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DEVELOPMENTS IN THE DELIVERY OF SULFASALAZINE: IMPROVING THE EFFECTIVENESS OF TREATMENTFOR INFLAMMATORY BOWEL DISEASE BY USING SPRAY DRYING TECHNIQUE AND PREFORMULATION STUDY OF SULFASALAZINE

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ABSTRACT

Inflammation of the gastrointestinal tract is caused by abnormal immune responses that are triggered by inheritance and environmental factors. 5-ASA has anti-inflammatory effects on the colonic mucosa once sulfasalazine is broken down into its active ingredients in the colon. Sulfasalazine's capacity to control the immune response and reduce inflammation is thought to be the reason for its therapeutic success in inflammatory bowel disease (IBD), however its precise mechanism is still unclear. The medication is most effective in keeping UC patients in remission and, to a lesser extent, CD patients. Sulfasalazine's physicochemical characteristics such as color, odor, melting point, and solubility are essential for creating potent formulations in preformulation investigations. The medication is identified and characterized using methods such as Fourier-transform infrared (FTIR) spectroscopy and UV spectrophotometry. The goal of developing Eudragit microparticles coated with sulfasalazine by spray drying techniques is to improve medication delivery and bioavailability. To guarantee their quality and effectiveness, these microparticles go through a thorough in vitro characterization process that includes optical microscopy, SEM, and zeta potential examination. In summary, sulfasalazine continues to be a vital component of IBD treatment, providing remarkable advantages in reducing inflammation and preserving remission. Its novel delivery methods, like microparticles, and ongoing pharmacokinetics research have the potential to improve patient outcomes in the treatment of inflammatory bowel disease.

KEYWORD: Sulfasalazine, IBD, Microparticle, Eudragit, Spray drying.

IBD: In addition to less common conditions such microscopic colitis, IBD encompasses Crohn's disease (CD) and ulcerative colitis (UC). IBD is typified by immune response dysregulation and gastrointestinal tract inflammation, usually accompanied by relapses & remissions. While dietarytherapies have historically received less attention, long-term use of immunosuppressive, biological, anti-inflammatory, and immunomodulatory drugs is frequently necessary for the management of the disease.[1] IBD is a collection of chronic relapsing illnesses characterized by inflammation of the gastrointestinal tract with varying phenotypic delivery. It mostly includes Crohn's disease (CD) and ulcerative colitis (UC). The etiology of IBD has been linked to a number of variables, including interactions between the immune system, microbiology, environment, and genetics. Even though significant work has been achieved in recent decades to clarify the complexity of IBD expression, the precise causes are still unknown.[1] Significant morbidity, ongoing prescription drugs, and gastrointestinal inflammation is the characteristic features of IBM. Globally, 84.3 out of every 100,000 people had IBD in 2017.[2]

IBD has a complicated, multivariate the cause of illness that includes disruptions to the gut microbiota, abnormal mucosal immune reaction to environmental stimuli, and a familial history. Together, epidemiological patterns, research using gnotobiotic mouse models, and genome-wide association studies that pinpoint genes related to microbial management provide evidence that the gut microbiota is crucial to the pathophysiology of IBD. Severe dysbiosis of the gut microbiome, characterized by the growth of harmful species and concurrent depletion of beneficial species, is a feature shared by ulcerative colitis and Crohn's disease. IBD is characterized by decreased abundances of bacterial genera (e.g., Faecalibacterium, Ruminococcus, Coprococcus, Dorea, Parabacteroides, Eubacterium, Oscillibacter, and Prevotella) that are involved in bio-transformations of bile acids, short-chain fatty acid production, and the synthesis of indole-based tryptophan compounds, and increased abundances of members of the phyla Proteobacteria and Actinobacteria. The resulting imbalance leads to increased immune response towards the microbial antigens, as well as changed metabolomic milieus that set off further inflammatory cascades.[3]

When the regulatory controls on mucosal immune responses to enteric microorganisms break down, it can lead to inflammatory bowel disease (IBD) in individuals who are at risk The characteristics of the inflammatory process are determined by the type of immune response

and the profile of cytokines produced, both of which are controlled by genetics.[4] The intestinal mucosa's action and regulatory cells, which have also been shown to be imbalanced in this patient population, must function in harmony for the immune system to be activated. The interaction of environmental factors on the microbiome and genetic predisposition results in the pathophysiology of inflammatory bowel disease (IBD). This breaks down the intestinal barrier, which in turn causes abnormal intestinal immune system stimulation.[5]

The digestive tract, particularly the ileum at the end of the small intestine, becomes inflamed, swollen, and irritated when a person has Crohn's disease. Inflammation brought on by Crohn's disease permeates the GI tract thoroughly, resulting in discomfort and diarrhea. Prolonged inflammation can lead to the formation of tissue scars within the intestine, narrowing the path and causing pain or cramping when food moves more slowly. Crohn's disease can affect any part of the digestive tract, from the mouth to the anus, and causes inflammation of the entire gut wall. Usually, the colon, the ileums, or the last segment of the small bowel are impacted. Ileitis, colitis, and ileo-colitis are the names given to these disease areas.[6]

PATHOLOGY OF IBD

It is unclear exactly why genetically susceptible people have impaired immune responses to gut flora, which is thought to be the cause of IBD. Deregulation of both innate and adaptive immune responses is a component of the atypical immunological response. IBD characteristics are thought to breach the intestinal epithelial barrier in particular zones, and non-resolving mucosal damage is thought to be a major feature of the illness. This injury's precise etiology is unknown, although potential causes include chemicals, infectious agents, and metabolic changes brought on by diet-mediated dysbiosis. It is believed that insufficient healing of the inflammatory response to the initial injury is what keeps the illness continuing. Tolerance to common pathogens or indications of autologous tissue damage may change, making it difficult for inflammation to effectively resolve. The issue to be addressed is whether changes to the epithelium barrier take place prior to or subsequent to inflammation in the lamina propria.[7]

Microparticle: Microparticles are structures with sizes between one and a thousand μm. They typically consist of the active ingredient mixed into a polymer matrix. Microparticles can be broadly classified into two forms based on how the drug molecules are incorporated:

microspheres, which are a homogeneous mixture of active material and polymer, and microcapsules, which are drug cores coated in polymer. The usage of polymer microparticles in the pharmaceutical business was initially patented in the 1970s by Holliday et al. They created microstructures for oral administration with modulated drug release by encasing acetylsalicylic acid in a continuous thin covering of ethylcellulose. The patient received an 8-hour analgesic benefit from the formulation, which released the medicinal component for 4 hours after ingestion.[8]

Since microparticles have so many uses and are so adaptable, they have drawn a lot of interest from scientists in many different fields. Numerous techniques, such as emulsification, spray drying, precipitation, and microfluidics, are used to create microparticles. Concerning the size, shape, and encapsulation efficiency of the particles, each method offers notable benefits. Both hydrophobic and hydrophilic payloads can be encapsulated in a polymer matrix through emulsification processes such solvent evaporation and diffusion. A rapid and scalable method for producing dry microparticles suitable for food encapsulation and pulmonary delivery is spray drying.[9]

Method and preformulation studies

Pre-formulation studiesOrganoleptic Properties

Pre-formulation is an integral part of the entire development process. A standard preformulation procedure ought to start with an explanation of the drug's composition. It is necessary to note the new drug's color, taste and smell using precise language. It is necessary to document the new drug's color, taste and odor using precise language. To prevent confusion among scientists using different phrases to express the same characteristic, it is crucial to create a common terminology to define these properties. The table provides a list of descriptive names for the colors, tastes and odors of medicinal powders that are most frequently encountered. All of the early batches of the new medication need to have their colors noted using the descriptive nomenclature The methodology for developing a safe, efficient and stable dosage form depends heavily on this research. The obtained drug sample was identified by various analytical techniques such as IR spectroscopy, UV spectroscopy, melting point etc.[10]

The Organoleptic studies like general appearance like nature, color, odor etc. were performed byvisual observations.

Color: Small quantity of drug was taken in butter paper and viewed in well illuminated place.

Odor: Very less quantity of drug was smelled to get the odor.[11].

