKa values (Shariati *et al.*, 2009; Kogawa *et al.*, 2014).

DXYH is used for treatment of malaria. However, it should not be used alone for initial cure of malaria. It is also used for the treatment of rickettsial infections, sexually transmitted infections, respiratory tract infections, specific bacterial infections, ophthalmic infections, anthrax, including inhalational anthrax (Post- Exposure) (Mistry and Menon, 2013). DXYH is a synthetic analogue of oxytetracycline which provides certain pharmacokinetic advantages over tetracycline being more active against some organisms. It is well known for its antibacterial action today and as a choice for pelvic inflammatory diseases, renal impairment and prophylaxis of travelers' diarrhea (Kaushik *et al.*, 2014).

It is also used to treat both intracellular and extracellular bacterial infections such as aerobic and anaerobic Gram-positive and Gram-negative bacteria, and other microorganisms such as chlamydia, protozoa, mycoplasma, mycobacteria, and spirochetes. Due to doxycycline's antibacterial effects on a wide range of pathogens, it is currently one of the most commonly prescribed antibiotics worldwide for treating infectious diseases (Cover *et al.*, 2012).

DXYH is said to be almost completely absorbed with a bioavailability of more than 80% with an average of ~95%. However, some authors comparing intravenous and oral doses of DXYH felt absorption was lower in the range 73–77 %. Absorption takes place in the duodenum. The half-life of absorption is 0.85–0.41 h. Food has less effect on absorption with DXYH serum concentrations being reduced by ~20 % by test meals compared with 50 % for tetracycline (Agwuh and MacGowan, 2006).

DXYH differs from oxytetracycline, chlortetracycline and tetracycline in that it is 5–10 times more lipophilic; hence, it possesses higher tissue penetration, larger volume of distribution and better antimicrobial properties, considering that entry into bacteria is not dependent on an active transportation mechanism as it occurs with the above referred tetracycline. Additionally, DXYH has amore prolonged half-life and a greater plasma protein binding rate, both in humans and animals (Gutiérrez *et al.*, 2012).

1.4 The present study

Doxycycline is a universal antibiotic use to treat both gram positive and gram negative infections where the susceptible organism was strongly proven to be present and also used to treat different microbial infections. It is a tetracycline antibiotic of half-life around 18 hours to 22 h and its absorption is through the lower parts of the intestine (Gutiérrez *et al.*, 2012).

Standard formulation of DXYH causes gastrointestinal upset, and thus does not give its desire pharmacological activity (Bryant *et al.*, 1987). Anorexia, vomiting, nausea, enterocolitis, glossitis, diarrhea, dyspepsia and inflammatory lesions in anogenital region are the major side effects. There are also esophagitis and esophageal ulcerations in some patients which had taken DXYH. Often, the choice of DXYH over other tetracycline in the treatment of infections is due to its adequate oral absorption and extended half-life, which allows fewer daily doses. Moreover, DXYH is considered the tetracycline of choice in patients with poor renal function due to its limited clearance by kidneys (Kogawa *et al.*, 2014). To overcome such disadvantage associated with the standard dosage forms, formulation of enteric coated tablets is important that reduces the rate of irritation in stomach, as well as rate of nausea and vomiting (Rama *et al.*, 2014).

There are marketed products of DXYH in the form of capsule in Ethiopia. But, there is a compliance of the dosage form having bitter taste, irritation in esophageal, gastric and sometimes cause nausea and vomiting.

Enteric coating tablets are designed in such a way that it by pass the gastric environment of stomach and release the active content in the basic pH of small intestine. The pH of small intestine in different regions was found to be around 5 to 7 pH in duodenum, 6 to 7 pH in jejunum and 7 pH in ileum. Hence, Eudragit-L-30-D-55® and Kollicot® MAE 100 P copolymers are selected where the dissolution properties are above pH 5.5 for both polymers (Thakral *et al.*, 2013). Furthermore, as DXYH is highly susceptible to light, it is necessary to use different formulation strategies in order to enhance its photo-stability (Kogawa *et al.*, 2014).

On the basis of the studies carried out till date, it is focusing that aqueous based enteric coating technology is nowadays very important in the field of pharmacy, particularly in formulation development, and the aqueous based coating and its various aspects which are giving the more benefits over the organic coating, which leads to non-toxicity, cost effectiveness and nonhazardous to environment (Obara and McGinity, 1995).

Since the method of manufacturing of DXYH tablets is direct compression, it has economic and time scale advantages. It requires less unit processes therefore needs less space, less equipment, lower labor cost, less processing time and less energy consumption compared to dry and wet granulations. The method eliminates stability issue for active ingredients that are sensitive to the moisture and heat. Another advantage of tablets prepared by direct compression is higher dissolution rate as the tablets disintegrate into the primary particle instead of into granules (Bolhuis and Chowhan, 1996; Agubata *et al.*, 2016).

Therefore, the aim of this study is to develop small intestine targeting tablets of DXYH aqueous based enteric coated tablets using conventional standard coated technique.

1.5 Objective of the Study

1.5.1 General Objective

To formulate, optimize and evaluate aqueous based enteric coated DXYH tablets and characterize *in vitro* drug release of coated tablets using Kollicoat® MAE 100 P and Eudragit-L30-D55® as an aqueous based enteric coating polymer.

1.5.2 Specific objectives

- To formulate and evaluate aqueous-based enteric coated DXYH tablets using Kollicoat® MAE 100 P and Eudragit-L-30-D-55® as enteric coating polymers;
- To optimize the formulation parameters of coated tablets using CCD;
- To evaluate the disintegrant effect on the core tablets;
- To evaluate the *in vitro* drug release characteristics of the enteric coated tablets; and
- To perform accelerated stability test for optimized batches of DXYH tablets.

2. EXPERIMENTAL

2.1 Materials

Hydrochloric acid and absolute ethanol (Carlo Erba Reagents, Italy), sodium hydroxide (BDH Laboratory Supplies, England), monobasic potassium phosphate (ERBA Pharma Reagents Group, Italy), magnesium stearate (S Kant Health Care Ltd., India), microcrystalline cellulose (JRS Pharma GmbH and Co.KG, Germany), maize starch (Roquette, Orkila, France), purified talc (B&S Global, India), titanium dioxide (Kronos International Inc., Germany), Eudragit-L-30-D-55® of 30% aqueous dispersion and Kollicot® MAE 100 P powder (BASF, The Chemical Company, Germany), 1,2-propylene glycol (Horst G.F. von Valtier GmbH and Co. KG, Germany), sodium starch glycolate (Huzhou Zhanwang Pharmaceutical Co. Ltd, China), Crospovidone (China Associate Group Co., Ltd., China), crosscarmelose sodium (Anhuisunhhere Pharmaceuticals Excipients Co. Ltd., China), aluminum foil and PVC/PVDC (Billcare, India) and doxycycline hyclate (Minxiaqiyauan Pharmaceuticals Co. Ltd, China) were kindly donated from EPHARM SC. They were used as received.

2.2 Methods

2.2.1 Pre-formulation studies

Direct compression method was employed for preparation of enteric coated tablets of DXYH with four different disintegrants in various concentrations. MCC was used as diluent and/or dry binder, talc was used as glidant and magnesium stearate as lubricant.

2.2.1.1 Formulation of core tablets

Twelve different tablet formulations were prepared using fore direct compression with different disintegrants and varied concentrations.

i) Assessment of Mixture Blend for Direct Compression

The core tablets of DXYH formulations were prepared. All the ingredients were accurately weighed, milled and passed through sieve no. 60 (250 microns) to get uniformly distributed and uniform sized particles. The ingredients (DXYH, MCC and disinitegrant) were blended in a

planetary mixer (Machines Collette, Belgium) for 10 min. The powder mix was then mixed with magnesium stearate and talc geometrically for additional 3 min. All the formulations were prepared with similar blending time. The composition of the core tablets is shown in Table 2.1. The effect of super-disintegrant SSG (2 % – 8 %,) (Guest, 2009), CCNa (0.5 – 5.0 %) (Hausler, 2009), CP (2 – 5 %) (Kibbe, 2009) and DS 1500 (3 – 15 %) (Young, 2009) were analyzed by varying the amount as well as the type of super-disintegrant to select best formulation eliciting good tableting properties.

ii) Compression of core tablets

The homogeneously blended mixture was then compressed on a 10 station rotary tablet press machine (Rimek Mini Press-II, India) using 11/32-inch stainless steel punches at a pressure of 6.5 to 10.5 kg/cm^2. Different formulations of core tablets were produced with drug equivalent to 100 mg of DXYH of average weight of 240.4 mg.

2.2.1.2 Evaluation of mixed powders

i) Angle of repose

The angle of repose of each powders batch was determined by fixed height funnel method. In this, 30 g powders was placed and allowed to flow through a stem-less funnel having 10 mm orifice from a fixed height of 10 cm. The duration of flow was recorded and used to calculate the flow rate. Angle of repose was determined according to Eq. 2.1.

$$\theta = \tan^{-1}\left(\frac{h}{r}\right) \qquad (2.1)$$

where θ = angle of repose, h = height of powders, r = radius of circle formed by the powders pile. The same procedure was repeated three times and the average value was taken.

Table 2.1: Formulations and compositions of powder mixes for direct compression (Avg. Wt. 240.4 mg).

Ingredients (mg/Tablet)	Formulation Codes											
	FSSG[1]	**FSSG[2]**	**FSSG[3]**	**FCP[1]**	**FCP[2]**	**FCP[3]**	**FCCNa[1]**	**FCCNa[2]**	**FCCNa[3]**	**FDS[1]**	**FDS[2]**	**FDS[3]**
DXYH	115.40	115.40	115.40	115.40	115.40	115.40	115.40	115.40	115.40	115.40	115.40	115.40
MCC (102)	95.77	102.98	110.19	102.98	106.59	110.19	102.98	108.9	113.80	78.94	93.36	107.79
SSG	19.23	12.02	4.81	--	--	--	--	--	--	--	--	--
CP	--	--	--	12.02	8.41	4.81	--	--	--	--	--	--
CCNa	--	--	--	--	--	--	12. 02	6.61	1.20	--	--	--
DS 1500	--	--	--	--	--	--	--	--	--	36.06	21.64	7.21
Talc	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50
Mg Stearate	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Quantity Per Tablet	240.400	240.400	240.400	240.400	240.400	240.400	240.400	240.400	240.400	240.400	240.400	240.400

FSSG: Formulation with Sodium Starch Glycolate
FCP: Formulation with Crospovidone
FCCNa: Formulation with Crosscarmelose Sodium
FDS: Formulation with Dried Starch

Superscript Level:
1: Maximum Level Concentration of Disintegrant
2: Mid-Level Concentration of Disintegrant
3: Minimum Level Concentration of Disintegrant

ii) Bulk and tapped density:

Both bulk and tapped densities were determined by taking a 30 g of granule/ powder mix in a 250 ml measuring cylinder and bulk volume occupied was read after light tapping. The cylinder was then tapped at a constant velocity using tapped densitometer (ERWEKA, Germany) for 500 times and then tapped volume was recorded. Bulk and tapped density were determined based on Eq. 2.2 and 2.3 respectively.

$$Bulk\ Density\ = \frac{Weight\ of\ powder}{Volume\ of\ packing} \tag{2.2}$$

$$Tapped\ Density = \frac{Weight\ of\ powder}{Tapped\ volume\ of\ packing} \tag{2.3}$$

iii) Hausner ratio:

The Hausner ratio was determined based on equation: Eq. 2.4.

$$\text{Hausner's ratio} = \frac{\rho_t}{\rho_b} \tag{2.4}$$

where ρt = tapped density, ρb = bulk density

iv) Compressibility index:

The compressibility index of granules was determined using Carr's compressibility index, and was determined based on equation: Eq. 2.5

$$\text{Carr's index} = \frac{\rho_t - \rho_b}{\rho_t} \times 100 \tag{2.5}$$

where ρ_t = tapped density, ρ_b = bulk density

2.2.2 Evaluation of uncoated Tablets

About 12 formulations were prepared, of which the formulation that provided good tablet parameters was selected for optimization of enteric coated tablets. The selected batches were subjected to evaluations as per USP36/NF31, 2013.

i) Weight variation test

Weight variation test was run by weighing 20 tablets individually; and average weight was calculated and standard deviation was determined.

ii) Friability test

For each formulation, pre-weighed tablet samples (20 tablets) were placed on the friabilator, which was then operated at 25 rpm for 100 revolutions. The tablets were then dusted and reweighed. Percent friability was calculated using Eq. 2.6.

$$\%\, Fribility = \frac{Initial\ Weight - Final\ Weight}{Initial\ Weight} \times 100 \qquad (2.6)$$

iii) Hardness, Thickness and Diameter

These parameters were measured by ERWEKA Hardness Tester (Model ERWEKA TBH-220, Germany) by taking ten tablets.

iv) Disintegration time test

Six tablets were placed in each tube of USP disintegration test apparatus (Model ERWEKA DT 700 GmbH, Germany) and the basket rack assembly was positioned in a 900 ml beaker of water at 37± 2 ^{0}C and run at 28 to 32 cycles per min for 15 min as per USP 36/NF31<701>, 2013.

v) Content Uniformity Test

Determination of the content of DXYH was carried out by standard UV/Vis spectroscope method under the operation conditions at wavelength of 276 ηm. Initially, accurately weighed 240.4 mg of the sample was taken (from 20 tablets) into a 100 ml amber volumetric flask; and then the samples was dissolved and diluted to volume with distilled water. After that, the solution was filtered through Whatman No.41 and the first 5 ml of filtrate was discarded. From the filtrate, 10 ml of the solution was transferred to a 100 ml volumetric flask for further dilution. Finally, 10 ml of the filtrate was diluted to 100 ml in the same manner and absorbance was measured for both working standard and sample using 1 cm cell. Purified water was used as a medium (Sreeram *et al.*, 2015).

2.2.3 UV- Calibration curve of DXYH

In the same manner, accurately weighed of 115.4 mg DXYH was transferred to a 100 ml volumetric flask and made up to volume with distilled water, 0.06 N HCL and 5.5 pH Phthalate buffer, separately. From these different solutions, 10 ml of each was withdrawn and further diluted to 100 ml with the respective solvents, and six different volumes of each (3.75, 5.00, 6.25, 7.50, 8.75 and 10 ml) of the solutions were transferred to a 100 ml volumetric flasks and diluted with the respective solvents to 100 ml. The UV-absorbance readings of these solutions were measured at 276 ηm for distilled water, 346 ηm for acid media and 345 ηm for 5.5 pH neutralized phthalate buffer with UV-Vis spectrophotometer (Model SHIMADZU UV-1800, Japan). Distilled water, 0.06 N HCl and 5.5 pH neutralized phthalate buffer were used as a blank, accordingly.

2.2.4 Enteric coating of tablets

i) Formulation of Kollicoat® MAE 100 P suspension

Aqueous coating suspension was prepared by adding required quantity of Kollicoat® MAE 100 P in 2 L of beaker containing sufficient amount of distilled water with continuous stirring for 10 min with a magnetic stirrer. Then required quantity of 1,2-PG was slowly poured into the suspension with continuous stirring to get the polymer suspension (Table 2.2). Pigment solution was prepared separately by adding slowly required quantity of titanium dioxide and talc in sufficient amount of distilled water with continuous stirring. The later suspension was finally poured into the former one and pulverized using colloidal mill (PUC Probst and Class, Germany) for 1.5 h. The proportion of polymer to plasticizer (1,2-PG:Kollicoat® MAE 100 P) varied from1:9 to 1.5:8.5 W/W.

ii) Formulation of Eudragit-L-30-D-55® suspension

Aqueous coating suspension was prepared first by adding slowly the Eudragit-L-30-D-55® suspension into the solvent system i.e., distilled water. The whole component was then stirred until the polymer completely formed uniform suspension for approximately 1 h (Table 2.3). Required amount of 1,2-PG and talc were mixed together separately and was stirred for 15 min with the solvent. The excipient suspension was then slowly added into the polymer solution while stirring for 1.5 h using colloidal mill. The proportion of polymer to plasticizer (1,2-PG:Eudragit-L-30-D-

55®) varied from 1.5:98.5 to 4.5:96.5 % W/W.**Table 2.2:** Three selected enteric coating compositions of Kollicoat® MAE 100 P for 0.5 kg of coating suspension

	Composition	Amount in g for minimum conc of 1,2-PG: of Kollicoat® MAE 100 P (1:9)	Amount in g for average conc of 1,2-PG: of Kollicoat® MAE 100 P (1.25:8.75)	Amount in kg for maximum conc of 1,2-PG: of Kollicoat® MAE 100 P (1.5:8.5)
Polymer suspension	**Kollicoat® MAE 100 P**	67.241	65.373	63.505
	Propylene glycol	7.471	9.339	11.207
	Water	360.901	360.901	360.901
Pigment suspension	**Titanium dioxide**	2.153	2.153	2.153
	Talc	17.176	17.176	17.176
	Water	45.059	45.059	45.059

iii) Tablet Coating

Tablet coating was done in a 12-inch conventional coating pan (Model ERWEKA TYPE AMD, Germany), charged with 0.5 kg of core tablets. The tablet bed was pre-warmed to 44−55 ºC. Coating solution was applied using automated external spray gun with low pressure air atomized liquid spray system. Coating parameters like pan speed, drying air temperature, nozzle distance from tablet bed, atomizing pressure, spray rate, coating time were kept constant during the optimization process. Upon completion of the coating, the tablets were allowed to rotate in the pan at a slower rate, to ensure complete drying of the tablets. The coating material deposit was increased to obtain different coating level, i.e., 5 %, to 12 % of the original weight. The tablets were selected at random and checked for the weight gain before and after the application of specified time of coating in order to verify the attainment of desired weight.

Table 2.3: Three selected enteric coating compositions of Eudragit-L-30-D-55® for 0.5 kg of coating suspension

Composition	Amount in g for minimum conc. of 1,2-PG:Eudragit-L-30-D-55® (1.5:98.5) (W/W)	Amount in g for average conc. of 1,2-PG:Eudragit-L-30-D-55® (3:97) (W/W)	Amount in kg for maximum conc. of 1,2-PG:Eudragit-L-30-D-55® (4.5:95.5) (W/W)
Eudragit-L30-D-55®	211.475	208.350	205.225
Propylene glycol	3.125	6.250	9.375
Talc	31.250	31.250	31.250
Water	254.150	254.150	254.150

2.2.5 Characterization of coated tablets

i. Disintegration time

Six tablets were placed in each tube of USP disintegration test apparatus (Model ERWEKA DT 700 GmbH, Germany) and the basket rack assembly was positioned in a 900 ml beaker of 0.1 N HCl at 37 $\pm$ 2 ºC and run at 28 to 32 cycles per min for 1 h for "Delayed Release Doxycycline Hyclate Tablets" as it is stated in USP36/NF31 <701>, 2013.

ii. *In vitro* Dissolution test

In-vitro drug release study was carried out using USP Type I dissolution apparatus (ERWEKA D-63150 GmbH, Germany) (USP 36/NF31, <711>, 2013) and the test was performed on six tablets. The dissolution medium was kept in thermostatically controlled water bath at 37 ± 0.5 ºC. Basket speed was adjusted to 50 rpm. In 0.06 N HCl, samples were removed from the dissolution media in different time intervals during 20 min of acid stage testing. And again, the filtered portion of samples was then analyzed after suitable dilution at 346 ηm. In neutralized phthalate buffer of pH 5.5, on separate tablets, selecting those that were not previously subjected to the acid stage testing, samples were removed from the dissolution media in different time intervals during 30 min of buffer stage testing. The filtered portion of samples was then analyzed after suitable dilution at 345 ηm. For both procedure, the tests were conducted under sink conditions and conducted with UV-Vis

spectrophotometer (Model SHIMADZU UV-1800, Japan). In the USP 36/NF31 monograph, for *"Delayed Release Doxycycline Hyclate Tablets"* it is stated that no individual value is more than 30 % of the labeled amount of doxycycline released in 20 min in 0.06 N HCl and no less than 90 % of the labeled amount of doxycycline released after 30 min in neutralized phthalate buffer of pH 5.5 is allowed.

iii. Percent weight gain of coated tablets

Tablets were initially dried (pre-warmed) in coating pan for 10 min with inlet temperature of coating pan with bed temperature around 40−45 °C, coating level of 2.5 % and good surface appearance. 50 tablets were randomly taken from different locations inside the pan with parameters shown in Table 2.4. The total weight of 50 tablets and the average weight of individual tablets were calculated. After completion of the spray coating process, the tablets were again dried in coating pan for 10 min with spray till the bed temperature reached and maintained at around 40−45 °C. 50 tablets were taken from different location of the pan. The coating level was calculated using Equation 2.7.

$$\text{Percent weight gain} = \frac{(wtF - wtI)}{wtI} * 100\% \quad (2.7)$$

where, wtI and wtF are tablet weights initial and final, respectively.

Table 2.4: Coating process parameters that were maintained during the coating of tablets.

S. No.	Fixed parameter	Specification
1	Atomizing pressure	1– 2 bar
2	Inlet air temperature	50–60 ^{0}C
3	Spray rate application	3–6 g/min/Kg
4	Coating pan speed	15 rpm
5	Number of spray gun	1
6	Distance tablet bed/spray gun	12 cm

2.3 Drug-excipients compatibility study by FT-IR

Fourier transformed infrared spectroscopy (FT-IR) spectra of pure DXYH, uncoated tablet of the four formulations (FSSG, FCP, FCCNa and FDS) and enteric coated of DXYH with Eudragit-L-30-D-55® and Kollicot® MAE 100 P were determined at room temperature using FT-IR spectrophotometer (Model SHIMADZU FT-IR-8400S, Japan) in transmittance mode. The spectra were recorded at a resolution of 8 cm^{-1} between wave numbers 4000 and 400 cm^{-1} with average of 20 scans using background spectrum corresponding to pure potassium bromide (KBr) plate. Samples were prepared and compressed with KBr on Minipress (Jasco, Japan) to form discs. The compressed discs were scanned and characteristic peaks were recorded and evaluated.

