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Advances in Bioresearch

ORIGINAL ARTICLE

Solubility Enhancement of Lycopene by Lyophilized Polymeric Nanoparticles

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ABSTRACT
Lycopene is a potent anti-oxidant which has been widely reported for its potential benefits at reducing the risks of certain type of cancer. The oral bioavailability of this highly lipophilic carotenoid is low and highly influenced by the extent of intestinal lymphatic uptake. The aim of the study was to develop an optimized formulation which allows for efficient absorption following oral administration. Solid dispersion of lycopene were developed initially, subsequently a polymeric nanoparticles was designed by using PVP K 30 and Eudragit L 100 solvent evaporation method. This polymeric nanoparticles was solidify by the Lyophilization techniques. This polymeric nanoparticles was evaluated by zeta potential measurement, entrapment efficiency solubility studies and In-vitro drug release studies. So this study concluded that the solubility of polymeric nanoparticles (i.e. lyophilized powder) is greater than the solid dispersion of the same ratios.
Keywords: *Lycopene, Solid Dispersion, Polymeric nanoparticles, Lyophilization.*

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INTRODUCTION

Lycopene has attracted considerable attention as a natural chemo preventive agent through various properties including its potent antioxidant properties. Number of epidemiological studies have demonstrated that high dietary intake of Lycopene is associated with reduced risk of various types of cancer [1]. Lycopene is a highly lipophilic carotenoid with the poor aqueous solubility, and previous studies have predicted that lycopene will display solubility rate limited absorption characteristics [2]. Hence formulation approaches which enhance solubility of Lycopene within the gastrointestinal tract (GIT) are considered crucial to increasing oral absorption. A number of formulation approaches have been utilised to enhance solubility of poorly soluble drug in GIT such as particle size reduction, or modification of crystal habit to enhance dissolution [3]. The solubility of a drug may be expressed as the parts, percentage, molarity, volume fraction, and mole fraction. Drug solubility is the maximum concentration of the drug solute dissolve in the solvent under specific condition of temperature, pH and pressure. The drug solubility in saturated solution is a static property where as the drug dissolution rate is dynamic property that relates more closely to the bioavailability rate. [5].
The main objective of the work was to enhance the solubility of lycopene with the help of suitable scientifically available novel methods.

MATERIAL AND METHODS

Materials
The drug Lycopene was received as a gift sample from Omni Active Health Technologies ltd. Supa, and Eudragit L100 was a gift sample from Evonik Degussa, Mumbai. Other chemicals and reagents used in the formulations and evaluation were of analytical grade, listed as below: 1.Lycopene (Omni Active Technologies Ltd. Supa), 2. Eudragit L 100 (Evonik Roehm Pharma, Mumbai), 3. Polyvinyl pyrolidone K 30 (Modern industries), 4. Dichloromethane (Modern industries), 5. N-Hexane (Merk life science Pvt. Ltd.

Mumbai), 6. Polyvinyl alcohol (Loba chem pvt. Ltd. Mumbai), 7. Potassium dihydrogen phosphate, 8. Sodium hydroxide, 9.Potassium chloride.

Instruments

1. Digital weighing balance (Shimadzu, Japan), 2. Mono quartz distillation unit (Borosil D.A.P.S.), 3. FT-IR (Bruker), 4. UV-Spectrophotometer (Shimadzu, Japan), 5. Remi Centrifuge, 6. Magnetic stirrer, 6. Digital ultrasonicater (Citizen), 7. Dissolution tester, USP, 8. Digital pH meter, 9. Probe sonicator.

Identification and characterization of drug

Organoleptic properties:

The organoleptic characteristics of Lycopene such as colour, odour and taste were studied.

Solubility study of Drug:

An excess amount of drug was added to 10 mL of water, 0.1 N HCl pH 1.2, and phosphate buffer of pH 7.4, 6.2, 6.8 and keep for the stirring for 24 h at room temperature with rotation speed of 100 rpm. and analyzed by UV spectrophotometer at obtained λ max all solubility measurements were performed in triplicate [8].

FT-IR spectra:

FT-IR spectra are used for functional group identification in compound. A small amount of drug in the form of powder was placed on selenium bromide crystal. FT-IR spectrum was run. Finally functional groups were detected by comparing the obtained IR ranges with reference ranges available.

4. UV Spectroscopy:

Determination of λ max of Lycopene:

From the stock solutions of Lycopene known concentrations of 10µg/ml was prepared by suitable dilution with n-hexane. The prepared solution was scanned against blank of n-hexane using UV spectrophotometer (Shimadzu1800).

2. Preparation of calibration curve for Lycopene:

From the 100 µg/mL prepared stock solutions, 0.5, 1, 1.5, 2, 2.5 ml solutions were pipetted into a series of 10 ml volumetric flask and were made-up to 10mL with Hexane to get 5, 10, 15, 20 and 25µg/mL solutions of Lycopene respectively. The absorbance of resulting solutions was measured at obtained λ max against the blank. Dilution for Calibration curves of Lycopene were performed in triplicate[27].

Drug –Excipient Compatibility Study

The purpose of drug/excipients compatibility consideration and practical studies is to define, as quickly as possible, real and possible interactions between potential formulation excipients and the API.Compatibility study was carried out both in absence and presence of moisture. The compatibility study was carried out at 45°C for 14 days with in hermetically sealed glass container of individual drug and Drug: Excipient (1:1).

Formulation development:

1 Selection of polymer:

Polymers was selected for preparation of Solid Dispersion and Nanoparticles, on the basis literature review, polyvinyl pyrolidone K30, Eudragit L100 compatible with Drug. It has been reported that have lower particle size and higher stability. It has shown enteric coating ability, pH-independent swelling and high drug loading ability.

2 Selection of Surfactants

PVA was selected for preparation of Nanoparticle, because, it has help to avoid particle aggregation. It helps to retention of drug particles.

Formulation of solid dispersion

Solid Dispersion of Lycopene was prepared by using polymer PVP K30 and Eudragit L 100 by solvent evaporation method. Both drug and polymer dissolved in organic solvent.(ratio are 1:1, 1:3, 1:5). The resultant solution kept for evaporation process at room temperature. After evaporation of solvent remaining solid material was collected.

Evaluation of solid dispersion

% Practical Yield:

Percentage practical yield was calculated to know about percent yield or efficiency of any method, thus its help in selection of appropriate method of production. SDs were collected and weighed to practical yield (PY) from the following equation.

