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PREFORMULATION AND SOLUBILITY STUDY OF ARTEMETHER IN DIFFERENT PHOSPHATE BUFFER SOLUTION.

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ABSTRACT

Malaria has been described since ancient times as seasonal periodic fever. In humans, malaria is caused by four distinct species of parasites: *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium malariae* and *Plasmodium ovale.* Existing treatments for malaria include a limited number of clinically effective antimalarial agents. World Health Organization guidelines for the treatment of uncomplicated falciparum malaria recommend the use of this Artemisinin-based combination therapy. Artemether is indicated for the treatment of P. falciparum malaria cases resistant to both Chloroquine and sulphadoxine, pyrimethamine combination. Artemether act as blood schizontocides. Artemether is a Sesquiterpine lactone derived from Artemisinin. Artemisinin is a compound derived from the sweet wormwood plant and has been used for centuries in traditional Chinese medicine to treat fever. Artemether is absorbed fairly rapidly with peak plasma concentrations reached about 2 hours after dosing. Artemether is poorly soluble in water. So current study was carried out for the determination of solubility of artemether in different phosphate buffer solution and also carried out study of standard calibration curve, melting point, FTIR and micromeritics property of artemether in preformulation study of drug. Results shows that in PH dependent solubility studies of Artemether, solubility was low at PH 6.8 and water and high at PH 4.5 and ethanol.

KEYWORDS: Artemether, solubility, antimalarial agent.

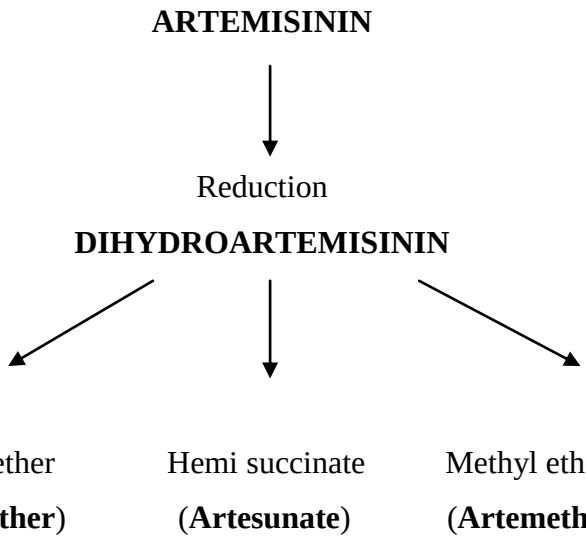
1. INTRODUCTION

Malaria has been described since ancient times as seasonal periodic fever. The name malaria is originated from Latin's '*mal aria'* which means bad air.[1] The symptoms shown by

malaria are fever, headache, muscle ache, back pain, joint pain, chest pain, nausea, vomiting, cough, in severe cases it leads to coma and finally it causes death.[2] Artemether and Lumefantrine are used to treat uncomplicated malaria caused by *Plasmodium falciparum* in a fixed ratio dosage of (1:6).[3]

Parasitic diseases are of immense global significance as around 30% of world's population experiences parasitic infections. Amongst various parasitic infections, malaria is the most life threatening disease and accounts for 1 million to 2 million deaths round the globe every year. The tropical countries such as India are more prone to the malaria and around 2million cases are reported annually. In humans, malaria is caused by four distinct species of parasites: *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium malariae* and *Plasmodium ovale*.[4] Amongst these, the most severe malaria is caused by blood-borne Apicomplexan parasite *P. falciparum* which is responsible for almost all malaria related deaths. Existing treatments for malaria include a limited number of clinically effective antimalarial agents. However, the clinical utility of the most of the antimalarial agents is hampered due to problems such as poor oral bioavailability and the emergence of drug-resistant parasite strains. Paradoxically, due to lack of economic incentives, there are not many initiatives for the development of new anti-malarial agents.[5]

Artemether is an antimalarial drug,[6] which acts against malarial parasites[7] and has a high activity against multi resistant lines of plasmodium falcipuram. Artemether is the methyl ether of dihydroartemisin. Artemisinin or Quinghaosu (.ching-how-soo.) is a sesquiterpene lactone containing endoperoxide bridge (c-o-o-c) that exhibits antimalarial activity. Artemisinin is obtained from the leaves of the herb Artemisia annua sweet wormwood or sweet wormwood belonging to the family Asteraceae was isolated in China in 1972. Artemisinin is poorly soluble in water and oil where as Artesunate is water soluble. Reduction of Artemisinin and esterification leads to the formation of the powerful derivatives as follows.[8][9]



World Health Organization guidelines for the treatment of uncomplicated falciparum malaria recommend the use of this Artemisinin-based combination therapy, and approved by Swissmedic in December 2008 and recently approved by the United States Food and Drug Administration.[10] Chemically, Artemether (ART), also called dihydro Artemisinin methyl ether, is a synthetic derivative of Artemisinin, widely used in malaria treatment in endemic areas.[11] This drug can be administered as an oily solution by intramuscular injection or in capsules orally. ART is active against the plasmodium genera that cause malaria. Its in vivo potency is 10 – 100 folds greater than other antimalarial drugs. [12][13][14]

Artemether (figure-1) chemically know as 3R, 5aS, 6R, 8aS, 9R, 10S, 12R, 12aR)-Decahydro-10-Methoxy-3, 6, 9-trimethyl-3, 12- epoxy-12H-pyrano [4, 3-j] -1, 2-benzodioxepin. Artemether is a compound with a molecular formula $C_{16}H_{26}O_5$ and molecular weight of 298.4 g mol-1 and is a white crystalline powder. Artemether is practically insoluble in water, very soluble in dichloromethane & acetone and freely soluble in ethyl acetate and dehydrated ethanol.[8] Artemether/Lumefantrine is a new and very well tolerated oral antimalarial drug effective even against multidrug resistance falciparum malaria. Many studies have shown that it is the most effective of the antimalarial compound in shortening the fever and parasite clearance times. It is also moisture and light sensitive drug.[15][16]