2.4 Accelerated stability study of the optimized batches

Samples of optimized batches of DXYH enteric coated tablets were blister packed in aluminum foil and PVC/PVDC. Tests were conducted at room temperature and accelerated stability conditions. The samples were designated as time 0, 1, 2 and 3 month for accelerated studies. The samples in the accelerated stability study were kept at 40 ± 2 °C and 75 ± 5 % RH in humidity chamber (BINDER Gmbh bergstr, Germany). Sample were withdrawn after one month interval and evaluated for change in *in-vitro* drug release pattern, assay, physical appearance, hardness and thickness, and disintegration time (ICH Stability testing, 2003).

2.5 Central Composite Design (CCD) of the experimental formulations

Response surface methodology (RSM) was used to study the effect of independent variables on response variables in conjunction with CCD (Dutka *et al.*, 2015).

2.6 Experimental design

As the coating level was varied between 5 to 12 %; three variables, namely, the concentration of polymer and plasticizer were varied based on weight by weight proportion to obtain the formulation that provides the required release profile and acidic resistance property.

CCD was chosen as it can spot any non-linearity in factor response relationship. Design Expert 6.0.8 Software was used to find the optimum area at which the desired responses are achieved.

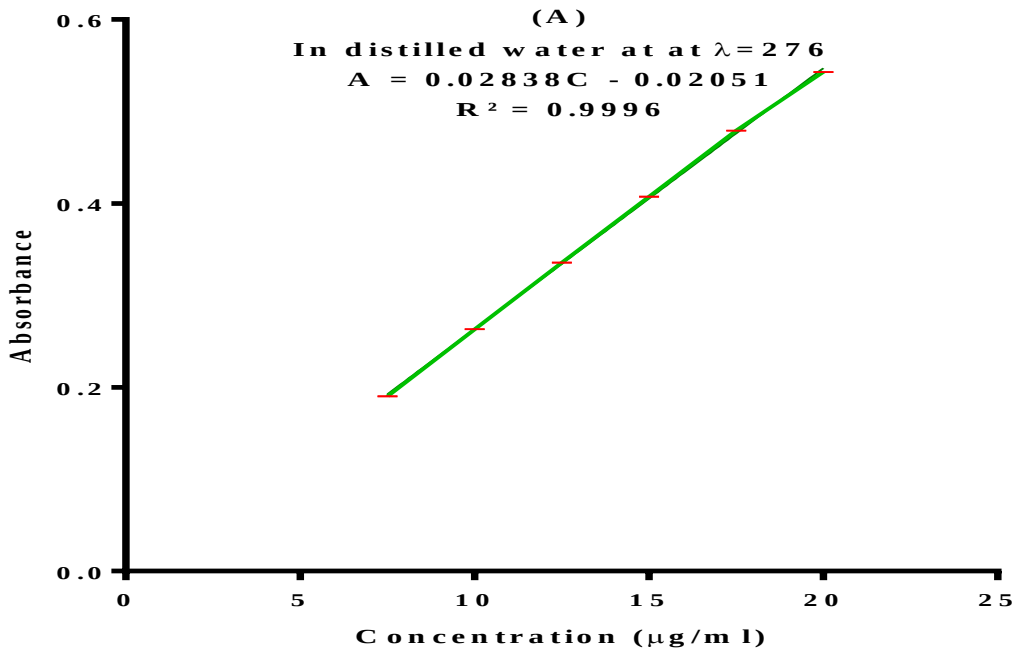
2.7 Statistical Analysis

Data was analyzed to compare individual differences in tablet properties using Analysis of Variance (ANOVA) on Design Expert 6.0.8 Portable (State-Ease, Inc, USA) statistical software. All the data measured and reported are averages of a minimum of triplicate measurements and the values are expressed as mean ± standard deviation (SD) at 95 % confidence interval, and p-values of <0.05 were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 UV Calibration curve of DXYH

The standard calibration curves for DXYH in distilled water, 0.06N HCl acid and 5.5 pH Phthalate buffer are shown in Fig. 3.1. The generated equations describing the relationship between concentration and absorbance in distilled water, 0.06N HCl acid and 5.5 pH Phthalate buffer, were A = 0.02838C + 0.02051, A = 0.03730C + 0.03665 and A = 0.03419C + 0.04161 with correlation coefficient (R^2) values of 0.9996, 0.9996 and 0.9997 at absorption maxima (λ_{max}) of 276ŋm (Sreeram *et al.*, 2015), 346ŋm and 345ŋm (USP 36/NF 31,2013) respectively. The linear regression equations generated were used for calculation of the concentration of DXYH in the three media.



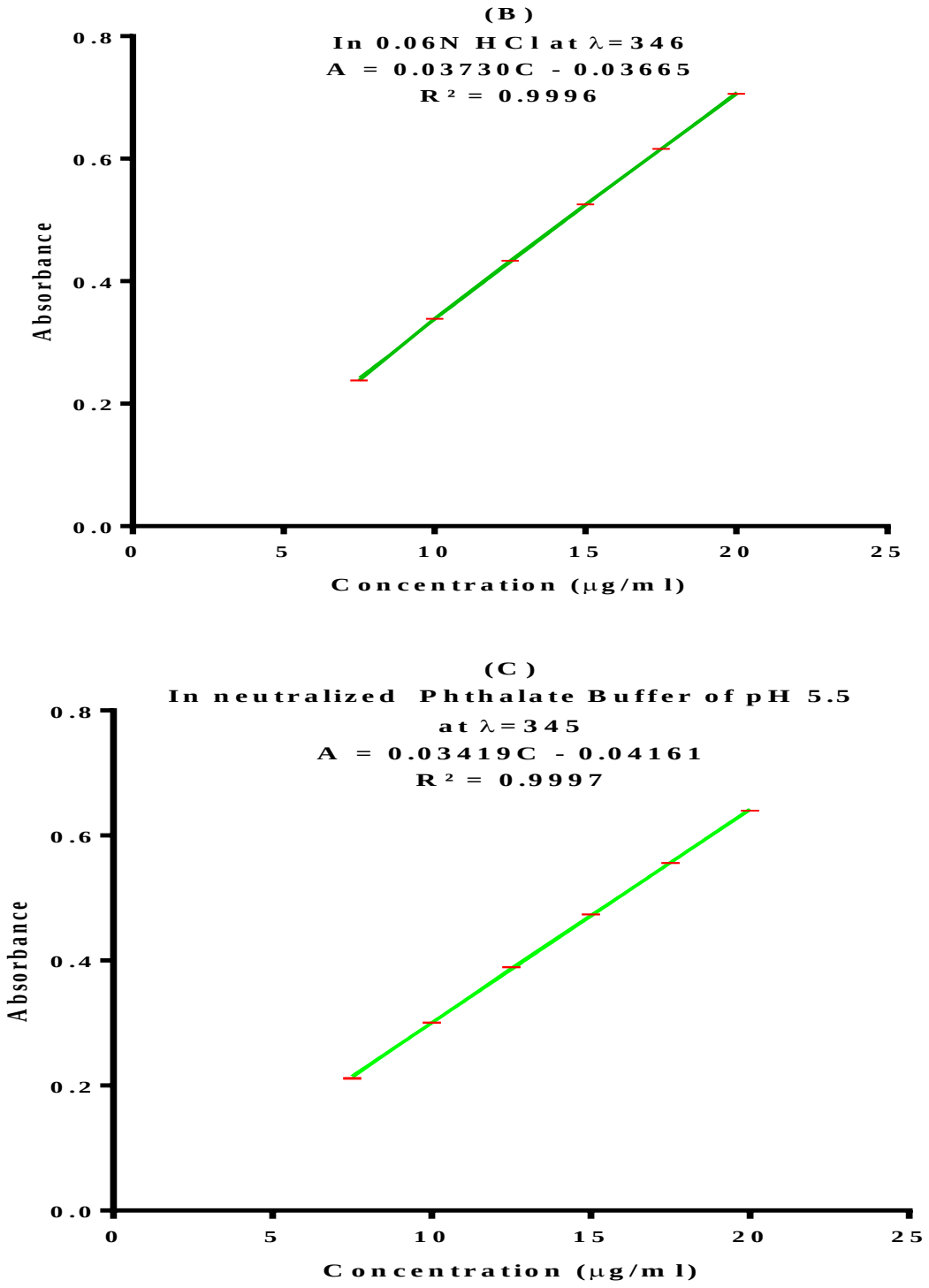


Fig. 3.1: UV absorption calibration curve for DXYH reference standard in distilled water at a correlation coefficient, R^2 = 0.9996 at 276 ηm (A); 0.06 N HCl at a correlation coefficient, R^2 = 0.9996 at 346 ηm (B) and 5.5 pH neutralized phthalate buffer at a correlation coefficient, R^2 = 0.9997 at 345ηm (C) with 95 % confidence interval.

3.2 Drug-excipients compatibility study by FT-IR

FT-IR spectral pattern of DXYH shows sharp peaks between 1600 and 1400 cm^{-1} corresponding to aromatic C═C bonds, peak at 1242 cm^{-1} corresponding to C-O bond, characteristic peaks at 3400 and 3200 cm^{-1}corresponding to primary OH and primary NH, respectively (Mishra and Mishra, 2011).

In Fig. 3.2a-h, the FT-IR (***APPENDIX I***) characteristic peaks for pure DXYH appeared in the polymer and/or excipients used and hence it is assumed that there was no incompatibility of the drug with any of the polymer and/or excipients in the formulations.

3.3 Preliminary study

3.3.1 Evaluation of Pre-Compression Parameters

Measurement of bulk density and specific surface area is very sensitive to sample preparation, especially if the powder used has a small particle size like DXYH. The flow-ability of the powder is related to the particle size and shape. Hausner ratio and Carr's index are measures of the relative importance of inter-particulate interactions. In a free-flowing powder, such interactions are generally less significant, and the bulk and tapped densities will be closer in value. For poorer flowing materials, there are frequently greater inter-particle interactions, and a greater difference between the bulk and tapped densities will be observed. These differences are reflected in the compressibility index. The bulk density, tapped density, compressibility index, Hausner's ratio and angle of repose of the blends of all the four batches are shown in Table 3.1. According to (USP36/NF31<1174>, 2013) when compressibility index, Hausner's ratio and angle of repose have (11-15 %), (1.12-1.18) and (30-35 º) values, respectively, the material is considered to have good rheological properties (USP36/NF31, 2013).

The angle of repose (º) and compressibility index (%) ranged from (24.27± 0.29 to 30.30±0.27) and (9.97±0.50 to 18.74±1.10), respectively. The bulk density and tapped density ranged from (0.46± 0.00 to 0.49± 0.44) and (0.52± 0.00 to 0.58±0.05), respectively. The Hausner's ratio ranged between (1.11±0.01 to 1.23±0.03).The results of angle of repose (<30º) indicate good flow properties of blends as indicated by lower compressibility index values, except for blends of dried starch (FDS^1, FDS^2 and FDS^3), which indicates excellent flow properties.

3.3.2 Evaluation of compressed uncoated tablets

The properties of compressed tablets such as weight variation, thickness, friability, hardness, disintegration time and dissolution are shown in the Table 3.2. The plain tablet mean weight variation, thickness, and diameter had values ranging from (238.48±2.14 to 241.92± 2.11) mg, (4.65± 0.01 to 4.72±0.08) mm and (8.68±0.01 to 8.74±0.04) mm, respectively. The result of the tablets indicates that die fill was uniform. The thickness depends upon the size of the punch and the weight of the tablet. Weight variation of the average percentage deviation of 10 tablets of each formulation was less than ±7. 5 %, indicating good uniformity.

Hardness ranged between (7.89± 1.03 to 10.30±0.42) kg/cm^2 in all the formulations indicating acceptable mechanical strength. In all the formulations the friability values ranged (0.18 to 0.82) % giving an indication that the formulated tablets were mechanically stable. However, formulations FDS^1, FDS^2 and FDS^3 that incorporate dried starch as disinitegrant generated excessive dust during compression process associated with chipping and capping as was evident during the friability test and the tablet had a tendency to cap. It is associated with the nature of starch which has insufficient flow and form segregation, does not compress well and tends to increase tablet friability and capping (Riley *et al.*, 2008; Hausler, 2009; Parmar and Rane, 2009).

Table 3.1: Micromeritic properties of various formulations of DXYH prepared by direct mixing

FORMULATION CODE	BULK DENSITY (g/ml)	TAPPED DENSITY (g/ml)	COMPRESSIBILITY INDEX (%)	HAUSNER RATIO (%)	ANGLE OF REPOSE (°)
FCCNa[1]	0.49± 0.44	0.57± 0.05	13.71± 0.31	1.16± 0.00	26.63± 0.25
FCCNa[2]	0.47± 0.01	0.54± 0.01	14.09± 0.60	1.16± 0.01	25.77± 0.32
FCCNa[3]	0.48± 0.00	0.55± 0.01	12.31± 1.29	1.14± 0.02	26.10± 0.17
FSSG[1]	0.49± 0.01	0.56± 0.01	12.59± 1.88	1.14± 0.02	24.83± 0.21
FSSG[2]	0.47± 0.00	0.53± 0.01	11.23± 1.38	1.13± 0.02	24.77± 0.21
FSSG[3]	0.49± 0.01	0.55± 0.01	12.39± 1.46	1.14± 0.02	24.44± 0.44
FCP[1]	0.47± 0.00	0.52± 0.01	9.97± 0.50	1.11± 0.01	24.33± 0.50
FCP[2]	0.46± 0.00	0.52± 0.00	10.95± 0.16	1.12± 0.00	24.27± 0.29
FCP[3]	0.47± 0.00	0.54± 0.01	12.55± 0.60	1.14± 0.01	24.70± 0.44
FDS[1]	0.47± 0.00	0.57± 0.01	18.49± 1.81	1.23± 0.03	29.57± 0.50
FDS[2]	0.46± 0.00	0.56± 0.00	17.74± 0.31	1.22± 0.01	30.00± 0.62
FDS[3]	0.47± 0.00	0.58± 0.05	18.74± 1.10	1.23± 0.02	30.30± 0.27

FSSG: Formulation with Sodium Starch Glycolate
FCP: Formulation with Crospovidone
FCCNa: Formulation with Crosscarmelose Sodium
FDS: Formulation with Dried Starch

Superscript Level:
1: Maximum Level Concentration of Disintegrant
2: Mid-Level Concentration of Disintegrant
3: Minimum Level Concentration of Disintegrant

Table 3.2: Tablet properties of DXYH formulations

Batch Code	Mean Weight (mg) N=10	Thickness (mm) N=10	Diameter (mm) N=10	Hardness (Kg/cm^2) N=10	Friability (%) N=20	Disintegration time (min) N=6	Assay (%)
FCCNa[1]	238.48± 2.14	4.72± 0.01	8.72± 0.03	8.48± 0.84	0.25± 0.00	13.37± 0.38	96.00
FCCNa[2]	240.17± 1.91	4.72± 0.01	8.74± 0.04	7.95± 1.14	0.23± 0.00	11.63± 0.20	97.40
FCCNa[3]	239.21± 2.51	4.71± 0.01	8.74± 0.04	8.28± 0.82	0.22± 0.00	9.35± 0.22	97.00
FSSG[1]	241.27± 2.66	4.69± 0.04	8.70± 0.01	8.53± 1.64	0.27± 0.00	4.30± 0.30	98.50
FSSG[2]	240.74± 4.18	4.71± 0.06	8.70± 0.02	7.89± 1.03	0.26± 0.00	5.15± 0.54	96.40
FSSG[3]	241.92± 2.11	4.68± 0.04	8.71± 0.02	6.84± 1.16	0.21± 0.00	7.15± 0.18	97.00
FCP[1]	241.68± 1.15	4.65± 0.08	8.72± 0.04	10.27± 0.56	0.20± 0.00	3.22± 0.20	101.50
FCP[2]	240.80± 1.13	4.65± 0.01	8.71± 0.01	10.30± 0.42	0.19± 0.00	3.50± 0.12	101.20
FCP[3]	241.46± 1.31	4.65± 0.01	8.68± 0.01	10.00± 0.58	0.20± 0.00	3.67± 0.41	99.50
FDS[1]	239.99± 6.16	4.71± 0.04	8.70± 0.04	8.70± 0.42	0.63± 0.00	12.35± 0.37	95.30
FDS[2]	239.25± 6.37	4.70± 0.01	8.69± 0.01	8.69± 0.01	0.82± 0.00	14.87± 0.41	95.00
FDS[3]	238.78± 5.78	4.71± 0.02	8.70± 0.03	8.70± 0.03	0.82± 0.00	>15	94.30

FSSG: Formulation with Sodium Starch Glycolate
FCP: Formulation with Crospovidone
FCCNa: Formulation with Crosscarmelose Sodium
FDS: Formulation with Dried Starch

Superscript Level:
1: Maximum Level Concentration of Disintegrant
2: Mid-Level Concentration of Disintegrant
3: Minimum Level Concentration of Disintegrant

The formulations FCP^1 to FCP^3 containing CP exhibited 99.5 to 101.5 % assay and 3.22±0.20 to 3.67±0.41 min disintegration time which were the highest assay and shortest disintegration time compared to all the other formulations.

The increase in concentration of super-disintegrants, typically, tend to disintegrate the tablets quicker. This is true with CP and SSG, whereas CCNa produced fast disintegrating tablets with lower concentrations, while at higher concentrations, the disintegration time was prolonged. This is attributed to ethers of cellulose at higher concentrations, form a hydrophilic barrier due to the gelling properties in aqueous media (De Castro *et al.*, 2006).

SSG and CCNa are sodium salt of carboxy methyl ether of starch and sodium salt of a cross linked, partly O-(carboxymethylated) cellulose, respectively, with their polymer backbones composed mostly of glucose repeat units (USP36/NF31, 2013). In contrast, CP is an insoluble, nonionic, densely cross-linked homo-polymers of *N*-vinyl-2-pyrrolidones. The repeat structure of CP is similar to that of N-methyl pyrrolidone, a water-miscible, polar aprotic solvent with high interfacial activity. Their porous particle morphology enables them to rapidly absorb liquids into the tablet by capillary action and to generate rapid volume expansion and hydrostatic pressures that result in tablet disintegration (Tanuwijaya and Karsono, 2013; USP36/NF31, 2013; Desai *et al.*, 2016).

Thus inclusion of CP was found to improve the disintegration of the tablets. For a similar concentration of super-disintegrants, CP induced faster disintegration than CCNa and SSG. This behavior could be attributed to the inherent properties of these materials such as their chemical structure, particle size and porosity. Furthermore, the tablets formulated with CP (FCP^1, FCP^2 and FCP^3) were found to be harder and thinner compared to other batches of tablets. Other components being unaltered, the reduction in thickness could be attributed to the highly compressible nature of CP compared to other super-disintegrants (Das *et al.*, 2013; Tanuwijaya and Karsono, 2013).

Among these formulations, the core tablet formulation of FCP^2 was mechanically stronger with least friability. The tablets were also rapidly disintegrate within 3.50±0.12 min. Thus on the basis of rheological properties of the post compression parameters, mechanical strength and its ability to generate the rapidly disintegrating tablets, FCP^2 was selected as an optimized core tablet formulation for enteric coating.

3.3.3 Optimization of the coating process

The preliminary study was performed to check the coating efficiency at specific coating level of 2.5 % for each polymer on FCP[2]. 0.5 Kg of coating suspension prepared as shown in Table 2.2 and 2.3 with 3 % and 12.5 % plasticizer concentration for Kollicot® MAE 100 P and Eudragit-L-30-D-55® were applied on plain tablets. Each coated tablet batch was evaluated for 2.5 % coating level and surface appearance as shown in Table 3.3. The data obtained from the preliminary study within the specified coating process parameters, all the tested criteria were found satisfactory. Hence, Core tablets were then coated as per the optimized parameters obtained and characterized to get the actual value and compared with the predicted value in the proposed formulations. It was observed that there was no significant difference between predicted and the actual value derived after successful characterization.

Table 3.3: Preliminary trial batch for optimization of coating process parameters Eudragit-L-30-D-55® and Kollicot® MAE 100 P

Parameters	Coated with Eudragit-L-30-D-55®	Coated with Kollicot® MAE 100 P
Initial average weight of tablets to be coated (mg) N=50	240.23±1.32	240.43±1.52
Final average weight of tablets after coating (mg) N=50	246.27±0.76	246.47±0.93
Initial total weight of tablets to be coated (mg)	12494.50	12507.50
Final total weight of tablets after coating (mg)	12807.66	12819.25
Final individual weight gain of tablets after coating (%) N=50	2.48±0.56	2.51±0.62
Final total weight gain of tablets after coating (%)	2.51	2.49

There was no twinning, tablet breakage, orange peel, picking and sticking, edge erosion and surface erosion during preliminary trial batches optimization of coating process parameters for Eudragit-L-30-D-55® and Kollicot® MAE 100 P.

From preliminary study, the composition of coating solution, namely, polymer and plasticizer percentage and coating level were taken as independent variables and their effects on disintegration time in 0.1 N HCl, drug release in 0.06 N HCl and drug release in neutralized phthalate buffer of pH 5.5 were considered as dependent variables and were further studied and optimized using RSM.

Other independent variables as atomizing pressure, spray rate application, inlet air temperature and coating pan speed were maintained constant during the coating operation (Table 2.4).

Using CCD as shown in Table 3.4 and Table 3.5 (***APPENDIX II***), low and high levels for each polymer and plasticizer concentration on the dry coating powder concentration were considered for 2^3 factorial design combined with six replicates of the center point and 2x2 axial points performed in two blocks.

3.4 Assessment of coated tablets

3.4.1 Tablets coated with Eudragit-L-30-D-55®

As shown in Table 3.6 (***APPENDIX II***), those tablets coated with Eudragit-L-30-D-55® at higher coating level extended the disintegration time and reduced release in 0.06 N HCl acid media and release in neutralized phthalate buffer pH 5.5 as well. This is because, the sealing effect of the film coatings naturally increases in proportion to the film thickness. In that case however, the release of active ingredients in digestive fluids with a pH of less than 5 is also delayed (Thakral *et al.*, 2013).

