Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Der Pharmacia Sinica, 2015, 6(5):36-54

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Solubility enhancement technique for an anti-malarial drug

Ram S. Pentewar[1*], Nasheer S. Shaikh[1], Aparna V. Bhalerao[2], Rohit Bharti[1] and Gajanan V. Pulgamwar[1]

[1]*Channabasweshwar Pharmacy College, Pimpri, Pune*
[2]*Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune*

ABSTRACT

Atovaquone is a competitive inhibitor of ubiquinol channel, specifically inhibiting the mitochondrial electron transport chain at the bcl complex. Inhibition of bcl activity results in a loss of mitochondrial function with poorly water soluble antiprotozoal drug, Atovaquone, by fabricating its solid dispersions were prepared by employing Kollidon VA-64 and Soluplus by solvent evaporation and spray drying method. Both solid dispersions significantly improved the dissolution profile of Atovaquone. Solid dispersions prepared by spray drying method showed more solubility enhancement with enhanced dissolution as compared to solid dispersions prepared by solvent evaporation method. IR and UV spectral analysis of solid dispersions indicated that there was no probable interaction between drug and carriers. Optimised solid dispersions were further evaluated by XRD and DSC. Dissolution rate of solid dispersions increased with increased concentration of polymer like Soluplus and Kollidon VA-64.

Keywords: Solid dispersion, Atovaquone, Solvent evaporation, Spray drying, solubility enhancement.

INTRODUCTION [1, 5-9]

Therapeutic effectiveness of a drug depends upon the bioavailability and in turn upon the solubility. The poor aqueous solubility and dissolution rate of API is one of the biggest challenges in pharmaceutical development. Several methods have been employed to improve the solubility of poorly water soluble drugs. A solid dispersion technique has been used by various researchers who have reported encouraging results with different drugs.

Solid dispersions represent a useful pharmaceutical technique for increasing the dissolution, absorption and therapeutic efficacy of drugs in dosage forms.

Factors responsible for observing faster dissolution of drug in solid dispersion [7]:

- Extremely small particle size of the solid, dispersed molecularly.
- Solubilization effect of carrier, which acts in the diffusion layer surrounding the particles.
- Absence of aggregation and agglomeration of particle.
- Improved wettability of drug due to carrier.
- Stabilization of metastable forms of drug.

Advantages [1]:

1. Enhancement of the rate and extend of absorption leading to reduction in dose.

2. Enhancement of dissolution and associated rapid absorption leading to reduction in proportion of presystemic drug metabolism.
3. Potential for acting as sustained release dosage form.

The rate of solvent removal can affect the particle size and hence physiochemical properties, such as the melting points and crystal structure of the solid dispersion.

MATERIALS AND METHODS

Table No. 1 List of chemicals/Material

Sr. No.	Name of Chemicals/Material	Manufacturer/Supplier
1.	Atovaquone	Glenmark Industries, Mumbai
2.	Soluplus	BASF India Pvt. Ltd., Mumbai
3.	Kollidon VA-64	BASF India Pvt. Ltd., Mumbai
9.	Dichloromethane	Research Lab. Mumbai
10.	Isopropyl alcohol	Loba Chemie, Mumbai
11.	Methanol	Research Lab. Mumbai
12.	Acetone	Loba Chemie, Mumbai

CHARACTERIZATION OF ATOVAQUONE AND EXCIPIENTS [11-16]:

A. CHARACTERIZATION OF ATOVAQUONE:

The characterization of drug was carried out by conducting various tests as follows;

i. Organoleptic properties:

ii. Spectral characteristics:

a. Determination of λ max in UV range:

The solutions of drug in methanol, PBS and distilled water were scanned in the range of 400 to 200 nm and respective λ max values were recorded.

b. Preparation of calibration curve of Atovaquone:

i) In methanol Absorbance was measured at 276.5 nm and calibration curve was plotted with different concentrations 5, 10, 15, 20, 25 µg/ml., in PBS Absorbance was measured at 277.5 nm and calibration curve was plotted with the concentrations 5, 10, 15, 20, 25 µg/ml and finally Absorbance was measured in distilled water at 277.5 nm and calibration curve was plotted concentrations 5, 10, 15, 20, 25 µg/ml.

b. Determination of infrared spectrum: IR absorption spectrum of Atovaquone was recorded by using FTIR spectrophotometer (FTIR-8400s) wherein 1-2 mg of drug sample was used.

ii) Determination of saturation solubility: For this excess quantities of Atovaquone were added into each of 3ml of distilled water, contained in glass vials. The solutions were shaken for 48 hours using orbital shaker incubator (temperature maintained at 37^0C ±1^0C). The solutions were filtered through membrane filter 0.45µ and the filtrate was diluted properly with respective solvents and the absorbance was recorded.

c. Powder X-ray diffraction pattern study: X-ray diffraction pattern of the selected complexes were compared with that of plain Atovaquone. The powder X-ray diffraction pattern of drug was carried out using Brukuer AXS D-8 Advance Diffractometer (Germany) with Cu line as a source of radiation. This was done by measuring the 2θ in the range of 3-50^0 with reproducibility of ±0.001 on a diffractometer. The XRD were recorded automatically using rate meter with time constant of 2×10^2 pulse / second and with the scanning rate of 2^0 min^{-1} over 2θ range.

d. Differential Scanning Calorimetric analysis: DSC analysis was carried out using (Mettler Tolido) instrument. Atovaquone was placed in a platinum crucible and the DSC thermogram was recorded at a heating rate of 10^0C/ min in the range of 40^0C to 300^0C. Nitrogen gas was purged at the rate of 30 ml/min to maintain inert atmosphere.

e. Scanning Electron Microscopy: The external morphology of drug was studied by Scanning Electron Microscopy (SEM). The sample for SEM was prepared by lightly sprinkling powder on a double adhesive tape stuck to an aluminium stub. Afterwards, the stubs containing the coated samples were placed in the Scanning Electron Microscope (JEOL JSM-6360, Japan) chamber. The sample was then randomly scanned and photomicrographs were taken at the acceleration voltage of 10 kV and the results of SEM were reported.

B. Characterization of excipients:
I. Determination of melting point: Melting points of excipients were determined by glass capillary method.
II. Determination of Infrared absorption spectrum: IR absorption spectrum of excipients like Kollidon VA64, Soluplus was recorded by using FTIR spectrophotometer (FTIR- 8000). The spectrum was recorded and the peaks belonging to major functional groups were identified.

a) Phase Solubility analysis of Atovaquone with Kollidon VA-64 and Soluplus:
Phase solubility studies were performed to determine stochiometric proportion of Atovaquone and Kollidon VA-64 and Soluplus. Also the data was used to determine the stability constant of complexes. For this, stock solutions of 1%, 2%, 3%, 4%, and 5% of KollidonVA-64 and Soluplus were prepared using distilled water. 5 ml of each stock solution was filled screw capped vials and the excess quantity of drug was added to each vial separately. The vials were shaken at ambient temperature for 48 hours using an orbital shaker. The supernatant solutions were collected carefully and were filtered through membrane filter 0.45µ and suitably diluted. The UV absorbance's of resultant solutions were recorded at 277.5 nm and were used to estimate the concentration of drug.

b) Preparation and Evaluation of solid dispersion of Atovaquone with Kollidon VA- 64 and Soluplus by solvent evaporation method:
Required quantities of Atovaquone, Kollidon VA-64, Soluplus, in ratio given in **Table No. 7.1** were accurately weighed and added in glass beaker containing Dichloromethane, Isopropyl alcohol and distilled water solvent system. The final solution was stirred until a clear solution was obtained and poured in the Petri dish. The solvent was then removed at 60^0C until dry solid mass was obtained. The product was then pulverized and sieved through 60 mesh. The sieved product was collected and stored in tightly closed containers and used for further study.

Table No. 2 Composition of solid dispersion prepared using Kollidon VA- 64 and Soluplus by solvent evaporation method

Carrier used	Solid dispersion system	Drug : Carrier Ratio
Kollidon VA-64	VA 1	1:1
	VA 2	1:3
	VA 3	1:5
Soluplus	SP 1	1:1
	SP 2	1:3
	SP 3	1:5

c) Preparation of solid dispersions of Atovaquone with Kollidon VA-64 and Soluplus by spray drying method:
Required quantities of Atovaquone, Kollidon VA-64 and Soluplus (the carrier) in ratio 1:3 and 1:5 were accurately weighed and added in glass beaker containing Dichloromethane, Isopropyl alcohol and distilled water solvent system. The final solution was stirred until a clear solution was obtained. The clear solution was spray dried to obtain a free flowing powder and stored in tightly closed containers until further used.