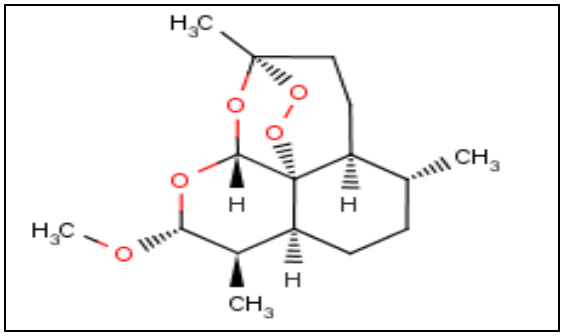


Fig. 1: Artemether.

The current study was conceded out determined solubility in different phosphate buffer solution of poorly water soluble Artemether [ARTM] [BCS class IV drugs].

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

ARTM obtained as a gift samples from mercury laboratories, Vadodara, India. Distilled water was used throughout the study. Sodium hydroxide, sodium chloride, hydrochloric acid, potassium hydrogen phosphate of analytical grades is available for the solubility study.

2.2 Instrumentation

UV- Visible double-beam spectrophotometer, Shimadzu model 1800 with spectral bandwidth of 1 nm, wavelength accuracy ± 0.3 nm and a pair of 10-mm matched quartz cells was used. All the Weighing was done on electronic balance.

Literature survey reveals that few analytical methods like HPLC, stability indicating, GCMS, HPTLC and UV spectrophotometer are reported. As each method suffers from its own limitations.[17]

3.1 PREFORMULATION STUDY OF DRUG

3.1.1 Identification of drug

A) Determination of λ_{max} by U V Spectroscopy.

B) Fourier transforms infrared spectroscopic studies (FTIR).

C) Melting point.

D) Micromeritics properties of drug.

3.1.2 Solubility studies

(A) Determination of λ max.

(B) Solubility Study at various PH & solvent.

3.1 PREFORMULATION STUDY OF DRUG

3.1.1 Identification of drug

(A) Determination of $\lambda_{max\ by}$ U V Spectroscopy[18]

Accurately weighed 10 mg of ART was transferred to a 100 ml volumetric flask. To it add 25 ml of 1 N HCl and this solution was heated on the water bath for 20 minutes at temperature 80 ± 2ºC. The solution was allowed to cool at room temperature and volume was then made up to the mark with distilled water to get concentration of 100 µg/ml and used it as a stock solution. The stock solution was further diluted with distilled water to get concentration. This solution was then scanned in the range of 200 – 400 nm where distilled water was used as a blank. The wavelength of maximum absorbance of ART was found at 254 nm.

(B) Fourier transforms infrared spectroscopic studies (FTIR)

FTIR spectra for the drug alone and with Excipients were recorded using a FTIR spectrophotometer with KBR pellets to study drug-excipient and excipient-excipient compatibility. Drug excipient interaction was determined by performing infrared spectroscopy using FTIR. The FTIR studies were carried out by the pressed pellet technique using a KBr press in which the KBr was taken and kept in a hot air oven for two hours for the removal any moisture. The above dried KBr was taken in the preparation of pellets of drug, and the selected formulations. The prepared pellet was placed in the sample holder and kept in the instrument to record the FTIR peaks.

(C) Melting Point

Melting point of Artemether has been carried out using melting point apparatus. Melting point of compounds was taken by open capillary method. A small amount of drug sample was transferred into capillary tube. Then capillary was placed in melting point test apparatus and noted down the temperature at which the drug started melting and was completely melted.

D) Micromeritics properties of drug

There are different micromeritics properties which are used for the flow property of the drug which are given below.

1. Angle of repose
2. Bulk density
3. Tapped density

4. Carr's compressibility index
5. Hausner's ratio

1. Angle of repose

It is the maximum angle possible between the tip of the pile and horizontal plane and it was measured by the fixed funnel method. It was measured by following a formula.

$\tan\theta = h/r$

Table. 3.1: Reference table for angle of repose.

Value	Types of flow
5 – 15	Excellent – free flowing granules
12 – 16	Good – free flowing powder granules
18 – 21	Fair powder granules
23 – 28	Poor – very fluid powders
28 – 35	Poor – fluid cohesive powder
35 – 38	Very poor – fluid cohesive
>40	Extremely poor – cohesive powder

2. Bulk density

It is the ratio of mass of the blend in bulk volume. It was measured by pouring the powder in measuring cylinder and measuring the volume occupied by powder.

3. Tapped density

It is the ratio of mass of the blend to tapped volume. It was measured by digital tap densitometer by measuring the volume occupied by powder after 100 standard tapping.

4. Carr's index: It was measured by below formula:

$$\%\text{compressibility} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

Table. 3.2: Reference table for Carr's index.

Carr's Compressibility Index	Types of flow
<10	Excellent
11-15	Good
16-20	Fair
21-25	Passable
26-31	Poor
32-37	Very Poor
>38	Very Very Poor

1. Hausner's ratio

It was measured by below formula

H.R= Tapped density

Bulk density

Table. 3.3: Reference tablet for Hausner's ratio.

Hausner's ratio	Type of flow
<1.25	Good flow
1.25 – 1.5	Moderate flow
>1.5	Poor flow

3.1.2 SOLUBILITY STUDIES[19][20]

(A) Determination of λ max.