It was also found that, with lower concentration of Eudragit-L-30-D-55® to plasticizer ratio, the coated tablets showed lower resistance to simulated gastric fluid and the release profile was higher in both 0.06 N HCl media and neutralized phthalate buffer pH 5.5. Furthermore, with the same coating level, increasing plasticizer concentration extended the disintegration time and decreased the release in 0.06 N HCl media and neutralized phthalate buffer pH 5.5 though the adhesion of Eudragit films is markedly increased when the concentration of the plasticizer is increased (Felton and McGinity, 1997; Felton and McGinity, 1999).

USP36/NF31 monograph stated that *"Delayed Release Doxycycline Hyclate Tablets"* should show no evidence of disintegration, cracking, or softening in simulated gastric fluid after 1 h. Whereas for release profile, no individual value is more than 30% of the labeled amount of DXYH released within 20 min in 0.06 N HCl and no less than 90% of the labeled amount of DXYH released after 30 min in neutralized phthalate buffer of pH 5.5 is allowed.

Therefore, by referring to Table 3.6 (***APPENDIX II***) of optimization results, formulation FCP^2E-14 fulfilled USP36/NF31 specification and was chosen as optimized tablets among the 20 trial

batches. This formulation showed 65 min disintegration time, 9 % and 99.3 % release profiles in 0.1N HCl acidic media, 0.06 N HCl acid, and 5.5 pH neutralized phthalate buffer, respectively.

3.4.2 Tablets coated with Kollicot® MAE 100 P

As shown in Table 3.7 (***APPENDIX II***), those tablets coated with Kollicot® MAE 100 P at higher coating level had longer disintegration time and reduced dissolution in 0.06N HCl media and dissolution in neutralized phthalate buffer pH 5.5 as well; again due to the sealing effect of the film coatings naturally increasing the proportion to the film thickness (Thakral *et al.*, 2013). Such an influence of polymer coating level on the release pattern of tablets was also observed by Missaghi and his team, who found a slower release for enteric coated rabeprazole tablets at 14 % coating level (Shahrzad *et al.*, 2010).

Furthermore, with the same coating level, increasing plasticizer concentration extended the disintegration time and decreased the release in 0.06 N HCl media and in neutralized phthalate buffer pH 5.5 though the adhesion of Eudragit films is markedly increased when the concentration of the plasticizer is increased (Felton and McGinity, 1997; Felton and McGinity, 1999).

Based on the optimizations conducted in this study, FCP^{2}K-18 showed a superior release profiles in neutralized phthalate buffer of pH 5.5 and disintegration time in 0.1 N HCl acid media and satisfactory release resistance in 0.06 N HCl media among all of the formulations as shown in Table 3.7 (***APPENDIX II***) and therefore, it was selected as optimized formulation. This formulation showed 67 min disintegration time, 9.5 % and 98.5 % release profiles in 0.1N HCl acidic media, 0.06 N HCl acid and 5.5 pH neutralized phthalate buffer, respectively. Thus, again it was complied with USP36/NF31 specification.

3.5 Mathematical model selection for response

From the fit summaries, suitable response model for the response was designated. As indicated in Table 3.8 and 3.9, (***APPENDIX II***) the fit summaries output of three responses: disintegration time in 0.1 N HCl, dissolution in 0.06 N HCl and dissolution neutralized phthalate buffer pH of 5.5. The fit summary analysis is based on p-values and R-squared values for comparing models for each response.

The software selects and suggests for further use the highest order polynomial where the additional terms are significant, the model is not aliased and adjusted R-squared and predicted R-squared are in reasonable agreement within 0.20 of each other and if the term's p-value is less than 0.05.

Hence, quadratic contribution was suggested with p=0.0002 and R^2=0.9470 for disintegration time in 0.1 N HCl, the linear contribution was suggested with p=0.0004 and R^2=0.8756 for dissolution in 0.06N HCl and quadratic contribution was suggested with p=0.0010 and R^2=0.9470 for dissolution in neutralized phthalate buffer pH 5.5 designed for those tablets coated with Eudragit-L-30-D-55® as shown in Table 3.8. For tablets coated with Kollicot® MAE 100 P, quadratic contributions were suggested with p=0.0025 and R^2=0.9633, p=0.0103 and R^2=0.9791 and p=0.0044 and R^2=0.9674 for disintegration time in 0.1N HCl, dissolution in 0.06 N HCl and dissolution in neutralized phthalate buffer pH 5.5, respectively as shown in Table 3.9 (***APPENDIX II***). Transformation was not used in this study, for fit summary output analysis.

3.6 Model adequacy checking

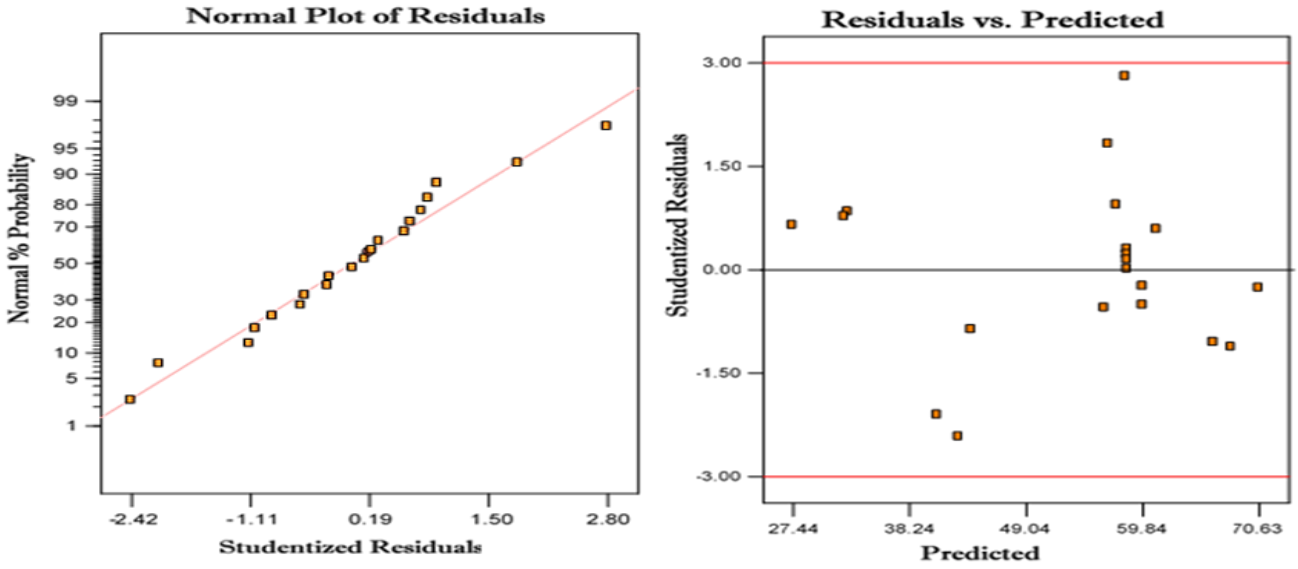
ANOVA was used to evaluate the best model fitting as well as the significance of the selected model for each response coated with specific coating materials. The regression model and terms in the model are considered to be significant when the p-value is less than 0.05. Model significance is desirable as it indicates that the terms in the model have a significant effect on the response.

As it is apparent from Tables 3.10 to 3.12 (***APPENDIX II***), models selected for Eudragit-L-30-D-55® coated tablets were found to be significant with p=0.0001 of quadratic model for disintegration time in 0.1N HCl, p<0.0001 of linear model for dissolution in 0.06 N HCl and p=0.0002 of quadratic model for dissolution in neutralized phthalate buffer pH of 5.5. ANOVA revealed that the main effects of parameters: coating level (B) (p< 0.0001) and 1,2-PG (C) (p=0.0031), quadratic effects of coating level (B^2) (p=0.0075) and 1,2-PG percentage (C^2) (p<0.0161) were found to be significant for disintegration time in 0.1 N HCl; and the main effects of parameters: coating level (B) (p<0.0001) and 1,2-PG percentage (C) (p=0.0027) were found to be significant for dissolution in 0.06 N HCl and the main effects of parameter: coating level (B) (p<0.0001) and quadratic effects of coating level (B^2) (p=0.0002) were found to be significant for dissolution in neutralized phthalate buffer pH of 5.5.

As shown in Table 3.13 to 3.15 (***APPENDIX II***) , models selected for Kollicot® MAE 100 P coated tablets were found to be significant with $p<0.0001$of quadratic models for disintegration time in 0.1 N HCl, $p<0.0001$of quadratic models for dissolution in 0.0 6N HCl and $p<0.0001$ of quadratic models for dissolution in neutralized phthalate buffer of pH 5.5. According to ANOVA, the main effect of model terms for disintegration time in 0.1 N HCl, the main effect of coating level (B) ($p<0.0001$) and the quadratic effect of coating level (B^2) ($p<0.0001$) were significant model terms for dissolution in 0.06 N HCl and main effect of coating level (B) ($p<0.0001$) and the quadratic effects of Kollicot® MAE 100 P percentage (A^2) ($p=0.0448$), coating level (B^2) ($p=0.0002$) and 1,2-PG percentage (C^2) ($p=0.0012$) were found to be significant model terms for dissolution in neutralized phthalate buffer of pH 5.5.

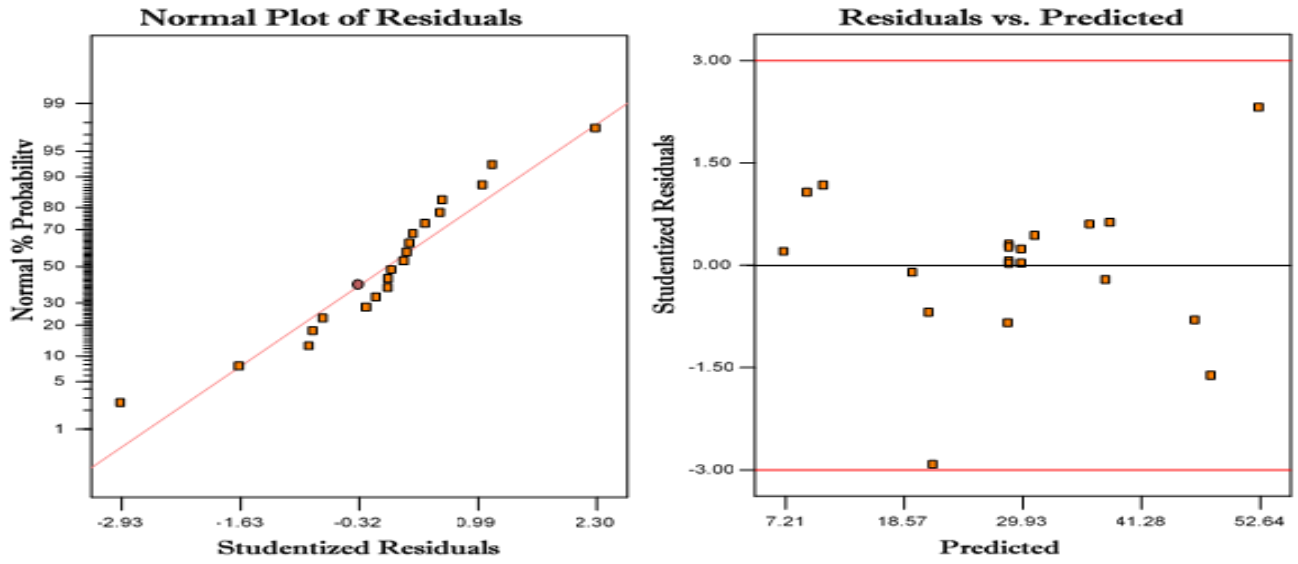
Conferring to the normal probability plot of residuals and the plot of internally studentized residuals and the plot of residuals versus the predicted value of responses for Eudragit-L-30-D-55® and Kollicot® MAE 100 P coated tablets depicted in (Fig. 3.3a-b to 3.5a-b and 3.6a-b to 3.8a-b) for disintegration time in 0.1 N HCl, dissolution in 0.06 N HCl and dissolution in neutralized phthalate buffer of pH 5.5, respectively. The data on the model efficiency was summarized in residuals providing a clear understanding of any variation in fit to the model. The two plots related to residuals were the normal probability plot of residuals and the plot of internally studentized residuals versus predicted values, which are considered as additional tests of model adequacy checking tools (Bari *et al.*, 2010).

Thus, based on the points or point clusters, which placed closely to the diagonal line in (Fig. 3.3a to 3.8a) concluding that the errors were distributed normally for all responses. Then again, the plot of internally studentized residuals versus predicted values in (Fig. 3.3b to 3.8b), indicated that the points are randomly scattered, with no obvious pattern and all the values lie within the recommended range of -3 and +3 which are considered as the top and bottom outlier detection limit. Hence, the selected models were adequate for their specific responses and there is no need for suspecting violation of independent or constant variance assumption.



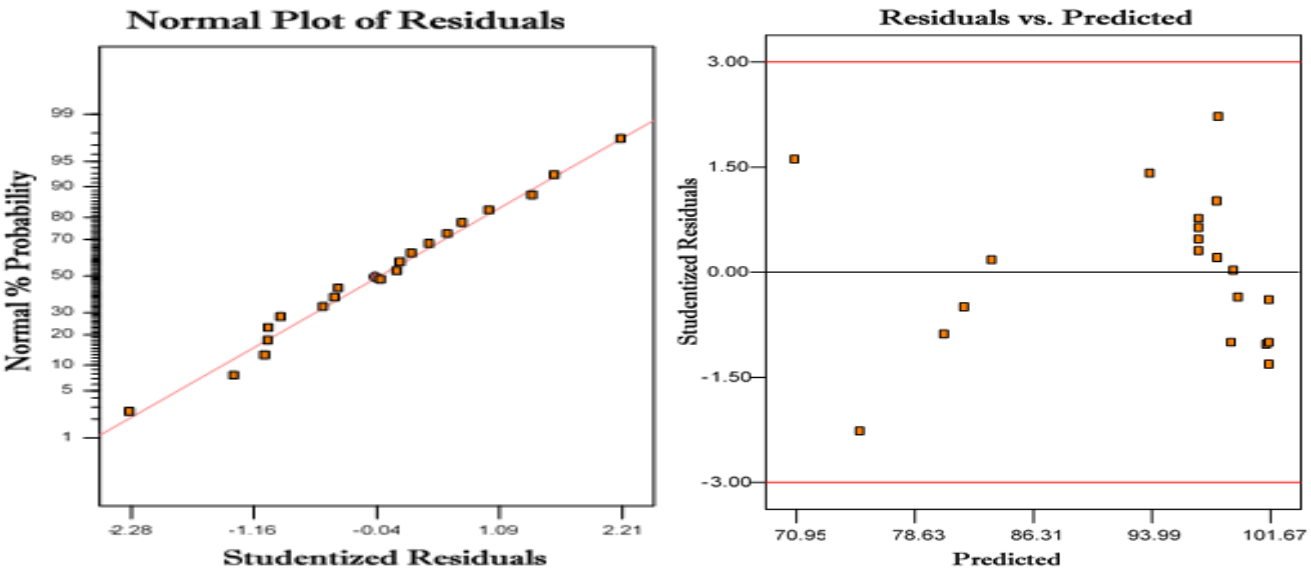
(a) (b)

Fig. 3.3: Normal probability plot of residuals (a), and plots of the residuals versus predicted response (b) for disintegration time in 0.1N HCl coated with Eudragit-L-30-D-55®.



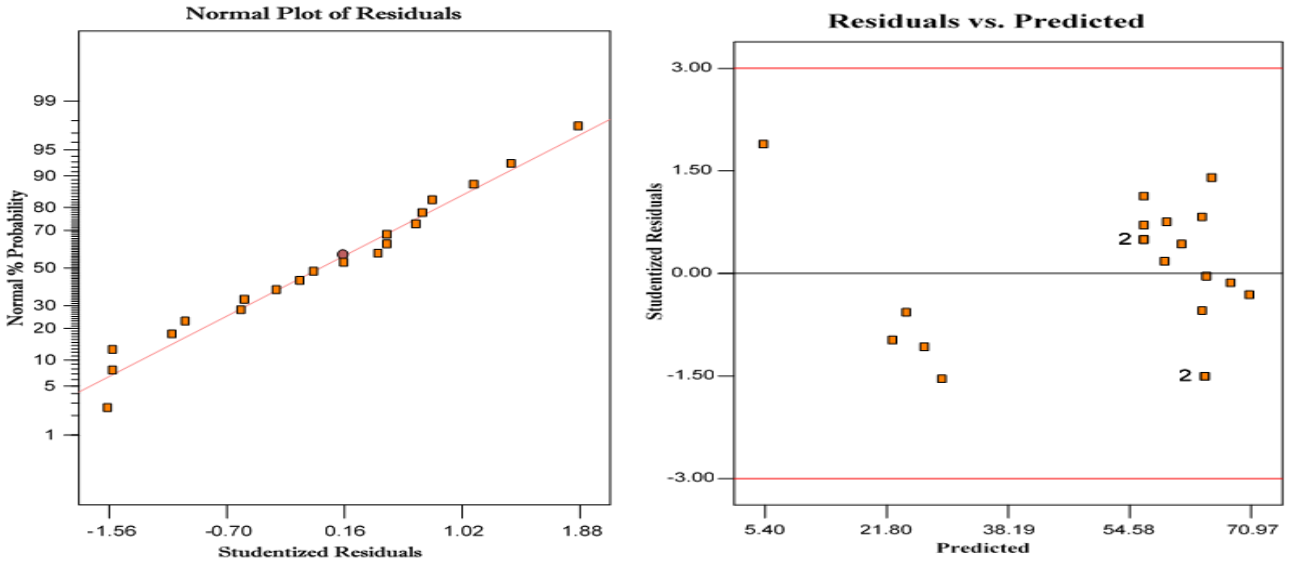
(a) (b)

Fig. 3.4: Normal probability plot of residuals (a), and plots of the residuals versus predicted response (b) for dissolution in 0.06 N HCl coated with Eudragit-L-30-D-55®.



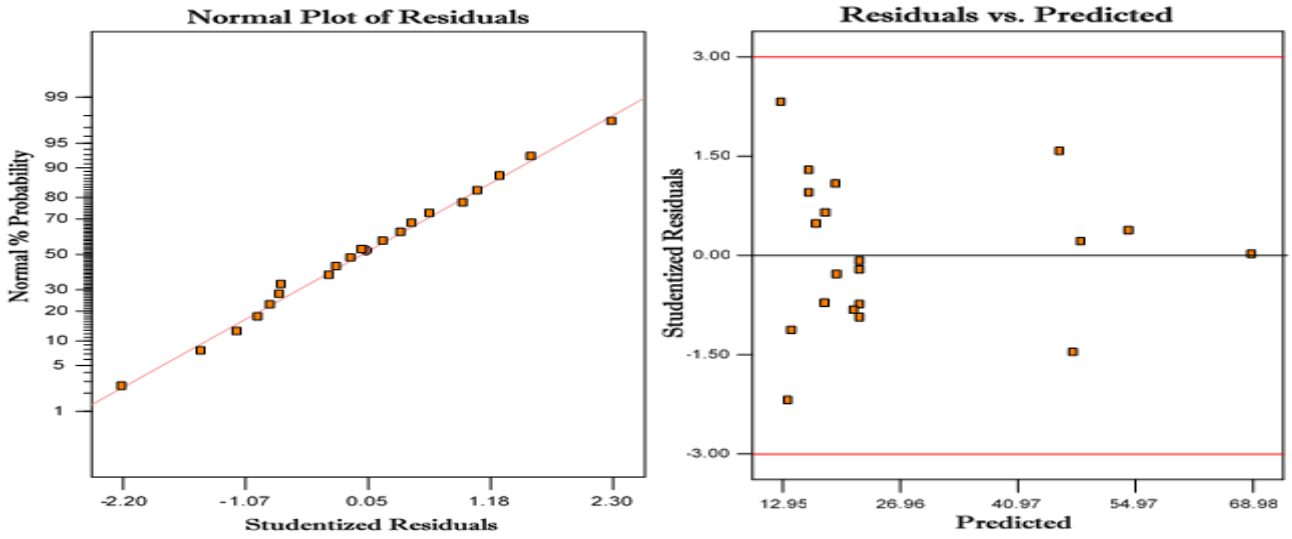
(a) (b)

Fig. 3.5: Normal probability plot of residuals (a), and plots of the residuals versus predicted response (b) for dissolution in neutralized phthalate buffer of pH 5.5 coated with Eudragit-L-30-D-55®.