Melting Point of Drug

For determination of melting point USP method was followed. Melting point of drugs wasdetermined by capillary fusion method. Small amount mixtures of drugs were filled in capillary and it was placing di n melting point apparatus. Then the temperature at which drug crystals started melting and turned into liquid was noted down.[12]

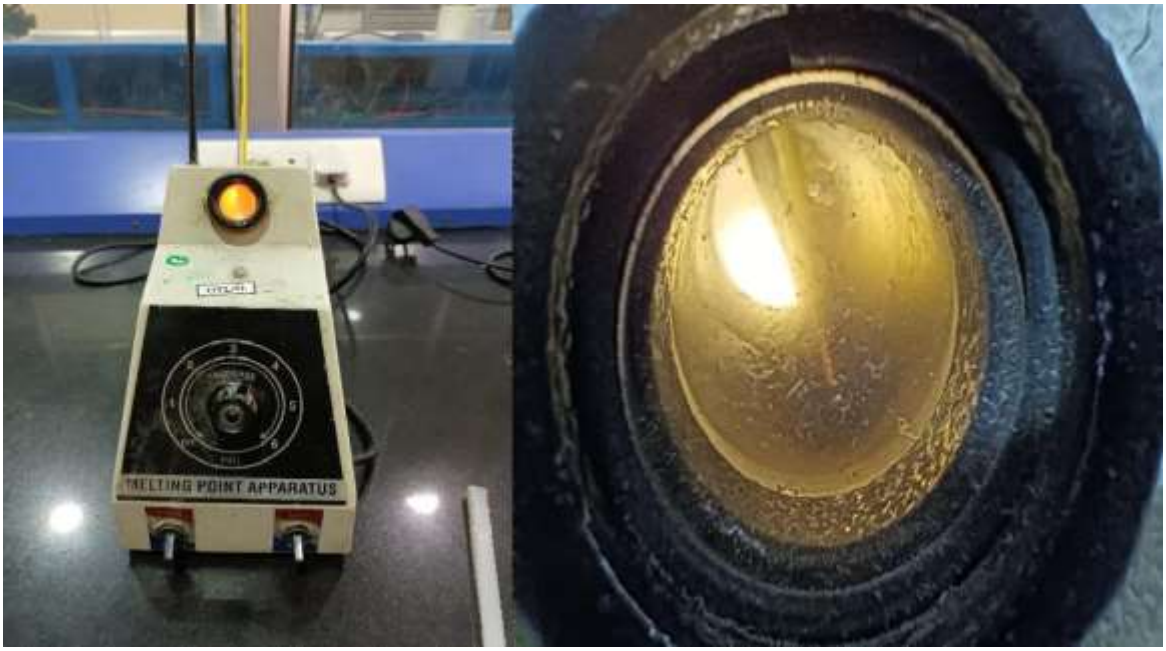


Fig. 6.1 Melting Point of pure Drug.

Determination of Absorption Maxima of sulfasalazine by UV- Spectrophotometer

Absorption maxima (λ_{max}) of drug were determined by UV Spectrophotometer (Shimadzu Pharma. Spec1800). Put 5 ml of methanol into a 10 ml volumetric flask. Pour in 10 milligrams ofsulfasalazine. In order to create a stock solution with a concentration of 1 mg/m, dissolve it and add a sufficient amount of water.

Preparation of Calibration Curve of sulfasalazine

Transfer a portion of 1–10 ml portions of the stock solutions into 500 ml volumetric flasks and then top them off with enough distilled water to reach the desired level. Solutions were scanned at 200–400 nm using the blank. The calibration curve was plotted using various

concentrations and the maximum wavelength observed was 364 nm when compared to blank. Different dilutions, such as 3 µg/ml, 6 µg/ml, 9 µg/ml, 12 µg/ml, 15 µg/ml, 18 µg/ml, 21 µg/ml, 24 µg/ml, 27 µg/ml and 30 µg/ml, were created in order to calculate the calibration curve.[13]

Solubility Studies

For quantitative solubility study, excess amount of drug was taken in thoroughly cleaned culture flasks containing 5 ml of different solvents (Methanol, Ethanol, Ether, Chloroform, water) and test tubes were tightly closed. These test tubes were shake on water bath shaker for 24 hrs. at room temperature. After 24 hrs., each sample was centrifuge for 15 minutes at 15,000 rpm and was suitably diluted and determined spectrophotometrically.[14][15][16]

Partition Coefficient of Drug

A drug's lipophilicity and capacity to cross cell membranes are indicated by its partition coefficient (oil/water). Its meaning is the proportion of unionized medication that is equally distributed between the aqueous and organic phases at equilibrium. The partition coefficient offers a way to describe how lipophilic or hydrophilic the medication is drug having values of P much greater than 1 are classified as lipophilic, where as those with values much less than 1 are indicative of a hydrophilic drug. The partition coefficient is commonly determined using an oil phase of n-octanol and water.[14]

Fig. 6.2: Partition Coefficient of Sulfasalazine.

Shake flask method

With the shake flask method, the partition coefficient determination investigation was carried out. Excess amounts of the drugs dissolved in 5 ml of two solvents (n-octanol: Water) together (1:1) and placed for 24 hrs. After 24 hrs., the two layers were separated and

centrifuge for 15 minutes at 15,000 rpm. The absorbance was taken in UV spectrophotometer at the respective λmax after appropriate dilution.[16]

FTIR spectroscopy of the drug

Fourier transform infrared Spectroscopies of different compounds were performed for identification of that particular compound. FT-IR Spectroscopy of pure drugs was done using KBr pellets. Various peaks in FT-IR Spectrum were interpreted for identification of different group in the structure of pure drugs. FT-IR Spectroscopy can also be used to investigate and predict any physicochemical interactions between different components.[14][17][18]

DEVELOPMENT OF SULFASALAZINE LOADED EUDRAGIT MICROPARTICLE USING SPRAYING DRYING METHOD

1. The spray-drying process was used to prepare the microparticles.
2. Eight distinct formulations were acquired, based on the quantity of SLZ present in theircomposition they were designated as F1, F2, F3, F4, F5, F6, F7 and F8.
3. Then Eudragit L100 we mixed in ethanol under the magnetic stirring until it mixed properly.
4. Then add the SLZ in it
5. Mix it properly.

Composition of different formulation Sulfasalazine microparticles with polymer EudragitL 100.

Sr. No	Formulation Code	Drug (mg)	Polymer (eudragit Ll00)
1	F1	1.1	2.9
		1.1	2.9
		1.1	2.9
2	F2	1.2	2.8
		1.2	2.8
		1.2	2.8
3	F3	1.3	2.7
		1.3	2.7
		1.3	2.7
4	F4	1.4	2.6
		1.4	2.6
		1.4	2.6
5	F5	1.5	2.5
		1.5	2.5
		1.5	2.5
6	F6	1.6	2.4

		1.6	2.4
		1.6	2.4
7	F7	1.7	2.3
		1.7	2.3
		1.7	2.3
8	F8	1.8	2.2
		1.8	2.2
		1.8	2.2

6. Then add purifier water.
7. The following operational parameters were used.

Table of operational parameters used in spray drying method parameters used in spray drying method

INLET TEMPERATURE (°C)	150.0
OUTLET TEMPERATURE (°C)	80.0
PLATE TEMPERATURE (°C)	10.0
INLET HIGH TEMPERATURE (°C)	200.0
OUTLET HIGH TEMPERATURE (°C)	180.0
COOLING TEMPERATURE (°C)	40.0
FEED PUMP FLOW RATE (ml/m)	2.0
'D' BLOCK ON TIME (sec.)	1
'D' BLOCK OFF TIME (sec.)	20
OXYGEN(N)	21
STIRRER SPEED (%)	11
ASPIRATOR FLOW RATE ($Nm^3/hr.$)	50
CYCLE TIME (min.)	425

8. The dried powders were carefully extracted, weighed and kept at room temperature in a dark,well-sealed glass vessel
9. Every formulation was taken from separate batches.[19]

Fig 6.3 Preparation of Sulfasalazine Microparticles Using Spraying Drying Method In vitro characterization of microparticles formulation.

Optical microscopy

Microscopes are used for measuring the size of the microparticles. In the process of spray drying each batch were collected on a glass side which was fixed with on a collector. The spherical appearance and quality were observed by optical microscopy.

Percentage yield: The already made microspheres from various formulas were gathered and weighed. The weight that was measured was divided by the entire quantity of non-volatile chemicals that went into making the microspheres. [20]

% Yield = Actual weight of microspheres /Total weight of drug and polymer x 100Angle of repose.

When a body is placed on an inclined surface and it starts to slide down, it is the smallest angle itmakes with the horizontal. Allow any inclined plane to be inclined at a θ angle with respect tothe horizontal, then watch as a body with mass starts to descend.[21]

Table 6.5: Angle of repose table.