Table No. 3 Composition of solid dispersion prepared by spray drying method using Kollidon VA-64 & Soluplus

Carrier used	Solid Dispersion system	Atovaquone: Carrier Ratio
Kollidon VA-64	SVA 1	1:3
	SVA 2	1:5
Soluplus	SSP 1	1:3
	SSP 2	1:5

Table No. 4 Parameters used for preparation of Solid dispersions of Atovaquone by spray drying method

Parameters	Conditions
Inlet temperature	60^0C
Outlet temperature	50^0C
Inlet high	70^0C
Outlet high	60^0C
Cool temperature	25^0C
Feed pump flow rate	45 nm/m^3
Aspiration flow rate	1 ml/min

EVALUATION OF SOLID DISPERSION OF ATOVAQUONE

The prepared solid dispersions were evaluated for

i. Saturation solubility:

To each of the glass vials containing 3 ml of distilled water, excess quantities of Atovaquone and each of the solid dispersions were added separately. These vials were shaken on orbital shaker for 48 hours. The resulting solutions were filtered through membrane filter 0.45µ; appropriate dilutions were made and the absorbance was recorded at 277.5 nm.

ii. Spectral characteristics:

a. UV –visible spectra:

Atovaquone powder and its solid dispersion with different carriers were dissolved in dichloromethane and phosphate buffer containing isopropyl alcohol solvent system and further diluted with PBS 7.4 pH containing 30% isopropyl alcohol. UV spectra of these solutions were recorded and λ max values were compared.

b. IR spectra:

The IR spectra of Atovaquone, individual carriers alone and solid dispersions of Atovaquone with each of carrier were recorded by FTIR-8000 by ATR technique. The presence or absence of major functional groups was noticed in the spectra.

iii. Drug content:

The percentage of Atovaquone content in each of the solid dispersions was estimated by dissolving quantity of solid dispersion equivalent to 10 mg of Atovaquone in 10 ml solvent system containing dichloromethane and isopropyl alcohol. The solutions were further diluted with methanol and the UV absorbance's were recorded at 276.5 nm. The contents were estimated using previously prepared calibration curve of Atovaquone in methanol.

iv. In-vitro drug release study:

Quantities of each type of solid dispersions equivalent to 20 mg of Atovaquone were subjected to dissolution test using USP XXII (Type- II) tablet dissolution test apparatus. Accurately weighed 20 mg Atovaquone was also subjected to similar test.

Parameters used for dissolution test of Atovaquone and its solid dispersions:

- Apparatus type : USP XXII(Type- II)

Dissolution medium : 500ml of PBS of pH7.4 +30% IPA

- Speed of paddle : 100 rpm
- Temperature of dissolution medium : $37^0C \pm 0.5^0C$
- Aliquots of sample withdrawn : 1 ml
- Dilution of aliquots : Up to 5 ml
- Frequency of sampling : Every 20 min up to 2 hours
- Wavelength for recording absorbance : 277.5 nm

v. X-Ray diffraction study:

X-ray diffraction pattern of the selected complexes were compared with that of plain Atovaquone. The powder X-ray diffraction pattern of drug was carried out using Brukuer AXS D-8 Advance Diffractometer (Germany) with Cu line as a source of radiation.

vi. Differential Scanning Calorimetric analysis:

DSC analysis was carried out using Mettler instrument.

vii. Scanning Electron Microscopy:

The external morphology of solid dispersion was studied by Scanning Electron Microscopy (SEM) (JEOL JSM-6360, Japan).

RESULTS AND DISCUSSION

A. Characterization of Atovaquone and Excipients:

Characterization of Atovaquone:

I. Organoleptic Properties:

Colour - Yellow crystalline powder
Odour - Odourless
Taste – Tasteless

II. Spectral analysis:

Table No. 5 Calibration data of Atovaquone

Solvent	λ max (nm)	Equation	R^2
Methanol	276.5 nm	0.062x	0.9929
PBS	277.5 nm	0.0709x	0.9982
Distilled water	277.5 nm	0.0499x	0.9988

c. Infrared spectrum of Atovaquone:

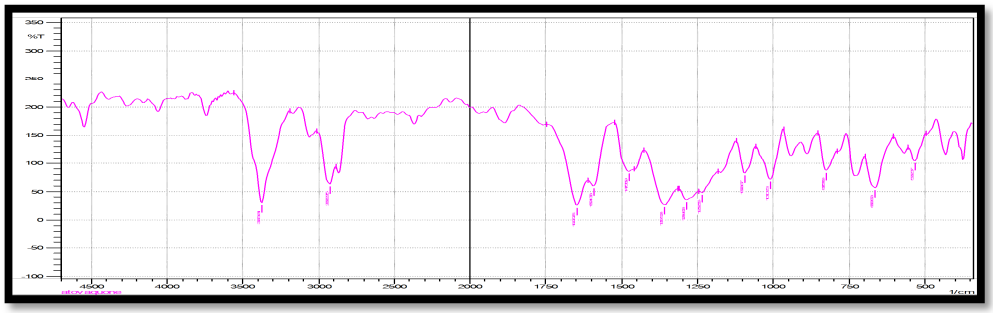


Fig No. 1 Infrared spectrum of sample of Atovaquone

Interpretation of infrared spectrum of sample of Atovaquone:

Table No. 6 Peaks observed in infrared spectrum of Atovaquone

IR Frequency (cm^{-1})	Assignments of bands
3366.67	O –H stretch (phenolic group)
2922.25	CH_2-, CH_3 alkanes
1647.26	C-C diketone group
1591.33	C= O stretch (primary amide)
663.53	C – Cl bending vibration

The IR spectrum of Atovaquone reveals the presence of major functional group in the structure of Atovaquone supporting its identity **(Table No. 6 and Fig. No. 1)**

III. Determination of saturation Solubility:

Saturation solubility of Atovaquone was found to be as given in **Table No. 7**

Table No. 7 Saturation solubility of Atovaquone

Solvent	Saturation solubility (µg/ml)
Distilled water	3.98
Phosphate buffer pH 7.4	4.12

IV. Thermal behaviour – Differential Scanning Colorimetery (DSC):

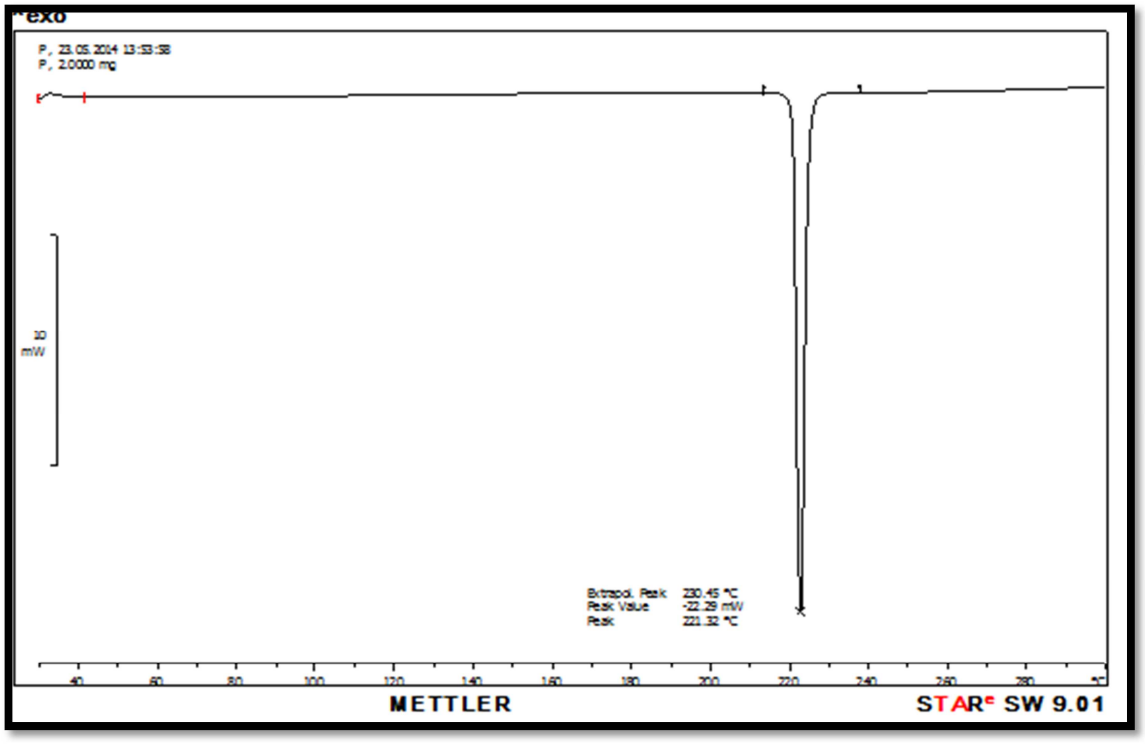


Fig No. 2 DSC pattern for Atovaquone (pure)

From **(Fig No. 2)** it shows that DSC thermogram of Atovaquone indicate the sharp endothermic at 221.32^{0}C due to its melting point.