The Standard drug solution of concentration of 100 µg/ml was prepared using following Media**,**

- Distilled Water
- 1N HCL
- 4.5 Ph acetate buffer
- 6.8 pH Phosphate buffer
- 7.4 pH Phosphate buffer

The Solution was scanned in UV visible spectrophotometer over wavelength range of 200-400 nm. From this scan, the peak of maximum λ max identified in each media and used for further analysis.

A.1) Standard calibration curve of Artemether in various PH.

Accurately weighed 10 mg of Artemether was transferred into 100 ml volumetric flask dissolved and diluted 100 ml up to mark with distilled water. This will give a stock solution having strength of 100µg/ml. Different aliquots were taken from their standard stock solution and diluted up to 10 ml with distilled water separately to prepare a series of concentrations from 10-60 µg/ml. Then the spectra were taken using a spectrophotometer (Shimadzu UV1800). In that distilled water was taken as a reference solution and sample solution with a linear series was taken and absorbance was checked on 210-254 λ max.

This procedure is further follow for the preparation of stock solution of ph 4.5, ph 6.8, ph 7.4 and 1N HCL.

(B) Solubility Study at various PH & solvent.

The solubility of artemether was determined in the various dissolution media as follows. Artemether (10 mg) were transferred into separate 100 ml conical flasks. Dissolution medium (50 ml) was added to each flask and the flasks were closed. These flasks were sonicated for 2 h at 37 °C with intermittent shaking. And closed flask were shaken for 24 h at 37±0.1 ^{0}C, speed 75 rpm using orbital shaking thermo stable incubator and The solutions were each filtered, through a 0.45 µ Whatman membrane filter. Each filtrate was analyzed separately by UV spectroscopy to determine the solubility of the drug in the particular dissolution medium.

4. RESULT AND DISCUSSION

4.1 PREFORMULATION STUDY OF DRUG

4.1.1 IDENTIFICATION OF DRUG

(A) Determination of λ_{max} by U V Spectroscopy

A standard curve of Artemether in 1 N HCL was analyzed in the range of 10-60 µg/ml. The selected range of Artemether was found to be linear. A regression coefficient (R^2) was found to be 0.999 at 254 nm. Results are shown in figure no. 4.1 and table no. 4.1.

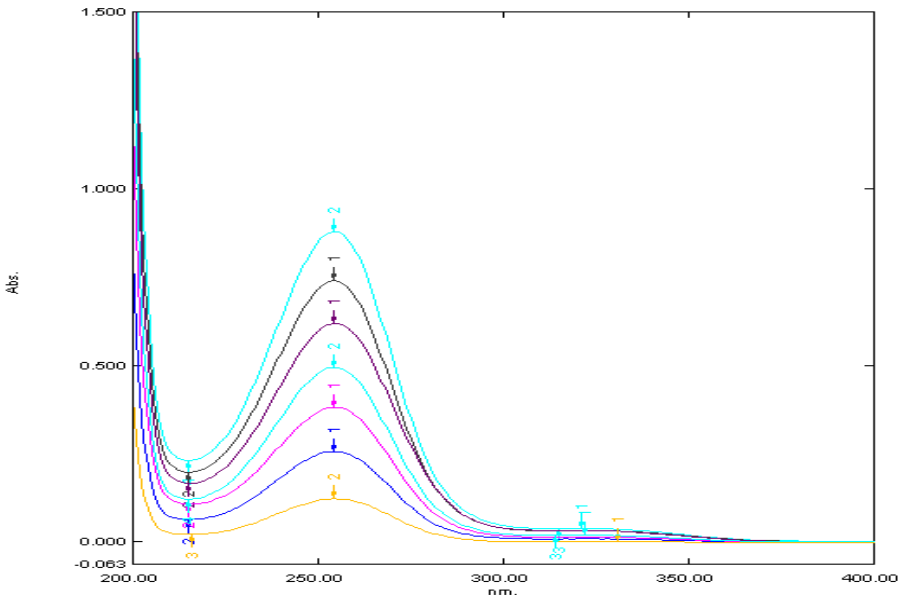


Figure 4.1: Standard calibration curve of artemether in 1N HCL.

Table 4.1: Standard calibration curve of artemether in 1N HCL.

Concentration	Absorbance
	1 N HCL
10	0.123
20	0.256
30	0.381
40	0.493
50	0.618
60	0.738

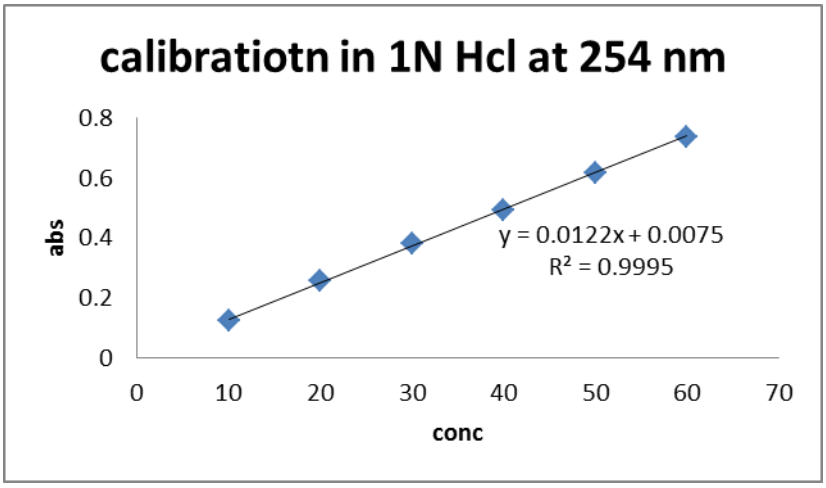


Figure 4.2: Standard calibration curve of artemether in 1N HCL, R^2=0.999.

