

3. Literature review

Khuhawar et al. (1998) studied the HPLC determination of rifampicin, isoniazid and pyrazinamide in the pharmaceutical preparations. Isoniazid alone and along with pyrazinamide and rifampicin has been estimated by HPLC. Rifampicin and pyrazinamide were separated from the derivative of isoniazid and are simultaneously determined. The chromatography is carried out from C18 column. These drugs were eluted with a mobile phase consisting of water, methanol, acetonitrile, isopropanol and sodium acetate and with flow rate of 1.7 ml/min by UV detection at 333 nm.

Saranjit Singh et al. (2001) reviewed the various causes for the decreased stability of rifampicin from anti TB FDC products and suggested the probable solutions for this issue. They stated that the increased degradation of rifampicin in presence of isoniazid in stomach after administration is the main factor behind this issue. They reported that rifampicin undergoes rapid degradation in presence of isoniazid in acidic medium, indicating the possibility of the concentration of rifampicin going below the required minimum therapeutic level, after intake of formulations containing both these drugs in combination. So, apart from the initial drug dose in the dosage form, stability of rifampicin in acidic conditions of stomach turns out to be an important factor in assuring therapeutic action of the drug.

In acidic conditions rifampicin decomposes to 3-formylrifamycin. It is suggested that in presence of isoniazid 3-formylrifamycin which is a degraded product of rifampicin in acidic medium reacts with isoniazid forming hydrazone by a very fast 2nd order reaction. This hydrazone is unstable under acidic conditions and forms back isoniazid and 3-formylrifamycin by a slow pseudo 1st order reaction (Fig. 3.1). As the 2nd order forward reaction is dominant over the backward reaction, the net reaction favors the formation of hydrazone. Because of this, the rate and extent of degradation of rifampicin to 3-formylrifamycin is increased. This reduction in the levels of rifampicin was affected only by the co-administration of isoniazid and was not affected by pyrazinamide and ethambutol (Saranjit Singh et al., 2001).

Bjorn Blomberg et al. (2001) studied the rationale for recommending fixed-dose combination tablets for treatment of tuberculosis. They suggested that only fixed-dose combination products of good quality and proven rifampicin stability and bioavailability should be used. They developed an effective protocol for assessing the rifampicin

bioavailability. Standardization of fixed-dose combination drug dosage forms has been suggested, which limits the preparations containing rifampicin to nine.