%PY= [actual mass (SD)/ theoretical mass]×100

Total Drug Content

The solid dispersion sample was dissolved in 10 ml n-hexane and stirred by vortex mixing, followed by membrane filtration technique using 0.45µm filter. The concentration of drug estimated by UV spectroscopy using hexane as blank at 470nm

Solubility study

An excess amount of SDs was added to 10 mL of water, 0.1 N HCl (pH 1.2), and phosphate buffer of pH 7.4, 6.2, 6.8 and keep for the stirring for 24 h at room temperature with rotation speed of 100 rpm and analyzed by UV spectrophotometer at obtained λ max all solubility measurements were performed in triplicate [8].

In Vitro Dissolution Study of SDs

The dissolution studies was accomplished by using type II dissolution apparatus using an Electrolab. The dissolution medium was 900ml of phosphate Buffer PH 6.8 maintained at 37± 0.5 0 c and 100 RPM stirring rate. The sample withdraw at specific time intervals filtered and concentration of drug was determined by spectrophotometricaly at obtained λ max.

FT-IR Spectroscopy

IR spectra of pure lycopene, PVP K30, Eudragit L100, and PVA with its solid dispersions were obtained by a Fourier transform infrared spectrophotometer.

Preparation of polymeric nanoparticles

Lycopene nanoparticles was prepared by using PVP K 30 and Eudragit L100 polymer by solvent evaporation method. The organic phase were prepared by dissolving the drug and different concentration of polymer. The resultant solution added in the aqueous phase containing PVA with continuous stirring then organic phase is evaporated. After evaporation of organic phase the remaining nanoparticle suspension was centrifuged at 3000-6000rpm for 15minutes to separate untrapped drug and the supernatant was collected. Again centrifuged supernatant by 10 k MWCO for separation of free drug collect the nanoparticles and wash with n-hexane.

Evaluation of polymeric nanoparticles

pH Measurement of formulation

5ml of each formulation was taken in a 10ml beaker. pH was recorded using calibrated pH meter.

Total Drug Content

The Nanoparticle sample was dissolved in 10 ml hexane and stirred by vortex mixing, followed by membrane filtration technique using 0.45µm filter. The concentration of drug estimated by UV spectroscopy using hexane as blank at obtained λ max.

Particle size analysis:

The mean particle size and particle size distribution of drug loaded polymeric nanoparticle were determined by Nano plus 3, Particulate System, Micromeritics USA, at 28°C.

Zeta potential measurement:

The zeta potential of Lycopene loaded Zeta potential is an indication of the stability of the nanoparticles. It is defined as the difference in potential between the surface of the tightly bound layer (shear plane) and electro-neutral region of the solution. Zeta potential has practical application in stability of systems containing dispersed particles since this potential, rather than the Nernst potential, governs the degree of repulsion between the adjacent, similarly charged, dispersed particles. If the zeta potential is reduced below a certain value (which depends on the particular system being used) the attractive forces exceeds the repulsive forces and the particles come together. This phenomenon is called flocculation. For a stable suspension stabilized only by electrostatic repulsion, higher zeta potential value, whether it is negative or positive, indicates long-term stability. Zeta potential analysis was performed by Nano plus 3, Particulate System, Micromeritics USA.

Entrapment efficiency measurement:

Entrapment efficiency (% EE) is expressed as fraction of drug incorporated into formulations relative to the total amount of drug used. Determination of % entrapment efficiency is an important parameter in case of polymeric nanoparticles as it may affect the drug diffusion. EE is determining the free drug (non-encapsulated) by UV method. 2 ml of Lycopene nanoparticles suspension placed in dialysis bag (mol weight of 12-14 kDa) and dialysis bag is placed inside in centrifuge tube in hanging position and centrifuged for 10 min at 4000 rpm. After centrifugation the free drug is collected at the bottom side of centrifuge tube. These free drug is analyzed in UV Spectrophotometer at obtained λ max.

$$\textbf{EE (\%)} = [\textbf{Totaldrug} - \textbf{Freedrug}/\textbf{Total drug}] \times 100$$

In- vitro drug release study:

The Lycopene formulation (2.0 mL) was enclosed in a dialysis bag (cellulose membrane, mw cutoff 12,000) and bag is placed in 100 mL release medium in beaker which was maintained at room temperature and at 100 rpm. Phosphate buffer (PBS, pH 6.8) was used as the release medium. At predetermined time intervals, 5 ml of the sample were withdrawn from the release medium and analyzed spectrophotometricaly at obtained λ max. After sampling, 5 mL of fresh medium was added in the release medium to maintain the sink condition.

Solubility study of lyophilized product Solubility Study:
An excess amount of lyophilized product (ratio 1:5) was added to 10 mL of water, 0.1N HCl (pH 1.2), and phosphate buffer of pH 7.4, 6.2, 6.8 and keep for the stirring for 24 h at room temperature with rotation speed of 100 rpm. The supernatant solution were then passed through a whatman filter paper. After equilibration, the samples were filtered through 0.45 µm pore size and analyzed by UV spectrophotometer at obtained λ max all solubility measurements were performed in triplicate [8].

RESULT AND DISCUSSION

Preformulation study:

Preformulation studies were completed.

Organoleptic characteristics

The organoleptic characteristics of Lycopene such as colour, odor, and taste were studied. Colour of drug was found to be pale yellowish. Taste of the drug was identified simply by taste sensation and odor by smelling.

Table 1: Organoleptic properties of Lycopene

Organoleptic Properties	Standard	Observation
Colour	Deep red	Deep red
Odour	Characteristics	Characteristics
Taste	Bitter	Bitter

The organoleptic characteristics of the drug were compared with the standard characteristics and both were found to be similar.

Melting point determination

Melting point of drug Lycopene was determined by capillary method using Thiele's tube. The temperature at which drug goes in the liquid state was consider as a melting point of Lycopene. Practically it was found that drug get melts at 173ºC. Reported melting point of the drug Lycopene is 172ºC-175ºC. The melting point of the drug was matches to the standard one hence the drug identified as Lycopene and the drug is in the pure form.

FT-IR Spectra

IR spectra interpretation study was performed for the identification of Lycopene. FT-IR study is important for determination of functional groups present n structure of sample. The IR spectrum of the pure Lycopene sample was recorded by FT-IR spectrometer.FTIR study is important for determination of functional groups present in structure of sample. The IR spectrum of the pure Lycopene sample was recorded by FT-IR Spectrometer as shown in Fig. 7.1.