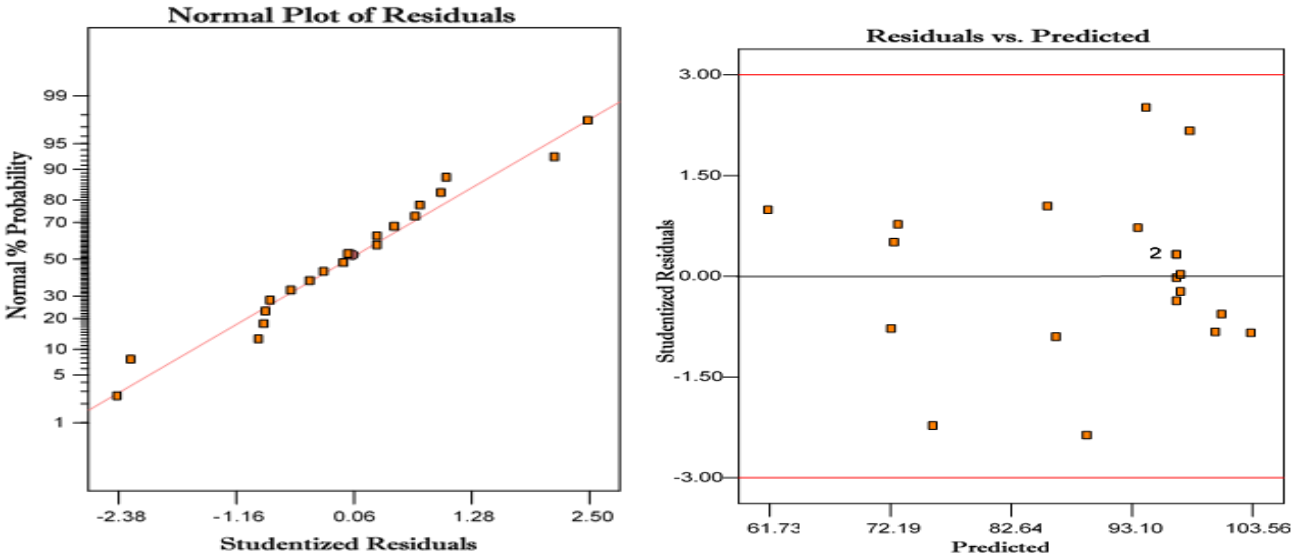
(a) (b)

Fig. 3.6: Normal probability plot of residuals (a), and plots of the residuals versus predicted response (b) for disintegration time in 0.1 N HCl coated with Kollicot® MAE 100 P.



(a) (b)

Fig. 3.7: Normal probability plot of residuals (a), and plots of the residuals versus predicted response (b) for dissolution in 0.06 N HCl coated with Kollicot® MAE 100 P.



(a) (b)

Fig. 3.8: Normal probability plot of residuals (a), and plots of the residuals versus predicted response (b) for dissolution in neutralized phthalate buffer of pH 5.5 coated with Kollicot® MAE 100 P.

For each tablets coated with the two polymers, all the three responses of significant mathematical regression models were generated in terms of coded factors and Equation 3.1a-b to 3.3a-b established from the model terms as shown in Table 3.16 and 3.17, presented the regression coefficients of the model terms in their respective models for each responses, respectively.

The polynomial equation was used to draw conclusion after considering the intensity of coefficient and the mathematical sign it carries, i.e., positive or negative. The sign of model coefficients has meanings on the response variables, that a coefficient implies the change in response, whenever that term is changed by one unit leaving the other terms constant. The magnitude implies the strength, whereas the sign indicates the direction factor varies the response. A positive sign indicates a positive effect where as a negative sign indicates a negative effect on the response (Sonar and Rawat, 2015).

Table 3.16: Estimated model term regression coefficients for the selected models for each response for tablets coated with Eudragit-L-30-D-55®.

	Coded Coefficients			Actual Coefficients		
	Disintegration time in 0.1 N HCl	**Dissolution in 0.06 N HCl**	**Dissolution in neutralized phthalate buffer of pH 5.5**	**Disintegration time in 0.1 N HCl**	**Dissolution in 0.06 N HCl**	**Dissolution in neutralized phthalate buffer of pH 5.5**
Intercept	+59.17	+29.34	+99.39	-4369.99668	+121.72541	-4954.13115
			Main effect			
Coating Level (B)	+12.84	-13.51	-9.13	-12.93914	-3.85970	-13.20041
1,2-PG (W/W) in % (C)	+4.59	-5.04	--	+42.73633	-3.35936	--
			Quadratic effect			
A^2	--	--	--	--	--	--
B^2	-3.83	--	-5.43	-0.31299	--	-0.44312
C^2	-3.30	--	--	-1.46837	--	--

Disintegration time in 0.1 N HCl (Y_1) = $+12.84B + 4.59C - 3.83B^2 - 3.30C^2 + 59.17$ (3.1a)

Dissolution in 0.06 N HCl (Y_2) = $-13.51B-5.04C + 29.34$ (3.2a)

Dissolution in neutralized phthalate buffer of pH 5.5 (Y_3) = $-9.13B - 5.43B^2 +99.39$ (3.3a)

Table 3.17: Estimated model term regression coefficients for the selected models for each response for tablets coated with Kollicot® MAE 100 P

	Coded Coefficients			Actual Coefficients		
	Disintegration time in 0.1 N HCl	**Dissolution in 0.06 N HCl**	**Dissolution in neutralized phthalate buffer of pH 5.5**	**Disintegration time in 0.1 N HCl**	**Dissolution in 0.06 N HCl**	**Dissolution in neutralized phthalate buffer of pH 5.5**
Intercept	+60.83	+19.26	+97.28	-1261.56852	+167.27334	-2354.47871
			Main effect			
Kollicot® MAE 100 P (W/W) in % (A)	--	--	--	--	--	--
Coating Level (B)	+19.49	-16.29	-12.44	+15.47837	+0.73153	+10.61286
1,2-PG (W/W) in % (C)	--	--	--	--	--	--
			Quadratic effect			
A^2	--	--	-1.97	--	--	-0.31532
B^2	-9.46	+8.96	-5.24	-0.77194	+0.73153	-0.42785
C^2	--	--	-3.95	--	--	-0.63211

Disintegration Time in 0.1N HCl (Y_1) = $+19.49B - 9.46B^2 + 60.83$ (3.1b)

Dissolution in 0.06N HCl (Y_2) = $-16.29B + 8.96B^2 + 19.26$ (3.2b)

Dissolution in neutralized phthalate buffer of pH 5.5 (Y_3) = $-12.44B -1.97A^2-5.24B^2 - 3.95C^2 + 97.28$ (3.3b)

Based on Equation 3.1a and Table 3.16, for tablets coated with Eudragit-L-30-D-55®, quadratic mathematical model for disintegration time in 0.1 N HCl indicate that the two factors: coating level (B) and 1,2-PG percentage (C), significantly affect the tablets positively, in such a way that increasing of these factors resulted in an increase disintegration time in 0.1 N HCl. In these cases, the effect of coating level was the strongest with higher coefficient of +12.84 than concentration of 1,2-PG percentage with coefficient of +4.59. But the quadratic effect of (coating level)^2 (B^2) and (percentage)^2 (C^2) had negative impact on disintegration time in 0.1 N HCl with higher coefficient of -3.83 strongly and no interaction effect was seen.

On the other hand, from Equation 3.2a and 3.3a and Table 3.16, linear and quadratic mathematical model was suggested for dissolution in 0.06 N HCl and dissolution in neutralized phthalate buffer of pH 5.5, respectively; and coating level (B) and 1,2-PG percentage (C) had negative impact for former response and only coating level (B) and quadratic effect of (coating level)^2 (B^2) revealed similar effect for the later response. Hence the increased in the coating level (B) and 1,2-PG percentage (C) resulted in reduced effect on dissolution in 0.06 N HCl and increased in the coating level (B) and quadratic effect of (coating level)^2 (B^2) resulted in reverse effect on dissolution in neutralized phthalate buffer of pH 5.5. But then, interactive effect for both responses and curvature effects for dissolution in 0.06 N HCl was not marked. For dissolution in 0.06 N HCl, coating level (B) had the strongest impact with coefficient of -13.51 and the same is true for dissolution neutralized phthalate buffer of pH 5.5 with coefficient of -9.13.

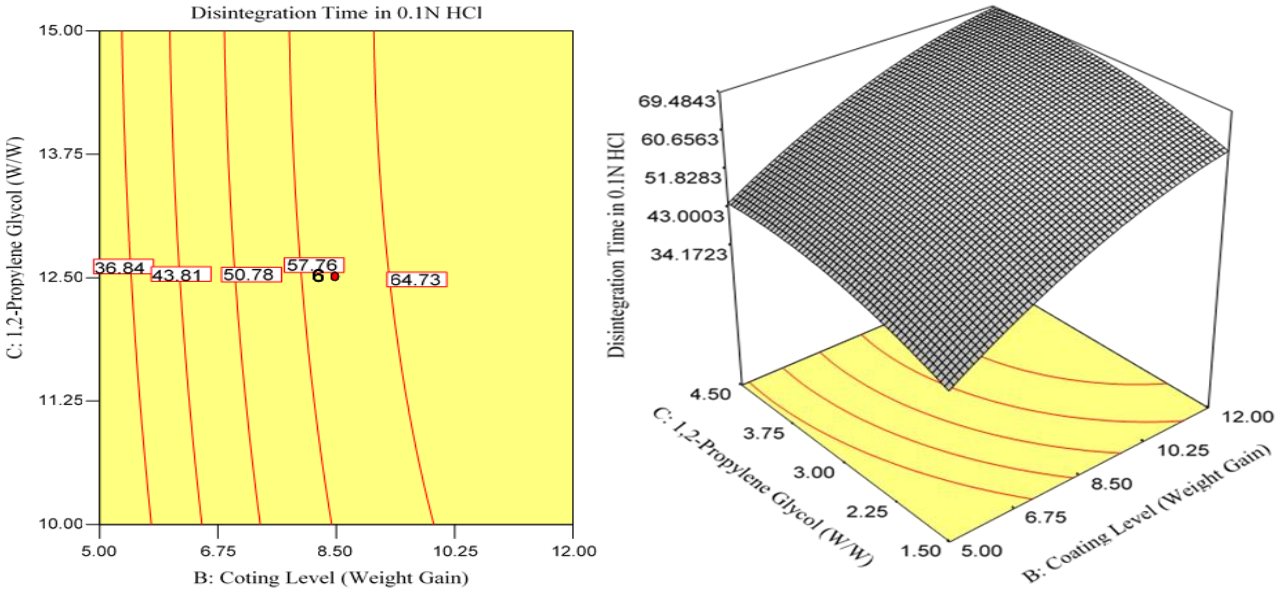
Considering tablets coated with Kollicot® MAE 100 P as indicated in Equation 3.1b and 3.2b, and Table 3.17, the increased in the coating level (B) resulted in the increased disintegration time in 0.1N HCl while it had negative impact on the dissolution in 0.06 N HCl and dissolution in neutralized phthalate buffer of pH 5.5 with greater impact on the response. There was no interaction effect for the three responses but there exists quadratic effect of (coating level)^2 (B^2) which had negative impact for disintegration time in 0.1 N HCl and dissolution in neutralized phthalate buffer of pH 5.5 and another quadratic effects of (Kollicot® MAE 100 P)^2 (A^2) and (1,2-PG percentage)^2 (C^2) had negative impact. From them, (coating level)^2 (B^2) exhibited the strongest impact with coefficient of -5.24.

3.7 Contour plot and surface response analysis

Contour plot helps in visualizing the response surface and for establishing desirable response values and operating conditions as surface plots does (Garg and Singhvi, 2015; Sarrai *et al.*, 2016). Three-dimensional (3D) plots and contour plots for the measured responses were formed, based on the model polynomial functions to assess the change of the response surface. Also the relationship between the factors and dependent variables can be further understood by these plots. Since the model has three factors, one factor was held constant for each diagram; therefore, a total of nine response surface diagrams was produced three for each response. In the analysis, figures were selected based on significant factors that were specified in the mathematical models.

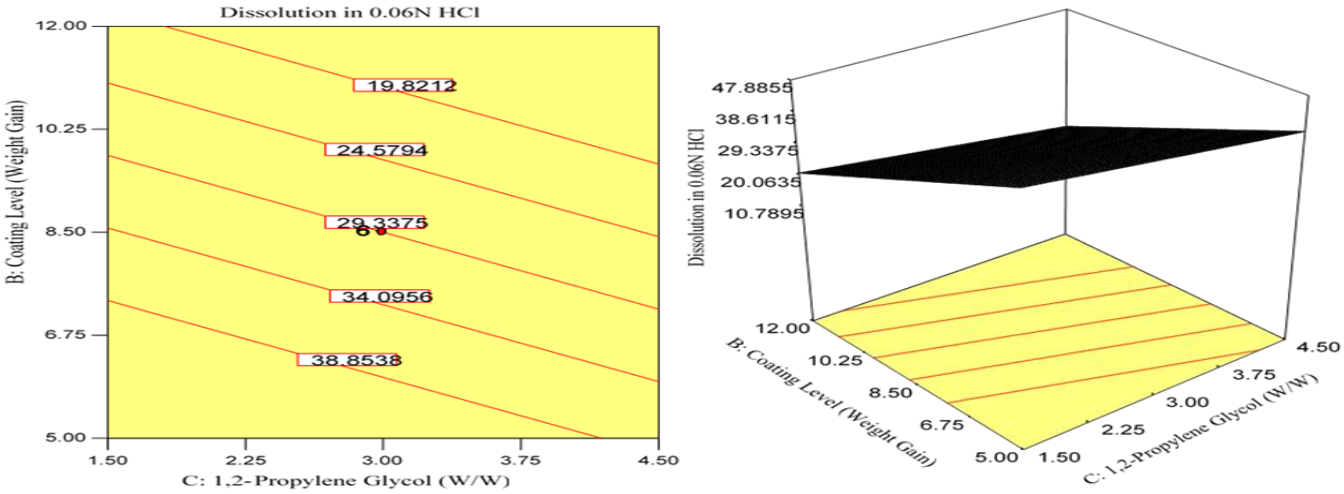
The contour plot and response surface plot for the response variables of disintegration time in 0.1 N HCl (Fig 3.9a and 3.9b) for tablets coated with Eudragit-L-30-D-55® show that there existed quadratic effect on disintegration time in 0.1 N HCl and as evident by nonlinear lines of contour plot and twisted line of response surface. Here the increase in disintegration time in 0.1 N HCl associated with an increase in coating level and 1,2-PG percentage at the same time. However, the effect of coating level was higher compared to 1,2-PG percentage. Similar result was obtained following ANOVA (Table 3.10, ***APPENDIX II***) with $p<0.0001$ and $p=0.0031$; and from the coefficients in the mathematical model generated for disintegration time in 0.1 N HCl (Eq. 3.1a), the coefficients were the same in sign (+12.84 and +4.59) respectively. Whereas for dissolution in 0.06 N HCl (Fig 3.10a and 3.10b), the plots show that there was linear effect on the dissolution in 0.06 N HCl with negative impacts as the effect of coating level was higher compared to 1,2-PG percentage to the-response as evident by the straight lines contour plot and non-twisted response surface. The same result is obtained with ANOVA (Table 3.11, ***APPENDIX II***) with $p<0.0001$ and $p=0.0027$ and from the coefficients in the mathematical model generated for dissolution in 0.06 N HCl (Eq. 3.2a), the coefficients were the same in sign (-13.51 and -5.04) in that order. However, for the dissolution in neutralized phthalate buffer of pH 5.5 (Fig 3.11a-b and 3.12a-b), the plots show that there existed quadratic effect on dissolution in neutralized phthalate buffer of pH 5.5 and as evident by nonlinear lines of contour plot and twisted line of response surface. Hence with the solitary decrease in coating level there was an increase in dissolution in neutralized phthalate buffer of pH 5.5 more pronouncedly. This was again confirmed by the coefficients in mathematical model

generated for dissolution in neutralized phthalate buffer of pH 5.5 (Eq. 3.3a) with the coefficient of -9.13 and the same result was with ANOVA (Table 3.12, ***APPENDIX II***) with $p<0.0001$.



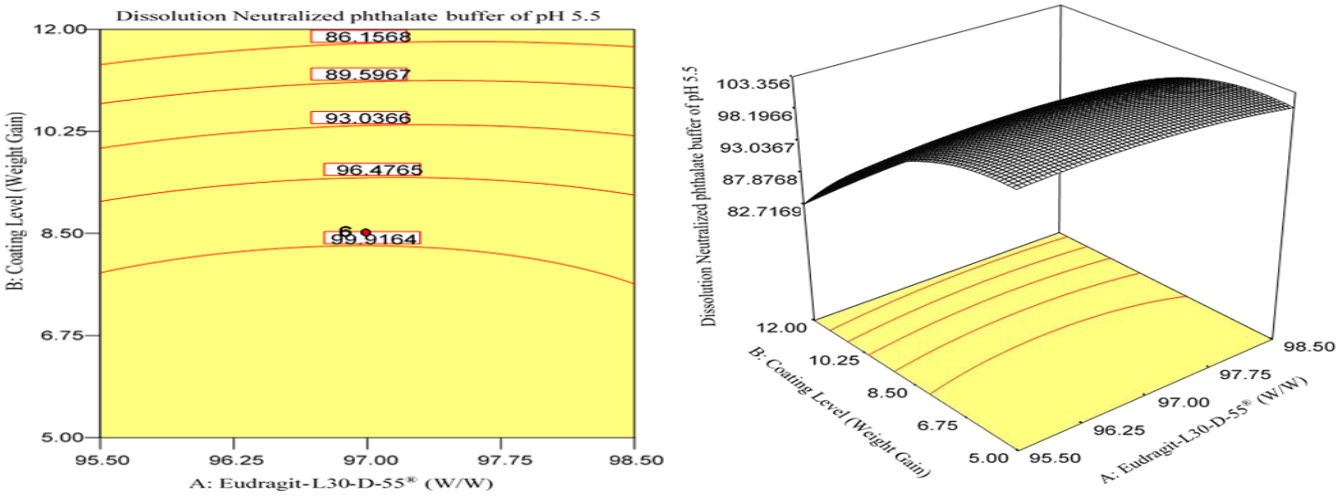
(a) (b)

Fig. 3.9: Contour plot (a) and response surface plot (b) of coating level and 1,2-propylene glycol percentage on disintegration time in 0.1 N HCl for tablets coated with Eudragit-L-30-D-55®



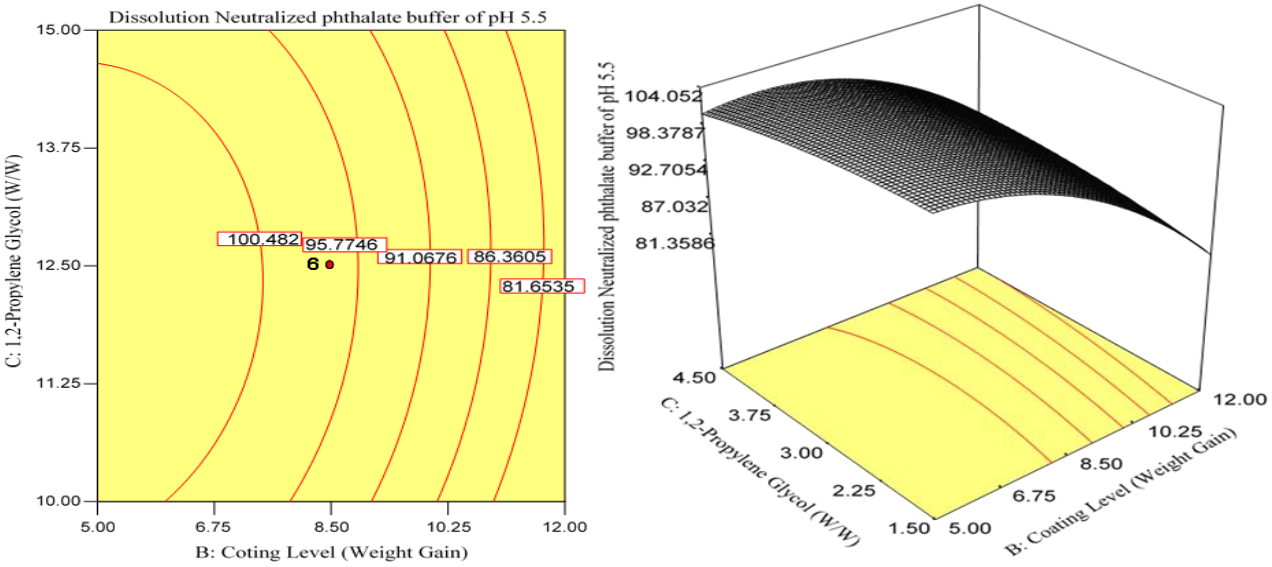
(c) (d)

Fig. 3.10: Contour plot (a) and response surface plot (b) of 1,2-propylene glycol percentage and coating level on dissolution in 0.06 N HCl for tablets coated with Eudragit-L-30-D-55®



(a) (b)

Fig. 3.11: Contour plot (a) and response surface plot (b) of coating level and Eudragit-L-30-D-55® percentage on dissolution in neutralized phthalate buffer of pH 5.5 for tablets coated with Eudragit-L- 30-D-55®.