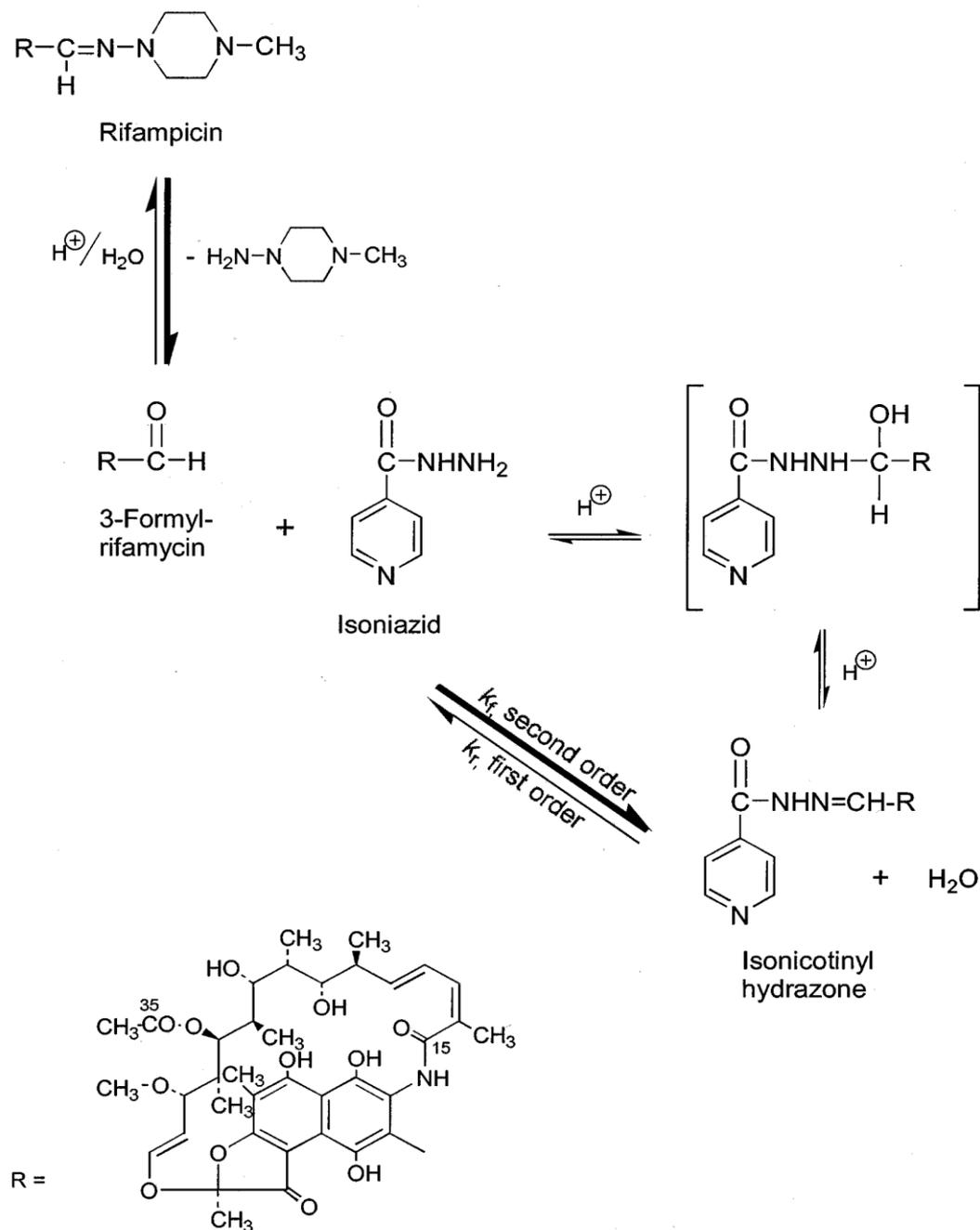


Fig. 3.1. Mechanism of enhancement of decomposition of rifampicin in the presence of isoniazid (Saranjit Singh et al., 2001)

Shishoo et al. (2001) studied the impaired bioavailability of rifampicin in presence of isoniazid from fixed dose combination formulations. They compared bioavailability of rifampicin after administration of a single component rifampicin (450 mg) capsule and rifampicin-isoniazid (450+300 mg) fixed dose combination (FDC) capsule formulations.

They confirmed our questions about the rifampicin stability in presence of isoniazid in acidic conditions of stomach, which is the key factor reducing the bioavailability of rifampicin from the combination dosage forms containing both these drugs. This study emphasizes the urgency to reconsider the FDC formulations to reduce or prevent the degradation of rifampicin in GIT.

Mariappan et al. (2003) studied the gastrointestinal permeability of rifampicin alone and in presence of isoniazid in rats. The results stated that rifampicin was well absorbed from the stomach due to its solubility, which was very high between pH 1 to 2. Absorption of isoniazid from stomach is very less when compared to intestine and it is well absorbed from all 3 portions of intestine. In combination, rifampicin disappearance was enhanced in the presence of isoniazid in the stomach and jejunum, but isoniazid disappearance was not influenced by rifampicin. This study concludes higher in situ rifampicin disappearance in the presence of isoniazid, attributable to drug degradation due to catalysis by isoniazid. As the two drugs show regional specific permeability, FDCs without reduced rifampicin bioavailability resulting from its decomposition in the presence of isoniazid can be designed by segregating delivery of the two drugs by around 3-4 h. Rifampicin should be released in the stomach and isoniazid in the intestine.

Sankar et al. (2003) studied the degradation of rifampicin in the pH range of 1-3 under isoniazid presence. They stated that rifampicin decomposed in presence of isoniazid to a greater extent at a pH of 2. This is the pH under which anti TB FDC formulations are administered. This study results clearly indicate that quite significant loss of rifampicin will occur because of the interaction between these 2 drugs at this pH of 2. This study also suggests that anti TB FDC products containing these drugs should be formulated in such a way that this interaction between these drugs is minimized when they are administered on fasting conditions.

Ramesh Panchagnula et al. (2004) studied the various biopharmaceutic and pharmacokinetic aspects of variable bioavailability of rifampicin. They stated that other anti TB drugs of FDC like isoniazid, pyrazinamide and ethambutol do not show any bioavailability problems. Rifampicin is the only lipophilic component of the FDC which belongs to BCS class II. Apart from this it displays pH-dependent solubility affecting its absorption from GIT.