Flow ability expected	Angle of repose
Excellent / Very Free Flow	25-30
Good / Free Flow	31-35
Fair (discharging aid may not be required)	36-40
Passable	41-45

Poor Flow / Cohesive (active discharging aid required)	46-55
Very Poor Flow / Very Cohesive	56-65
Approximate no flow	> 66

Fourier Transform Infrared Spectrometers (FTIR)

Fourier Transform Infrared Spectrometers, or FTIR spectrometers, are extensively utilized in the pharmaceutical business, food testing, petrochemical engineering, organic synthesis and polymer science. Furthermore, because FTIR spectrometers can be separated with chromatography, these instruments can be used to study the mechanism of chemical reactions as well as the identification of unstable chemicals.[22]

In Vitro Release Studies

- The drug release study was assessed by USP method II, also known as the dissolution testmethod.
- Then the microparticle was placed in a 900 mL of dissolution medium and after that therotation of the paddle fluid was maintained at 100 rpm.
- The dialysis bag method was used to compare the drug release of Pure SlZ (100 mg) dispersed in distilled water (10 ml) and sonicated for 30 minutes, as well as SSZ microparticle.
- A dialysis bag containing 5 milliliters of each sample was submerged in 900 milliliters of phosphate-buffered saline (PBS, pH 7.4) and continuously stirrer at a speed of 100 revolutions per minute. Five milliliters of the sample were taken out of the dissolution media at predefined intervals (0, 1, 2, 3, 4, 5, 6, 7, 8, 12 and 24 hours) as long as the sink conditionswere maintained.
- UV-visible spectroscopy was used to measure the SL. 6Z concentration of the samples at 364nm.[23]

SEM

SEMs, or scanning electron microscopes, are strong instruments for examining the structure & surface structures of microparticles. A concentrated beam of high-energy electrons is employedin SEM to interact with the material. X-rays, backscattered electrons and secondary electrons are among the signals produced when the electron beam strikes the material. The signals are recognized and processed to provide surface-level high-resolution pictures of the sample.[24]

Tap density

The mass of a powder divided by its volume after it has been tapped for a predetermined amount of time is known as the powder's "tapped density." A powder's random dense packing is represented by its tapped density. Equation can be used to determine the tapped density, where M is the mass in grams and Vf is the tapped volume in milliliters.

Bulk Density

The mass of an untapped powder sample divided by its volume, which includes the interparticle void volume contribution, is the powder's bulk density. As a result, the density of the powder particles and their spatial arrangement within the powder bed both affect the bulk density. Because the measurements are done using cylinders, the bulk density is represented in grams per milliliter (g/mL), even though the international unit is kilogram per cubic meter (1 g/mL = 1000 kg/m3). Another way to describe it is as grams per cubic centimeter, or g/cm3.

$$\text{Bullk Density} = \frac{\text{Weight (W)}}{\text{Volume(V1)}}$$

Hausner Ratio (%): Hausner ratio is the ratio of tapped density to the bulk density. The Hausner ratio (HR) is a measure of powder flow easiness that is indirect. It is calculated by the following formula:[25] Hausner Ratio = Tapped density/ Bulk density Flow properties corresponding to Hausner's ratio S.No. Hausner's Ratio Property

1. 0-1.25 Free flowing
2. 1.25-1.6 Cohesive powder

Zeta Potential

The particles' surface charge affects their physical characteristics, such as their tendency to collect, but it also greatly affects how well they function biologically. Negatively charged surfaces, such as those of natural polysaccharide molecules like polymethacrylic acid, hyaluronic acid and polyalginic acid, attract and are hemocompatible with tissues in inflammations. On the other hand, positively charged microparticles—such as chitosan and poly-l-lysine—have a mucoadhesive quality and are not hemo compatible. Photon correlation spectroscopy is a useful tool for determining particle size and zeta potential. Zeta potential measurements provide useful information on the ability of surface-charged microparticles to aggregate in a suspension (above±30 mV stable suspended particles due to repulsion), as

well as whether the surface-charged molecule is adsorbed or encapsulated.[26]

Drug release kinetic studies

In the present study, raw data obtained from in vitro release studies was analyzed, wherein data was fitted to different equations and kinetics model to calculate the percent drug release and release kinetics of sulfasalazine from fast dissolving film. The kinetic models used were a Zero- order equation, First-order, Higuchi's model and Korsmeyer-Peppas equation.

Zero-order kinetics

A zero-order release would be predicted by the following equation.

$A_t = A_0 - K_0 t$ (1)

Where:

A_t = Drug release at time 't' A_0 = Initial drug concentration

K_0 = Zero order rate constant (hr^{-1})

When the data is plotted as cumulative percent drug release versus time, if the plot is linear thenthe data obeys zero-order release kinetics, with a slope equal to K_0.

First order kinetics

A first-order release would be predicted by the following equation.

$\text{Log } C = \text{Log } C_0 - Kt/2.303$ (2)

Where:

C = Amount of drug remained at time 't'C_0 = Initial amount of drug

K= First-order rate constant (hr^{-1})

When the data is plotted as log cumulative percent drug remaining versus time yields a straight line, indicating that the release follows first-order kinetics. The constant 'K' can be obtained by multiplying 2.303 with slope values.

Higuchi's model

Drug release from the matrix devices by diffusion has been described by following Higuchi's classical diffusion equation.

$Q = [D\varepsilon/\tau\ (2A - \varepsilon C_S)]\ C_S t^{1/2}$ (3)

Where

Q= Amount of drug released at time't'

D= Diffusion coefficient of the drug in the matrix A = Total amount of drug in unit volume of matrix.

C_S = The solubility of drug in the diffusion mediumε = Porosity of the matrix

τ = Tortuosity

t = Time (hrs.) at which 'Q' amount of drug is released.

Equation may be simplified if one may assume that Dε, Cs and A are constant. Then equationbecomes.

$Q=K.t^2$ (4)

When the data is plotted according to equation i.e., cumulative drug released versus square root of time, yields a straight line, indicating that the drug was released by diffusion mechanism. The slope is equal to 'K'.

Korsmeyer-Peppas Model

The release rates from controlled release polymeric matrices can be described by the equation:$Q = K1t^n$ (5)

Where:

Q = Percentage of drug released at time 't'

K = Kinetic constant incorporation structural and geometric characteristics and 'n' is the diffusion exponent indicative of the release mechanism.

For Fickian release, n = 0.45 while anomalous (non-Fickian) transport, n ranges between 0.45and 0.89 and for zero-order release, n = 0.89.[27]

RESULTS AND DISCUSSION

PREFORMULATION STUDIES

The aim of preformulation studies is to investigate the physical and chemical properties of a drugsubstance. Preformulation studies are essential to the entire process of developing new drugs. It is the examination of the drug's chemical and physical characteristics before the compounding procedure. These investigations concentrate on the drug's physicochemical characteristics that may have an impact on how well it works and how to create an effective dose form. In the end, a full comprehension of these characteristics might justify formulation design or lend credence to the necessity of molecular modification. In the most straightforward scenario, these preformulation studies might only verify that the compound's development is not significantly hampered. The methodology for developing a safe, efficient and stable dosage form depends heavily on this research. A variety of analytical methods, including solubility, melting point, UV and IR spectroscopy, were used to identify the drug

sample that was acquired.

The selected drug sulfasalazine was subjected for investigation of physical characterization parameters such as.

- Organoleptic properties
- Melting point
- UV-visible spectra
- Solubility
- Partition coefficient
- FT-IR spectra

Organoleptic Properties

Organoleptic properties of were Sulfasalazine found to be as per literature. The Organolepticproperties of Sulfasalazine were found to the given in **Table 1.1**

Table 1.1: Organoleptic Properties of Sulfasalazine.

Sr. No.	Properties	Inferences
1.	Colour	Yellow or orange
2.	Form	Powder
3.	Taste	Tasteless

Melting Point

The melting point of a substance is the temperature at which the solid phase gets converted to liquid phase under the one atmosphere of pressure. Determining the melting point shows the drug's purity. Melting point of Sulfasalazine was determined by capillary tube method and was found to be quite similar to the reported melting point as shown in **Table 1.2**

Table 1.2: Melting Point of Sulfasalazine.

Drug	Reference M.P.	Observed M.P.
Sulfasalazine	240 - 260°C	257°C

Discussion: The melting point of sulfasalazine was found to be 257°C which is in the range ofthe pure drug. As a result, the drug sample had been cleared of all contaminants.

UV Spectroscopy

Determination of absorption maxima in Methanol

For the drug's quantitative analysis, a double beam UV-visible spectrophotometer was used.

A 60 µg/ml solution of sulfasalazine ethanol was scanned in the range of 364 nm.

The result of UV spectrum of sulfasalazine shown in **Figure 1.1**.