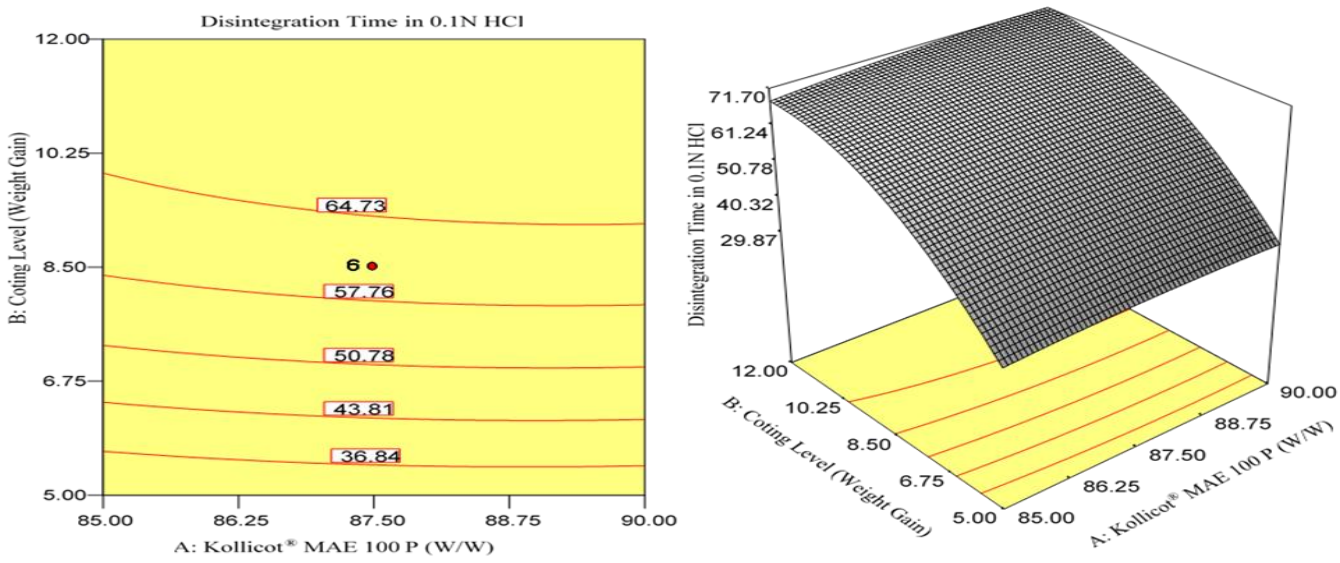


(a) (b)

Fig. 3.12: Contour plot (a) and response surface plot (b) of coating level and 1,2-propylene glycol percentage on dissolution in neutralized phthalate buffer of pH 5.5 for tablets coated with Eudragit-L-30-D-55®

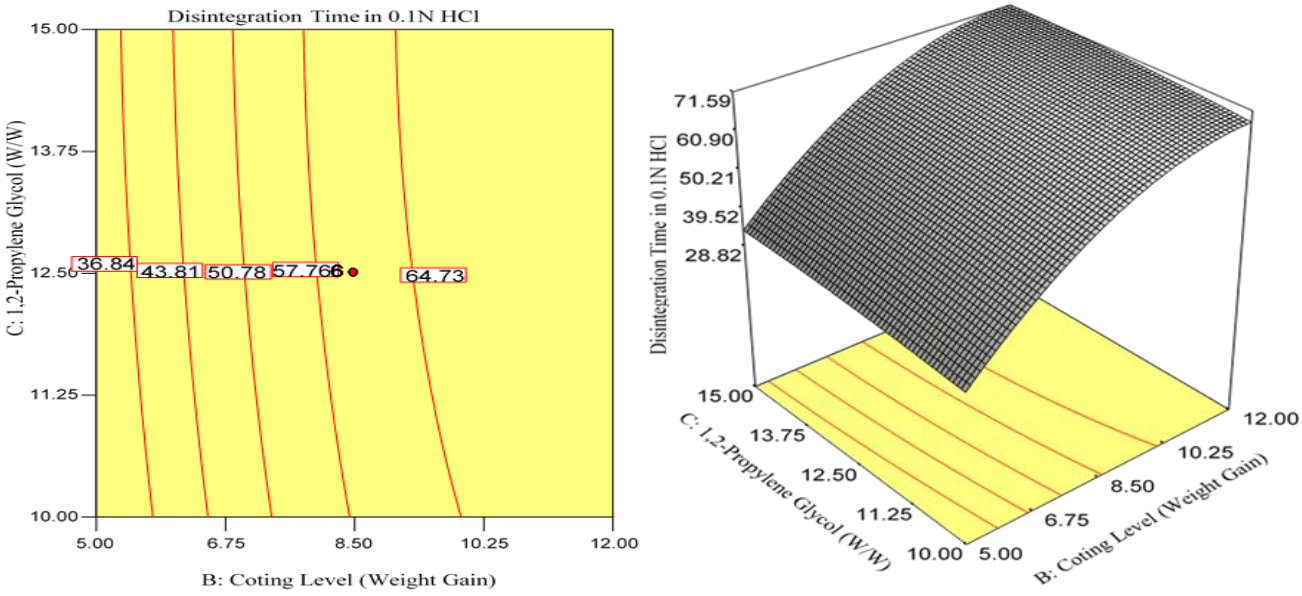
Considering tablets coated with Kollicot® MAE 100 P, the contour plot and response surface plot for the response variables of disintegration time in 0.1 N HCl (Fig 3.13a-b and 3.14a-b) showed that increase in coating level resulted in increase in disintegration time in 0.1 N HCl prominently with quadratic effect on disintegration time in 0.1 N HCl and as evident by nonlinear lines of contour plot and twisted line of response surface. The same result was obtained with ANOVA (Table 3.13, ***APPENDIX II***) with $p<0.0001$ and from the coefficients in mathematical model generated for disintegration time in 0.1 N HCl (Eq. 3.1b) with positive sign of +19.49. However, for the dissolution in 0.06 N HCl (Fig 3.15a-b and 3.16a-b), the plots show that there existed quadratic effect on dissolution in 0.06 N HCl and as evident by nonlinear lines of contour plot and twisted line of response surface. Henceforth with the solely decreased coating level, there was an increase in dissolution in 0.06 N HCl more pronouncedly. This was again confirmed by the coefficients in mathematical model generated for dissolution in 0.06 N HCl (Eq. 3.2b) with the negative sign coefficient of -16.29 and the same result was obtained in the ANOVA result (Table 3.14, ***APPENDIX II***) with $p<0.0001$. Likewise, for the dissolution in neutralized phthalate buffer of pH 5.5 (Fig 3.17a-b and 3.18a-b), the plots show that there existed quadratic effect on dissolution in neutralized phthalate buffer of pH 5.5 and as evident by nonlinear lines of contour plot and twisted line of response surface.

Hence with the solitary decrease in coating level, there was an increase in dissolution in neutralized phthalate buffer of pH 5.5 more prominently. This was again confirmed by the coefficients in mathematical model generated for dissolution in neutralized phthalate buffer of pH 5.5 (Eq. 3.3b) with the negative sign coefficient of -12.44 and the same result was obtained in the ANOVA result (Table 3.15, ***APPENDIX II***) with $p<0.0001$.



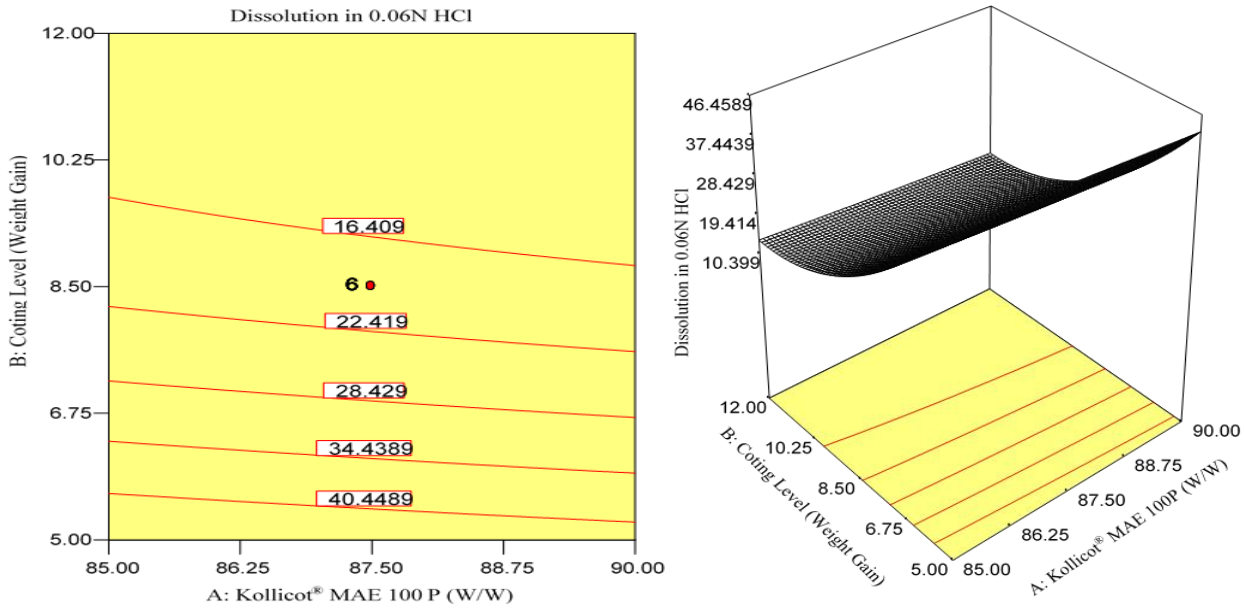
(a) (b)

Fig. 3.13: Contour plot (a) and response surface plot (b) of Kollicot® MAE 100 P percentage and coating level on disintegration time in 0.1 N HCl for tablets coated with Kollicot® MAE 100 P.



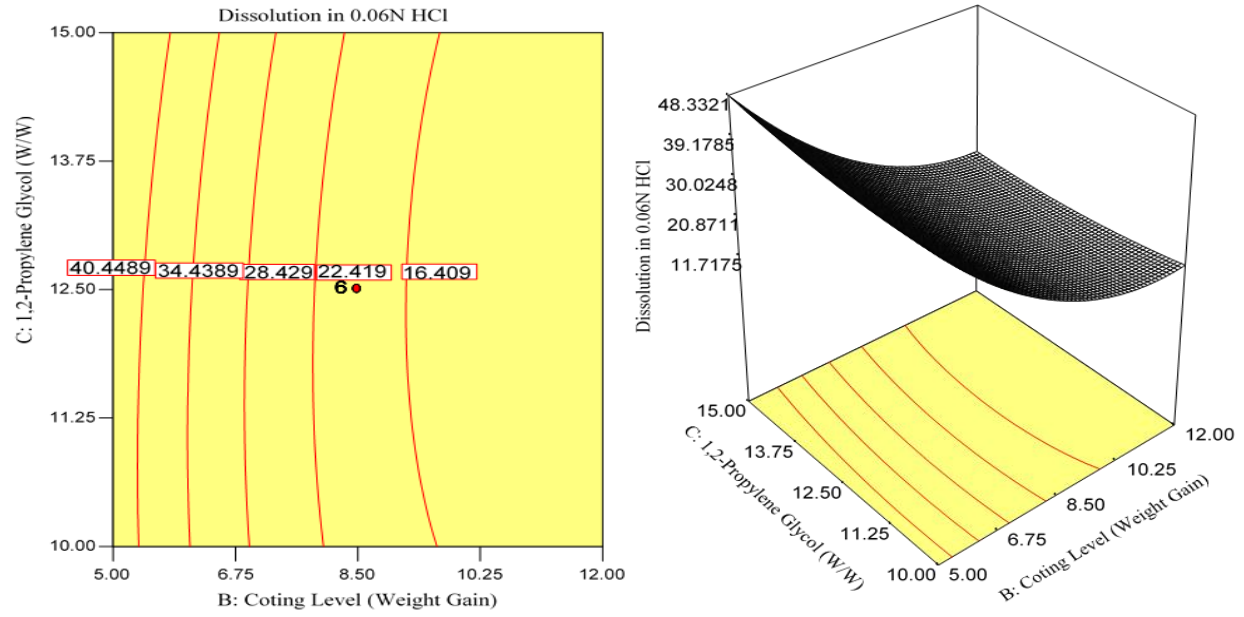
(a) (b)

Fig. 3.14: Contour plot (a) and response surface plot (b) of coating level and 1,2-propylene glycol percentage on disintegration time in 0.1 N HCl for tablets coated with Kollicot® MAE 100 P.



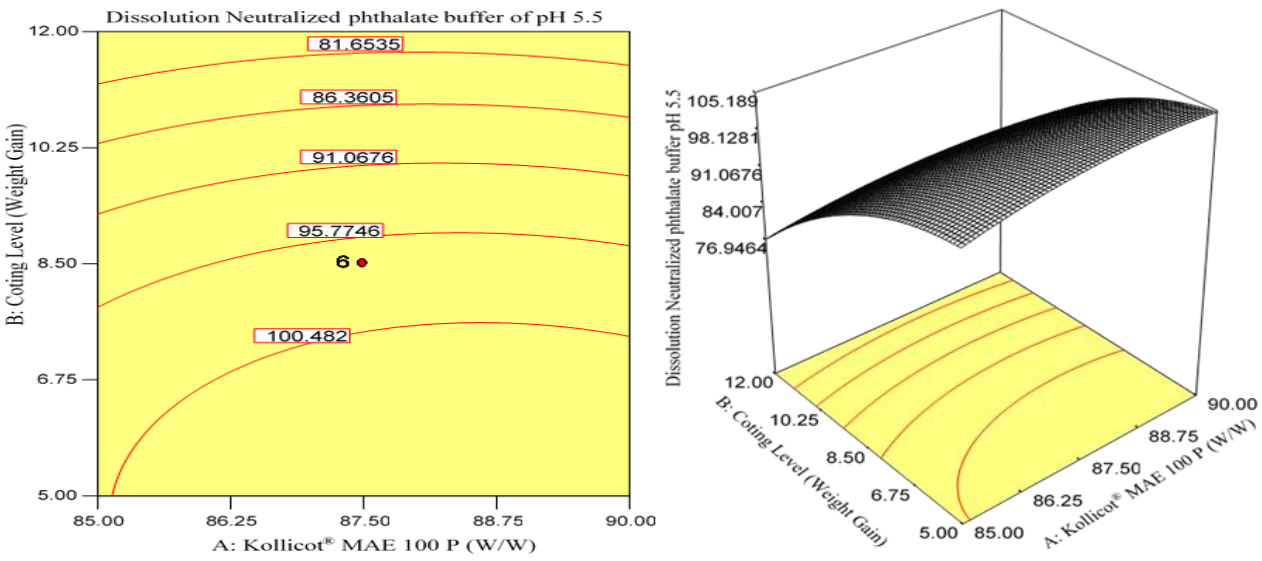
(a) (b)

Fig. 3.15: Contour plot (a) and response surface plot (b) of Kollicot® MAE 100 P percentage and coating level on dissolution in 0.06 N HCl for tablets coated with Kollicot® MAE 100 P.



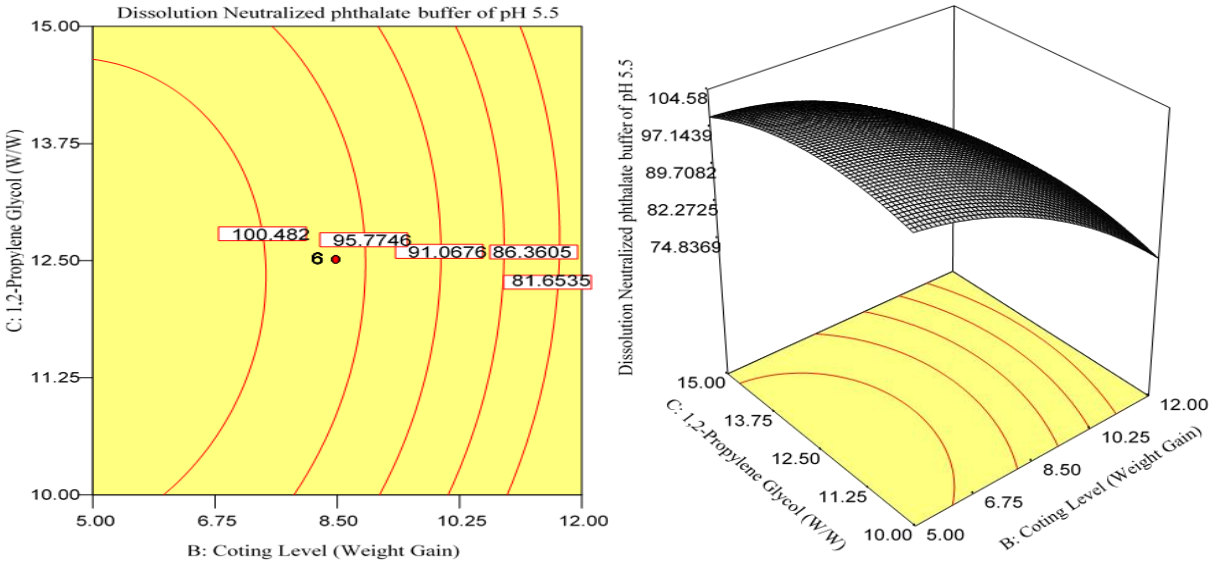
(a) (b)

Fig. 3.16: Contour plot (a) and response surface plot (b) of Coating level and 1,2-propylene glycol percentage on dissolution in 0.06 N HCl for tablets coated with Kollicot® MAE 100 P.



(a) (b)

Fig. 3.17: Contour plot (a) and response surface plot (b) of Kollicot® MAE 100 P percentage and coating level on dissolution in neutralized phthalate buffer of pH 5.5 for tablets coated with Kollicot® MAE 100 P.



(a) (b)

Fig. 3.18: Contour plot (a) and response surface plot (b) of coating level and 1,2-propylene glycol percentage on dissolution in neutralized phthalate buffer of pH 5.5 for tablets coated with Kollicot® MAE 100 P.

3.8 Simultaneous optimization of response variables for coating

The formulation was then optimized for the response variables: disintegration time in 0.1 N HCl, dissolution in 0.06 N HCl and dissolution in neutralized phthalate buffer of pH 5.5 simultaneously. For the purpose, both graphical and numerical techniques were used in an attempt to compromise various responses by satisfying the requirements for each response by setting factor ranges to the actual levels and the "target" at maximum values. The lower limit and the upper limit were set as indicated in Table 3.18 to get the desirability equation.

3.8.1 Numerical optimization

A numerical optimization technique, focusing on the desirability approach, was used to create the optimum settings for the desired formulation. Using the RSM method the global desirability function was obtained with the intention of optimizing multiple responses. With this method numerical optimization is used in order to find the specific points that maximize the overall desirability function. Based on Table 3.18, using design expert version 6.0.8 the predicted optimum values and the corresponding levels of parameters according to the set goals were obtained as presented in Fig 3.19a-c to 3.20a-c, where the dots indicate the best solution found by the design expert solver. In desirability approach the general desirability function which indicates how well the combined goals for all responses are satisfied was determined. Desirability function ranges from 0 to 1, with value closer to one indicates a higher satisfaction of response goals (Zou and Yuan, 2008; Khayet *et al.*, 2010).

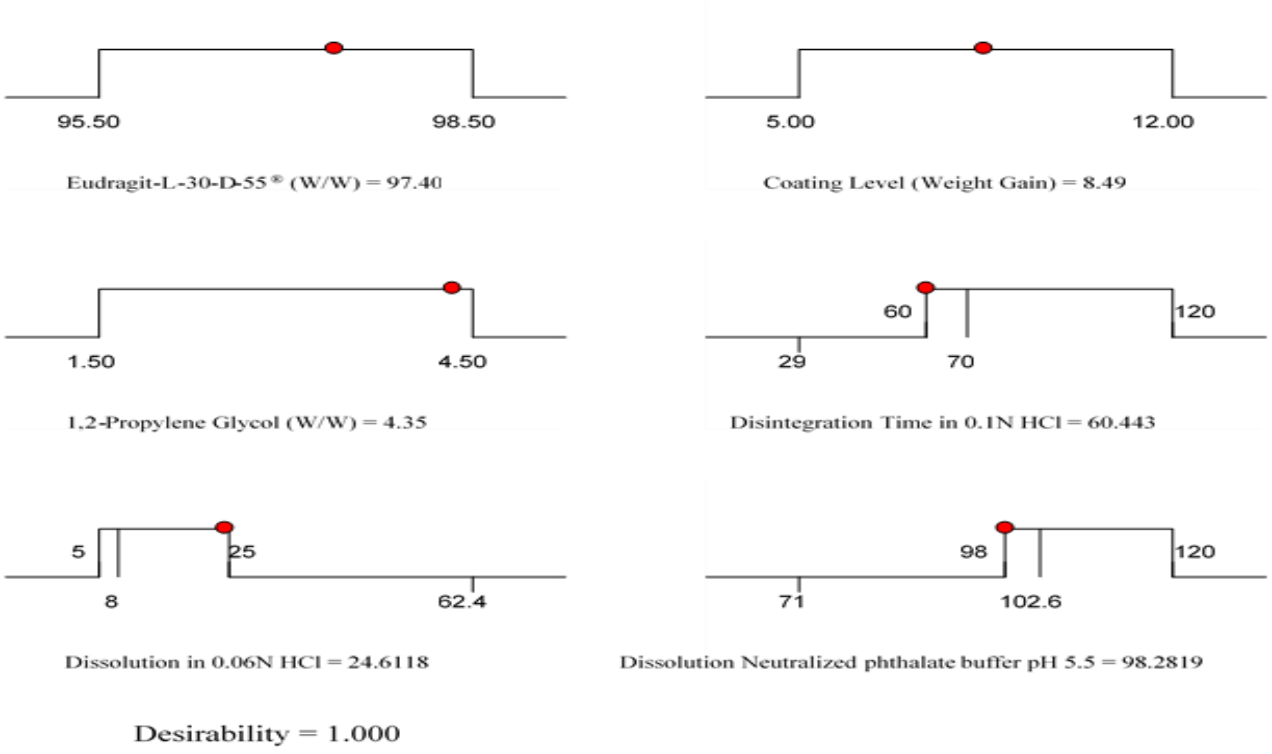
Thus, considering tablets coated with Eudragit-L-30-D-55®, the overall desirability was found to be 1.000 as calculated from the optimal points 97.40 for Eudragit-L-30-D-55® Percentage, 8.49 for coating level and 4.35 for plasticizer concentration (Fig. 3.19a). Likewise, for tablets coated with Kollicot® MAE 100 P, again the overall desirability was found to be 1.000 as calculated from the optimal points 89.63 for Kollicot® MAE 100 P percentage, 8.27 for coating level and 12.60 for plasticizer concentration (Fig. 3.20a).