Brijesh et al. (2004) studied the formulation and in vitro evaluation of ranitidine hydrochloride gastroretentive drug delivery systems. HPMC, guar gum and xanthan gum

polymers were used for this study. Sodium bicarbonate was included for its carbon dioxide generating properties.

Hemanth kumar et al. (2004) studied high-performance liquid chromatography method for the determination of rifampicin and desacetyl rifampicin in plasma and urine. The retention times of desacetyl rifampicin, rifampicin and rifapentine, the internal standard was 2.9, 4.8 and 10.5 min.

Niklas Sandler et al. (2005) studied pellet manufacturing by extrusion-spheronization using process analytical technology. They investigated the phase transitions occurring in nitrofurantoin and theophylline formulations during pelletization by extrusion-spheronization.

Mahesh Chavanpatil et al. (2006) prepared sustained release gastroretentive dosage forms for ofloxacin preferably once daily. Different polymers, such as psyllium husk, HPMC K100M, crosspovidone and its combinations were tried in order to get the desired sustained release profile over a period of 24 h. The optimized formulation was subjected to stability studies at different temperature and humidity conditions as per ICH guidelines.

Alaa Eldeen et al. (2006) prepared chitosan beads for the drug verapamil. They studied the kinetics of drug release. They exhibited a kinetic model of combined mechanism of diffusion partially through a swollen matrix and partially through water-filled pores.

Ali et al. (2007) developed and validated a selective, simple, precise stability-indicating HPTLC method for estimation of isoniazid and rifampicin as pure drugs and in dosage forms.

Glass et al. (2007) developed and validated stability indicating HPLC method for the simultaneous estimation of pyrazinamide, isoniazid and rifampicin in a FDC product using artificial neural networks. The effect of concentration, solvent type, nature of buffer in mobile phase and type of column were studied. Most effective separation and shorter retention times were attained using C-18 μ -bondapak column with the dimensions of 4.6×250 mm, $10 \mu\text{m}$ and 125 \AA with acetonitrile – tetrabutyl ammonium hydroxide mixture as mobile phase and with pH of 3.10.

Amitha Joshi et al. (2007) studied dissolution testing of isoniazid pellets by USP apparatus 3 using reciprocating cylinder. They optimized the formulations using response surface methodology.

Gohel et al. (2007) developed a new formulation of rifampicin and isoniazid to reduce the decomposition of rifampicin in stomach conditions and to segregate rifampicin and isoniazid release in stomach and intestinal part of GIT respectively. Rifampicin gastroretentive tablets were prepared by wet granulation technique using HPMC, calcium carbonate and PEG 4000. Dissolution studies revealed that a significant amount of rifampicin was decomposed from the immediate release formulations (capsules) that contained both rifampicin and isoniazid. This is mainly because of accumulation of rifampicin in the dissolution bowl and isoniazid presence. This decomposition of rifampicin to 3-formylrifampicin was reduced by minimizing the physical contact between these drugs and by controlling rifampicin release in acidic environment using modified Rossett-Rice apparatus.

Podczeck et al. (2008) prepared the non-ionic surfactant containing pellets by extrusion-spheronization process using microcrystalline cellulose. They incorporated hydrophilic surfactant and hydrophobic surfactants with a concentration range of 5-95% in the granulating liquid used to prepare the pellets. The extrusion pattern was affected by these surfactants and enhanced the sphericity of the pellets.

Jianfang Liu et al. (2008) studied the determination of rifampicin and related compounds in pharmaceuticals by HPLC using monolithic column. For the estimation of rifampicin along with rifampicin quinone, rifamycin, rifampicin N-oxide and 3-formylrifamycin, an accurate and precise HPLC method was developed and validated.

Gohel et al. (2008) explored cold extrusion technique for the preparation of mini tablets of isoniazid. These minitables were coated using HPMC phthalate. Full factorial design was adopted to optimize the formulation. The optimized batch showed more than 90% of drug release in phosphate buffer in 15 min.

Patel et al. (2009) formulated and evaluated the gastroretentive beads for famotidine. They evaluated these beads with respect to various physico-chemical properties like percent drug loading, drug entrapment efficiency, buoyancy and in vitro release.