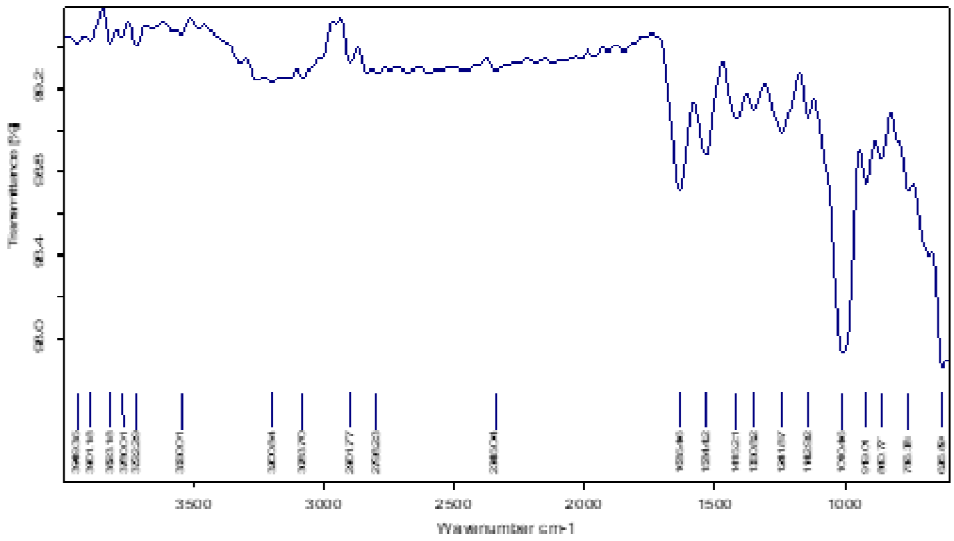


Figure 3: FT-IR spectra of Lycopene

From the results it is clear that the frequencies of the standard Lycopene matches with the functional group present in the drug sample, hence the drug is confirmed and is in pure form.

UV Spectroscopy

The Determination of Absorption Maxima (λmax):

The absorption maxima (λmax) of drug Lycopene Hexane was found to be 470 nm when scanned from 800 nm to 200 nm.

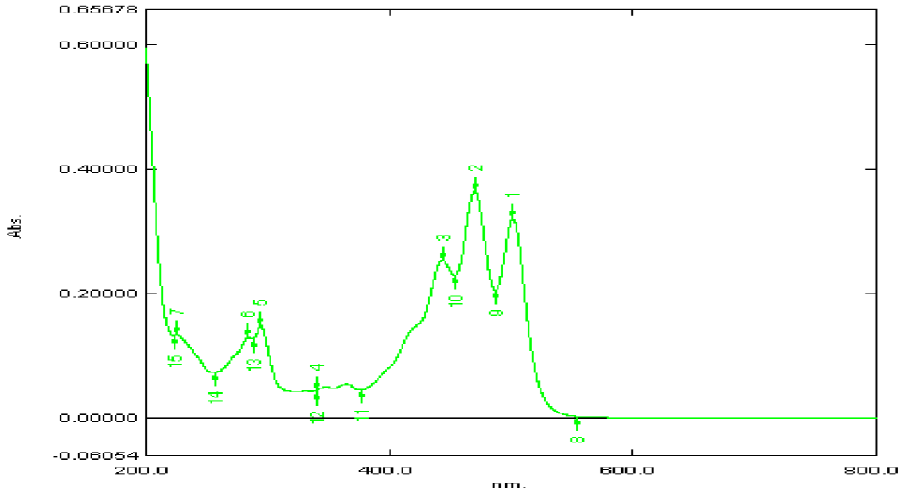


Figure 4: Absorbance spectrum of Lycopene.

Preparation of calibration curve for Lycopene:

From the prepared stock solutions were 0.5, 1, 1.5, 2.0, 2.5 ml solutions were pipette into a series of 10 ml volumetric flask and were made-up to 10mL with n-Hexane to get 5, 10, 15, 20 and 25µg/mL solutions of Lycopene respectively. The absorbance of resulting solutions was measured at 470 nm against the blank. Dilution for Calibration curves of Lycopene were performed in triplicate. A graph was plotted by taking concentration on X-axis and absorbance on Y-axis.

Absorbance of Lycopene

The calibration curve for Lycopene in Hexane was prepared by plotting absorbance versus concentration at practically obtained λmax 470 nm. Concentration ranges selected were of 5,10,15,20, & 25µg/ml. The calibration curve of Lycopene in Hexane was found as follows.

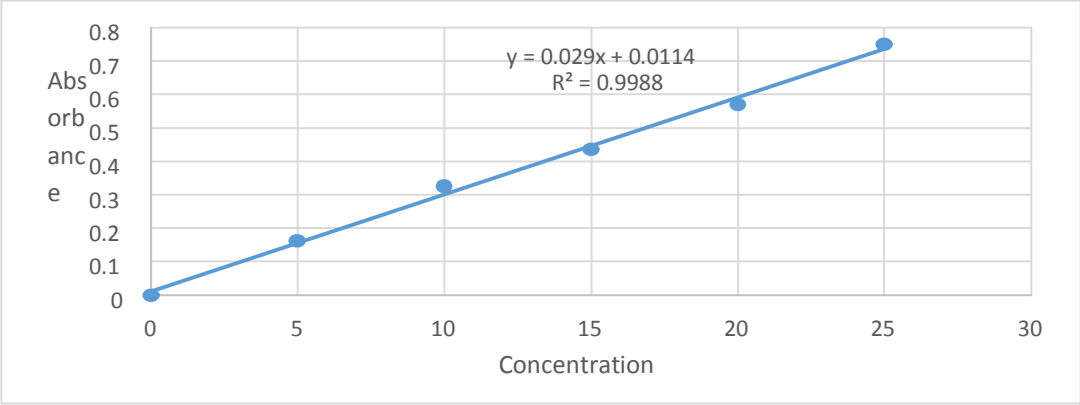


Figure 5: Calibration curve for Lycopene in Hexane

Identification and Characterization of excipients

Before going for formulation development study it is necessary to check the quality of raw materials so each excipients characterized for organoleptic behavior, and IR spectroscopy.

Organoleptic Characterization

All the observations were summarized in following table.

Table 2: Organoleptic properties of excipients.