Table 3.18: Criterion setting of factors and responses used during numerical and graphical optimization

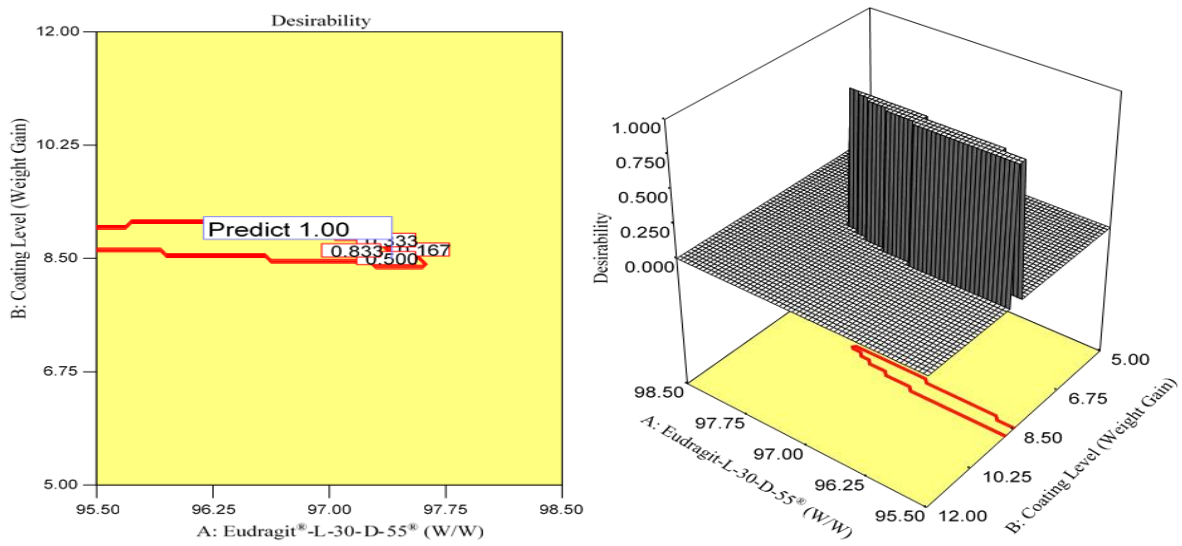
Formulation	Variables		Limit		Goal	Importance
	Factor	Response	Low	Higher		
FCP²E	Eudragit-L-30-D-55®(%)		95.5	98.5	In range	3
	Coating Level		5	12	In range	3
	1,2-Propylene Glycol (%)		1.5	4.5	In range	3
		Disintegration Time in 0.1N HCl	60	120	In range	3
		Dissolution in 0.06N HCl	5	25	In range	3
		Dissolution in Neutralized phthalate buffer of pH 5.5	98	120	In range	3
FCP²K	Kollicot®MAE 100 P (%)		85	90	In range	3
	Coating level		5	12	In range	3
	1,2-Propylene glycol (%)		10	15	In range	3
		Disintegration time in 0.1N HCl	60	120	In range	3
		Dissolution in 0.06N HCl	5	25	In range	3
		Dissolution in neutralized phthalate buffer of pH 5.5	98	120	In range	3

3.8.2 Graphical optimization

Considering the criteria established in Table 3.18 and overlaying of the models obtained from CCD, graphical optimization was done to definitely indicating out the optimal conditions of disintegration time in 0.1 N HCl, dissolution in 0.06 N HCl and dissolution in neutralized phthalate buffer of pH 5.5. Thus as depicted in Fig.3.21, the overlay plot in which the yellow area denotes the area complying the set criteria, which was plotted using 1,2-PG percentage and coating level for tablets coated with Eudragit-L30-D-55® and Kollicot® MAE 100 P and coating level for tablets coated with Kollicot® MAE 100 P. The area identified by yellow color was preferred as representative of the optimized area that was obtained by numerical optimization in Fig. 3.19a and 3.20b.

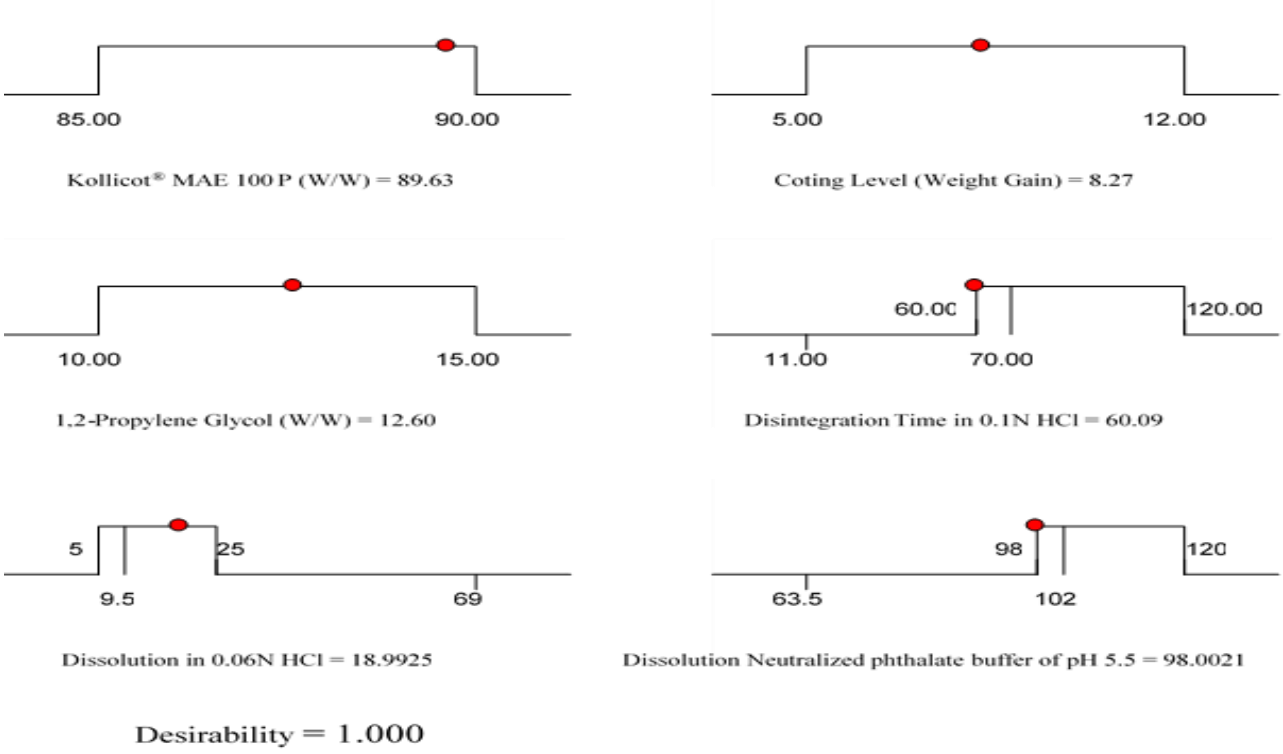


(a)

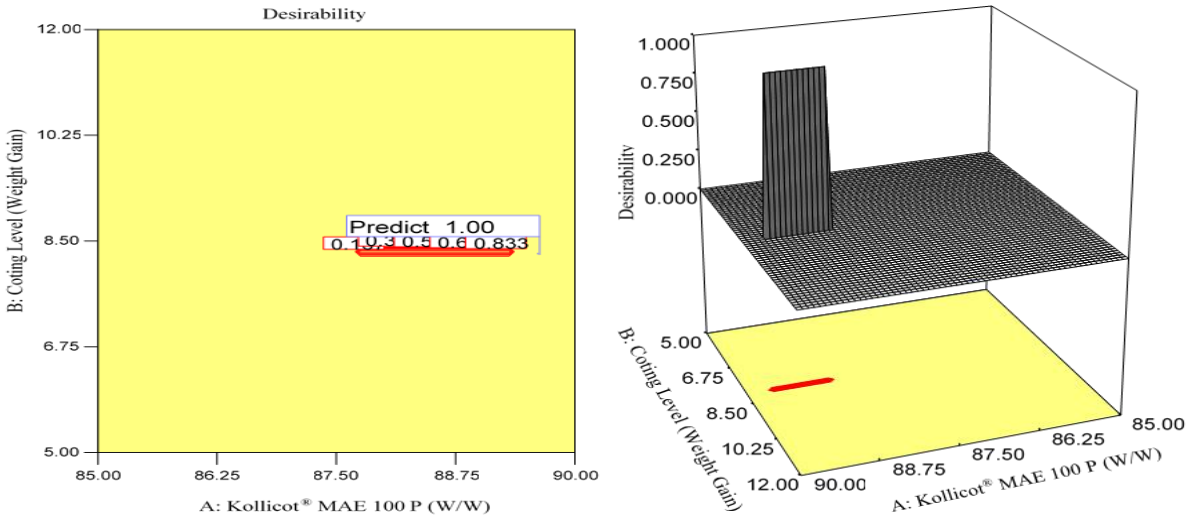


(b) (c)

Fig. 3.19: Numerical optimization results of predicted optimum values and the corresponding levels of parameters (a), contour plot (b) and the surface response plot (c) of the overall desirability function for tablets coated with Eudragit-L-30-D-55®.

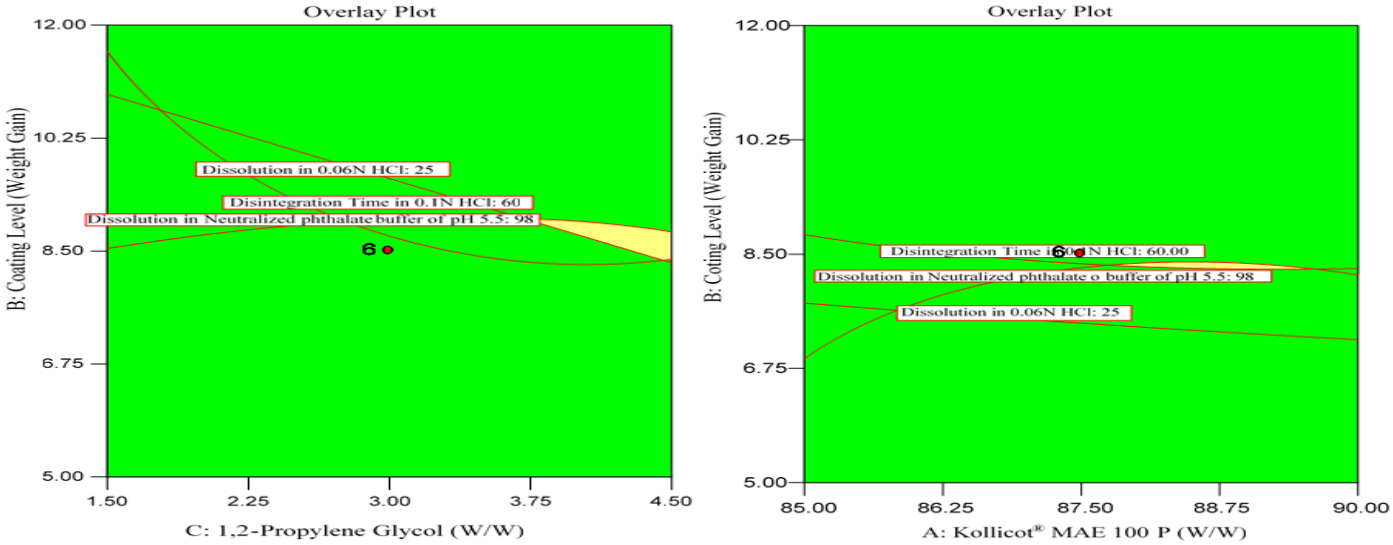


(a)



(b) (c)

Fig. 3.20: Numerical optimization results of predicted optimum values and the corresponding levels of parameters (a), contour plot (b) and the surface response plot (c) of the overall desirability function for tablets coated with Kollicot® MAE 100 P.



(a) (b)

Fig. 3.21: Overlay plot of disintegration time in 0.1 N HCl, dissolution in 0.06 N HCl and dissolution in neutralized phthalate buffer of pH 5.5 as a function of 1,2-propylene glycol percentage and coating level for tablets coated with Eudragit-L-30-d-55® (a) and as a function of Kollicot® MAE 100 P and coating level for tablets coated with Kollicot® MAE 100 P (b).

3.8.3 Confirmation test

Confirmation experiments were conducted in a triplicate at the optimal combinations of the factors that coated with specified polymer to confirm the validity of achieved optimal points during optimization. Tablets were prepared based on the optimization formulation and the coated tablets were evaluated for disintegration time in 0.1 N HCl, dissolution in 0.06 N HCl and dissolution in neutralized phthalate buffer of pH 5.5. As shown in Table 3.19, the predicted values and experimental results were in agreement and the percentage error values obtained at optimal levels of the factors were within 5 %, confirming that the experimental values of the optimized formulations agreed with the predicted values.

Table 3.19: Experimentally prepared formulations based on the predicted values and the evaluation of disintegration time in 0.1 N HCl, dissolution in 0.06 N HCl and dissolution in neutralized phthalate buffer of pH 5.5 for the two polymers

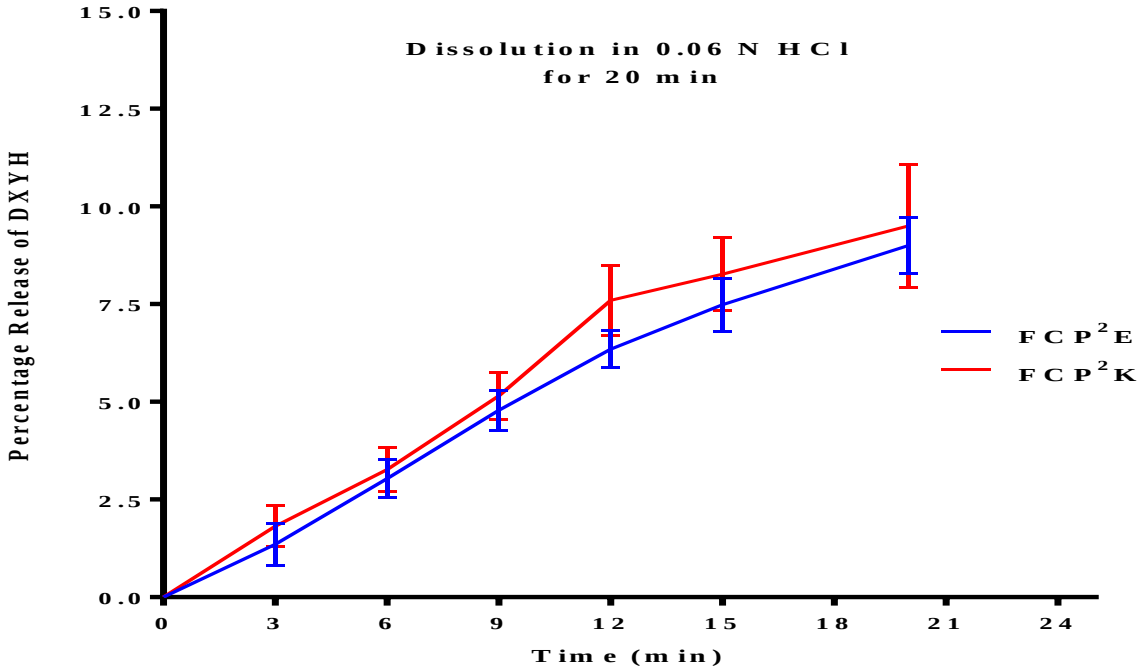
Responses	**Predicted Value for FCP^2E**	**Experimental Value for FCP^2E**	**Percentage error**
Disintegration time in 0.1 N HCl	60.4*	62.2	2.9
Dissolution in 0.06 N HCl	24.6*	23.7	3.8
Dissolution in neutralized phthalate buffer of pH 5.5	98.3*	99.5	1.2
Responses	**Predicted Value for FCP^2K**	**Experimental Value for FCP^2K**	**Percentage error**
Disintegration time in 0.1 N HCl	60.1*	61.7	2.6
Dissolution in 0.06N HCl	19.0*	18.5	2.7
Dissolution in Neutralized phthalate buffer of pH 5.5	98.0*	98.9	0.9

** The predicted values are rounded to the nearest digit.*

According to the result in Table 3.19, the predicted values and experimental results were in agreement and the percentage error values obtained at optimal levels of the factors were within 5%, confirming that the experimental values of the optimized formulations agreed with the predicted values.

3.9 Release profile of optimized formulations

According to Fig 3.22 A, the cumulative release profile was observed from those tablets coated with Eudragit-L-30-D-55® (9± 0.71%) and Kollicot® MAE 100 P (9.5± 1.58 %) in 0.06 N HCl acid media. Similarly, as shown in Table 3.22 B, the cumulative release profile in neutralized phthalate buffer of 5.5 pH was observed from tablets coated with Eudragit-L-30-D-55® (99.3± 1.89 %) and Kollicot® MAE 100 P (98.5± 2.58 %). Comparable release profiles were observed from both formulations and hence, were complied with USP 36/NF31, <711>, 2013.



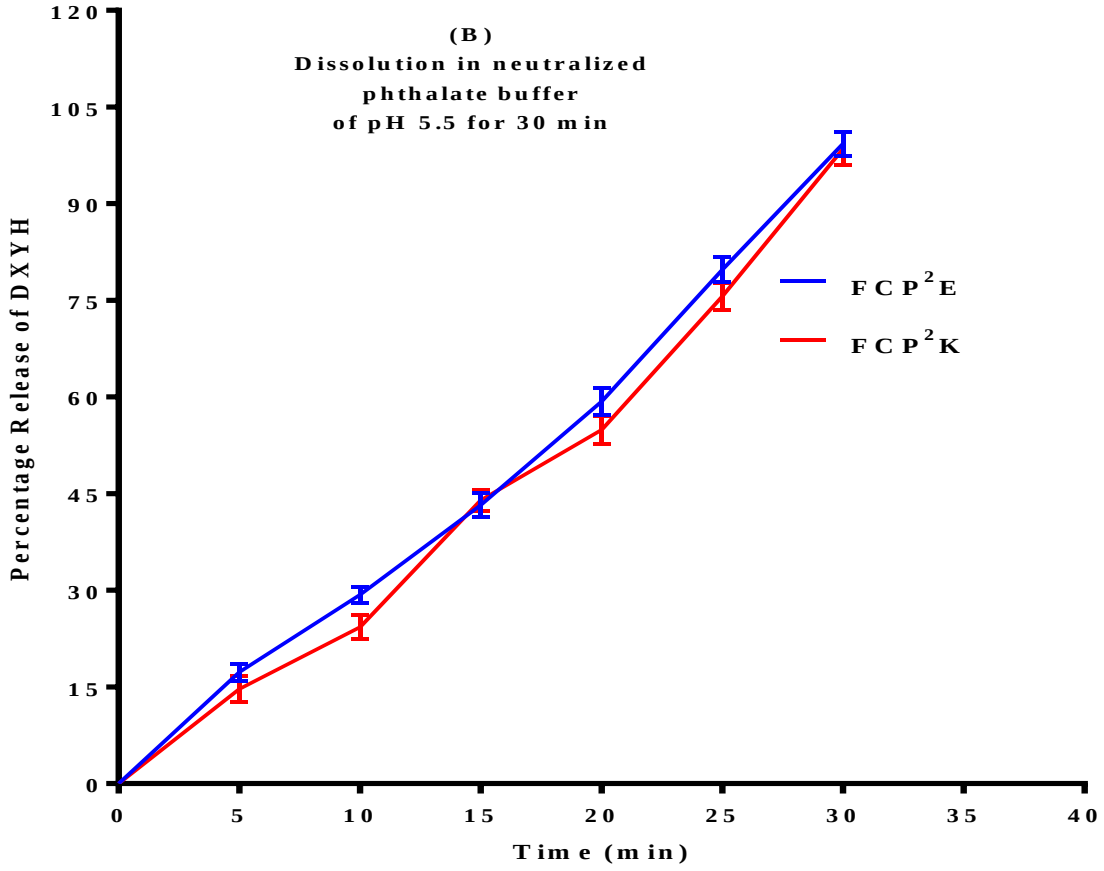


Fig. 3.22: *In vitro* release profile of test Doxycycline Hyclate coated with Eudragit-L-30-D-55® blue and Kollicot® MAE 100 P red A) in Dissolution in 0.06 N HCl within 20 min B) in neutralized phthalate buffer of pH 5.5 within 30 min (n=6, Mean ± SD**)**

3.10 Accelerated Stability study of the optimized batches

The accelerated stability study results clearly demonstrated that the optimized batches: FCP^{2}E-14 and FCP^{2}K-18 coated tablets were robust and all the tested parameters were remained without any significant changes within the studied stability period as reported in Table 3.20 and 3.21. The result in the tables clearly indicate that accelerated study showed no significant variation from initial in physical characteristics, hardness, no obvious defects or signs of peeling or chipping and the coated tablets showed complete disintegration and dissolution at the end of 1st, 2nd and 3rd month. Hence

it is concluded that the optimized enteric coated tablets were stable and the data obtained could be used to predict the shelf life of the product.

Table 3.20: Accelerated stability study of optimized batch (FCP^2E-14) carried out at 40ºC ± 2ºC / 75% RH ± 5% RH

	Study period			
Parameters	**0 time (Initial)**	**1st month**	**2nd month**	**3rd month**
Avg. Wt. (mg) N=10	260.65±1.02	260.42±0.92	260.57±1.32	260.62±1.41
Hardness (kg/cm2) N=10	10.90± 0.42	10.97± 0.63	10.42± 1.02	10.23± 1.39
Thickness (mm) N=10	5.05±0.01	5.04±0.02	5.04±0.01	5.06±0.02
Disintegration Time in 0.1N HCl N=6	65.00±0.64	66.0±0.73	65.52±0.94	68.34±1.35
Dissolution in 0.06 N HCl in 20 min N=6	9.00± 0.71	9.50± 0.95	10.50± 1.03	11.50± 1.23
Dissolution in 5.5 neutralized phthalate buffer in 30 min N=6	99.30± 1.89	98.92± 1.49	98.47± 1.28	97.98± 1.36
Assay (%)	102.5	101.9	100.7	100.1

Table 3.21: Accelerated stability study of optimized batch (FCP^2K-18) carried out at 40ºC ± 2ºC / 75% RH ± 5% RH

	Study period			
Parameters	**0 time (Initial)**	**1st month**	**2nd month**	**3rd month**
Avg. Wt. (mg) N=10	260.87±1.33	260.62±1.69	260.47±1.21	260.91±1.68
Hardness (kg/cm2) N=10	11.14± 0.99	11.62± 1.03	10.94± 0.89	11.05± 1.10
Thickness (mm) N=10	5.08±0.01	5.06±0.01	5.07±0.01	5.08±0.01
Disintegration Time in 0.1N HCl N=6	67.00±0.94	66.50±0.93	66.00±1.12	65.00±1.21
Dissolution in 0.06 N HCl in 20 min N=6	9.50± 1.58	10.50± 1.23	11.32± 1.30	11.98± 1.42
Dissolution in 5.5 neutralized phthalate buffer in 30 min N=6	98.50± 2.58	98.05± 2.12	97.81± 2.35	97.02± 2.49
Assay (%)	102.0	101.7	101.1	100.2

4. CONCLUSION

The investigation was intended to exploit the aqueous based coating composition to develop an enteric tablet of DXYH, which can resist the release of drug in the acid milieu and immediately release its contents on contact with the basic pH. Crospovidone, among other disintegrants was ascertained to be the appropriate ingredient to initiate rapid disintegration, better hardness and friability of the core tablets.