Swati Pund et al. (2009) studied the multivariate approach of optimization of formulation for studying the various process variables affecting the physical and mechanical properties of isoniazid pellets. In this current study, site-specific release isoniazid pellets were prepared with a good drug loading capacity using extrusion and spheronization technique followed by Sureteric® aqueous coating. They used two level full factorial design for the optimization of the formulations.

Janardhan et al. (2009) formulated gastroretentive tablets of Ofloxacin by wet granulation technique. Different formulations were prepared using various concentrations of HPMC K4M (rate retarding polymer), HPMC 5cps (promotes floating), sodium carboxy methyl cellulose (channeling agent), sodium bicarbonate and citric acid anhydrous (effervescent agents). The formulations were evaluated for quality control tests and subjected to in vitro buoyancy and dissolution studies compared to the marketed formulation. It was found that the formulation with sodium carboxy methyl cellulose along with citric acid and sodium bicarbonate in equal quantities before granulation and during lubrication increased the drug release of the drug from the dosage form.

Arza et al. (2009) developed swellable, floating sustained release tablets of ciprofloxacin hydrochloride by wet granulation method using a combination of hydrophilic polymer (different grades of hydroxyl propyl methyl cellulose), different swelling agents (crosspovidone, sodium starch glycolate, and croscarmellose sodium) and gas generating agent (sodium bicarbonate).

Vani et al. (2010) formulated and evaluated gastroretentive floating beads of ranitidine hydrochloride. They used HPMC as the rate controlling polymer and attained a release of 81% of ranitidine hydrochloride in 8 h in simulated gastric fluid.

Shiva Kumar Yellanki et al. (2010) formulated and evaluated gastroretentive floating alginate beads of riboflavin. They achieved a floating time of 10 h and they studied the kinetics for the drug release by applying various kinetic models for drug release.

Jignyasha et al. (2011) developed muco adhesive gastroretentive tablets of amoxicillin using PEG, hydroxypropyl cellulose and HPMC. Formulations were tested for their in vitro release profile of drug, swelling characteristics and in vitro muco adhesion property.

Vishal Gupta et al. (2011) formulated, evaluated and studied the stability of olanzapine matrix pellets for controlled release.

Srikanth et al. (2012) they applied statistical design for the preparation of a gastric floating tablet of Propranolol HCl and to investigate the effect of formulation variables on drug release and the buoyancy properties of these delivery systems. The contents of polyethylene oxide WSR coagulant and sodium bicarbonate were used as independent variables in central composite design of the best formulation. Main effects and interaction terms of the formulation variables were evaluated quantitatively using a mathematical model approach showing that both independent variables have significant effects on floating lag time, % drug release at 1 h and time required to release 90% of drug (t_{90}).

Various studies in literature state reduction in the dose of rifampicin to below 9 mg/kg will lead to failure of therapy and can lead to drug resistance. Presently, the dose of rifampicin prescribed is 10 mg/kg, which indicates a very narrow margin of only 10% in actual delivered dose and the minimum necessary for therapeutic action (Jakko et al., 2013).

3.1. Optimization process

Optimization process involves the following steps:

- Determination of the independent variables (factors) along with their levels. This is dependent on the earlier reports and studies.
- Depending on the factors and their levels, suitable model and design is selected.
- The various experimental runs are determined and performed
- The obtained responses are statistically analyzed by using a suitable and appropriate statistical tool (like analysis of variance) and interpreted accordingly.
- The effect of each and every factor over the responses is studied.
- The responses are screened, by using multiple criteria to get the values of independent variables.

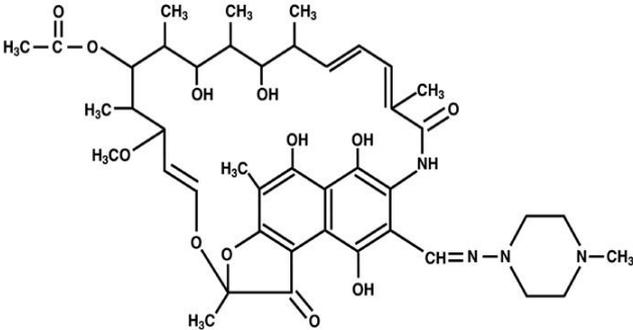
3.1.1. Software for design and optimization