Excipients	**Standard**	**Observation**
PVP K 30	Colour-White Odour- Odorless	Colour- White Odour- Odorless
Eudragit L 100	Colour-White Odour- faint amine-like	Colour-White Odour- faint amine-like
PVA	Colour-White Odour-Odorless Taste- Tasteless	Colour-White Odour-Odorless Taste- Tasteless

The organoleptic characteristics of the excipients were studied and compared with standard characteristics and both were found to be similar.

FT-IR study

Eudragit L100:

FT-IR spectra are used for functional group identification in compound. A small amount of Eudragit RL100 in the form of powder was placed on selenium bromide crystal and spectrum was run. The FT-IR spectrum of Eudragit RL100 is given below.

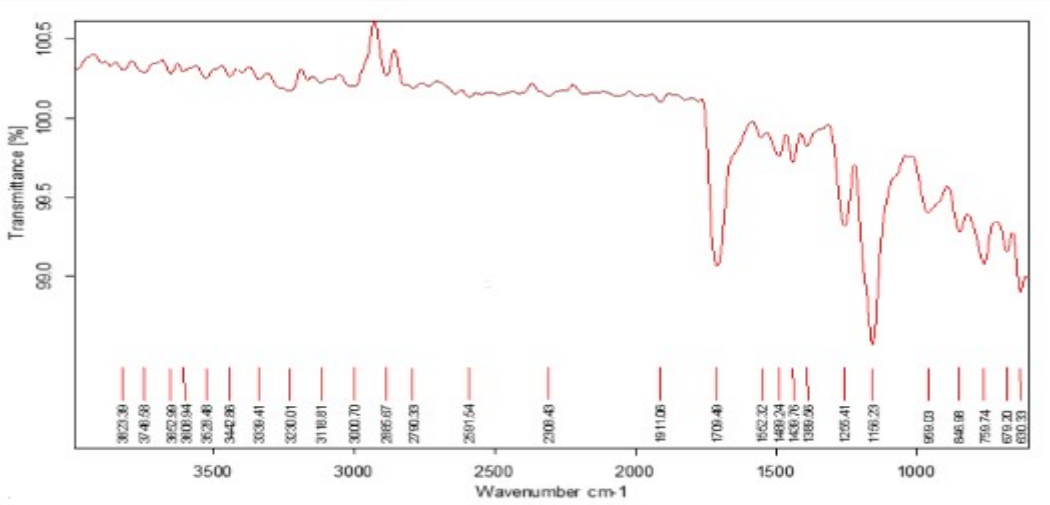


Figure 6: FT-IR spectra of Eudragit L100.

Table 3: Interpretation of Eudragit L100

Functional group	Types of vibration	Frequency (cm-1)
		Standard
C-H(-CH2)	Bending	1465
C-H(-CH3)	Bending	1450-1375
C=O	Stretching	1725-1700
C-O	Stretching	1300-1000
O-H(H-bonded)	Stretching	3400-3200

The IR peaks of Eudragit L100 were compared with standard graph of Eudragit RL100 and found to be similar.

PVP K 30:

FT-IR spectra are used for functional group identification in compound. A small amount of PVP K 30 in the form of powder was placed on selenium bromide crystal and spectrum was run. The FT-IR spectrum of PVP K 30 is given below.

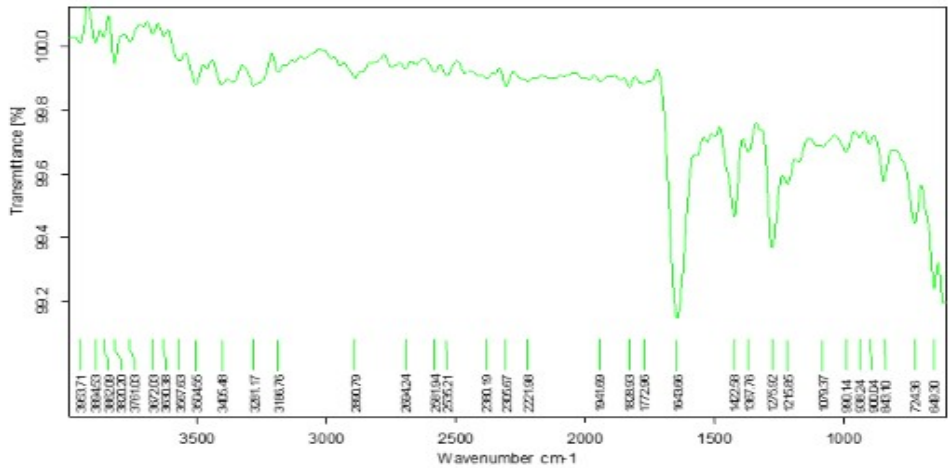


Figure 7: FT-IR spectra of PVP K 30.

Table 4: IR Interpretation of PVP K 30.

Functional group	Types of vibration	Frequency (cm^{-1})
		Standard
C-H(-CH_2)	Bending	1465
C-H(aromatic)	Stretching	3150-3050
C=O	Stretching	1725-1700
C-N(Amines)	Stretching	1300-1000

The IR peaks of PVP K 30 were compared with standard graph of Eudragit RL100 and found to be similar.

Poly vinyl Alcohol:

FT-IR spectra are used for functional group identification in compound. A small amount of PVA in the form of powder was placed on selenium bromide crystal and spectrum was run. The FT-IR spectrum of PVA is given below.

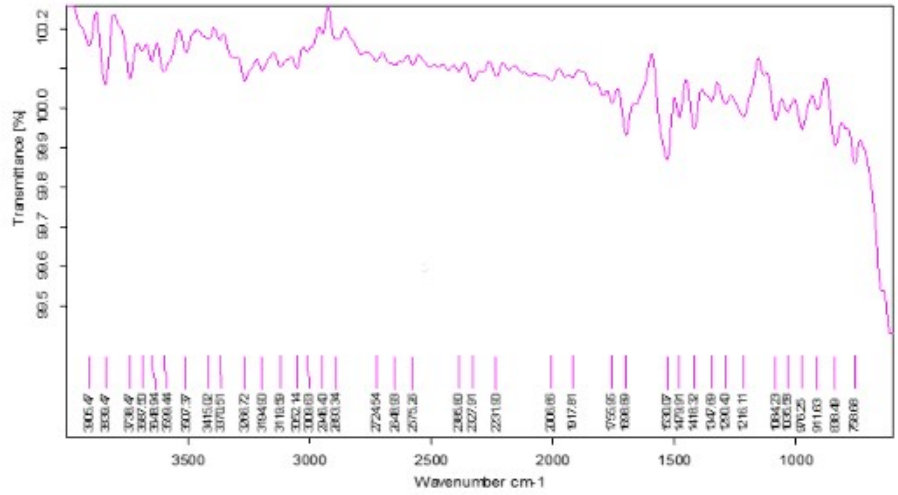


Figure 8: FT-IR spectra of PVA.