B) Fourier transforms infrared spectroscopic studies.

(FTIR):

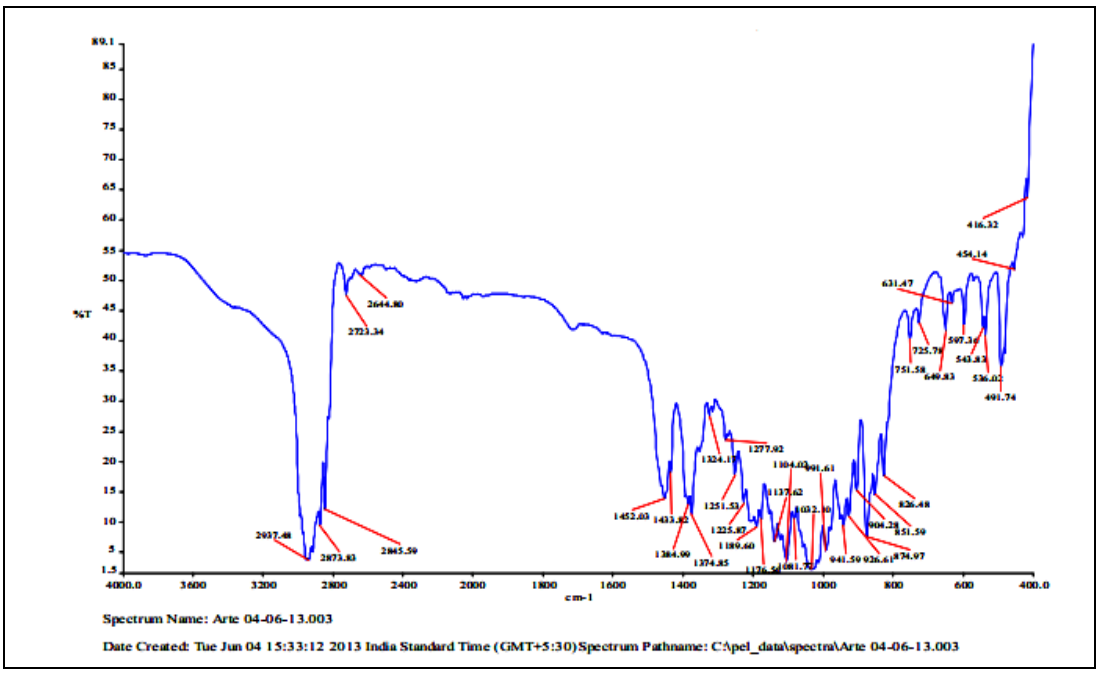


Figure 4.3: Reference spectra of Artemether.

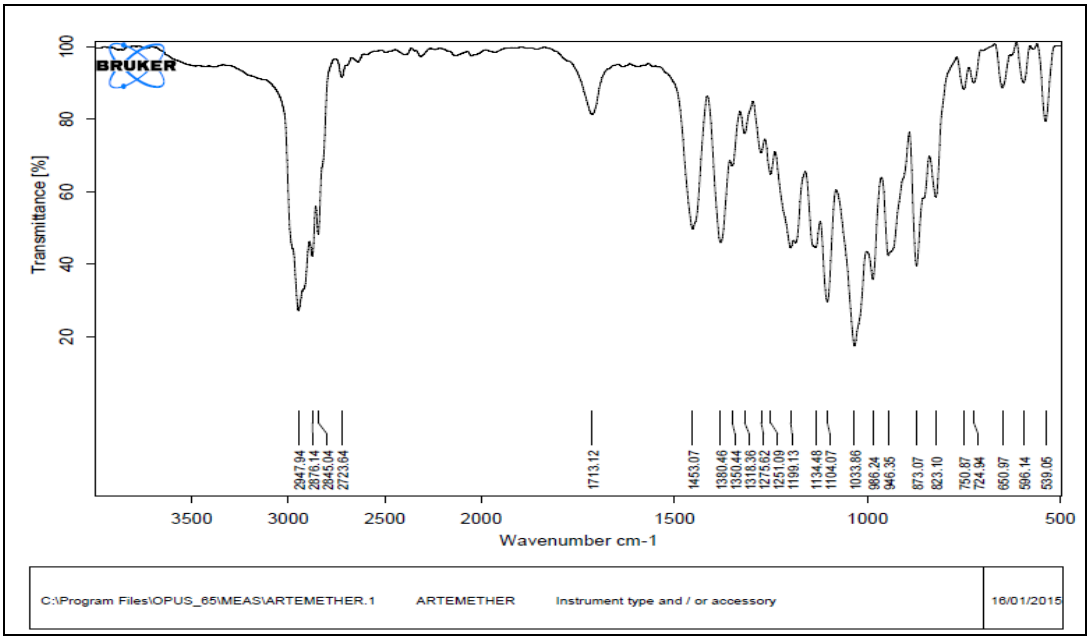


Figure 4.4: FTIR Spectra of pure Artemether.

Table 4.2: Interpretation of Artemether IR spectra.

Sr no	Wave no(cm^{-1})	Interpretation (Stretching)
1.	2947,2876	CH(Aliphatic)
2.	1713	C-O-O-C (peroxide)
3.	1033	C-O
4.	1104	C-O-C (ether)

(C) Melting Point

Table 4.3: Comparison of melting point of Artemether with reported melting point.

Melting point of artemether	Reported Melting Point	Observed Melting Point
	86-90 ^{o}C	85-88 ^{o}C

From this Melting point method, it was conclude that drug melts between the range of 85 to 88 ^{o}C, which is between the range of reported melting point value of Artemether. So it confirms the procured drug Artemether is pure.

(D) Micromeritics Property.

Table 4.4: Micromeritics Property.