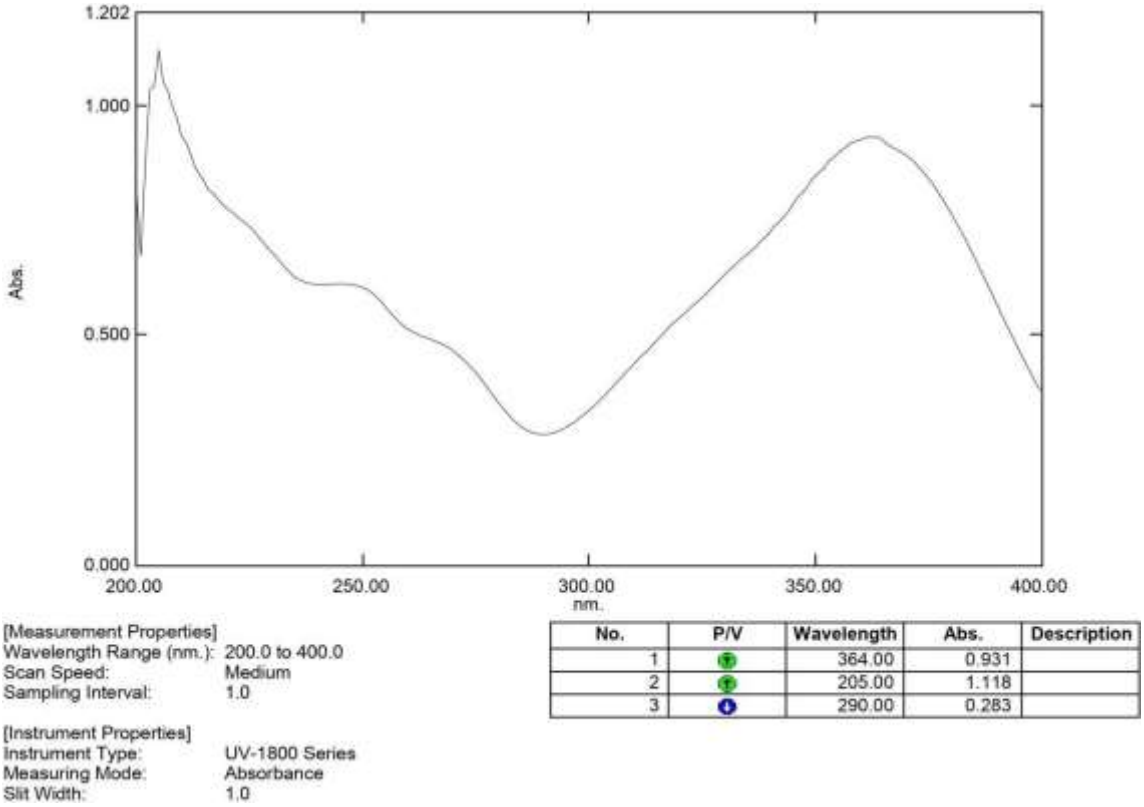


Figure 1.1: UV Spectrum of sulfasalazine Table 1.3: Absorption maxima (λ max) of sulfasalazine.

Name of drug	Absorption maxima (λ max)	
	Observed	Reference
Sulfasalazine	364	359

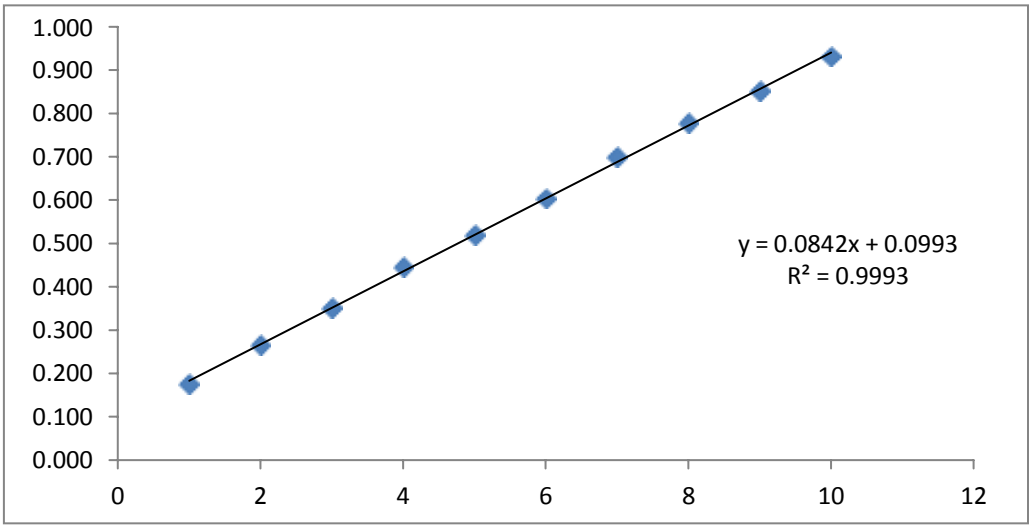
Discussion: The maximum wavelength of sulfasalazine observed at 364 nm similar to literature(**Table 1.3**).

Preparation of standard curve of Sulfasalazine

Table 1.4: Calibration curve of Sulfasalazine (λ max= 364 nm).

Sr. No.	concentration. (µg/ml)	Mean ± SD
1	3	0.175±0.004
2	6	0.265±0.008
3	9	0.352±0.008
4	12	0.445±0.023
5	15	0.520±0.026
6	18	0.604±0.003

7	21	0.698±0.003
8	24	0.779±0.004
9	27	0.852±0.003
10	30	0.931±0.004



Value is expressed as mean ± SD; n = 3

Figure 1.2: Standard calibration curve of Sulfasalazine

Table 1.5: Result of regression analysis of UV method.

Statistical parameters	Results
λmax	364 nm
Regression equation (y =mx+ c)	y = 0.084x + 0.099
Slope (m)	0.084
Intercept (C)	0.099
Correlation coefficient (R^2)	0.999

Discussion: - The calibration curve for sulfasalazine was obtained by using the 3 to 30 µg/ml concentration of sulfasalazine methanol. The absorbance was measured at 364 nm. The calibration curve of sulfasalazine shows in graph indicated the regression equation.

y = 0.084x + 0.099 and R^2 value 0.999 which shows good linearity as shown in **Table 1.5** and Figure 1.2.

Solubility Studies

Drug solubility tests in a range of solvents were performed to identify the components that wouldbe used in the formulation development process. Analysis of the drug was carried out on UV Spectrophotometer at 364 nm.

Table 1.6: Solubility studies of sulfasalazine for different solvents.

Sr. No.	Name of Solvents	Solubility (mg/ml)	Solubility
1	Ethanol	10.35±0.74	Slightly soluble
2	Methanol	0.067±0.002	Practically insoluble
3	Chloroform	0.074±0.008	Practically insoluble
4	Water	0.035±0.004	Practically insoluble
5	Ether	0.053±0.004	Practically insoluble

Value is expressed as mean ± SD; n = 3

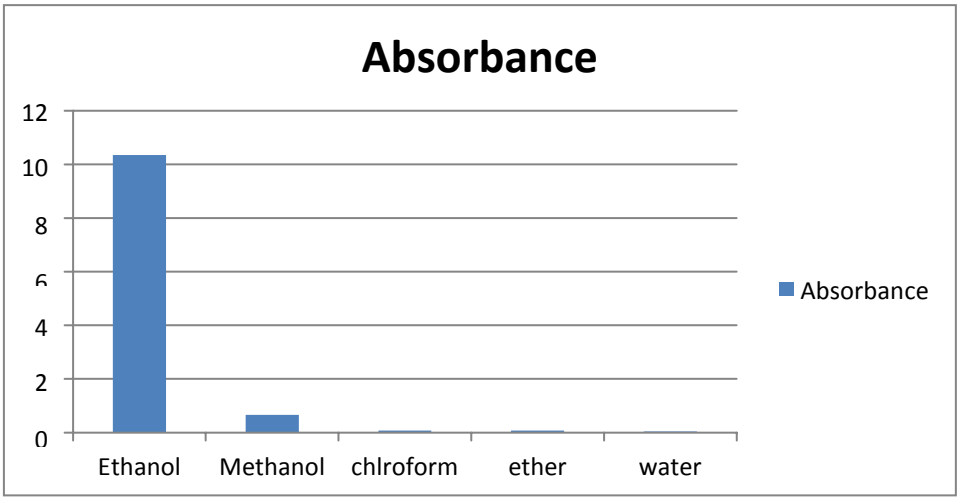


Figure 1.3: Solubility study of drug in different solvents.