Due to complicated technical challenges that are encountered by the researchers during formulation development, it is important to have efficient software for design and optimization process. Design of experiments (DoE) has been widely used during formulation development and also in product and process optimization. Main benefit of applying DoE is that it considers all the factors and evaluates them simultaneously, rapidly and systematically. Using this DoE, the effect of each factor on each and every response can be found out and based on statistical analysis; the critical factors can be identified. Once these factors are detected, the optimal formulation can be described by applying proper DoE to optimize the various levels of all these variables. Even the various processes like manufacturing etc. can also be developed and optimized in similar way. One more advantage of DoE is the process validation and scale-up can be very effective because of the ruggedness of the formulation and manufacturing method.

There are various software packages available in the market which facilitates the design, optimization and statistical interpretation of various experiments. Some of the software packages which are mainly useful for the experimental design and their statistical analysis are Design expert, Multi simplex, Echip and Nemrodw.

3.2. Drug profiles

3.2.1. Rifampicin

- **Mol. formula** $C_{43}H_{58}N_4O_{12}$
- **Chemical structure**

- **Generic name** 5,6,9,17,19,21-hexahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-8-[N-(4-methyl-1-piperazinyl)formimidoyl]-2,7-(epoxypentadeca[1,11,13]trienimino)naphtha[2,1-b]furan-1,11(2H)-dione 21-acetate
- **Mol. weight** 822.94
- **Solubility** It is freely soluble in chloroform and dimethyl sulfoxide; soluble in ethyl acetate, methanol, tetrahydrofuran; slightly soluble in acetone, water, carbon tetrachloride
- **Polarity** It has a log P value of 3.719
- **pKa** pKa values 1.7 and 7.9 because of 4-hydroxy and 3-piperazine nitrogen
- **Stability** Highly stable in dimethyl sulfoxide and stable in water
- **Melting point** 183 °C
- **Human dose** Daily dose of 10 mg/kg, in a single oral administration and should not to exceed 600 mg per day.
- **Mechanism of action** Rifampicin inhibits the essential *rpoB* gene product β -subunit of DNA dependent RNA polymerase activity, acting early in transcription. It is thought to bind to the β -subunit, close to the RNA/DNA channel, and physically blocks the transit of the growing RNA chain after nucleotides have been added (Wehrli et.al., 1968)

- **Indications** The primary indications for rifampicin are for treatment of tuberculosis (pulmonary and extrapulmonary lesions) and for leprosy. It has recently been used for brucellosis (Petri, 2001).
- **Contraindications** In case of hypersensitivity to the drug, during pregnancy except in presence of a disease such as severe tuberculosis, in alcoholics with severely impaired liver function and with jaundice (Petri, 2001).
- **Adverse effects** Hepatitis, serious hypersensitivity reactions which includes thrombocytopenia, hemolytic anemia, renal failure, liver toxicity, hypotension and shock, shortness of breath, nausea, vomiting, diarrhoea. Rifampicin causes orange-red staining of all body fluids (Petri, 2001).

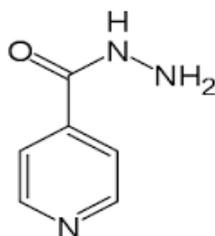
❖ Pharmacokinetics

- **Absorption** Rifampicin is readily absorbed from the GIT. C_p is attained at 1.5 to 4 h after oral dosing (Kebrele, 1970). After an oral dose of 450 mg, plasma concentration reaches from 6 to 9 $\mu\text{g/ml}$.
- **Distribution** Rifampicin rapidly distributes into most organs, tissues, bones and body fluids, including exudates into tuberculosis infected lung cavities (Acocella, et.al., 1967)
- **Metabolism** The principal pathways of metabolism of rifampicin involve desacetylation and hydrolysis.
- **Excretion** Approximately 50% of the rifampicin dose is eliminated within 24 h and 6 to 30% of the drug is excreted unchanged in the urine, while 15% is excreted as active metabolite. Approximately 43 to 60% of oral dose is excreted in the faeces.