Sr. no	Parameter	S1	S2	S3	AVG
1.	Bulk density (gm/ml)	0.23	0.25	0.21	0.23
2.	Tapped density(gm/ml)	0.43	0.41	0.39	0.41
3.	Compressibility (%)	46.51	39.02	46.15	43.89
4.	Hausner's ratio	1.86	1.64	1.85	1.79
5.	Angle of repose (θ)	48.91	55.54	54.54	52.99

From this micromeritics property, it was concluded that Artemether have very poor powder flow property. Artemether is extremely poor cohesive powder.

4.1.2 SOLUBILITY STUDY IN VARIOUS PH SOLUTION

(A) Determination of λ max.

A.1) Standard calibration curve of Artemether in various PH.

A.1.1) Standard calibration curve of artemether in distilled water.

A standard curve of Artemether in distilled water was analyzed in the range of 10-60 µg/ml. The selected range of Artemether was found to be linear. A regression coefficient (R^2) was found to be 0.995 at 211 nm. Results are shown in figure no. 4.5, 4.6 and table no.4.5.

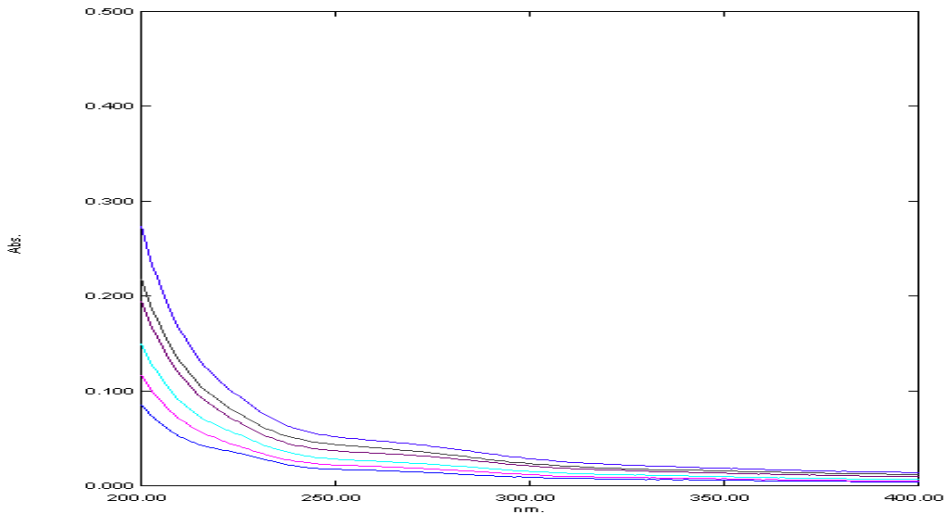


Figure 4.5: Standard calibration curve of artemether in distilled water.

Table 4.5: Standard calibration curve of artemether in distilled water.

Concentration	absorbance
	water
10	0.050
20	0.067
30	0.086
40	0.113
50	0.132
60	0.157

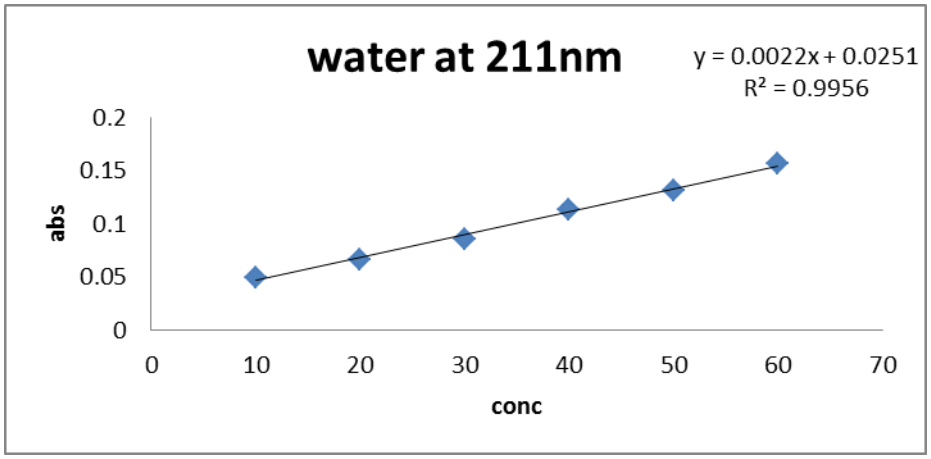


Figure 4.6: Standard calibration curve of artemether in distilled water, R^2=0.995.

A. 1.2) Standard calibration curve of artemether in 1 N HCL

A standard curve of Artemether in 1N HCL was analyzed in the range of 10-60 µg/ml. The selected range of Artemether was found to be linear. A regression coefficient (R^2) was found to be 0.995 at 254 nm. Results are shown in figure no. 4.7, 4.8 and table no.4.6.

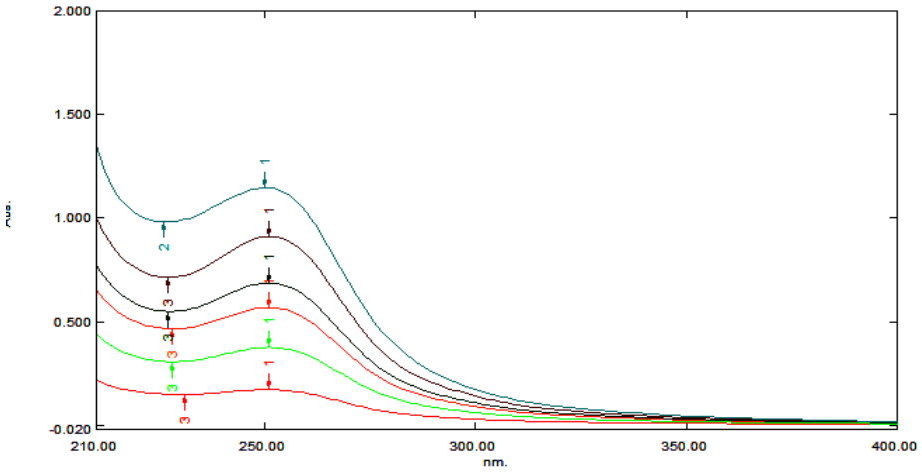


Figure 4.7: Standard calibration curve of artemether in 1N HCL.