V. Powder X-ray diffraction pattern:

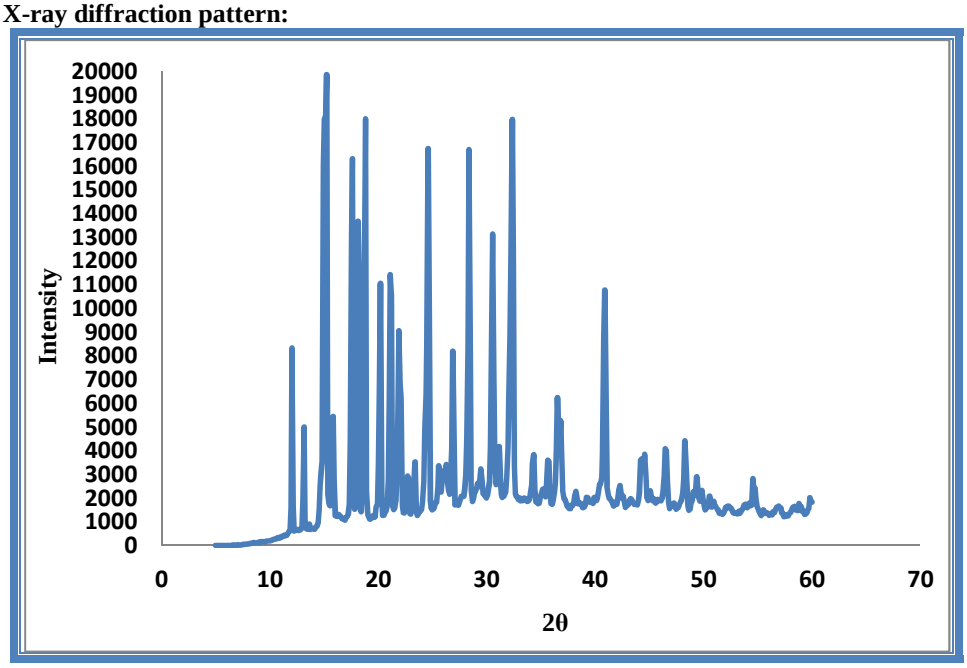
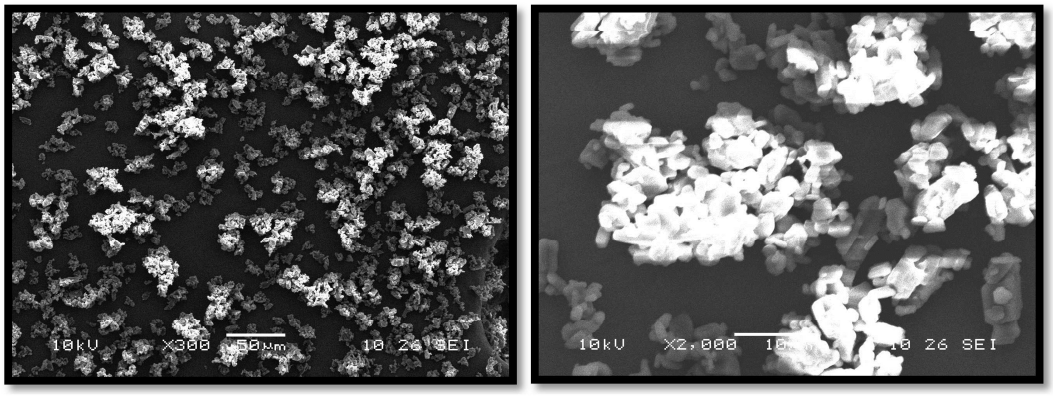


Fig No. 3 XRPD pattern for Atovaquone (pure)

The X-ray diffractogram of Atovaquone **(Fig No. 3)** exhibited intense and sharp peaks corresponding to crystalline nature of Atovaquone.

VI. Scanning Electron Microscopy of Atovaquone (SEM):



[A] **[B]**

Fig No. 4 Scanning Electron microphotograph of Atovaquone (pure)

The SEM of Atovaquone pure drug shown in **Fig No. 4** appeared as crystalline in shape and rod shaped with rough edges.

The findings of melting point, saturation solubility, spectral analysis, DSC thermogram, X-ray diffraction pattern and SEM support the identification of the sample of Atovaquone.

B. Characterization of Excipients:

I. Melting point determination:

Table No. 8 Melting points of polymers

Name of polymer	Reported Melting point	Observed Melting point
Kollidon VA-64	138^0C - 140^0C	138^0C
Soluplus	120^0C	119^0C

Phase solubility analysis of Atovaquone with Kollidon VA-64 and Soluplus:

Results of phase solubility analysis reported of Atovaquone with Kollidon VA-64 and Soluplus is given in **figure No. 5** and data for the same is given in **Table No. 9**

Table No. 9 Result of phase solubility analysis of Atovaquone with Kollidon VA-64 and Soluplus

Conc. Of polymer	Conc. of Atovaquone in Kollidon VA-64 (ug/ml)	Conc. of Atovaquone in Soluplus (ug/ml)
0	3.98	3.98
1%	5.64	9.87
2%	14.56	19.51
3%	22.88	33.55
4%	40.29	44.56
5%	45.52	53.24

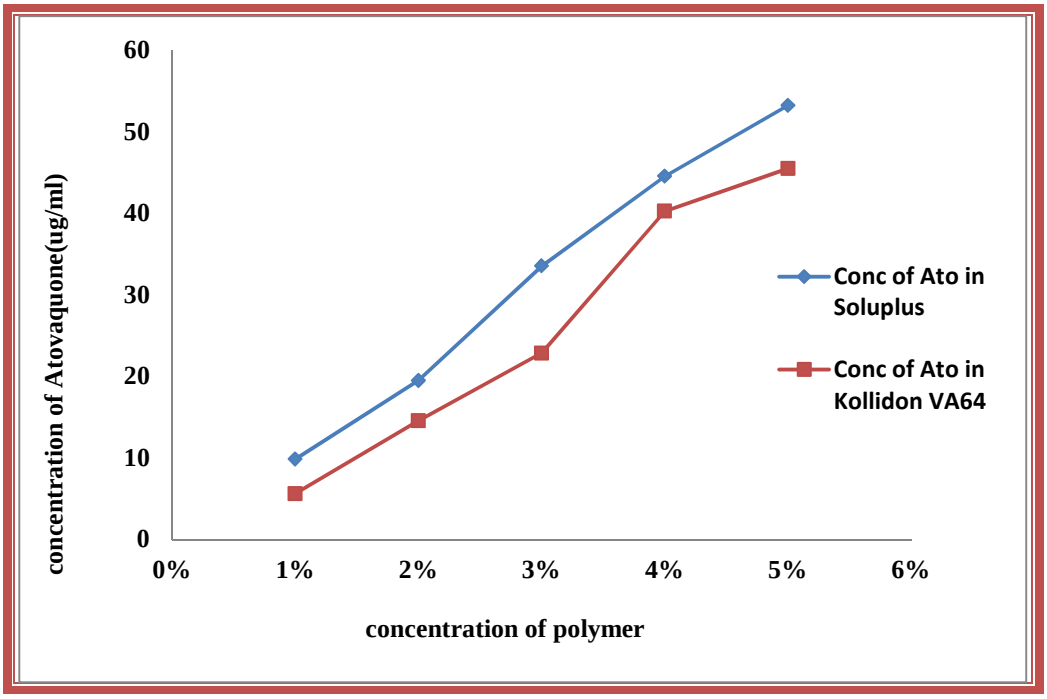


Figure No. 5 Phase solubility diagram of Atovaquone with Kollidon VA-64 and Soluplus

EVALUATION OF ATOVAQUONE SOLID DISPERSIONS:

I. Saturation solubility study in D/W by solvent evaporation method:

a. Saturation solubility of solid dispersion of Atovaquone prepared with Kollidon VA 64 and Soluplus in distilled water

Improved dissolution behaviour of solid dispersion of Atovaquone can be attributed to increase in saturation solubility of Atovaquone as per Noyes Whitney equation. Solid dispersion systems lead to reduction particle size of Atovaquone because of which there is an enhancement of saturation solubility. This change was confirmed by conducting similar saturation solubility studies on untreated Atovaquone as control.