Discussion: From the above data, it is clearly seen that sulfasalazine is slightly soluble in methanol and practically insoluble in ethanol, ether, chloroform and water. (**Figure 1.3 and Table 1.6.**

Partition coefficient determination

Partition coefficient of the sulfasalazine was determined using n-octanol and water. Log P greater than one indicates that the drug is lipophilic in nature, whereas those with partition coefficients less than one are indicative of a hydrophilic drug. This indicated the lipophilicity andpurity of drug.

Table 1.7: Partition coefficient determination of Sulfasalazine.

Partition coefficient ofdrug	Solvent system	Log P Values	Reference
Sulfasalazine	n-octanol: water	2.34±0.002	2.5

Value is expressed as mean ± SD; n = 3

Discussion: The partition coefficient of Sulfasalazine n-octanol: water was found to be, this 2.34±0.002 indicates that the drug is lipophilic in nature (**Table 1.7**) which is similar to the literature.

FTIR Studies

FTIR study of Sulfasalazine

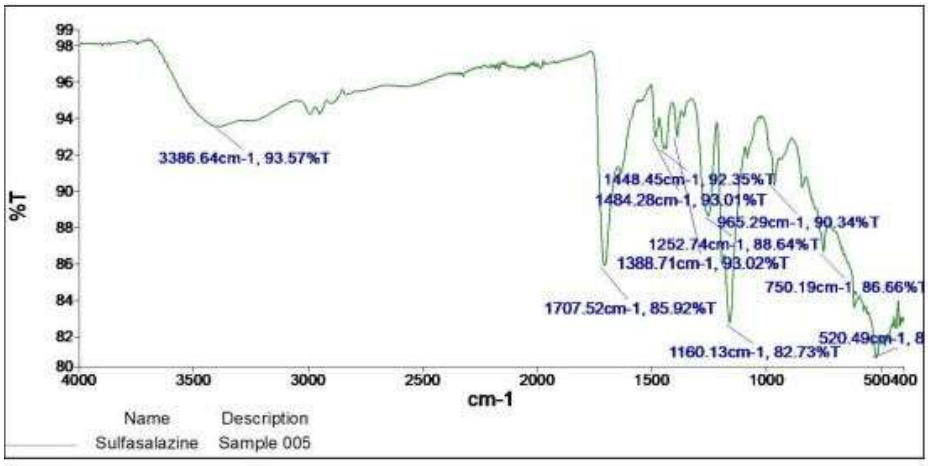


Figure 1.4: FTIR interpretation of Sulfasalazine.

Table 1.8: FTIR interpretation of Sulfasalazine.

Sr. No.	Characteristics Peak	Reference (cm^{-1})	Observed (cm^{-1})
1.	N-H	3394	3386.64
2.	OH Bending	1100 -1070	1160.13
3.	C-N stretching	1350 – 1100	1252.74
4.	C–N	1174.51	1160.13

Discussion: The FTIR spectra of Sulfasalazine were shown in the **Figure 1.4; Table 1.8**. The principal IR absorption peaks of Sulfasalazine at 3386.64 cm^{-1}(N-H),1160.13 cm^{-1}(OH Bending),1252.74 cm^{-1}(C-N stretching),1160 cm^{-1} (C-N) were all observed in the spectra of Sulfasalazine. These observed principal peaks confirmed the purity and authenticity of the Sulfasalazine.

FTIR spectrum of EudragitL100

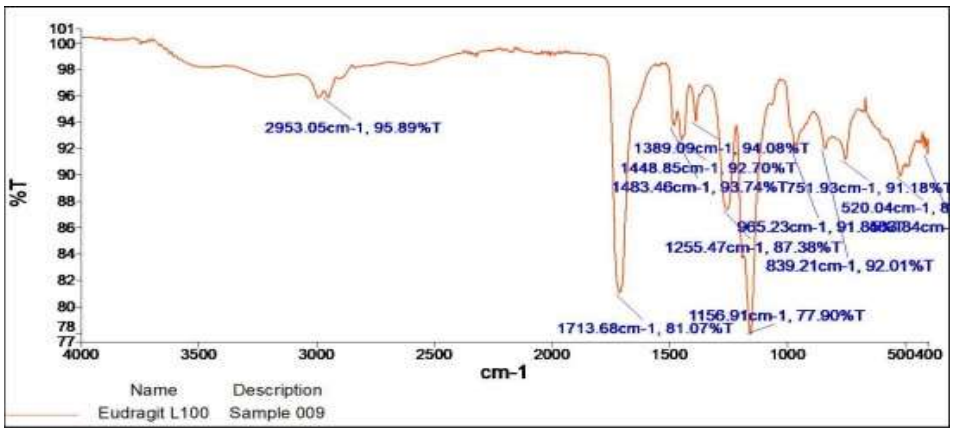


Figure 1.5: FTIR spectrum of eudragit L100.

Table 1.9: FTIR interpretation of eudragit L100.

Sr. No.	Characteristics Peak	Reference (cm^{-1})	Observed (cm^{-1})
1.	C=O	1709.28	1713.68
2.	Ar-CH (In plane Bending)	1158.96	1156.91
3.	Al-CH-bend	1255.31	1255.47

Discussion: The FTIR spectra of eudragit L100 were shown in the **Figure 1.5; Table 1.9**. The principal IR absorption peaks of eudragit L100 at 1719.68 cm^{-1}(C=O), 1156.91cm^{-1} 1255.47(Ar- CH (In plane Bending), Al-CH-bend were all observed in the spectra of eudragit Ll00. These observed principal peaks confirmed the purity and authenticity of the eudragit L100.

FTIR spectrum of Sulfasalazine with Eudragitl100

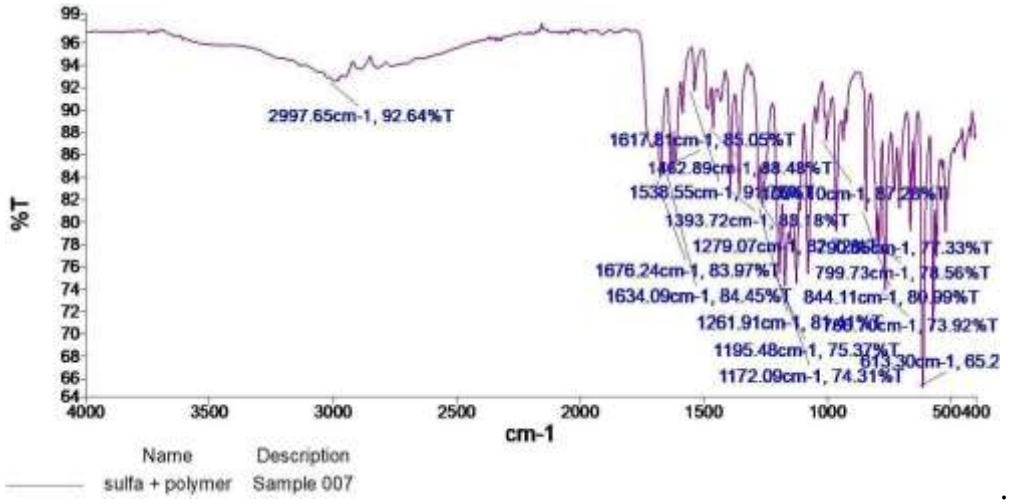


Figure 1.6: FTIR spectrum of drug with polymer.

Discussion: The physical mixture of API and polymer show no interaction.

Preparation, optimization and characterization of microparticle

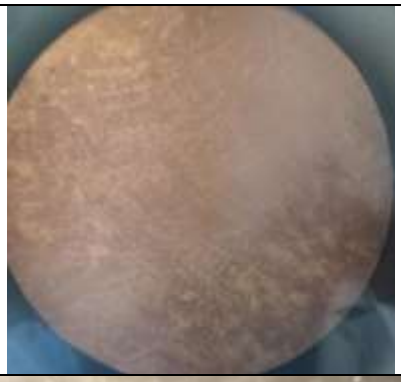
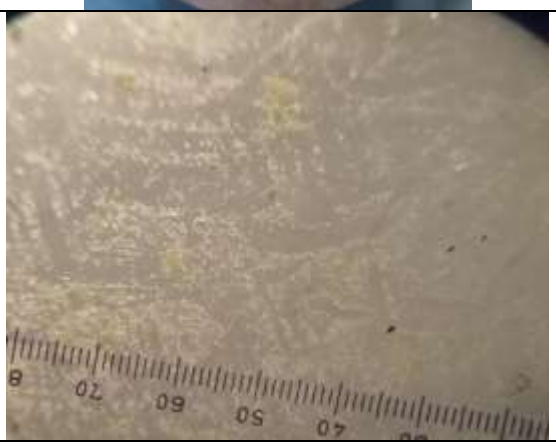
A number of novel drug delivery systems have emerged to achieve sustained and targeted drug delivery. The poor bioavailability of Sulfasalazine leads to poor variation in bioavailability. Preparation of the microparticles with eudragit L100 to improve the poor bioavailability and the dissolution behaviors. This system is more stable, having higher drug entrapment capacity, easy to scale up, better for intestine delivery. Microparticle loaded with Sulfasalazine successfully prepared using spray drying technique. Multiple formulations were made with varying polymer ratios. These microparticles were characterized % Drug entrapment and Appearance.