Table 5: IR Interpretation of PVA.

Functional group	Types of vibration	Frequency (cm^{-1})
		Standard
C-H(-CH_2)	Bending	1465
C=O	Stretching	1725-1700
C-O	Stretching	1300-1000
O-H(Alcohols)	Stretching	3650-3600

The IR peaks of PVA were compared with standard graph of PVA and found to be similar.

Solubility study of Drug

The solubility study of the plain drug in the various solvents is as follows:

Table 6: Solubility study of Drug.

Drug	Solvent	Concentration (µg/ml)
Lycopene	H_2O	6.51
	pH 7.4	11.91
	pH 6.2	10.43
	pH 6.8	0.549
	pH 1.2	0.459

Formulation Development

% Practical Yield

The %practical yield of the solid dispersion was found is as follows:

Table 7: % Practical Yield.

Ratio	Practical Yield (mg)	Practical Yield (%)
1:1	178	89
1:3	350	87.5
1:5	480	80

Total Drug Content

The total drug content of the various ratios of solid dispersion was found to be:

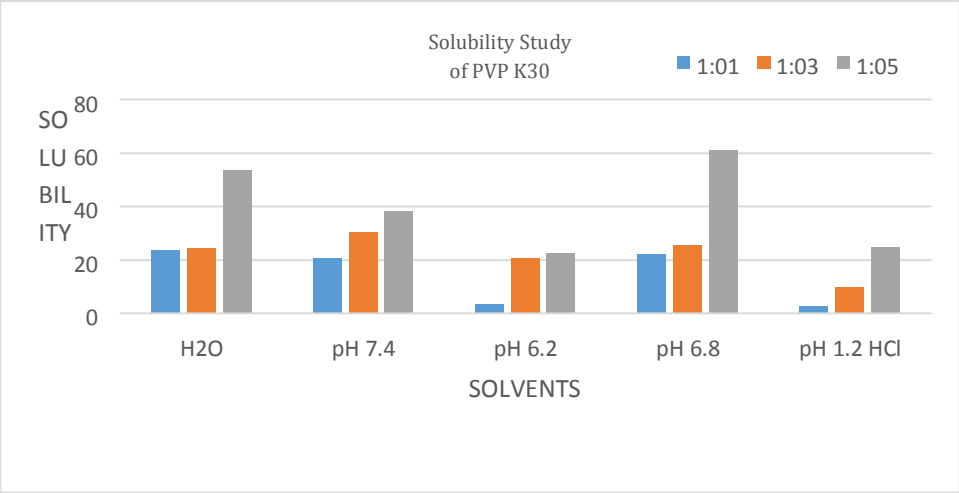
Table 8:Total Drug Content

Polymer	Total Drug Content (%)		
	1:1	**1:3**	**1:5**
PVP K 30	90.5	81.2	61.8
Eudragit L 100	100	55.6	58.19

Solubility study of Solid Dispersion

The solubility study of solid dispersion batches of both polymers in various pH solutions is as follows:

Figure 9: Solubility Study of Solid Dispersion of PVP K 30



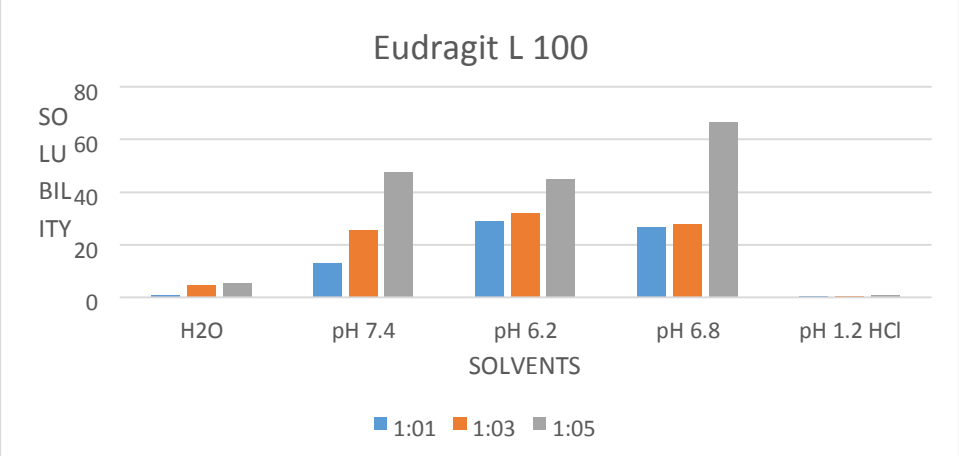


Figure10 Solubility study of Solid Dispersion of Eudragit L 100.

The solubility study of solid dispersion was found in the above solvents and SD shows the higher solubility in PBS pH 6.8.