Table No. 10 Saturation solubility of solid dispersion of Atovaquone prepared with Kollidon VA 64 and Soluplus in distilled water

Carrier used	Solid Dispersion system	Drug: carrier	Saturation solubility µg/ml ± S.D.
----	Pure drug	--	3.98 ± 0.26
Kollidon VA- 64	VA 1	1:1	9.88 ± 0.46
	VA 2	1:3	14.42 ± 0.29
	VA 3	1:5	27.88 ± 0.52
Soluplus	SP 1	1:1	43.53 ± 0.40
	SP 2	1:3	47.19 ± 0.46
	SP 3	1:5	51.87 ± 0.52

All values are expressed as mean ±SD, n=3

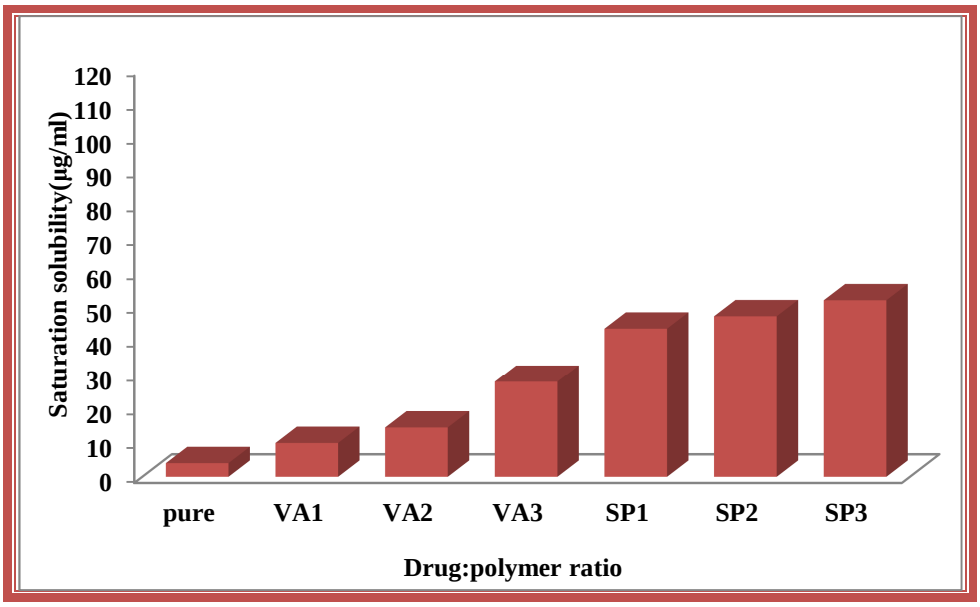


Figure No. 6 bar diagram for saturation solubility of solid dispersion of Atovaquone prepared with Kollidon VA 64 and Soluplus in distilled water by solvent evaporation method

ii. Spray drying method:

a. Saturation solubility of solid dispersions of Atovaquone prepared with Kollidon VA-64 and Soluplus in distilled water:

Table No. 11 Saturation solubility of solid dispersions of Atovaquone prepared with Kollidon VA-64 and Soluplus in distilled water by spray drying method

Carrier used	Solid Dispersion system	Drug: carrier ratio (Drug: polymer)	Saturation solubility µg/ml ± S.D.
--	**Pure drug**	--	**3.98 ± 0.26**
Kollidon VA-64	**SVA 1**	**1:3**	**72.81 ± 0.40**
	SVA 2	**1:5**	**75.43 ± 0.46**
Soluplus	**SSP 1**	**1:3**	**81.49 ± 0.49**
	SSP 2	**1:5**	**88.81 ± 0.82**

All values are expressed as mean ±SD, n=3

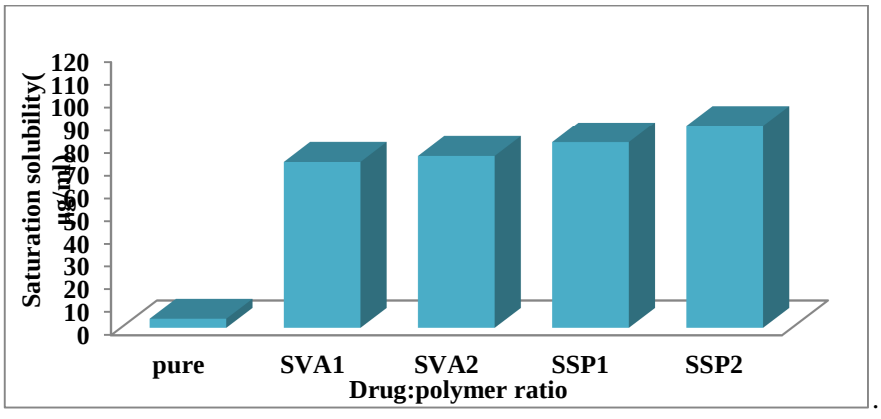


Figure No. 7 bar diagram for saturation solubility of solid dispersion of Atovaquone prepared with Kollidon VA-64 and Soluplus by spray drying method in distilled water

II. Spectral analysis:

a. IR spectral analysis:

b.

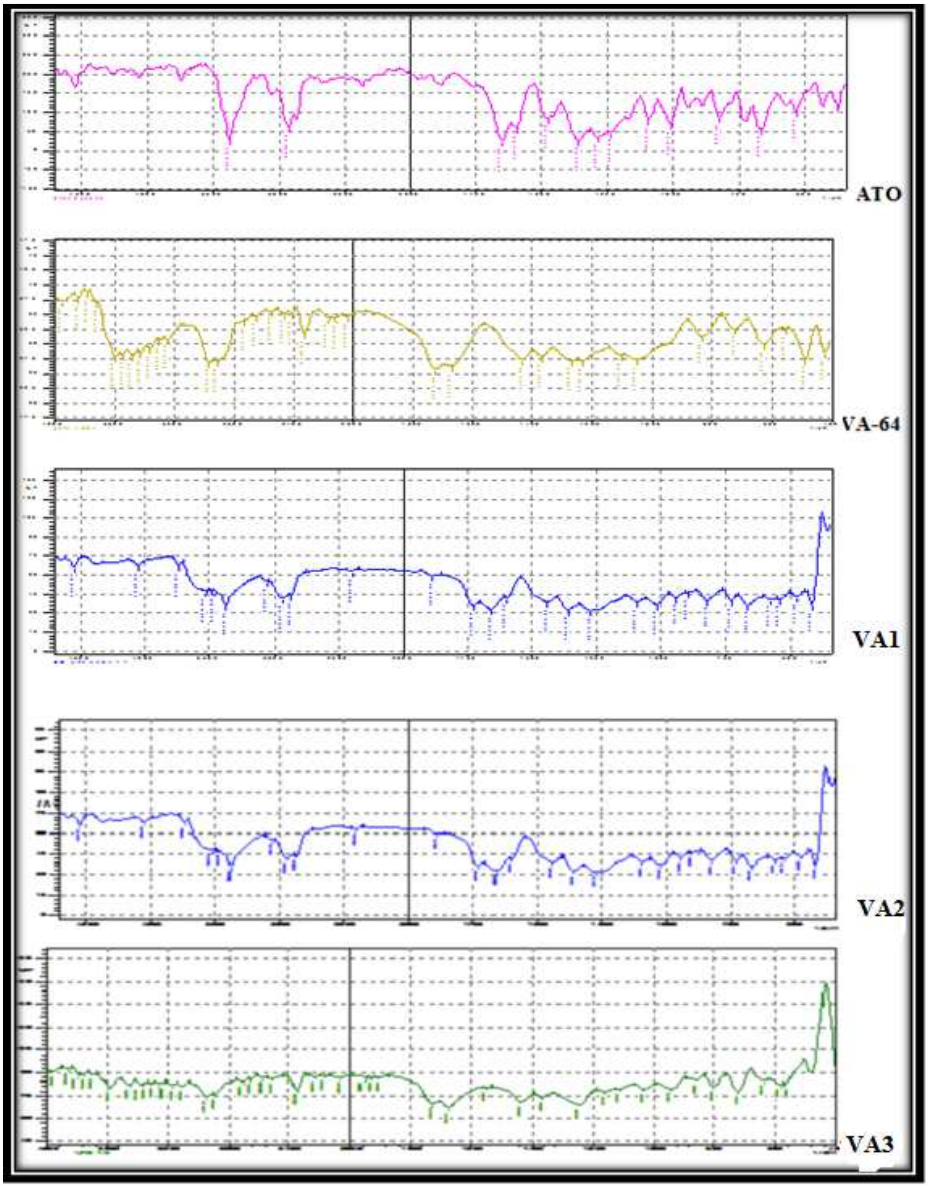


Fig No. 8 IR spectra of Atovaquone (pure), Kollidon VA-64 and solid dispersions prepared by solvent evaporation method