Evaluation & characterization of microparticlesOptical Microscopy Analysis

Table 1.10: Optical Microscopy Analysis.

S.no.	Formulation code	Appearance
1	F1	Less Spherical Microparticles was formed
2	F2	Less Spherical shape Microparticles was formed
3	F3	Spherical shape Microparticles was formed
4	F4	Spherical shape Microparticles was formed
5	F5	Spherical shape Microparticles was formed
6	F6	Perfect Spherical shape Microparticles was formed
7	F7	Spherical shape Microparticles was formed
8	F8	Spherical shape Microparticles was formed

Table 1.11: Optical Microscopy picture of F5, F6 formulation Morphology characterization by scanning electron microscopy (SEM) analysis.

FORMULATION CODE	RESULT	MICROSCOPIC IMAGE
F5	**Spherical shape**	
F6	**PERFECT FORMATION**	

SEM was used for the examination of morphology of sulfasalazine loaded microparticles. The SEM of sulfasalazine loaded microparticles show smooth and non-porous surface as shown in **fig.1.7**

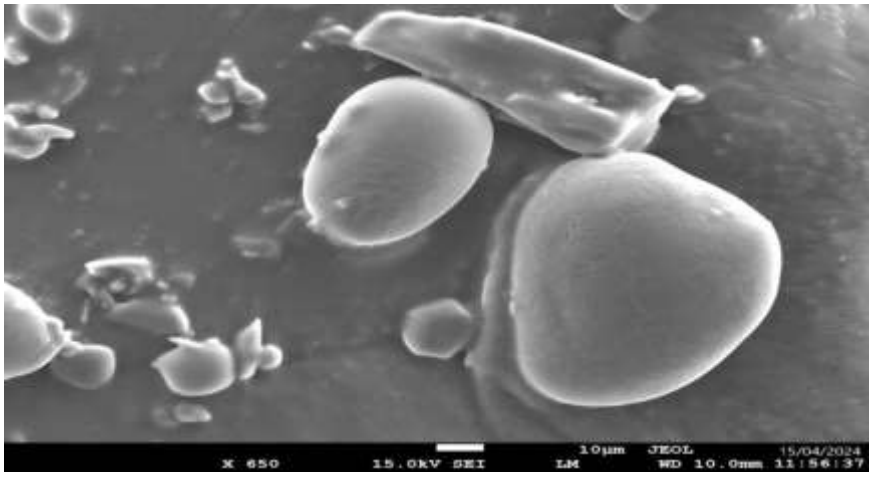


Figure 1.7: Scanning electron microscopy (SEM).

Percentage Yield

The production of the microparticles was determined by calculating the initial weight of raw material and the final weight of the microparticles obtain. All the experiment are performed in triplicate and the result are reported in the **table 1.12.**

Table 1.12: Percentage yield of formulation.

Percentage Yield								
Sr. No	**Formulation Code**	**Drug (mg)**	**Polymer (eudragitL100)(mg)**	**total**	**microparticles (mg)**	**% Yield**	**Average**	**S. D**
1	F1	1.1	2.9	4	2.44	61.00	61.417	0.722
		1.1	2.9	4	2.49	62.25		
		1.1	2.9	4	2.44	61.00		
2	F2	1.2	2.8	4	2.51	62.75	62.583	0.289
		1.2	2.8	4	2.51	62.75		
		1.2	2.8	4	2.49	62.25		
3	F3	1.3	2.7	4	2.56	64.00	63.833	0.289
		1.3	2.7	4	2.56	64.00		
		1.3	2.7	4	2.54	63.50		
4	F4	1.4	2.6	4	2.6	65.00	65.583	0.520
		1.4	2.6	4	2.64	66.00		
		1.4	2.6	4	2.63	65.75		
5	F5	1.5	2.5	4	2.88	72.00	70.917	0.946
		1.5	2.5	4	2.82	70.50		
		1.5	2.5	4	2.81	70.25		
6	F6	1.6	2.4	4	2.88	72.00	71.667	0.577
		1.6	2.4	4	2.84	71.00		
		1.6	2.4	4	2.88	72.00		
7	F7	1.7	2.3	4	2.65	66.25	66.250	0.250
		1.7	2.3	4	2.64	66.00		
		1.7	2.3	4	2.66	66.50		
		1.8	2.2	4	2.44	61.00		

8	F8	1.8	2.2	4	2.43	60.75	61.583	1.233
		1.8	2.2	4	2.45	63.00		

Discussion: The production yield was found to be between 61.417 to 71.667 for F1 and F8. The highest yield was found to be in formulation F6 i.e. 71.667 %.

Entrapment

Several batches of eudragit L100 microspheres containing sulfasalazine were prepared varying the drug to polymer ratio. Values of the drug entrapment efficiency in to eudragit L100 microspheres are presented in **(Table No. 1.13).**

Table 1.13 Entrapment efficiency of drug in microparticle.

DRUG Entrapment		
Formulation Code	**Entrapment**	**MEAN±SD**
F1	66.07	66.1±0.18
	66.31	
	65.95	
F2	73.69	73.51±0.03
	73.69	
	73.15	
F3	72.5	72.34±0.15
	72.2	
	72.32	
F4	79.1	78.78±0.32
	78.45	
	78.81	
F5	82.38	82.36±0.09
	82.26	
	82.44	
F6	85.59	85.53±0.27
	85.77	
	85.23	
F7	78.03	77.83±0.20
	77.61	
	77.85	
F8	73.51	74.2±0.71
	74.94	
	74.16	

It can be concluded that the highest entrapment efficiency of drug loaded microspheres was found to be 85.53 ±0.27 for formulation F6. The entrapment efficiency was found to be 66.1±0.18%, 73.51±0.03%, 72.34±0.15%, 78.78±0.32%, 82.36±0.09%, 85.53±0.27%, 77.83±0.20 %, 74.2±0.71%, for F1, F2, F3, F4, F5, F6, F7, F8 formulation respectively.

Discussion: The entrapment efficacy of formulation F6 (85.53±0.27) is found to be high then other. This may be due to increase the amount of eudragit L 100 for some time after adding morepolymer the entrapment of the drug is decrease.

Evaluate the flow properties

Table 1.14: Pre-compression parameters.

Formulation Code	Angle of repose (θ) (Mean ±SD)	Bulk density (g/cm^3) (Mean ±SD)	Tap density (g/cm3) (Mean ±SD)	Hausner's Ratio (Mean ±SD)	Carr's index (%) (Mean ±SD)
F1	21.44±0.73	0.39±007	0.42±005	1.06±0.06	5.88±0.90
F2	24.72±0.31	0.42±0.44	0.44±0.02	1.04±0.10	3.97±2.00
F3	26.42±35	0.44±0.45	0.45±0.01	1.03±0.06	2.85±0.98
F4	22.61±17	0.49±0.008	0.51±0.01	1.05±0.10	4.31±0.99
F5	18.22±30	0.51±0.02	0.54±0.02	1.05±0.20	4.66±0.89
F6	21.04±15	0.49±0.007	0.52±0.003	1.07±0.12	6.27±0.88
F7	21.87±21	0.43±0.008	0.45±0.01	1.05±0.10	4.84±0.08
F8	20.16±30	0.43±0.01	0.45±0.01	1.05±0.15	4.64±0.93

Evaluate the flow properties

Initially all the formulation blends were evaluated for various pre-compression parameters to evaluate the flow properties. The observations are tabulated in table 1.14. The values of angle of repose, bulk density, tapped density, Hausner's ratio and Carr's index for all F1 to F8 formulations are 18.22±30 to 26.42±35, 0.39±007 to0.51±0.02, 1.03±0.06 to 1.07±0.12 and 2.85±0.98 to 6.27±0.88 respectively.