Table 4.6: Standard calibration curve of artemether in 1N HCL.

Concentration	Absorbance
	1 N HCL
10	0.041
20	0.064
30	0.090
40	0.123
50	0.139
60	0.166

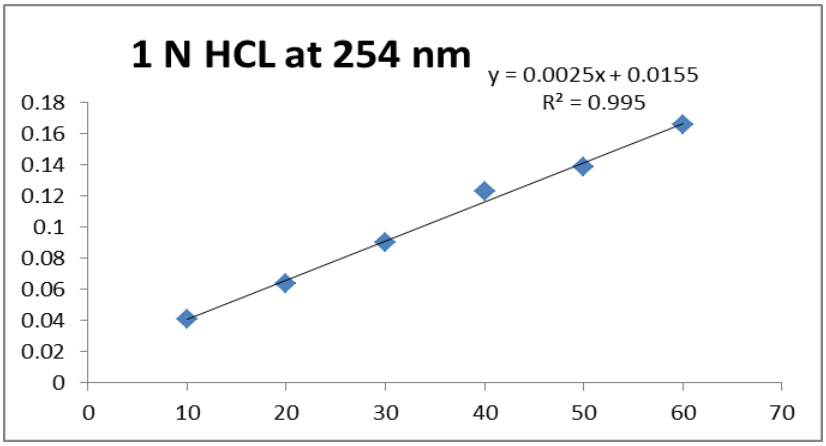


Figure 4.8: Standard calibration curve of artemether in 1N HCL, R^2=0.995.

A.1.3) Standard calibration curve of artemether in PH 4.5.

A standard curve of Artemether in PH 4.5 was analyzed in the range of 10-60 µg/ml. The selected range of Artemether was found to be linear. A regression coefficient (R^2) was found to be 0.961 at 254 nm. Results are shown in figure no. 4.9, 4.10 and table no.4.7.

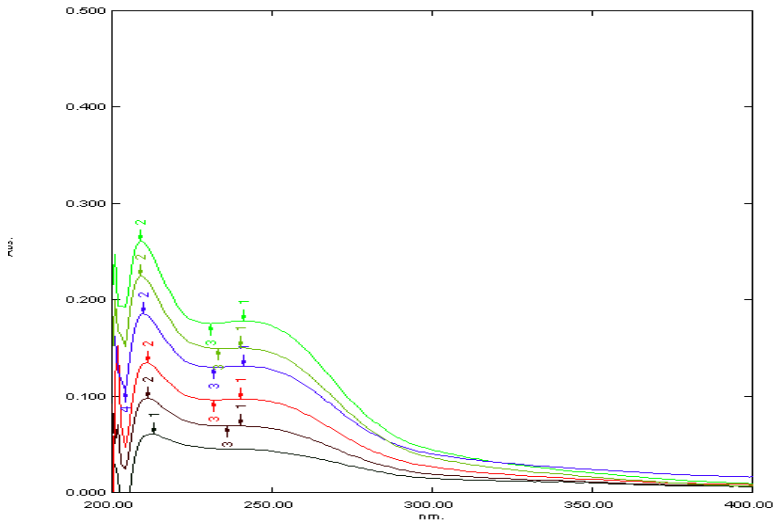


Figure 4.9: Standard calibration curve of artemether in PH 4.5.

Table 4.7: Standard calibration curve of artemether in PH 4.5.

Concentration	absorbance
	PH 4.5
10	0.014
20	0.030
30	0.129
40	0.275
50	0.387
60	0.425

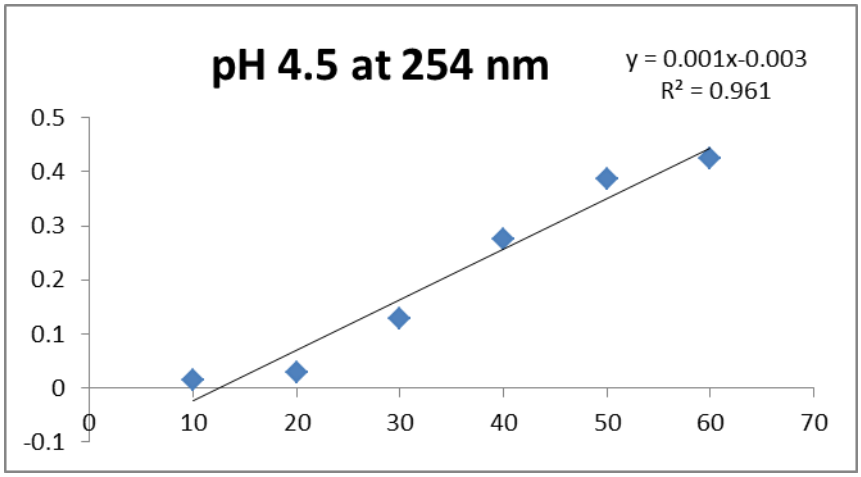


Figure 4.10: Standard calibration curve of artemether in PH 4.5, R^2=0.961.