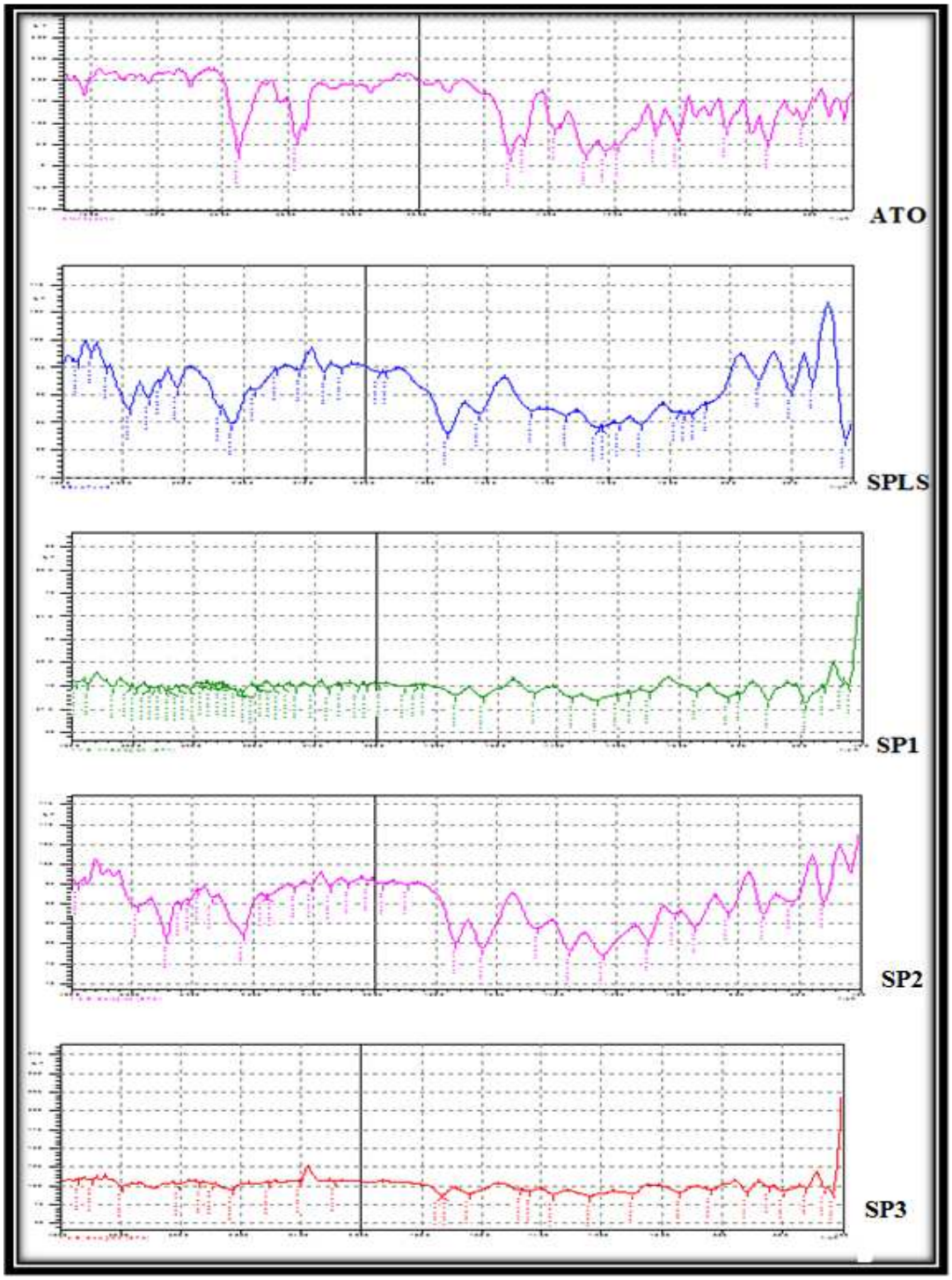


Fig No. 9 IR spectra of Atovaquone (pure) and Soluplus solid dispersions prepared by solvent evaporation method

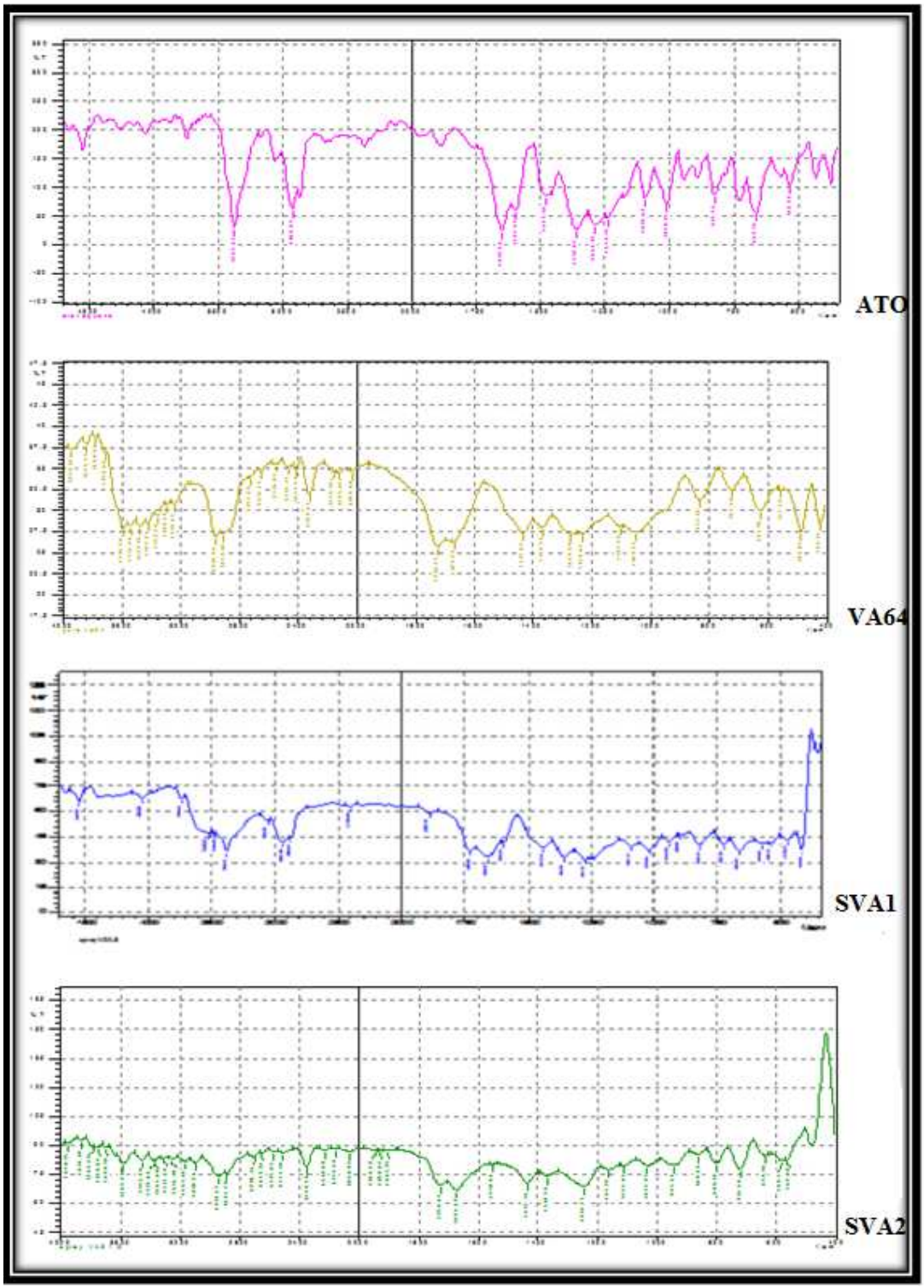


Fig No. 10 IR spectra of Atovaquone (pure), Kollidon VA-64 and solid dispersions prepared by spray drying method