FTIR Fourier Transform Infrared Spectrometers

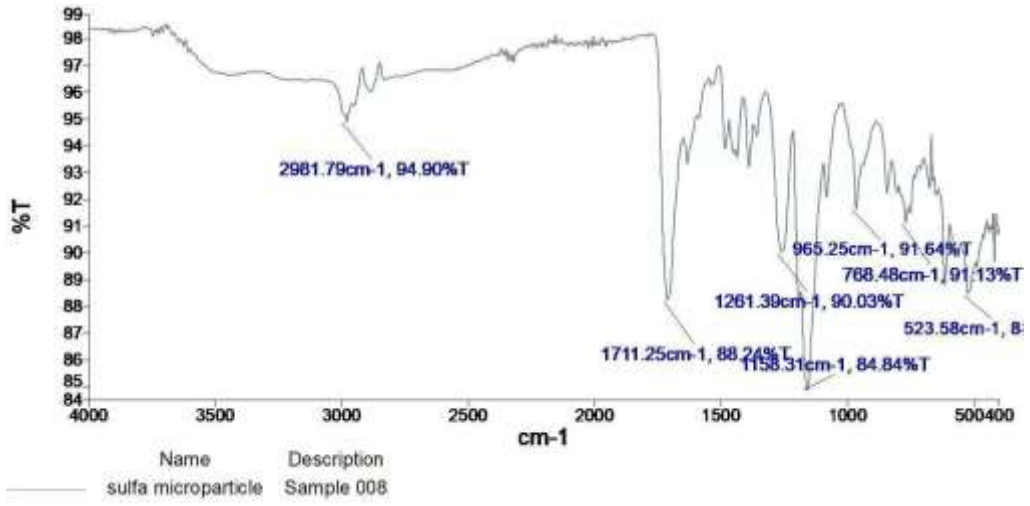


Figure 1.8: FTIR spectrum of microparticles.

As seen from Figure 1.8, FTIR spectrum of microparticles displayed some peaks of Sulfasalazine with reduced intensity. FTIR spectrum of pure drug Sulfasalazine showed the characteristic peaks at wave number of 1158.31 cm^{-1}, 1261.30 cm^{-1} representing to the CH stretching, C-N stretching respectively.

Zeta potential

The charge that is present on the surface of the particle is displayed by the Zeta Potential. Either +30 or -30 mV is typically used as the overall level to distinguish between stable and unstable microparticle. Typically, particles are regarded as stable when their zeta potential is greater than +30 mV or greater than -30 mV.

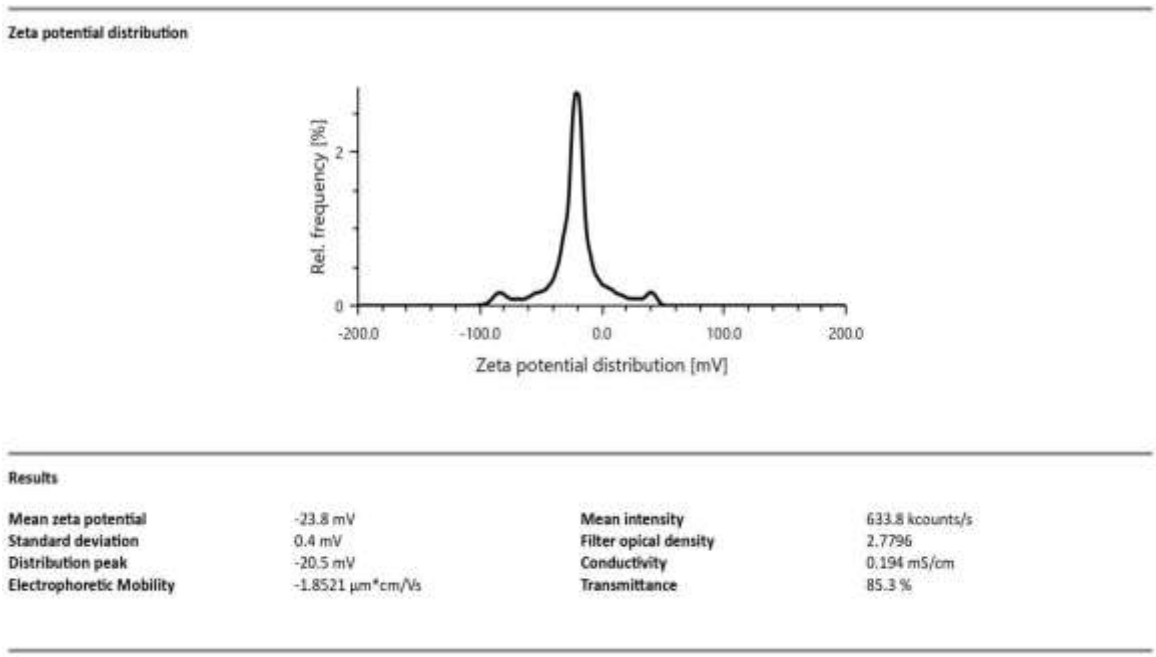


Figure1.9: Zeta potential of sulfasalazine microparticles.

Discussion: The zeta potential of sulfasalazine microparticles is -23.8 mV which lies between the stable range and close to the reference value. The sharp peak indicates that the drug taken of desirable purity, no change in Zeta potential pattern, even after pure drug converted to microparticles.

In vitro release studies

Drug release graphs for pure drugs are displayed in Figure 1.9 Formulation containing drug-loaded microparticle showed good characteristic delay drug release in comparison to the pure drug. Of the formulations, F6 showed approximately 86.242% drug release in the medium within 24 hours; aside from that, in vitro release of pure drug showed 47.61% drug release

within 24 hours (Table 1.15 and Figure 1.11). The microparticle provided defined surface area to entrap drug molecules.

Table 1.15 In vitro release profile of pure drug and drug containing microparticle F6.

Time	DRUG RELEASE OF PURE DRUG	DRUG RELEASE OF MICROPARTICLE
0	0	0
0.25	5.6	1.01
0.5	12.73	5.85
1	21.85	9.19
2	23.94	13.82
3	27.99	25.71
4	31.47	41.19
6	33.5	59.61
8	34.56	74.839
10	36.94	80.523
12	41.01	84.593
24	47.61	86.242

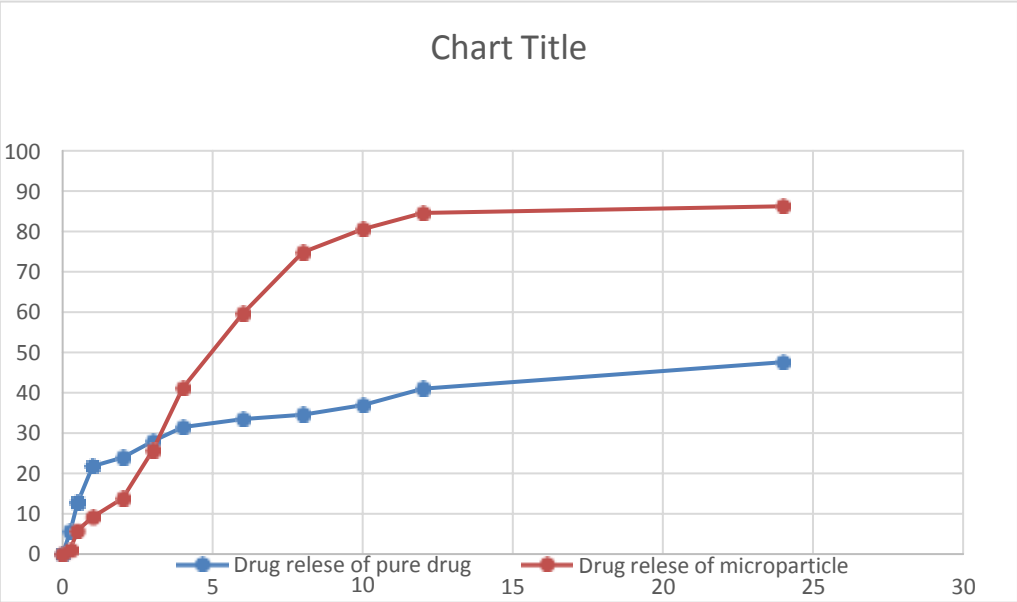


Fig. 1.10 Drug release graph for pure drug and formulation F6.