Following optimization of coating process, formulations FCP^2E-14 and FCP^2K-18 were found to have comparable release profile in both neutralized phthalate buffer of pH 5.5 and 0.06 N acidic media. The release profile of FCP^2E-14 was 99.3± 1.89 % and 9± 0.71 % whereas for FCP^2K-18 was 98.5± 2.58 % and 9.5+ 1.58 % within 30 min and 20 min in neutralized phthalate buffer of pH 5.5 and 0.06 N acidic media, respectively. Apart from this, both formulations was resistant in simulated gastric acid (0.1 N HCl) for 1 h and complied with USP 36/NF 31, 2013 pharmacopeial specification.

Confirmatory experiment revealed that the predicted values and experimental results were in agreement and the percentage error values obtained at optimal levels of the factors were within 5 %, confirming that the experimental values of the optimized formulations agreed with the predicted values. In addition to that, the coating process for both systems was free of problems and resulted in highly smooth coated tablets with no visible defects.

The results of accelerated stability studies for optimized batches showed no significant changes in the physical parameters of the tablets, drug content and *in-vitro* dissolution data until the end of 3 months from the initial values. Hence it is concluded that the formulated DXYH enteric coated tablets were stable and this study fulfilled all the pharmacopoeial specifications.

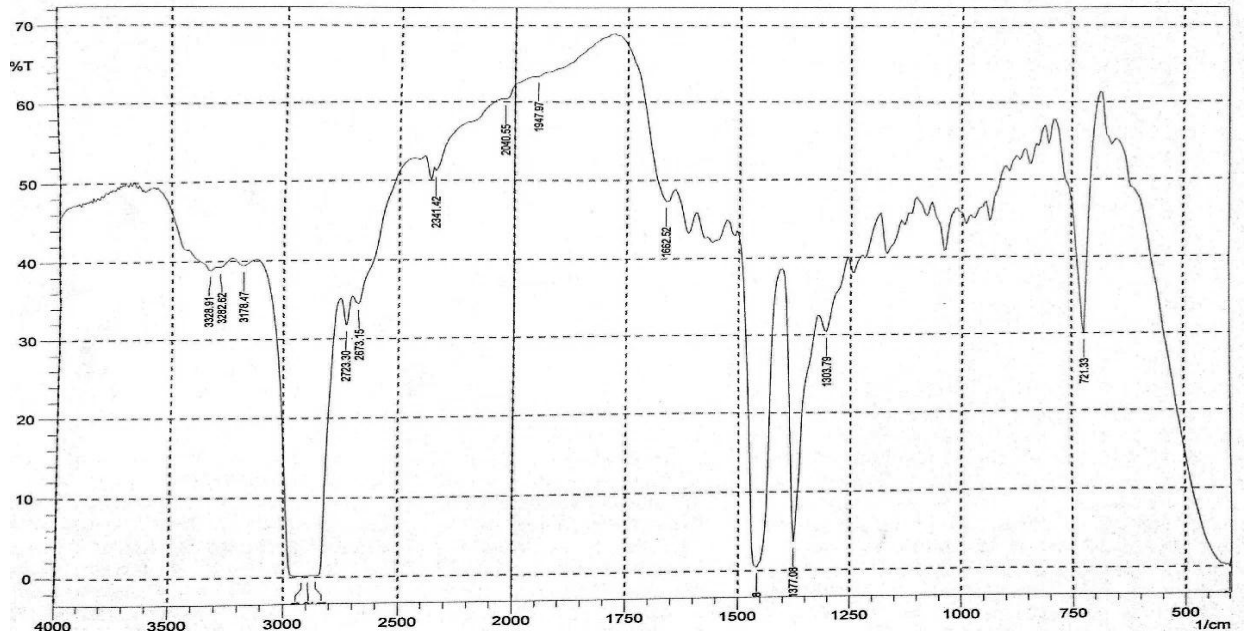
SUGGESTIONS FOR FURTHER WORK

Further investigations in the following direction are suggested:

- Real time stability studies of the coated tablets.
- Pilot scale and production scale trial.
- Similarity factor (f_2) study with commercial enteric coated product as no enteric coated tablets of Doxycycline Hyclate was available in the country during the study.

APPENDIX I

(a)



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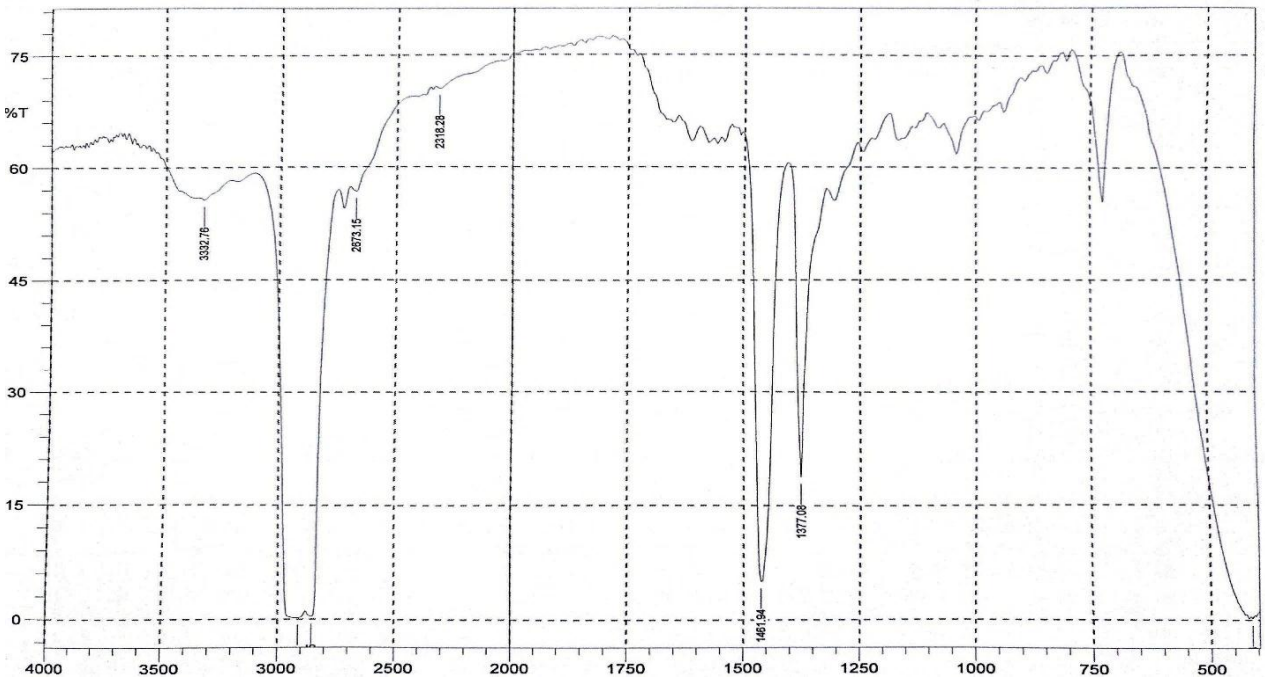

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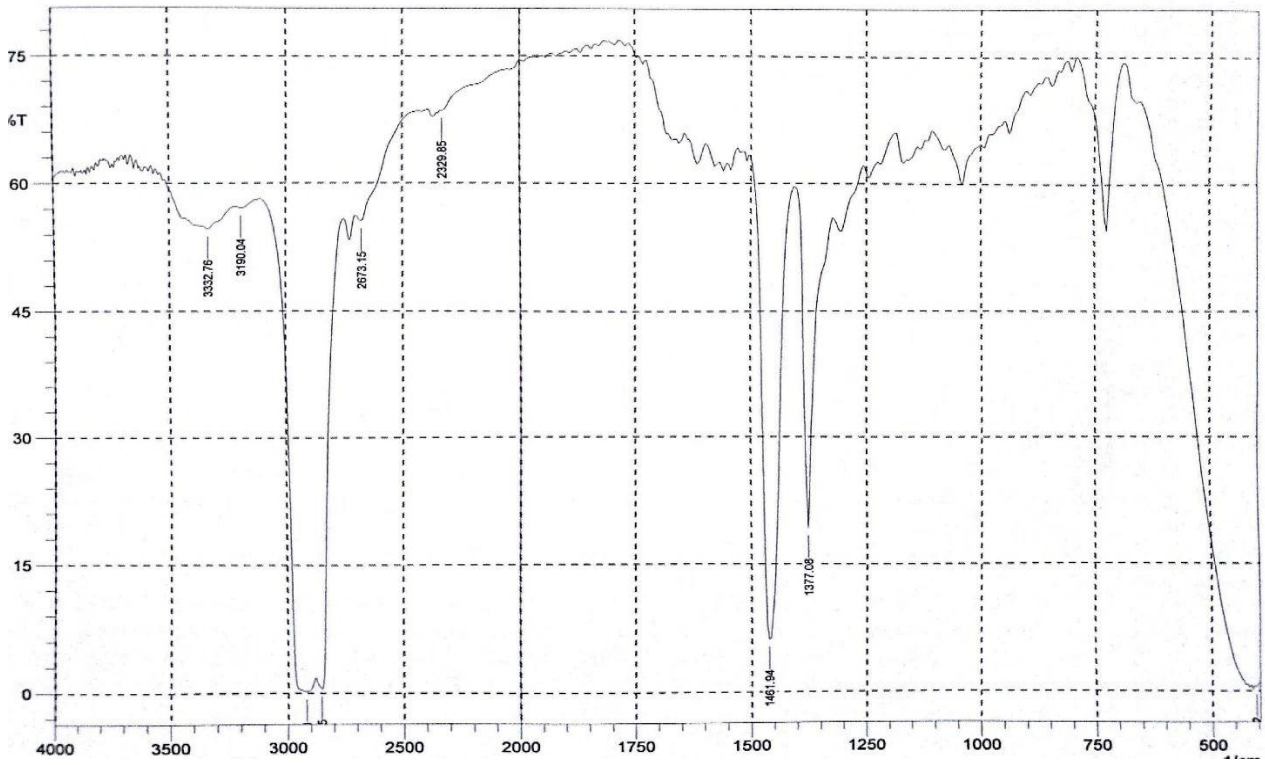
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75
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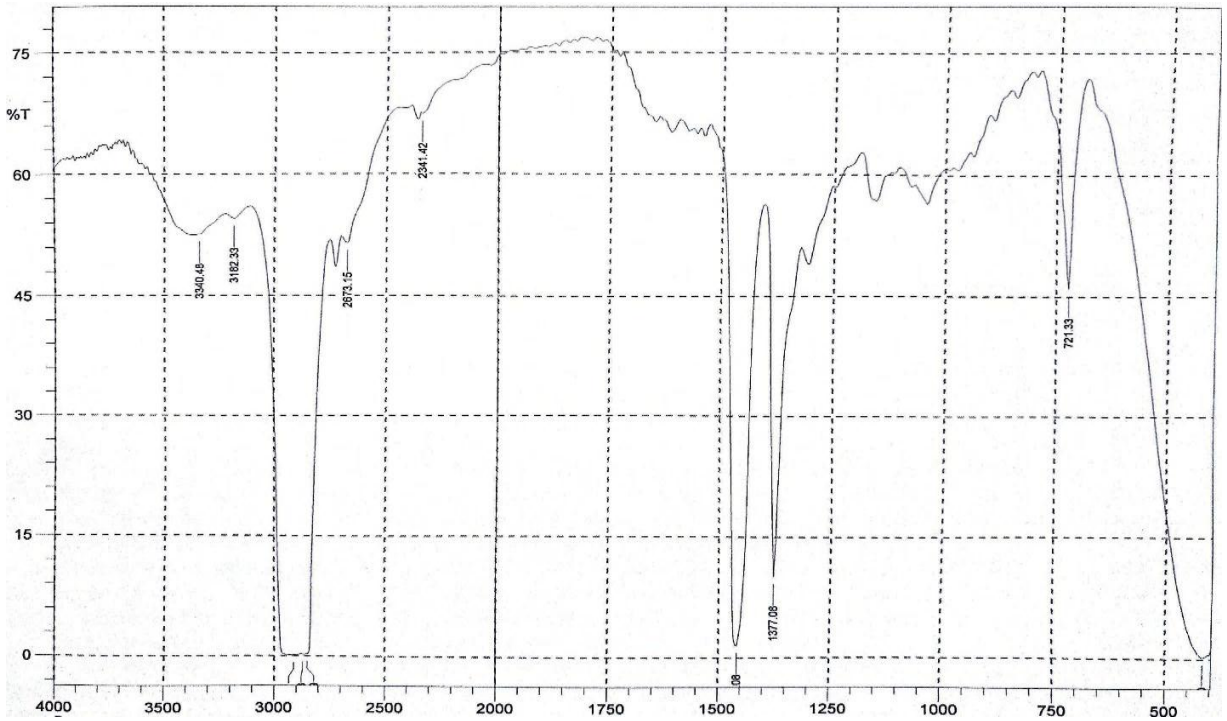
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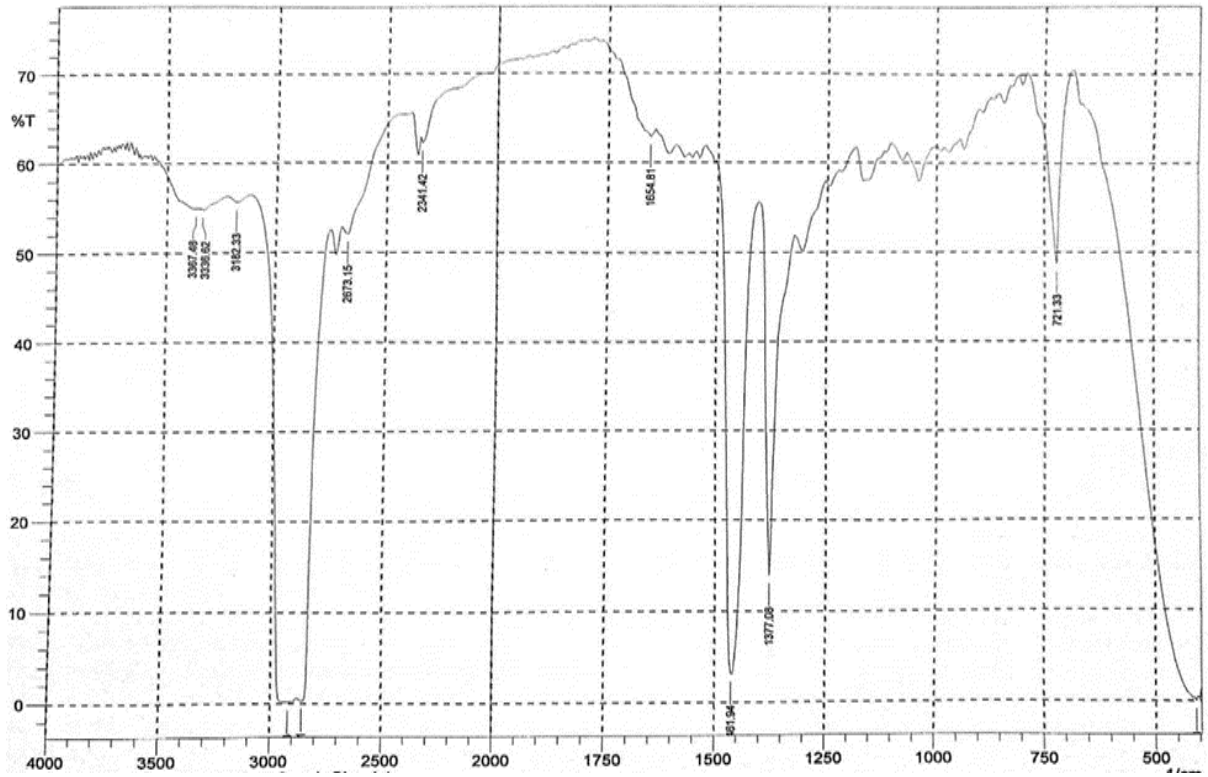
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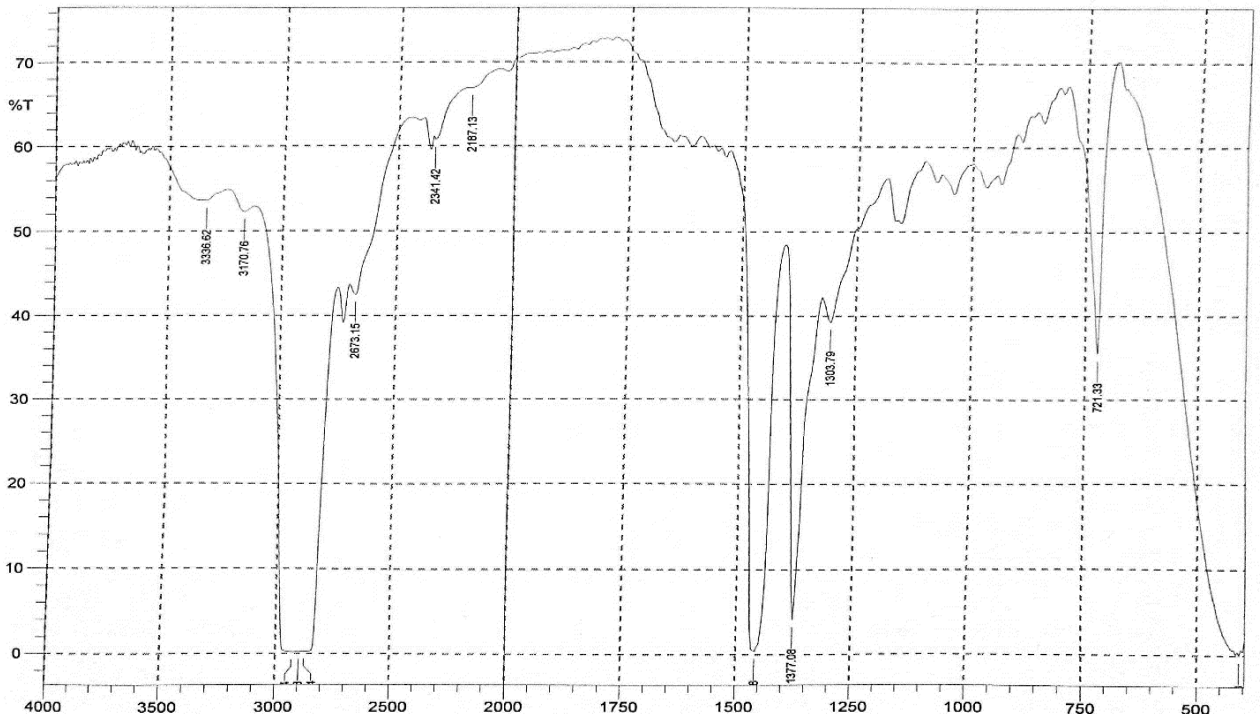
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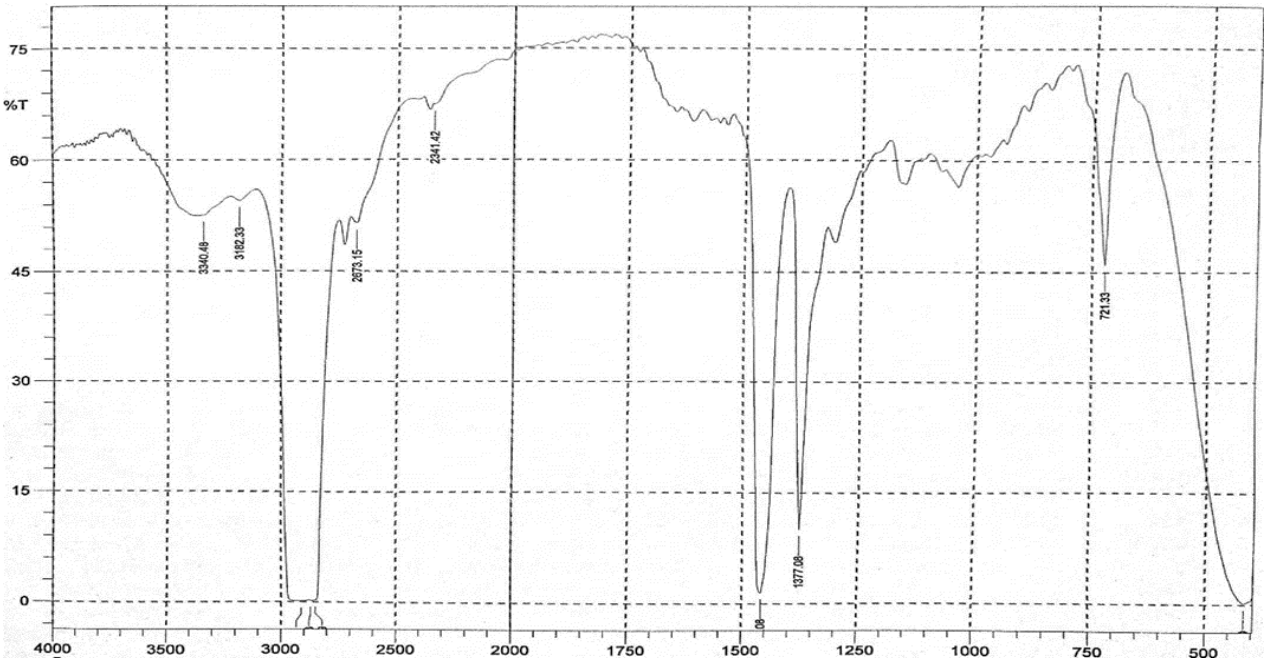
(g)