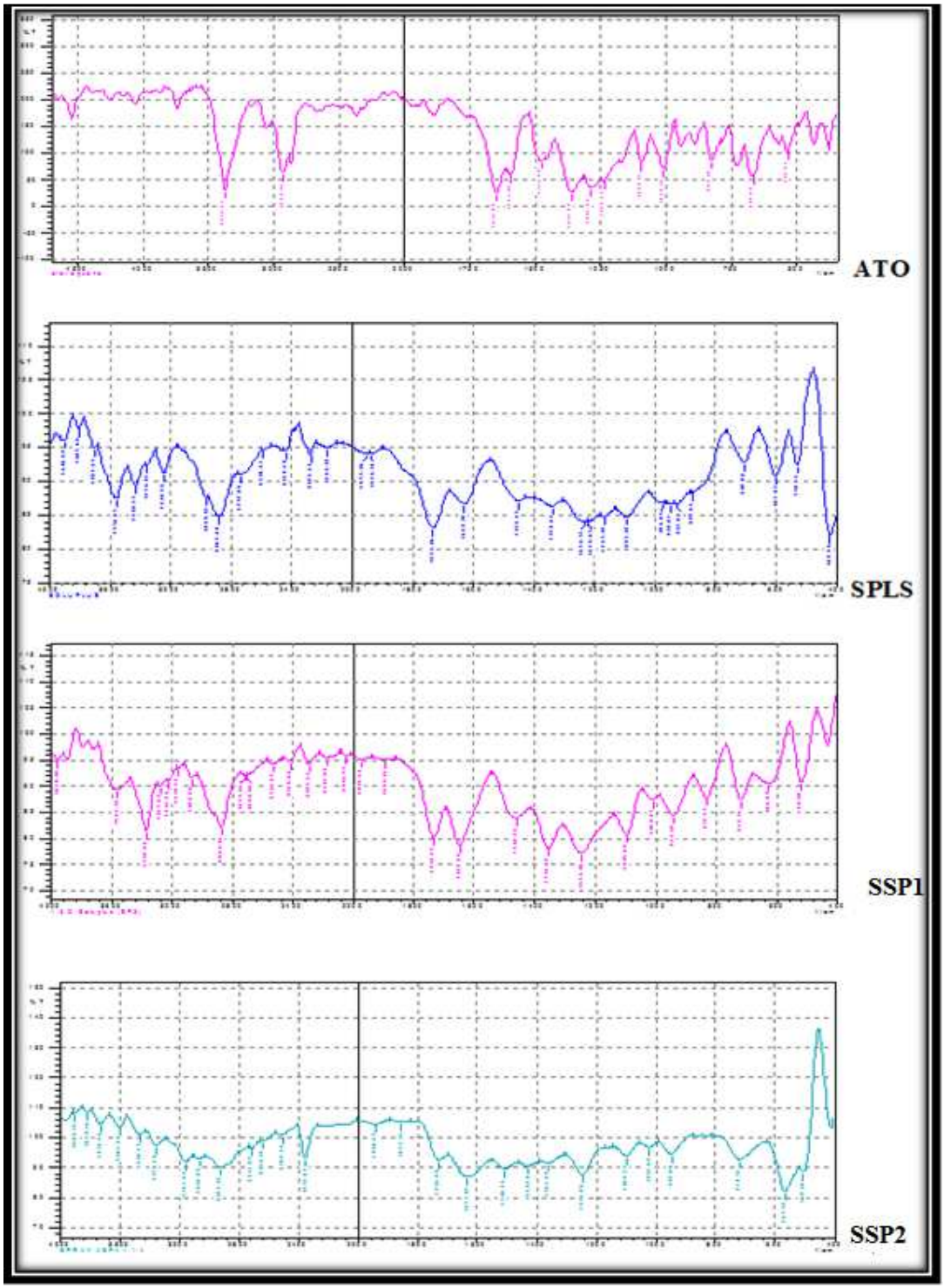


Fig No. 11 IR spectra of Atovaquone (pure) and Soluplus solid dispersions prepared by spray drying method

III. Atovaquone content estimation:

Table No. 12 Percent Atovaquone content of various solid dispersions systems prepared by solvent evaporation method

Carrier used	Solid dispersion system	Drug: Carrier ratio (Drug: Polymer)	% Atovaquone content ± S. D.
KollidonVA-64	VA 1	1:1	96.81 ± 0.26
	VA 2	1:3	97.60 ± 0.30
	VA 3	1:5	99.20 ± 0.42
Soluplus	SP 1	1:1	98.40 ± 0.34
	SP 2	1:3	98.72 ± 0.36
	SP 3	1:5	99.75 ± 0.22

All values expressed as mean ±SD, n=3

Table No. 13 Percent Atovaquone content of various solid dispersions systems prepared by spray drying method

Carrier used	Solid dispersion system	Method	Drug: Carrier ratio	% Atovaquone content ± S. D.
Kollidon VA64	SVA 1	SD	1:3	98.40 ± 0.44
	SVA 2	SD	1:5	99.20 ± 0.40
Soluplus	SSP 1	SD	1:3	99.52 ± 0.18
	SSP 2	SD	1:5	99.68 ± 0.30

All values expressed as mean ±SD, n=3

Table No. 14 Dissolution data for solid dispersions of Atovaquone prepared with Kollidon VA-64 by solvent evaporation method in PBS pH 7.4 + 30% Isopropyl alcohol

Time (min.)	Cumulative Atovaquone release (%)			
	Atovaquone (pure)	**VA 1**	**VA 2**	**VA 3**
20	2.9 ± 0.50	4.8 ± 0.22	8.1 ± 0.32	12.1 ± 0.22
40	10.3 ± 0.29	16.1 ± 0.28	22.9 ± 0.48	38.9 ± 0.51
60	19.7 ± 0.42	28.6 ± 0.36	34.3 ± 0.66	49.4 ± 0.64
80	29.6 ± 0.35	38.5 ± 0.56	46.7 ± 0.72	64.6 ± 0.82
100	38.2 ± 0.38	51.8 ± 0.62	54.9 ± 0.81	68.3 ± 0.28
120	46.7 ± 0.26	56.2 ± 0.58	66.5 ± 0.56	72.1 ± 0.88

All values are expressed as mean ±SD, n=3

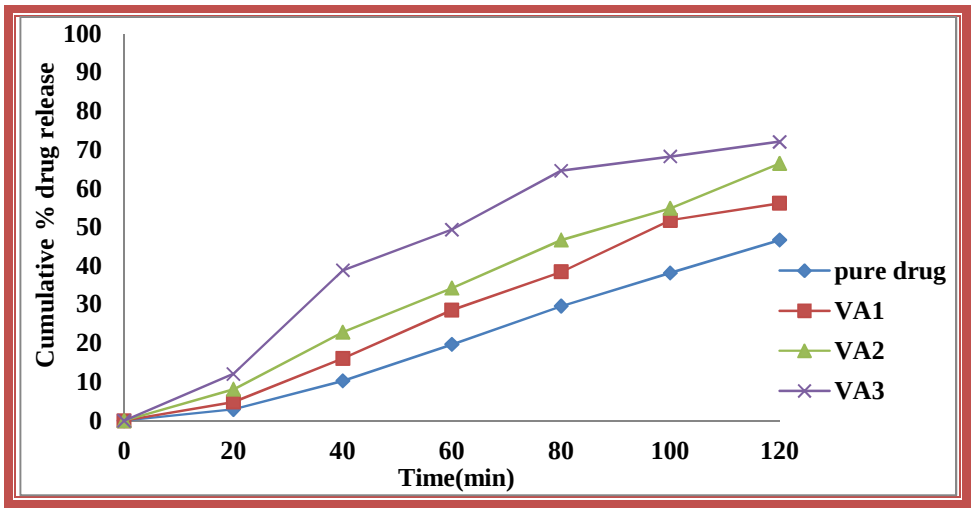


Figure No. 12 Dissolution data for solid dispersion of Atovaquone in different ratios with Kollidon VA-64 prepared by solvent evaporation method

Formulations VA1-VA3 were studied for *in vitro* drug release. Results shown that solid dispersions prepared with Kollidon VA64 by solvent evaporation method shown the increase in dissolution as compare to plain drug. At 120 min batch VA1 gave 56.2% & batch VA3 gave 72.1% drug release. Dissolution of solid dispersion shown that dissolution rate increases with increase in concentration of Kollidon VA-64.