A. 1.4) Standard calibration curve of artemether in PH 6.8

A standard curve of Artemether in PH 6.8 was analyzed in the range of 10-60 µg/ml. The selected range of Artemether was found to be linear. A regression coefficient (R^2) was found to be 0.994 at 254 nm. Results are shown in figure no. 4.11, 4.12 and table no.4.8.

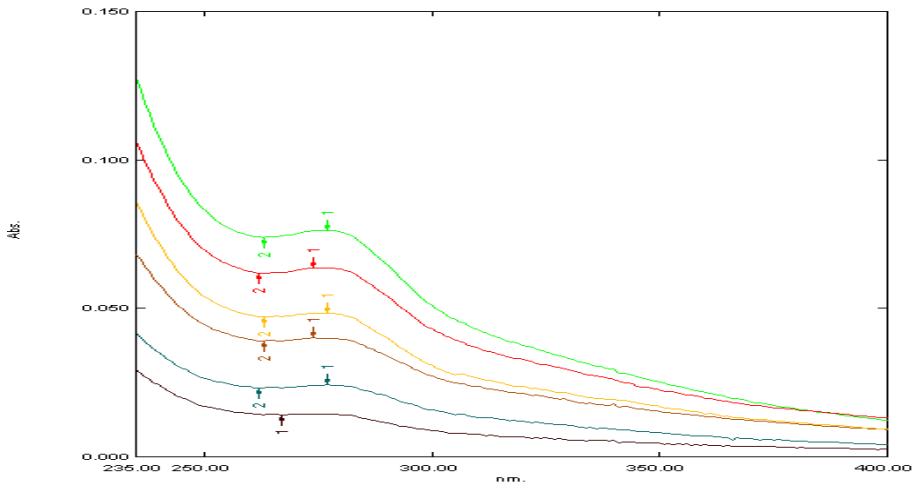


Figure 4.11: Standard calibration curve of artemether in PH 6.8.

Table 4.8: Standard calibration curve of artemether in PH 6.8.

1Concentration	Absorbance PH 6.8
10	0.073
20	0.099
30	0.159
40	0.208
50	0.247
60	0.299

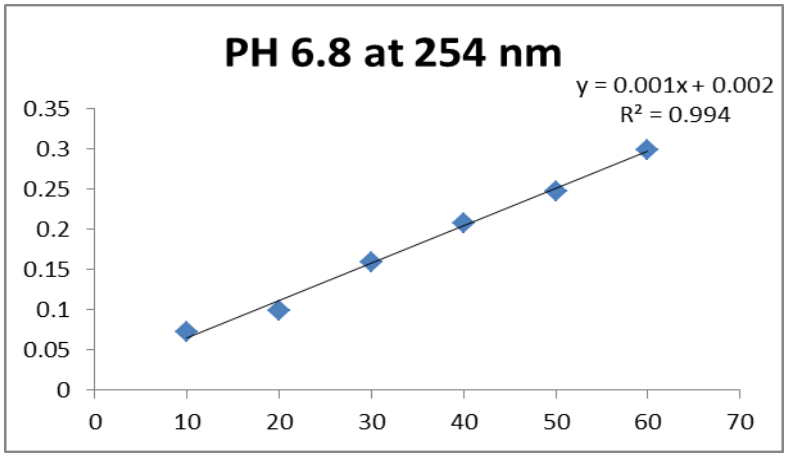


Figure 4.12: Standard calibration curve of artemether in PH 6.8, R^2=0.994.

A.1.5) Standard calibration curve of artemether in PH 7.4

A standard curve of Artemether in PH 7.4 was analyzed in the range of 10-60 µg/ml. The selected range of Artemether was found to be linear. A regression coefficient (R^2) was found to be 0.943 at 211 nm. Results are shown in figure no. 4.13, 4.14 and table no.4.9.

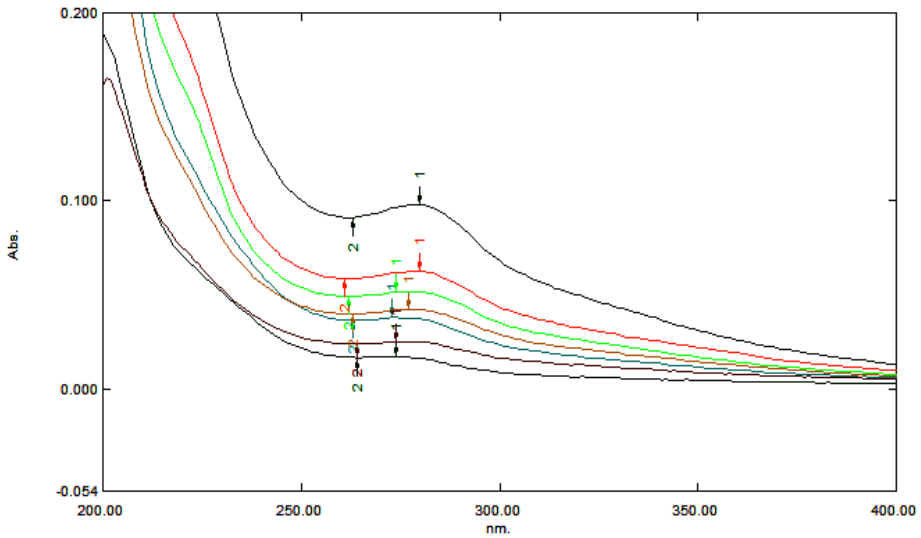


Figure 4.13: Standard calibration curve of artemether in PH 7.4.

Table 4.9: Standard calibration curve of artemether in PH 7.4.