3.2.2. Isoniazid

- **Mol. formula** $\text{C}_6\text{H}_7\text{N}_3\text{O}$

- **Chemical structure**



-
- **Generic name** Isonicotinic acid hydrazide; isonicotinoyl hydrazine; isonicotinyl hydrazine
 - **Mol. weight** 137.14
 - **Solubility** It is soluble in water (~14% at 25 °C, ~26% at 40 °C) ethanol: (~2% at 25 °C), boiling ethanol (~10%), chloroform (~0.1%). Practically insoluble in ether, benzene.
 - **Polarity** It has a log P value of 0.64
 - **Acidity/basicity** pH of a 1% aqueous solution 5.5 to 6.5
 - **Stability** It is highly stable in dimethyl sulfoxide and stable in water
 - **Melting point** 171.4
 - **Human dose** 5 mg/kg for adults, 10-20 mg/kg for children.
 - **Mechanism of action** Isoniazid is a prodrug activated by catalase-peroxidase hemoprotein, KatG. Isoniazid inhibits InhA, a nicotinamide adenine dinucleotide (NADH)-specific enoyl-acyl carrier protein reductase involved in fatty acid synthesis.
 - **Indications** The primary indications for isoniazid is for the treatment of tuberculosis (pulmonary and extrapulmonary lesions)
 - **Contraindications** Isoniazid is contraindicated in known cases of hypersensitivity to the drug. It is contraindicated in alcoholics with severely impaired liver function and with jaundice.
 - **Adverse effects of isoniazid** Peripheral neuropathy, other neurotoxic effects like convulsions, toxic encephalopathy, optic neuritis and atrophy, memory impairment and toxic psychosis, hepatic effects like liver toxicity, common prodromal symptoms of hepatitis are anorexia nausea, vomiting, fatigue, malaise, and weakness, gastrointestinal effects like nausea and vomiting.
- ❖ **Pharmacokinetics**
- **Absorption** Isoniazid is readily absorbed when administered orally. C_p of 3-8 $\mu\text{g/ml}$ develop 1-2 h after fasting dose of 300 mg orally.
 - **Distribution** Isoniazid diffuses readily into all body fluids and cells. Isoniazid is
-

not considered to be bound appreciably to plasma proteins.

- **Metabolism** The plasma half-life of isoniazid ranges from 1-4 h. Those who are fast acetylators because of genetic variations have short half-lives. The primary metabolic route is acetylation of isoniazid to acetylisoniazid by N-acetyltransferase
- **Excretion** Excretion is primarily renal. From 75% to 95% of dose of isoniazid is excreted in the urine within 24 h, mostly as metabolites.

3.3. Excipient profile

3.3.1 Hydroxy propyl methyl cellulose

Synonyms: HPMC, Methocel, methylcellulose propylene glycol ether, methyl hydroxy propyl cellulose, hypromellose.

Chemical name: Cellulose hydroxypropyl methyl ether.

Description: It is an odorless and tasteless, white or creamy-white fibrous or granular powder. It is soluble in cold water forming a viscous colloidal solution, practically insoluble in chloroform, ethanol (95%), and ether, but soluble in mixtures of ethanol and dichloromethane, mixtures of methanol and dichloromethane, and mixtures of water and alcohol. It is a stable material and should be stored in a well-closed container, in a cool, dry place.

Applications: HPMC is primarily used as binder in tablets, in film-coating, and as a matrix for use in controlled release formulations. Levels from 2% and 5% w/w can be used as a binder in granulation techniques. Higher viscosity grades are used to control and sustain the release of drugs controlled drug delivery systems. It is used at arrange of 10–80% w/w for extended release of drugs.

3.3.2. Polyethylene oxide

Synonyms: POLYOX, Polyoxirane, Polyoxyethylene

Molecular weight: 100000-7000000 g/mol

Description: It occurs as white to off-white, free flowing powder, soluble in water, acetonitrile, chloroform and methylene chloride. It is insoluble in ethylene glycol and most alcohols. The exposure to high temperatures can result in reduction in viscosity, so stored in tightly sealed containers in a cool and dry place. It is incompatible with strong oxidizing

agents. The POLYOX grade which is also known as POLYOX WSR 301 used for the study has a viscosity of 5500 cps for a 1% solution.

Applications: Polyethylene oxide is used as a tablet binder at lower concentrations. The higher molecular weight grades provide controlled drug release and low levels of polyethylene oxide are effective thickeners. It is categorized as mucoadhesive, tablet binder, and thickening agent.

3.3.3. Sodium alginate

Synonyms: Algin, alginic acid, sodium polymannuronate

Source: Alginate is a non-toxic, biodegradable, natural polysaccharide obtained from marine brown algae and some species of bacteria.