Table No. 15 Dissolution data for solid dispersions of Atovaquone prepared with Soluplus by solvent evaporation method in PBS pH 7.4 + 30% Isopropyl alcohol

Time (min.)	Cumulative Atovaquone release (%)			
	Atovaquone (pure)	**SP 1**	**SP 2**	**SP 3**
20	2.9 ± 0.50	15.5 ± 0.22	19.8 ± 0.32	21.1 ± 0.22
40	10.3 ± 0.29	42.5 ± 0.28	47.1 ± 0.48	54.4 ± 0.51
60	19.7 ± 0.42	54.8 ± 0.36	57.2 ± 0.66	65.2 ± 0.64
80	29.6 ± 0.35	66.3 ± 0.56	68.3 ± 0.72	76.9 ± 0.82
100	38.2 ± 0.38	69.1 ± 0.62	76.9 ± 0.81	80.6 ± 0.28
120	46.7 ± 0.26	76.4 ± 0.58	79.8 ± 0.56	82.4 ± 0.88

All values are expressed as mean ±SD, n=3

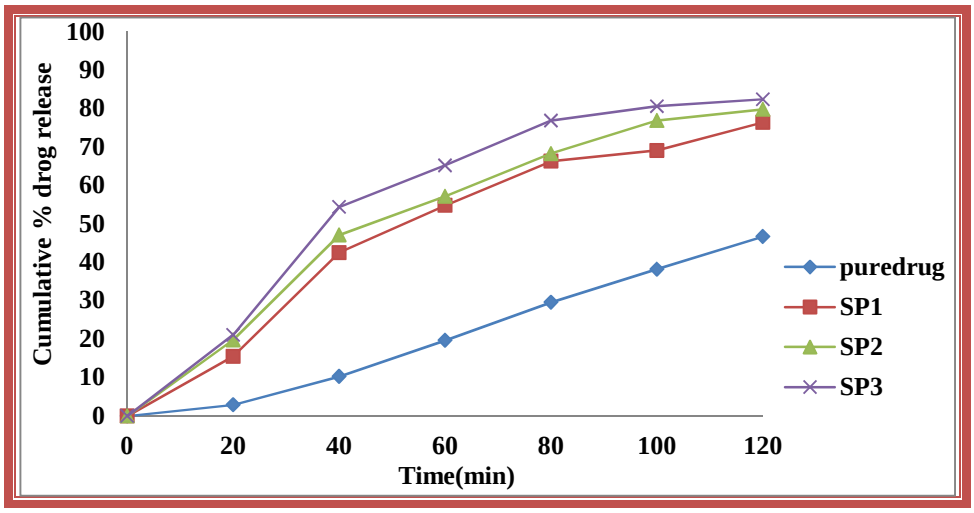


Figure No. 13 Dissolution data for solid dispersion of Atovaquone in different ratios with Soluplus prepared by solvent evaporation method

Formulations SP1-SP3 were studied for *in vitro* drug release. Results shown that solid dispersions prepared with Soluplus by spray drying showed the increase in dissolution as compare to plain drug. At 120 min batch SP1 gave 76.4% & batch SP3 gave 82.4% drug release. Dissolution of solid dispersion shown that dissolution rate increases with increase in concentration of Soluplus.

Table No. 16 Dissolution data for solid dispersions of Atovaquone prepared with Kollidon VA-64 by spray drying method in PBS pH 7.4 + 30% Isopropyl alcohol

Time (min.)	Cumulative Atovaquone release (%)		
	Atovaquone (pure)	**SVA 1**	**SVA 2**
20	2.9 ± 0.54	30.7± 0.22	33.8± 0.20
40	10.3 ± 0.22	70.1± 0.26	81.3± 0.60
60	19.7 ± 0.44	80.5± 0.38	84.4± 0.55
80	29.6 ± 0.38	83.1± 0.66	86.2± 0.28
100	38.2 ± 0.34	87.2± 0.78	89.4± 0.48
120	46.7 ± 0.28	90.3± 0.70	92.5± 0.44

All values are expressed as mean ±SD, n=3

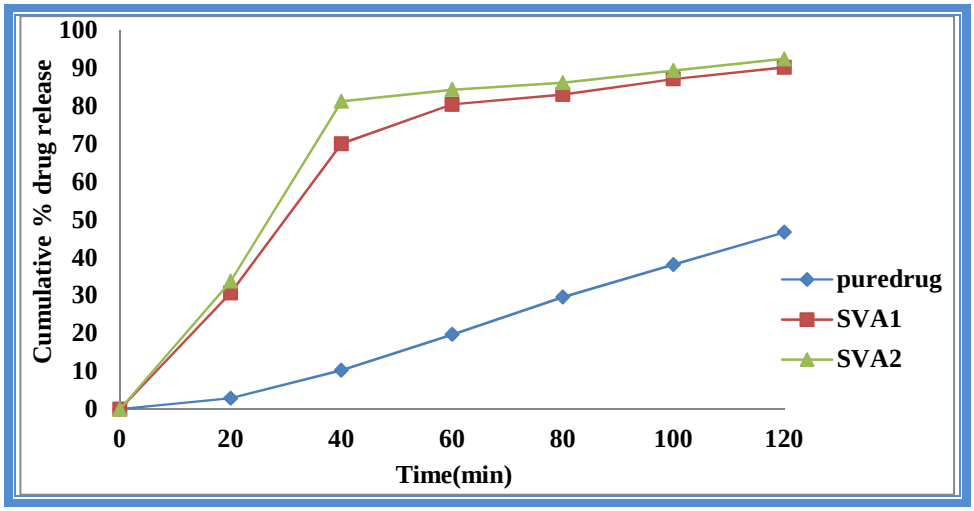


Figure No. 14 Dissolution data for solid dispersion of Atovaquone in different ratios with Kollidon VA-64 prepared by spray drying method

Formulations SVA1-SVA2 was studied for *in vitro* drug release. Results shown that solid dispersions prepared with Kollidon VA64 by spray drying showed the increase in dissolution as compare to plain drug. At 120 min batch SVA1 gave 90.3% & batch SVA2 gave 92.5% drug release. Dissolution of solid dispersion shown that dissolution rate increases with increase in concentration of Kollidon VA-64.

Table No. 17 Dissolution data for solid dispersions of Atovaquone prepared with Soluplus by spray drying method in PBS pH 7.4 + 30% Iso propyl alcohol

Time (min.)	Cumulative Atovaquone release (%)		
	Atovaquone (pure)	**SSP 1**	**SSP 2**
20	2.9± 0.54	37.1± 0.22	40.1 ± 0.20
40	10.3 ± 0.22	84.3± 0.26	87.4 ± 0.60
60	19.7 ± 0.44	86.2± 0.38	91.2 ± 0.55
80	29.6 ± 0.38	90.4± 0.66	93.2 ± 0.28
100	38.2 ± 0.34	92.1± 0.78	95.1 ± 0.48
120	46.7 ± 0.28	95.3± 0.70	97.4 ± 0.10

All values are expressed as mean ±SD, n=3

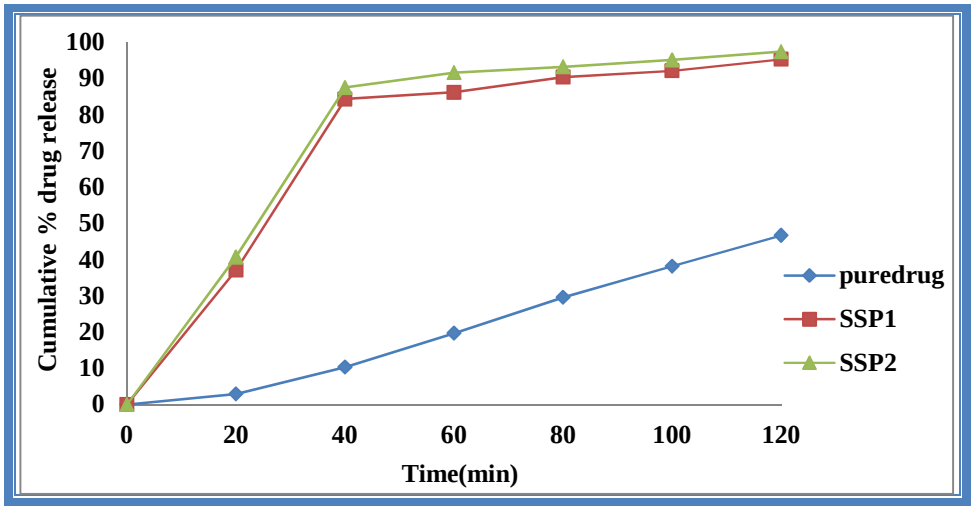


Figure No. 15 Dissolution data for solid dispersion of Atovaquone in different ratios with Soluplus prepared by spray drying method