***In vitro* drug dissolution study:**

Dissolution Study of Lycopene

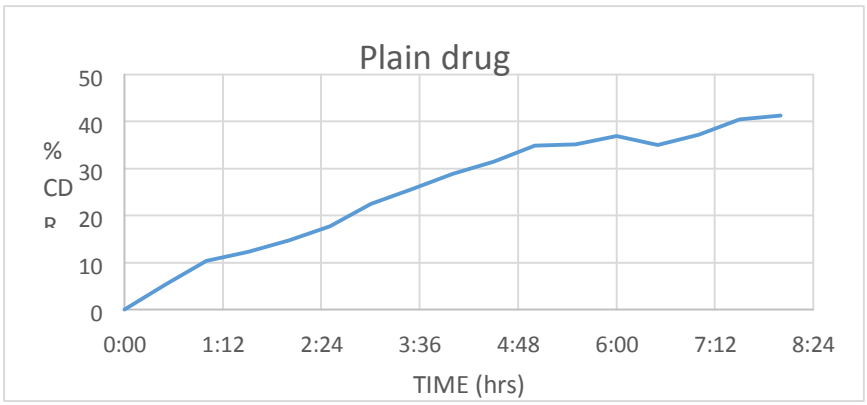


Figure 11: Dissolution Study of Lycopene

Dissolution study of ratios of SDs of PVP K 30

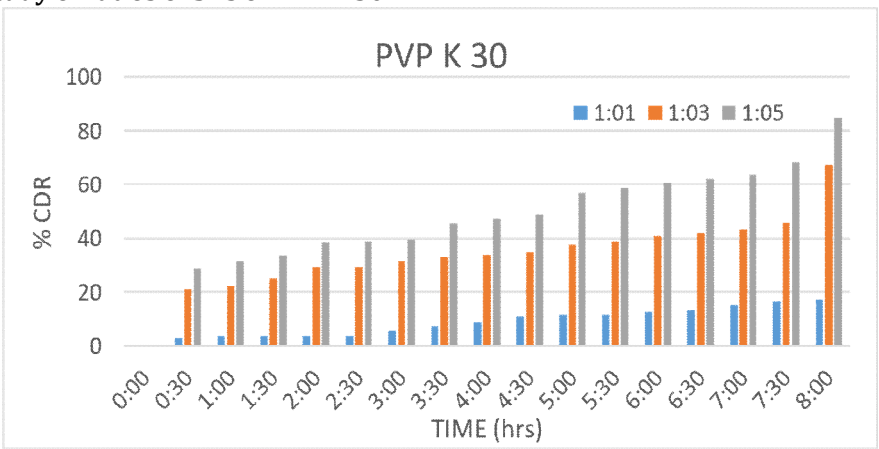


Figure 12: Dissolution study of ratios of SDs of PVP K 30

Evaluation of polymeric nanoparticles:

Particle size analysis:

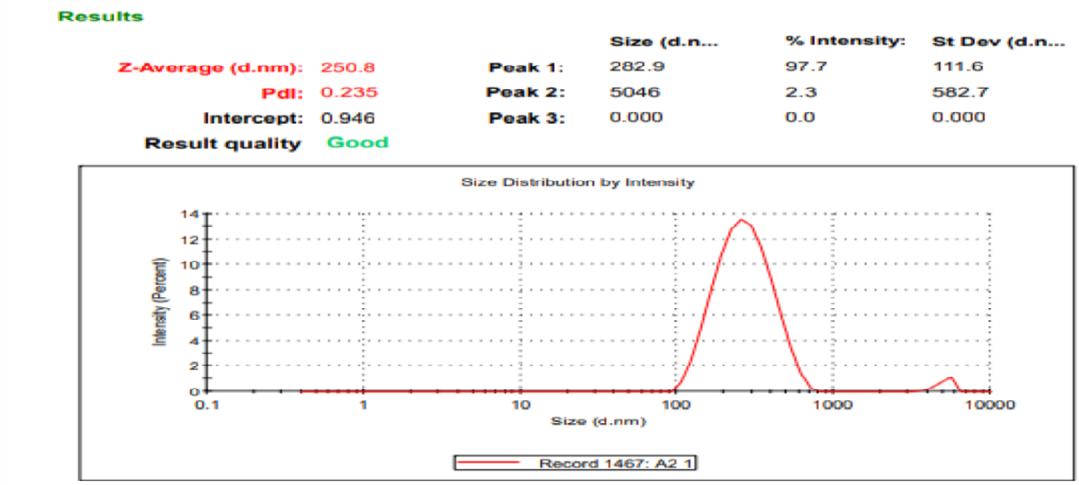


Figure 13: Particle size of Nanoparticales of PVP K 30

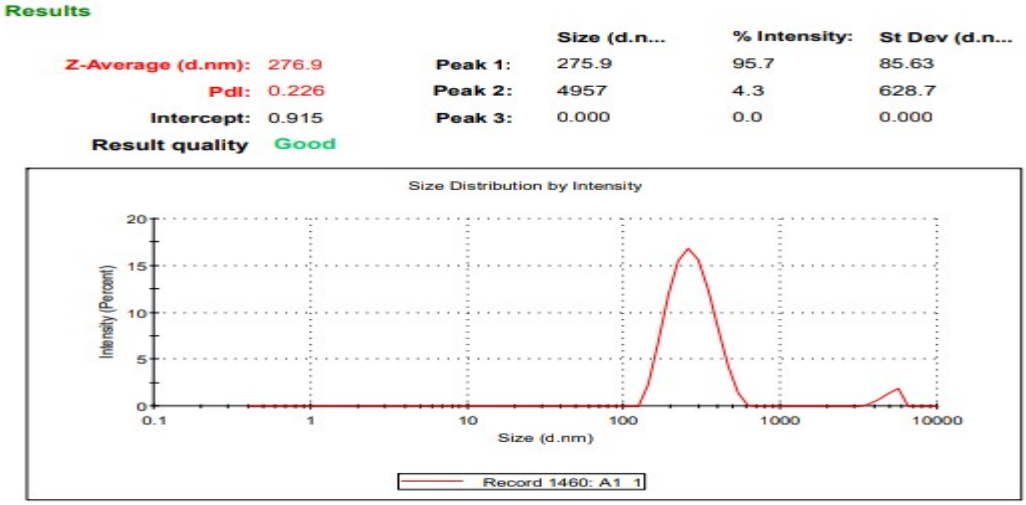


Figure 14: Particle size of Nanoparticales of Eudragit L 100.

Zeta potential measurement:

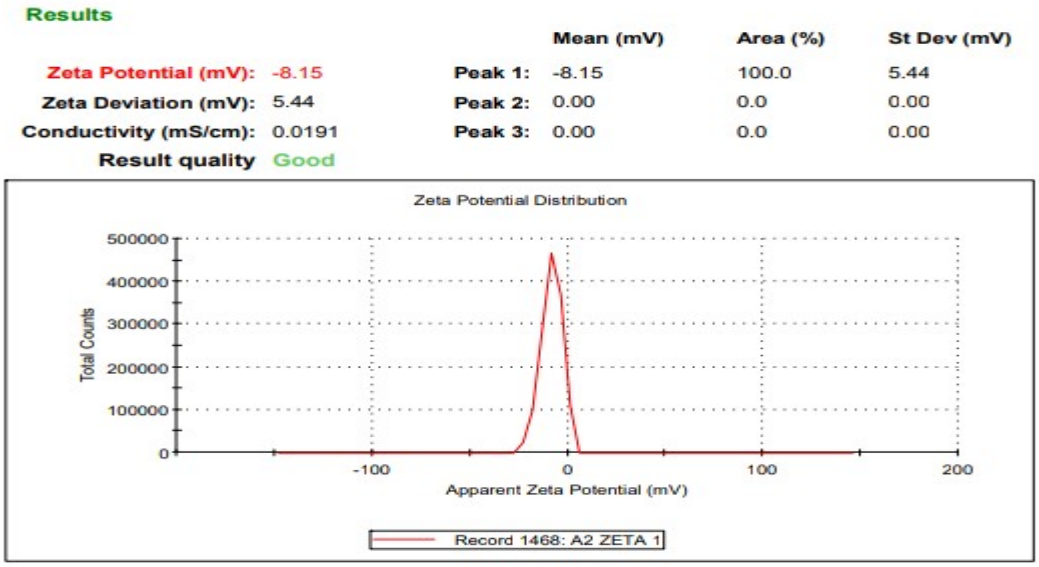


Figure 15: Zeta potential of Nanoparticales of PVP K 30

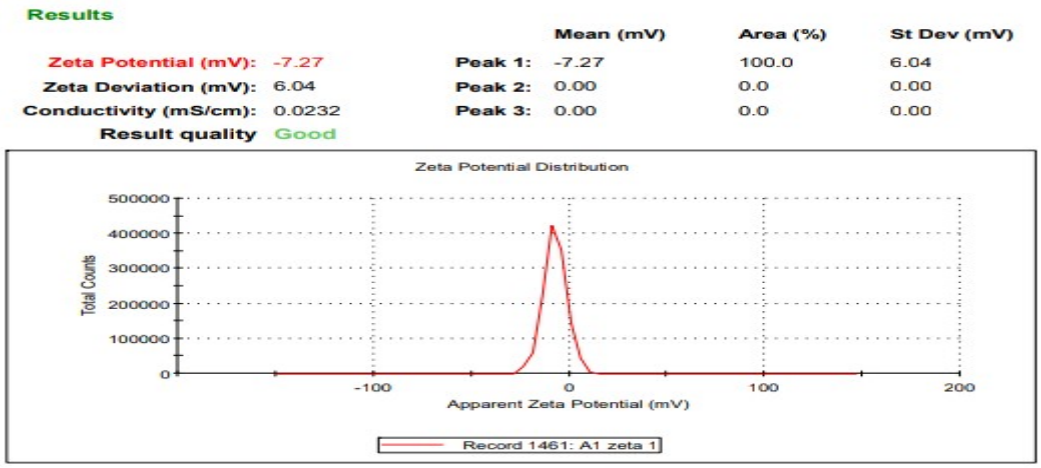


Figure 16: Zeta potential of Nanoparticales of Eudragit L 100

Entrapment efficiency measurement:

Table 9: Entrapment efficiency of Nanoparticle

Trial batches	Polymer	Entrapment Efficiency
1	PVP K 30	82.94%
2	Eudragit L 100	80.89%

The nanoparticles of drug with both polymers shows the good entrapment efficiency

pH Measurement of formulation

The pH measurement of each formulation of nanoparticles was found to be weakly acidic in nature at room temperature.

Total Drug Content

Table 10: Total Drug Content

Polymer	Total Drug Content (%)
PVP K 30	57.0
Eudragit L 100	53.48

In- vitro drug release study:

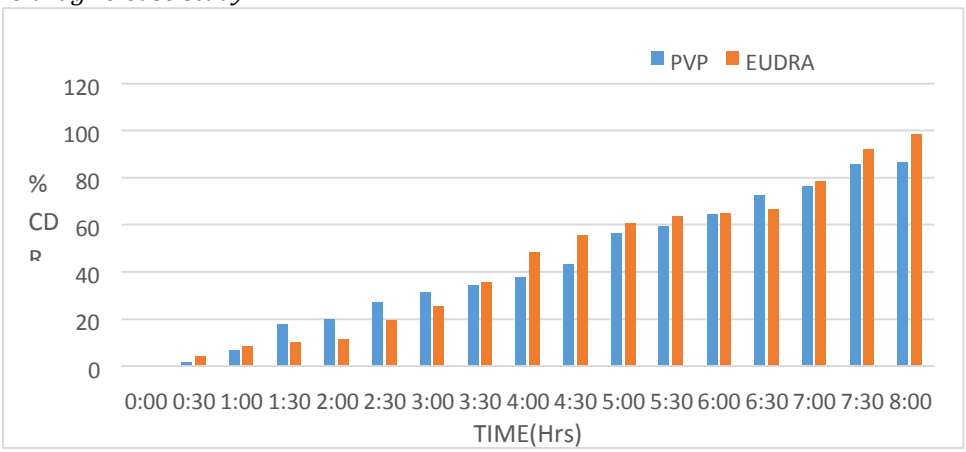


Figure 17: In - vitro drug release study of nanoparticles

Comparative drug release study of Drug, SDs, and Nanoparticles

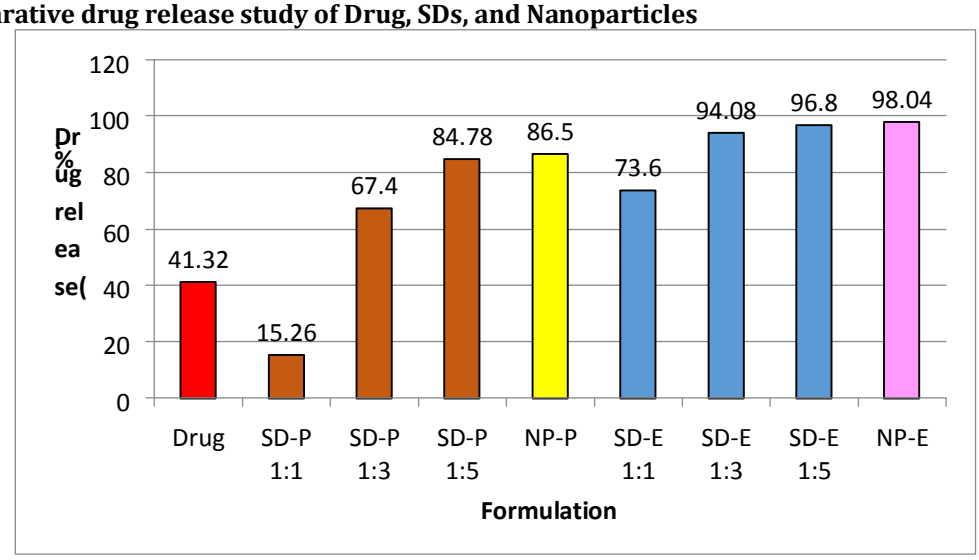


Figure 18: Comparative drug release study of Drug, SDs, and Nanoparticles.