Description: Odorless and tasteless, white to pale yellowish brown coloured powder which is soluble in water. It is a sodium salt of alginic acid which is a natural polysaccharide and a linear polymer consisting of 1, 4 - linked β -D-mannuronic acid and α -D-gluronic acid residues in varying proportions and arrangements.

It forms a reticulated structure which can be cross-linked with divalent or polyvalent cations to form insoluble meshwork. Calcium and zinc cations have been reported for cross-linking of acid groups of alginate.

Applications: Stabilizing agent, suspending agent, tablet binder, viscosifying agent and in the preparation of pharmaceutical beads.

3.3.4. Lactose anhydrous

Synonyms: Super Tab anhydrous, Super Tab 11SD, anhydrous lactose NF for direct compression, lactopress anhydrous and lactosum.

Molecular weight: 342.30

Description: Lactose occurs as white to off-white crystalline particles or powder and soluble in water, sparingly soluble in ethanol (95%) and ether. Lactose may develop a brown coloration on storage, the reaction being accelerated by warm, damp conditions, incompatible with strong oxidizers and is stored in well closed container in a cool and dry place.

Applications: It is mainly used in direct compression technique and also as a tablet, capsule filler and binder. Anhydrous lactose can be used for drugs which are moisture sensitive

because of its low moisture content. It is categorized as binder, directly compressible tableting excipient, lyophilization aid, and tablet and capsule filler.

3.3.5. Magnesium stearate

Synonyms: Magnesium octadecanoate, octadecanoic acid

Chemical name: Octadecanoic acid magnesium salt

Functional category: Tablet and capsule glidant

Description: Magnesium stearate is a fine, light white precipitated or milled powder of low bulk density. It has a faint odor of steric acid and characteristic taste. It is greasy to touch and readily adheres to skin. It is practically soluble in ethanol, water and ether, slightly soluble in warm benzene and warm ethanol (95%). It is very stable material

Applications: It is mainly applied in cosmetics, food products and pharmaceutical dosage forms, as glidant in tablets and capsules (0.25% - 0.5%) and also used in barrier creams.

3.3.6. Talc

Synonyms: hydrous magnesium calcium silicate, soapstone, stealite

Description: It occurs as very fine, white to grayish-white, odorless, impalpable, unctuous and crystalline powder. It is practically insoluble in dilute acids and alkali, organic solvents and water. It is a stable material should be stored in a well-closed container in a cool dry place.

Applications: It is applied as diluent and lubricant, also as a dissolution retardant in the development of controlled release products.it is also used as a dusting powder and categorized as anticaking agent, glidant, tablet and capsule diluents, and tablet and capsule lubricant.

3.3.7. Sodium bicarbonate

Synonyms: Baking soda, effer-soda, monosodium carbonate, sodium acid carbonate, sodium hydrogen carbonate

Description: It is a colorless or white, odorless crystalline powder with a saline, slightly alkaline taste. It is practically insoluble in ether and ethanol (95%), soluble in water. Sodium bicarbonate is stable in dry air but slowly decomposes in moist air and should therefore be stored in a well-closed container in a cool, dry place.

Applications: Sodium bicarbonate is generally used as gas generating agent in the pharmaceutical preparations. It is also widely used to produce or maintain an alkaline pH in a preparation.

3.3.8. Eudragit L 100

Chemical name/IUPAC name: Poly (methacrylic acid-co- methyl methacrylate) 1:1

Ph. Eur.: Methacrylic acid – methyl methacrylate copolymer (1:1)

USP/NF: Methacrylic acid copolymer, Type A – NF

JP: Methacrylic acid copolymer L

Description: It is solid substance in form of a white powder with a faint characteristic odor. It is an anionic copolymer based on methacrylic acid and methyl methacrylate.

Applications: For highly effective and stable enteric coats which rapidly dissolve in the upper intestinal part of GIT. Used for site specific and targeted drug delivery to intestine.

3.3.9. Calcium Chloride

Description: Calcium chloride occurs as a white or colorless crystalline powder, granules or crystalline mass. It is deliquescent in nature. Because of its deliquescent nature, it should be stored in air tight containers.

Applications: Important applications of calcium chloride include its use as cross-linker for certain polymers in the bead formation. Other applications include antimicrobial preservative and as desiccant.