Formulations SSP1-SSP2 was studied for *in vitro* drug release. Results shown that solid dispersions prepared with Soluplus by spray drying have shown the increase in dissolution as compare to plain drug. At 120 min batch SSP1 gave 95.3% & batch SSP4 gave 97.4% drug release. Dissolution of solid dispersion shown that dissolution rate increases with increase in concentration of Soluplus.

v. X-Ray powder diffraction studies of solid dispersions:

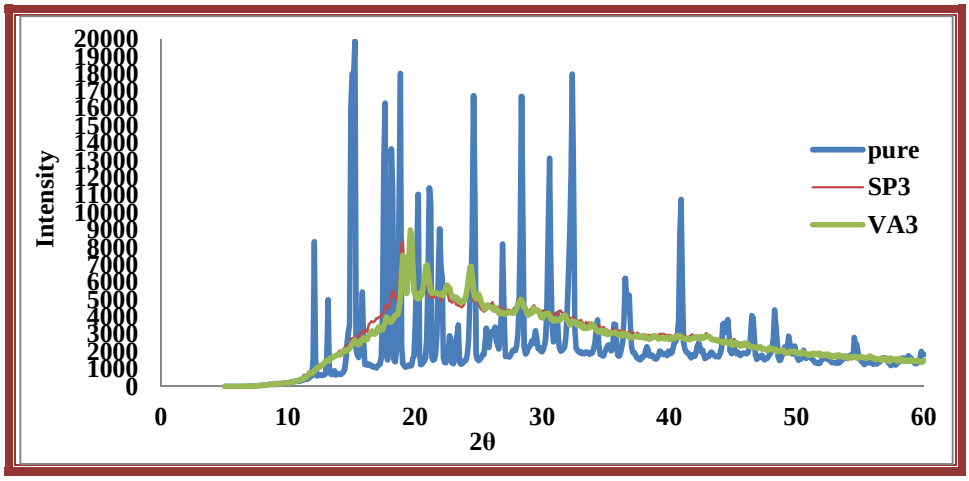


Fig No. 16 XRD pattern for Atovaquone (pure) and solid dispersion of Atovaquone prepared using Kollidon VA-64 (VA3) and Soluplus (SP3) by solvent evaporation method

The X-Ray diffractogram of pure Atovaquone and solid dispersion of Atovaquone using Kollidon VA-64 and Soluplus by solvent evaporation method was shown in **Fig No. 12**. The diffractogram of solid dispersions with Kollidon VA64 (1:5) and Soluplus (1:5) prepared by solvent evaporation method showed broad, diffused peaks with low intensities. It was indicated that the characteristic peaks of drug was disappeared and Atovaquone was completely transformed into amorphous nature.

Dissolution studies of solid dispersions:

The dissolution behaviour of pure Atovaquone and solid dispersions prepared with Kollidon VA-64, Soluplus using Dichloromethane, Isopropyl alcohol and water solvent system by solvent evaporation and spray drying method shown in Table No 14 to 17 and Figure No. 12 to 15. The release rate for pure Atovaquone was found to be 46.7% in 2 hours and rate was increased as the Atovaquone: polymer ratio increased from 1:1 to 1:5. For Kollidon VA-64 it was increased from 56.2% to 72.1% and for Soluplus release rate was increased from 76.4% to 82.4%.

The release rate for Kollidon VA-64 and Soluplus was increased from 90.3% to 92.5% and 95.3% to 97.4% respectively by spray drying method.

It was clear that the pure Atovaquone has the lowest dissolution rate and all solid dispersion formulations showed a higher dissolution rate. This may be attributed to improved wettability of the drug particles, significant reduction in drug particle size during formation of solid dispersion and the intrinsically higher rate of dissolution of the selected soluble carriers, which could pull insoluble but finely mixed drug particles into bulk of dissolution medium.

The dissolution rate was higher for spray dried dispersion because in spray drying particle size was reduced into very fine particle size with spherical shape.

Summary:

Malaria is a disease caused by a parasite called Plasmodium species that lives part of its life in humans and part in mosquitoes. Malaria remains one of the major killers of humans worldwide, threatening the lives of more than one-third of the world's population. Each year 350 to 500 million cases of malaria occur worldwide.

Atovaquone is used as a fixed-dose combination with proguanil (Malarone) for treating children and adults with uncomplicated malaria or as chemoprophylaxis for preventing malaria in travellers.

Its use in pharmaceutical field is limited because it suffers from low aqueous solubility and belongs to class II of the biopharmaceutical classification system (BCS). Due to low solubility, it exhibits poor dissolution and insufficient oral bioavailability. Thus, an efficient oral formulation of Atovaquone with an enhanced dissolution rate and hence, an improved bioavailability is highly desired.

Hence it was decided to improve solubility of Atovaquone by using Solubilization techniques such as Solid Dispersion. The various for preparation of solid dispersions, Solvent evaporation & spray drying methods were applied and polymers like Kollidon VA-64, Soluplus in the ratios 1:1, 1:3, 1:5 were used. . The effects of several variables to solid dispersion preparation were investigated. IR and UV spectral analysis, DSC were used to characterize solid dispersions.

The solid dispersions prepared by solvent evaporation method showed satisfactory improvement but the dissolution rate was higher for spray dried dispersion along with fast release.

Solid dispersions prepared by various methods were evaluated by methods like Saturation solubility, percent drug content, and by *in -vitro* dissolution method for percent cumulative drug release.

CONCLUSION

From the findings of various physical and chemical tests, it can be concluded that

1. Both solid dispersions significantly improved the dissolution profile of Atovaquone.
2. Dissolution rate of solid dispersions increased with increased concentration of polymer like Soluplus and Kollidon VA-64.
3. The relative efficiency of different carriers to improve the dissolution profile of Atovaquone was in order, Soluplus > Kollidon VA64
4. Solid dispersions prepared by spray drying method showed more solubility enhancement with enhanced dissolution as compared to solid dispersions prepared by solvent evaporation method.
5. SEM studies showed well separated, dense spherical particles with a smooth surface of Atovaquone solid dispersion prepared with Soluplus by spray drying method.

REFERENCES

[1] Chiou W.L., Riegelman S., *Journal of Pharmaceutical sciences*. **1971**; 60(9):1281-1302.
[2] Vasconcelos T. *et al*. *Drug Discovery Today*. Vol-12: **2007**; 1068-1075.
[3] Shamma R.N., Basha M., *Powder technology*.237, **2013**; 406-414.
[4] Ravishankar K., Chowdary K.P.R., *World Journal of Pharmaceutical Research*. Volume 2: Issue 3. **2000**; 578-586.
[5] Mydral P.B., Yalkowsky S.H., "Solubilization of drugs in aqueous media." Encyclopedia of pharmaceutical technology. 3rd edition: Marcel Dekker Inc., New York, **2007**; 1:1104-1114, 3311-3333.
[6] Dressman J., Leuner C., *European Journal of Pharmaceutics and Biopharmaceutics*. **2000**; 50:47-60.
[7] Rowe R.C., Sheskey P.J., Quinn M.E., "Handbook of pharmaceutical excipients." 6th edition, London, Pharmaceutical press: **2009**; 210-214.
[8] Aulton M.E., "Pharmaceutics: The science of dosage form design." 2 nd edition. Churchill Livingstone, London. **2002**; 15-42, 113-138, 234-252.
[9] Bramhankar D.M., Jaiswal S.B., "Biopharmaceutics and Pharmacokinetics-A treatise". Delhi, Vallabh Prakashan, **2003**; 27.29.
[10] Indian Pharmacopoeia, volume 1, Ministry of Health and Family welfare, Government of India, The controller of publications,**1996**; 7.
[11] Material Safety Data Sheet of PVP VA-64.
[12] Profile of Atovaquone assessed on 15 December 2013 [Internet] URL: www.drugsupdate.com
[13] Material Safety Data Sheet of Atovaquone.
[14] Thomas R., "Solubility enhancement with BASF Pharma polymers." Germany: BASF pharma ingredients & services: **2011**; 68-78.

[15] Buhler V. Kollidon®, "polyvinyl pyrrolidone excipients for the pharmaceutical industry." 9th edition. Germany: BASF pharma ingredients & services: **2008**; 207-252.
[16] Technical information on Soluplus®, BASF pharma ingredients & services: **2010**; 1-8.