Solubility study of lyophilized product

The solubility study of lyophilized powder of nanoparticles batches of both polymers in various pH solutions is as follows:

Table 11: Solubility study of lyophilized powder of NPs of PVP K 30

Polymer	**Solvent**	**Amount of soluble drug (µg/ml)**
		1:5
PVP K 30	H_2O	57.83
	pH 7.4	45.38
	pH 6.2	26.86
	pH 6.8	65.78
	pH 1.2 HCl	29.90

Above solubility study shows the highest solubility in phosphate buffer pH 6.8.

Table 12: Solubility study of lyophilized powder of NPs of Eudragit L 100

Polymer	**Solvent**	**Amount of soluble drug (µg/ml)**
		1:5
PVP K 30	H_2O	11.40
	pH 7.4	51.78
	pH 6.2	49.90
	pH 6.8	73.60
	pH 1.2 HCl	2.33

Above solubility study shows the highest solubility in phosphate buffer pH 6.8.

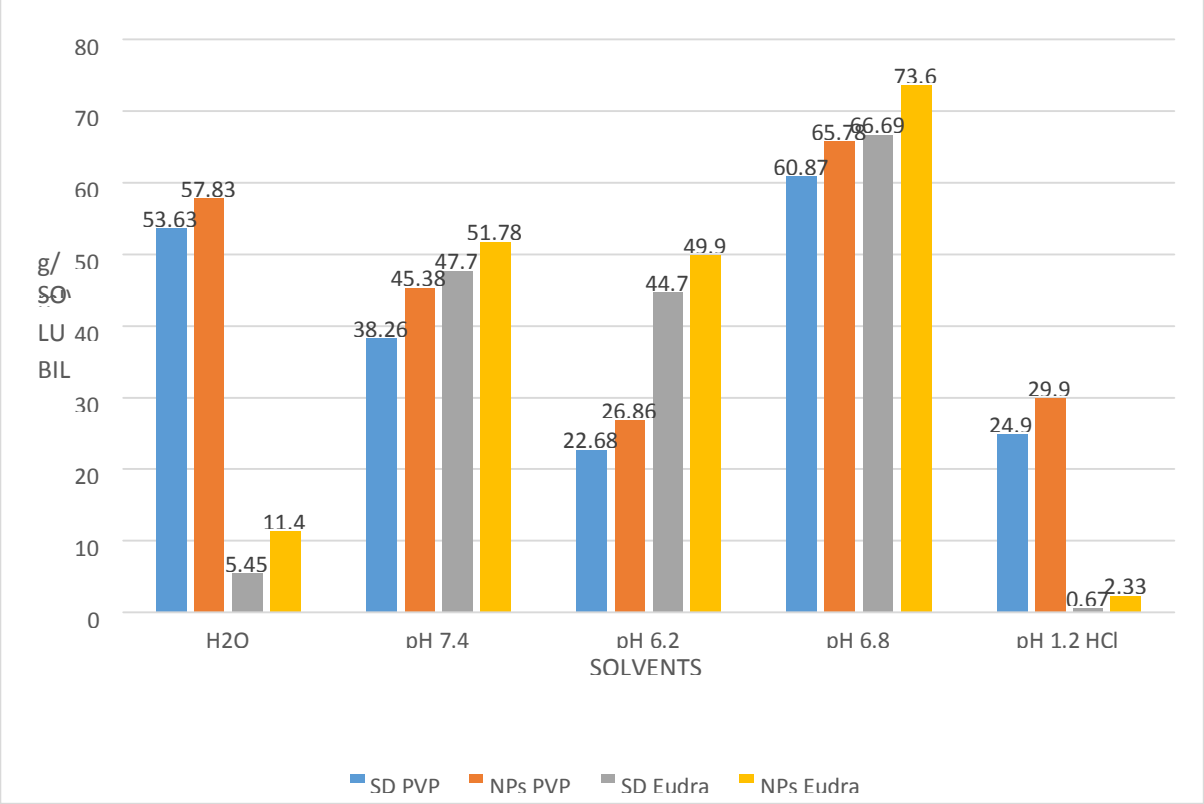


Figure 19: Comparative solubility study of SDs and NPs of both polymers

The above comparative solubility study of SDs & lyophilized powder of both polymer shows the highest solubility in phosphate buffer pH 6.8. And also shows the greater solubility of lyophilized powder than the solid dispersions of both polymers.

DISCUSSION

Lycopene is practically insoluble in water. According to the bio pharmaceutical classification, it comes under Class II drug and its oral bioavailability is 0.6- 3.4%of the administered dose due to poor aqueous solubility and dissolution is rate limiting step in the absorption of poorly water soluble drug.

Solubility and dissolution of lycopene can be improved by using the solid dispersion technique. Solid dispersion of lycopene is done by solvent evaporation method. PVP K 30 and Eudragit L 100 are selected for the preparation of solid dispersion. The prepared combinations were characterized by FT-IR, saturation solubility, and dissolution studies.

The solid dispersion of Lycopene is prepared in three different ratios with PVP K30and Eudragit L 100. Lycopene and PVP K30 (1:5) and Lycopene and Eudragit L 100(1:5) were selected according to their solubility study. The selected combinations of both polymers were formulated into polymeric nanoparticles.

The result from all the above mentioned activities have been summarized as follows: The effect of several variables related to dispersion preparation were investigated by IR and UV spectral analysis. For this drug solvent evaporation method proved to be the most effective in improving aqueous solubility of the drug. The solid dispersion prepared in different ratios was showed solubility enhancement in the order of 1:1> 1:3> 1:5 of both polymers. FT-IR confirmed that there were no apparent chemical interactions between drug and carriers. These studies also indicated the physical state of the drug in the solid dispersions or complex. Dissolution study confirmed that dissolution rate for solid dispersions increased with increased concentration of carriers. Polymeric Nanoparticles prepared by combination of PVP K 30 and Eudragit L 100 by using solvent evaporation method.

CONCLUSION

In this research endeavour has been made to study the effect of concentration of polymer and surfactant on the solubility and dissolution profile of nanoparticles. In that the selected nanoparticales batches were lyophilized then this lyophilized powder was obtained. This obtained powder was used for the solubility

study in various solvents, which showed the greater solubility compared to the batches of solid dispersions of both polymers.

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