Concentration	absorbance
	PH 7.4
10	0.062
20	0.084
30	0.087
40	0.098
50	0.102
60	0.120

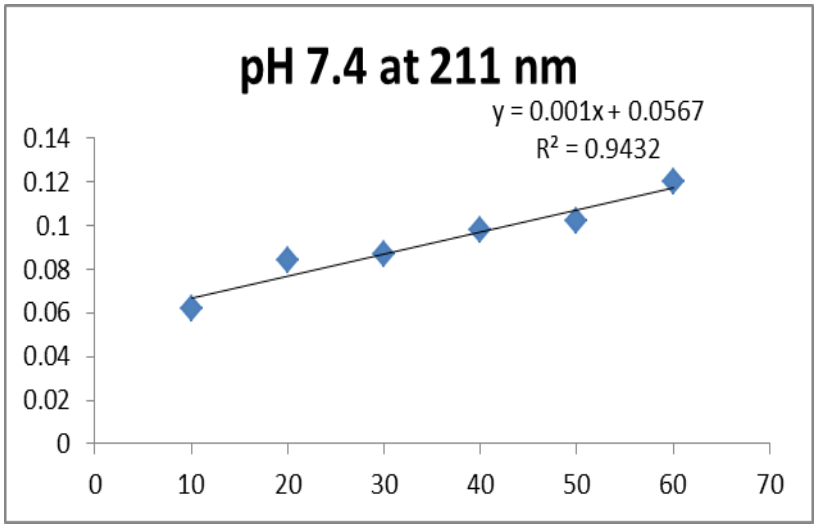


Figure 4.14: Standard calibration curve of artemether in PH 7.4, R^2=0.943.

From the above graph of the Artmether in different buffer solution, the λ max of Artemether selected to be is 210-254 λ max.

(B) Solubility Study at various PH & solvent

Weakly basic drugs and weakly acidic drugs or salts thereof demonstrated PH dependent solubility. For weak acids, as the PH value increases, the solubility of the acid also increases due to the contribution from the ionized form. If log P of any drug is exceeding 1, indicating that the compound has a rather hydrophilic character at physiological PH. Artemether have logP value is 3.53 and it is strongly basic drug, pKa is -3.9.The solubility of the Artemether was tested in different pH medium, water and ethanol maximum solubility was determined in 4.5 pH buffer 4.72 µg/ml and in ethanol 230 mg/ml. The solubility of different pH medium show in table no. 4.10.

In PH dependent solubility studies of Artemether, solubility was low at PH 6.8 and water and high at PH 4.5 and ethanol.

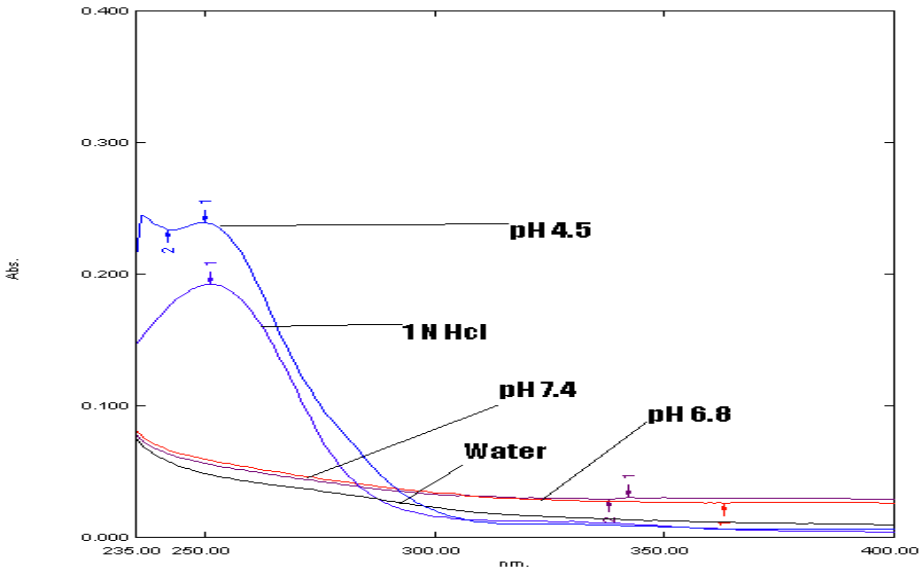


Figure 4.15: overlay graph of Artemether in various PH solutions.

➢ **Solubility Results Of Artemether:**

Table 4.10: Solubility Results of Artemether.

Sr. No	Media	Drug Added(mg)	Sample Absorbance	Solubility (µg/ml)
1	Purified Water	10 mg	0.176	1.51 µg/ml
2	1 N HCL	10 mg	0.190	1.75 µg/ml
3	Ph 4.5	10 mg	0.233	4.72 µg/ml
4	Ph 6.8	10 mg	0.057	1.1 µg/ml
5	Ph 7.4	10 mg	0.166	2.2 µg/ml
6	Ethanol	-	-	230 mg/ml

DISCUSSION

The solubility study of artemether in phosphate buffer solution was carried out that described as above. The solubility of the Artemether was tested in different pH medium, Water, 1 N HCL and Ethanol, maximum solubility was determined in 4.5 pH buffer 4.72 µg/ml and in ethanol 230 mg/ml. In PH dependent solubility studies of Artemether, solubility was low at PH 6.8 and water and high at PH 4.5 and ethanol. That can be concluded by this experimental study. Artemether have logP value is 3.53 and it is strongly basic drug, pKa is -3.9. Weakly basic drugs and weakly acidic drugs or salts thereof demonstrated PH dependent solubility. For weak acids, as the PH value increases, the solubility of the acid also increases due to the contribution from the ionized form. If log P of any drug is exceeding 1, indicating that the compound has a rather hydrophilic character at physiological PH.

CONCLUSION

From this study it can be concluded that artemether is poor soluble drug in water. Its solubility in different PH medium that describe above and that mainly used for determined in vitro release of formulation in different medium.

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