7.3.9.1 Zero order kinetics models

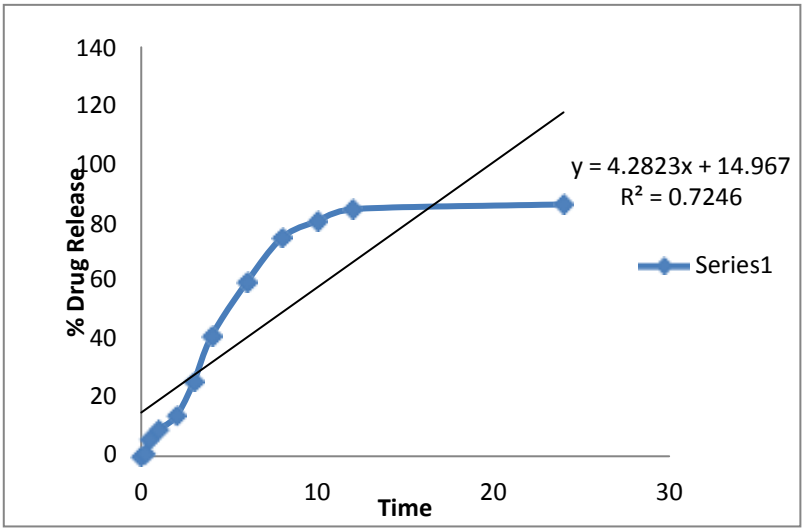


Fig. 1.11 Zero order graph of formulation F6First Order kinetics model.

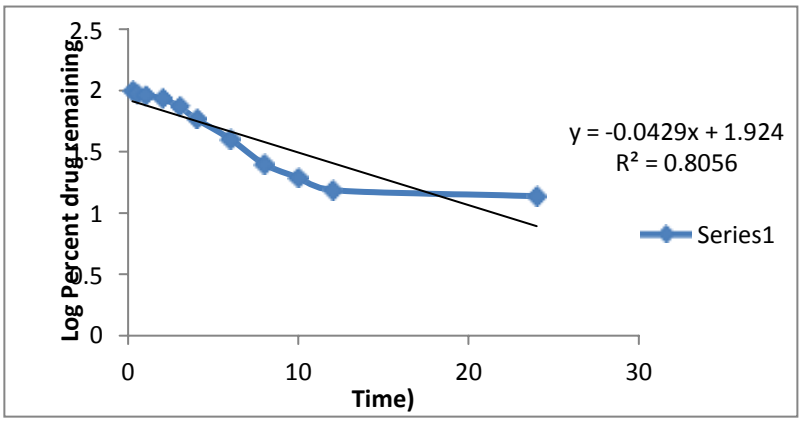


Figure 1.12: First order graph of formulation F6Higuchi kinetics model.

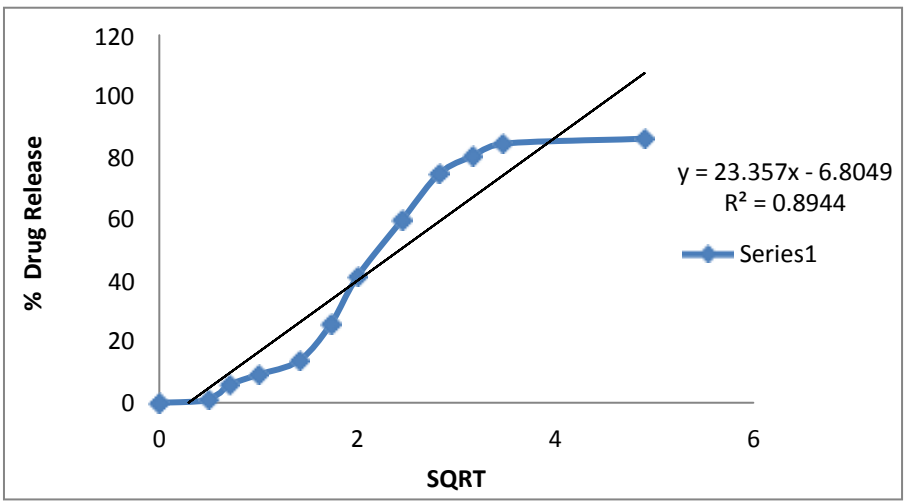


Figure 1.13: Higuchi order graph of formulation F6.

Korsmeyer peppas kinetics model

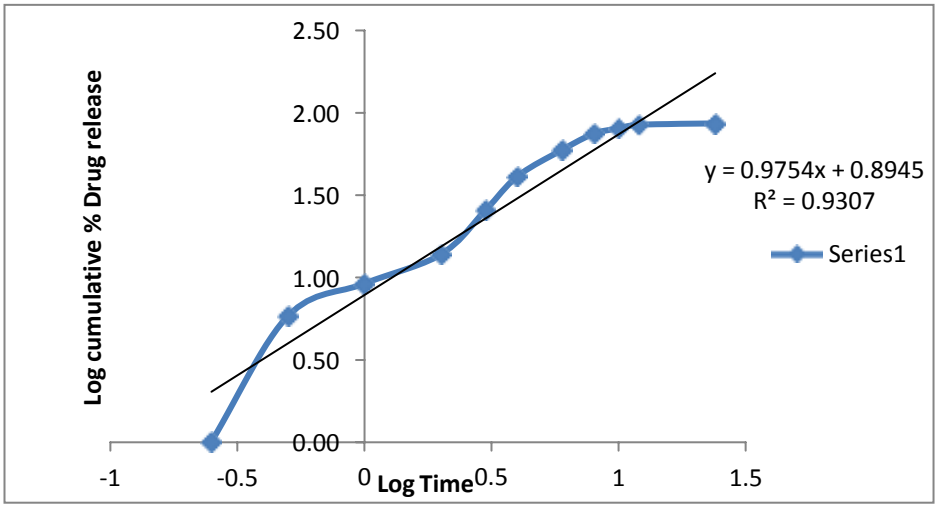


Figure 1.14: **Korsmeyer peppas order graph of formulation F6.**

Discussion: The *in vitro* drug release of formulation F6 was best explained by, as First order, theplots showed the linearity (R^2=0.8), followed by Higuchi kinetics (R^2=0.89), Korsmeyer-Peppas show highest linearity (R^2=0.93), zero order (R^2=0.72) and suggesting that the diffusion plays an important role in the conventional release.

Table 1.16: **Kinetic equation parameter of formulation F6.**

Formulation Code	Zero order		First order		Higuchi		K. Peppas	
	K0	R^2	K0	R^2	K0	R^2	K0	R^2
F6	4.28	0.72	-0.04	0.8	23.35	0.89	0.97	0.93

The data obtained for in vitro release shown in table 1.16 were fitted into equation for the zero order, first order and higuchi and Korsmeyer-peppas release models. The interpretation of data was based on the value of the resulting regression coefficients.

The zero order rates described the system where the drug release independent of its concentration showed the percent drug release Vs time for zero order kinetics. The higuchi order rate described the release from systems where the release of drugs from a matrix as a square root of a time- dependent process based on Fickian diffusion.

The calculated regression coefficients for zero order, first order and higuchi models and Korsmeyer were shown in table 1.16. It was found that in vitro drug release of F6 Formulation was best explained by Korsmeyer-Peppas model as the plot showed the highest linearity. The value of R^2 found to be highest for the Korsmeyer-Peppas model.

CONCLUSION

In conclusion, a thorough analysis using a variety of approaches was conducted to evaluate and characterize the sulfasalazine-loaded microparticles. Formulations F3 through F8 created spherical microparticles, with F6 obtaining a perfect spherical shape, according to optical microscopy. These microparticles' smooth and non-porous surface morphology was validated by scanning electron microscopy (SEM). The formulation F6 had the highest yield, with the manufacturing yield ranging from 61.417% to 71.667%. The formulations' drug entrapment efficiency varied, with F6 exhibiting the highest efficiency at 85.53 ±0.27% once more. This implies that, up until a particular threshold is achieved, a higher concentration of Eudragit L100 correlates to a higher rate of drug entrapment. All formulations showed good flow qualities according to pre-compression parameters, with Carr's index, Hausner's ratio, bulk density, tappeddensity, and angle of repose all falling within allowable bounds. The existence of sulfasalazine in the microparticles was verified by FTIR spectroscopy, which showed distinct peaks, but with less strength. The microparticles' stable surface charge of -23.8 mV, as revealed by the zeta potential study, suggests good stability.

When compared to the pure medication (47.61%), formulation F6 exhibited a significantly higher drug release rate (86.242% within 24 hours) according to in vitro release experiments. Formulation F6 most closely matched the Korsmeyer-Peppas model, according to kinetic modeling of the in vitro drug release data, suggesting that diffusion is an important factor in drugrelease from these microparticles. Formulation F6 most closely matched the Korsmeyer-Peppas model, according to kinetic modeling of the in vitro drug release data, suggesting that diffusionis an important factor in drug release from these microparticles. Due to its ideal spherical morphology, maximum production yield, exceptional drug entrapment efficiency, steady zeta potential, and advantageous drug release profile, formulation F6 was found to be the most promising contender overall.