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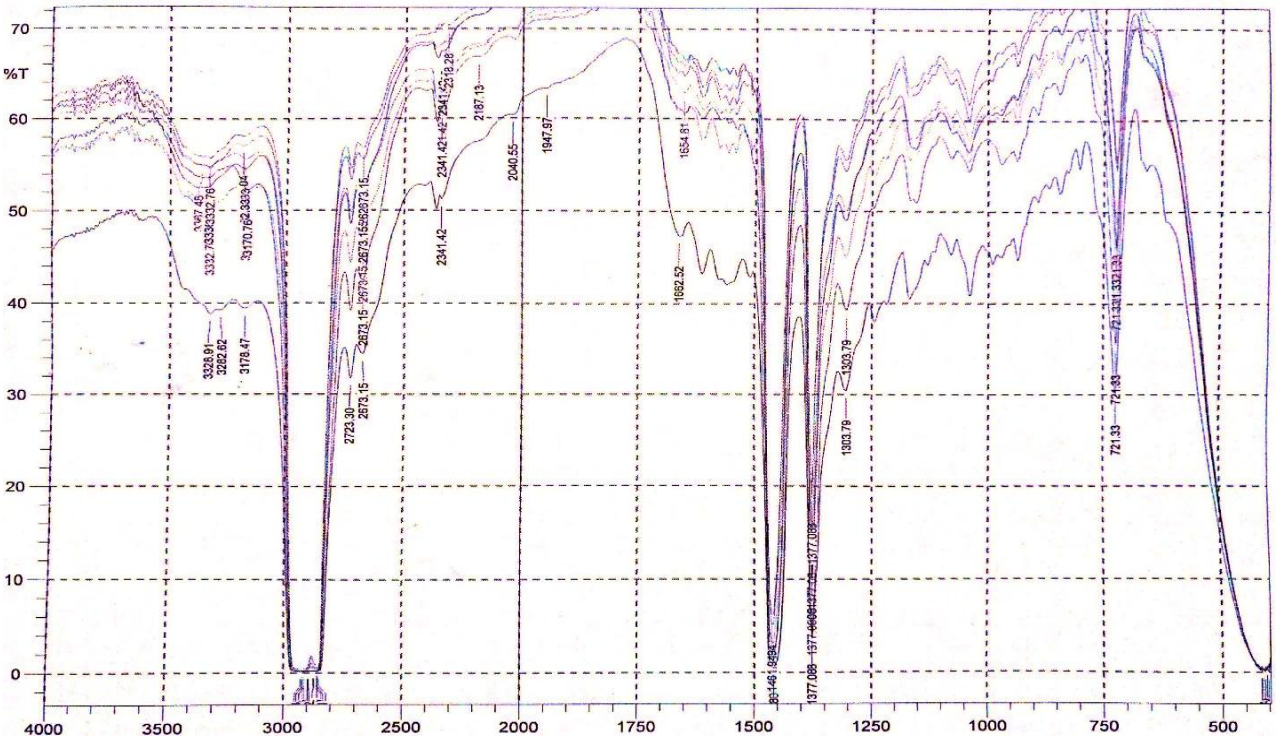

Fig. 3.2: FT-IR spectra of pure DXYH (a); Uncoated DXYH of FCCNa (b), FSSG (c), FDS (d) and FCP (e); coated with Kollicot® MAE 100 P (f), and coated Eudragit-L-30-D-55® (g); merged FT-IR spectra of all (a-g) (h)

Appendix II

Table 3.4: Coating design matrix coated with Kollicot® MAE 100P

Formulation Code	Point Type	Block	Factors (Actual and coded Value)		
			A: Kollicot® MAE 100P (W/W) (%)	B: Coating level (Weight gain) (%)	C: 1,2-PG (W/W) (%)
FCP^{2}K-1	Fact	1	90.00 (+1)	12.00 (+1)	15.00 (+1)
FCP^{2}K-2	Fact	1	85.00 (-1)	12.00 (+1)	15.00 (+1)
FCP^{2}K-3	Fact	1	85.00 (-1)	5.00 (-1)	10.00 (-1)
FCP^{2}K-4	Fact	1	90.00 (+1)	12.00 (+1)	10.00 (-1)
FCP^{2}K-5	Fact	1	90.00 (+1)	5.00 (-1)	15.00 (+1)
FCP^{2}K-6	Fact	1	90.00 (+1)	5.00 (-1)	15.00 (-1)
FCP^{2}K-7	Center	1	87.50 (0)	8.50 (0)	12.50 (0)
FCP^{2}K-8	Fact	1	85.00 (-1)	12.00 (+1)	10.00 (-1)
FCP^{2}K-9	Center	1	87.50 (0)	8.50 (0)	12.50 (0)
FCP^{2}K-10	Fact	1	85.00 (-1)	5.00 (-1)	15.00 (+1)
FCP^{2}K-11	Center	1	87.50 (0)	8.50 (0)	12.50 (0)
FCP^{2}K-12	Center	1	87.50 (0)	8.50 (0)	12.50 (0)
FCP^{2}K-13	Center	2	87.50 (0)	8.50 (0)	12.50 (0)
FCP^{2}K-14	Axial	2	87.50 (0)	14.39 (+α)	12.50 (0)
FCP^{2}K-15	Axial	2	83.30 (-α)	8.50 (0)	12.50 (0)
FCP^{2}K-16	Axial	2	87.50 (0)	2.61 (-α)	12.50 (0)
FCP^{2}K-17	Center	2	87.50 (0)	8.50 (0)	12.50 (0)
FCP^{2}K-18	Axial	2	91.70 (+α)	8.50 (0)	12.50 (0)
FCP^{2}K-19	Axial	2	87.50 (0)	8.50 (0)	8.30 (-α)
FCP^{2}K-20	Axial	2	87.50 (0)	8.50 (0)	16.70 (+α)

Table 3.5: Coating design matrix coated with Eudragit-L-30-D-55®.

Formulation Code	Point Type	Block	Factors (Actual and coded Value)		
			A: Eudragit-L-30-D-55® (W/W) (%)	B: Coating level (Weight gain) (%)	C: 1,2-PG (W/W) (%)
FCP^{2}E-1	Center	1	97.00 (0)	8.50 (0)	3.00 (0)
FCP^{2}E-2	Fact	1	98.50 (+1)	5.00 (-1)	1.50 (-1)
FCP^{2}E-3	Fact	1	95.50 (-1)	5.00 (-1)	1.50 (-1)
FCP^{2}E-4	Fact	1	98.50 (+1)	5.00 (-1)	4.50 (-1)
FCP^2 E-5	Center	1	97.00 (0)	8.50 (0)	3.00 (0)
FCP^{2}E-6	Fact	1	95.50 (-1)	12.00 (+1)	4.50 (+1)
FCP^{2}E-7	Center	1	97.00 (0)	8.50 (0)	3.00 (0)
FCP^{2}E-8	Center	1	97.00 (0)	8.50 (0)	3.00 (0)
FCP^{2}E-9	Fact	1	95.50 (-1)	5.00 (-1)	4.50 (+1)
FCP^{2}E-10	Fact	1	98.50 (+1)	12.00 (+1)	4.50 (+1)
FCP^{2}E-11	Fact	1	98.50 (+1)	12.00 (+1)	1.50 (-1)
FCP^{2}E-12	Fact	1	95.50 (-1)	12.00 (+1)	1.50 (-1)
FCP^{2}E-13	Center	2	97.00 (0)	8.50 (0)	3.00 (0)
FCP^{2}E-14	Axial	2	97.00 (0)	8.50 (0)	5.52 (+α)
FCP^{2}E-15	Axial	2	97.00 (0)	8.50 (0)	0.48 (-α)
FCP^{2}E-16	Axial	2	97.00 (0)	2.61 (-α)	3.00 (0)
FCP^{2}E-17	Center	2	97.00 (0)	8.50 (0)	3.00 (0)
FCP^{2}E-18	Axial	2	99.52 (+α)	8.50 (0)	3.00 (0)
FCP^{2}E-19	Axial	2	97.00 (0)	14.39 (+α)	3.00 (0)
FCP^{2}E-20	Axial	2	94.48 (-α)	8.50 (0)	3.00 (0)

Table 3.6: Trial batches for optimization of process parameters and evaluation responses of tablets coated with Eudragit-L-30-D-55®

Formulation code	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
	A: Eudragit-L-30-D-55® (W/W) (%)	B: Coating level (Weight gain) (%)	C: 1,2-PG (W/W) (%)	Disintegration time in 0.1 N HCl (min)	Dissolution in 0.06 N HCl (%)	Dissolution in neutralized phthalate buffer of pH 5.5 (%)
FCP^{2}E-1	97.00	8.50	3.00	58.50	30.00	99.00
FCP^{2}E-2	98.50	5.00	1.50	34.00	43.00	99.60
FCP^{2}E-3	95.50	5.00	1.50	34.50	41.00	99.00
FCP^{2}E-4	98.50	5.00	4.50	36.00	39.00	96.50
FCP^2 E-5	97.00	8.50	3.00	59.00	28.80	98.50
FCP^{2}E-6	95.50	12.00	4.50	64.00	16.00	84.00
FCP^{2}E-7	97.00	8.50	3.00	59.60	29.00	98.00
FCP^{2}E-8	97.00	8.50	3.00	59.30	30.20	99.40
FCP^{2}E-9	95.50	5.00	4.50	42.00	37.00	99.40
FCP^{2}E-10	98.50	12.00	4.50	65.50	14.00	81.00
FCP^{2}E-11	98.50	12.00	1.50	62.50	19.00	79.00
FCP^{2}E-12	95.50	12.00	1.50	60.90	18.00	71.00
FCP^{2}E-13	97.00	8.50	3.00	58.00	30.00	97.80
FCP^{2}E-14	97.00	8.50	5.52	65.00	9.00	99.30
FCP^{2}E-15	97.00	8.50	0.48	37.00	41.00	102.60
FCP^{2}E-16	97.00	2.61	3.00	29.00	62.40	100.90
FCP^{2}E-17	97.00	8.50	3.00	59.00	31.00	98.70
FCP^{2}E-18	99.52	8.5	3.00	59.70	25.00	98.70
FCP^{2}E-19	97.00	14.39	3.00	70.00	8.00	74.00
FCP^{2}E-20	94.48	8.50	3.00	55.00	33.00	100.20

Table 3.7: Trial batches for optimization of process parameters and evaluation responses of tablets coated with Kollicot® MAE 100 P

Formulation code	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
	A: Kollicot MAE 100P (W/W) (%)	B: Coting level (Weight Gain) (%)	C: 1,2-PG (W/W) (%)	Disintegration rime in 0.1 N HCl (Min)	Dissolution in 0.06 N HCl (%)	Dissolution in neutralized phthalate buffer of pH 5.5 (%)
FCP^{2}K-1	90.00	12.00	15.00	68.00	17.20	72.10
FCP^{2}K-2	85.00	12.00	15.00	63.00	16.80	73.50
FCP^{2}K-3	85.00	5.00	10.00	20.00	45.00	102.00
FCP^{2}K-4	90.00	12.00	10.00	65.00	18.00	71.00
FCP^{2}K-5	90.00	5.00	15.00	25.00	49.00	99.00
FCP^{2}K-6	90.00	5.00	10.00	23.00	49.00	100.00
FCP^{2}K-7	87.50	8.50	12.50	59.00	21.60	98.00
FCP^{2}K-8	85.00	12.00	10.00	63.00	19.40	74.30
FCP^{2}K-9	87.50	8.50	12.50	60.00	22.00	96.00
FCP^{2}K-10	85.00	5.00	15.00	24.00	55.00	95.00
FCP^{2}K-11	87.50	8.50	12.50	59.00	20.00	97.00
FCP^{2}K-12	87.50	8.50	12.50	62.00	19.40	98.00
FCP^{2}K-13	87.50	8.50	12.50	58.00	20.00	97.50
FCP^{2}K-14	87.50	14.39	12.50	70.00	12.00	63.50
FCP^{2}K-15	83.30	8.50	12.50	62.00	21.50	85.00
FCP^{2}K-16	87.50	2.61	12.50	11.00	69.00	102.00
FCP^{2}K-17	87.50	8.50	12.50	58.00	19.00	96.80
FCP^{2}K-18	91.70	8.50	12.50	67.00	9.50	98.50
FCP^{2}K-19	87.50	8.50	8.30	60.00	19.00	85.00
FCP^{2}K-20	87.50	8.50	16.70	70.00	20.00	87.80

Table 3.8: Fit summary statistics for Eudragit-L-30-D-55® enteric coated tablets

Response	Source	R-squared	Adjusted R-squared	Predicted R-squared	p-value	Remark
Disintegration time in 0.1N HCl	Linear	0.8308	0.7970	0.6940	< 0.0001	
	2FI	0.8364	0.7545	0.3568	< 0.0001	
	Quadratic	0.9470	0.8939	0.4620	0.0002	Suggested
	Cubic	0.9971	0.9896	-0.9865	0.0068	Aliased
Dissolution in 0.06 N HCl	Linear	0.8756	0.8507	0.7298	0.0004	Suggested
	2FI	0.8770	0.8154	0.4499	0.0002	
	Quadratic	0.9107	0.8215	0.0696	0.0002	
	Cubic	0.9994	0.9977	0.9779	0.6975	Aliased
Dissolution in neutralized phthalate buffer of pH 5.5	Linear	0.6527	0.5833	0.3541	0.0001	
	2FI	0.6940	0.5410	-0.0643	< 0.0001	
	Quadratic	0.9417	0.8834	0.5619	0.0010	Suggested
	Cubic	0.9724	0.9007	-20.1846	0.0004	Aliased

Table 3.9: Fit summary statistics for Kollicot® MAE 100 P enteric coated tablets

Response	Source	R-squared	Adjusted R-squared	Predicted R-squared	p-value	Remark
Disintegration time in 0.1N HCl	Linear	0.7737	0.7285	0.5723	0.0003	
	2FI	0.7741	0.6611	0.2897	0.0002	
	Quadratic	0.9633	0.9266	0.8022	0.0025	Suggested
	Cubic	0.9709	0.8951	-21.4201	0.0003	Aliased
Dissolution in 0.06tN HCl	Linear	0.7372	0.6847	0.4894	0.0003	
	2FI	0.7435	0.6152	0.2248	0.0002	
	Quadratic	0.9791	0.9582	0.8408	0.0103	Suggested
	Cubic	0.9904	0.9655	-5.7771	0.0046	Aliased
Dissolution in neutralized phthalate buffer of pH 5.5	Linear	0.7539	0.7047	0.5838	0.0003	
	2FI	0.7617	0.6425	0.1903	0.0002	
	Quadratic	0.9674	0.9348	0.6678	0.0044	Suggested
	Cubic	0.9988	0.9957	0.8855	0.5030	Aliased

Table 3.10: Summary of ANOVA for response surface quadratic model of disintegration time in 0.1 N HCl for Eudragit-L-30-D-55®

Source	Sum of squares	df	Mean square	F-value	p-value	Remark
Model	2895.88	9	321.76	17.85	0.0001	Significant
Eudragit-L-30-D-55® (A)	1.49	1	1.49	0.082	0.7805	Insignificant
Coating Level (B)	2251.53	1	2251.53	124.93	< 0.0001	Significant
1,2-Propylene Glycol (C)	287.77	1	287.77	15.97	0.0031	Significant
A^2	16.14	1	16.14	0.90	0.3687	Insignificant
B^2	211.69	1	211.69	11.75	0.0075	Significant
C^2	157.18	1	157.18	8.72	0.0161	Significant
AB	11.52	1	11.52	0.64	0.4446	Insignificant
AC	3.92	1	3.92	0.22	0.6520	Insignificant
BC	1.45	1	1.45	0.080	0.7835	Insignificant
Residual	162.20	9	18.02			
Lack of Fit	161.04	5	32.21	111.06	0.0002	Significant
Pure Error	1.16	4	0.29			
Core Total	3063.94	19				

Table 3.11: Summary of ANOVA for response surface quadratic model of dissolution in 0.06 N HCl for Eudragit-L-30-D-55®

Source	Sum of squares	df	Mean square	F-value	p-value	Remark
Model	2847.03	3	949.01	35.20	< 0.0001	Significant
Eudragit-L30-D-55® (A)	8.00	1	8.00	0.30	0.5939	Insignificant
Coating Level (B)	2492.26	1	2492.26	92.44	< 0.0001	Significant
1,2-Propylene Glycol (C)	346.77	1	346.77	12.86	0.0027	Significant
Residual	404.41	15	26.96			
Lack of Fit	402.43	11	36.58	73.91	0.0004	Significant
Pure Error	1.98	4	0.49			
Core Total	3258.07	19				

Table 3.12: Summary of ANOVA for response surface quadratic model of dissolution in neutralized phthalate buffer of pH 5.5 for Eudragit-L30-D-55®

Source	Sum of squares	df	Mean square	F-value	p-value	Remark
Model	1645.06	9	182.78	16.16	0.0002	Significant
Eudragit-L30-D-55® (A)	0.00230	1.00000	0.00230	0.00020	0.98890	Insignificant
Coating Level (B)	1139.36	1	1139.36	100.71	< 0.0001	Significant
1,2-Propylene Glycol (C)	0.84	1	0.84	0.074	0.7914	Insignificant
A^2	20.24	1	20.24	1.79	0.2138	Insignificant
B^2	424.31	1	424.31	37.51	0.0002	Significant
C^2	14.66	1	14.66	1.30	0.2844	Insignificant
AB	6.66	1	6.66	0.59	0.4625	Insignificant
AC	26.28	1	26.28	2.32	0.1618	Insignificant
BC	39.16	1	39.16	3.46	0.0957	Insignificant
Residual	101.82	9	11.31			
Lack of Fit	100.30	5	20.06	53.05	0.0010	Significant
Pure Error	1.51	4	0.38			
Core Total	1833.24	19				

Table 3.13: Summary of ANOVA for response surface quadratic model of disintegration time in 0.1 N HCl for Kollicot® MAE 100 P

Source	Sum of squares	df	Mean square	F-value	p-value	Remark
Model	6556.57	9	728.51	26.26	< 0.0001	Significant
Kollicot® MAE 100 P (A)	27.58	1	27.58	0.99	0.3448	Insignificant
Coating Level (B)	5189.79	1	5189.79	187.07	< 0.0001	Significant
1,2-Propylene Glycol (C)	48.81	1	48.81	1.76	0.2174	Insignificant
A^2	13.58	1	13.58	0.49	0.5019	Insignificant
B^2	1287.66	1	1287.66	46.41	< 0.0001	Significant
C2	9.08	1	9.08	0.33	0.5812	Insignificant
AB	1.13	1	1.13	0.041	0.8449	Insignificant
AC	0.13	1	0.13	0.00451	0.9480	Insignificant
BC	1.13	1	1.13	0.041	0.8449	Insignificant
Residual	249.68	9	27.74			
Lack of Fit	243.68	5	48.74	32.49	0.0025	Significant
Pure Error	6.00	4	1.50			
Core Total	7094.55	19				

Table 3.14: Summary of ANOVA for response surface quadratic model of dissolution in 0.06 N HCl for Kollicot® MAE 100 P

Source	Sum of squares	df	Mean square	F-value	p-value	Remark
Model	4871.61	9	541.29	46.87	< 0.0001	Significant
Kollicot® MAE 100 P (A)	39.35	1	39.35	3.41	0.0980	Insignificant
Coating Level (B)	3623.78	1	3623.78	313.78	< 0.0001	Significant
1,2-Propylene Glycol (C)	5.02	1	5.02	0.43	0.5261	Insignificant
A^2	0.22	1	0.22	0.019	0.8943	Insignificant
B^2	1156.36	1	1156.36	100.13	< 0.0001	Significant
C^2	34.00	1	34.00	2.94	0.1203	Insignificant
AB	0.13	1	0.13	0.011	0.9194	Insignificant
AC	8.40	1	8.40	0.73	0.4157	Insignificant
BC	22.44	1	22.44	1.94	0.1967	Insignificant
Residual	103.94	9	11.55			
Lack of Fit	98.77	5	19.75	15.28	0.0103	Significant
Pure Error	5.17	4	1.29			
Core Total	5126.97	19				

Table 3.15: Summary of ANOVA for response surface quadratic model of dissolution in neutralized phthalate buffer of pH 5.5 for Kollicot® MAE 100 P

Source	Sum of squares	df	Mean square	F-value	p-value	Remark
Model	2752.13	9	305.79	29.67	< 0.0001	Significant
Kollicot® MAE 100 P (A)	31.82	1	31.82	3.09	0.1128	Insignificant
Coating Level (B)	2112.40	1	2112.40	204.97	< 0.0001	Significant
1,2-Propylene Glycol (C)	0.66	1	0.66	0.064	0.8066	Insignificant
A^2	55.93	1	55.93	5.43	0.0448	Significant
B^2	395.56	1	395.56	38.38	0.0002	Significant
C^2	224.75	1	224.75	21.81	0.0012	Significant
AB	5.61	1	5.61	0.54	0.4794	Insignificant
AC	7.80	1	7.80	0.76	0.4069	Insignificant
BC	8.61	1	8.61	0.84	0.3845	Insignificant
Residual	92.75	9	10.31			
Lack of Fit	89.76	5	17.95	23.97	0.0044	Significant
Pure Error	3.00	4	0.75			
Core Total	2844.92	19				

DECLARATION OF ORIGINALITY

I, the undersigned, declare that this thesis is my original work and has not been presented for a degree in any University.

Name: Seife Shimels Teklearegay

Signature _____

This thesis has been submitted for examination with my approval as a University advisor.

Name: Prof. Tsige Gebre-Mariam

Signature _____

Dr. Anteneh Belete

Signature _____

Place and date of submission: Addis Ababa, Ethiopia, May, 2018

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