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Development and evaluation of a new spectrophotometric method for determination of Amlodipine in its pure and pharmaceutical forms using zinc oxide nanoparticles

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Abstract

The research includes developing a simple and accurate spectrophotometric method for the determination of amlodipine (AMD) in its pure and pharmaceutical preparations with zinc oxide nanoparticles (ZnO) using water as a solvent to form a complex that gave the highest absorption at the wavelength 364 nm. Beer's law limits were 10-100 µg.ml^{-1}. The correlation coefficient was 0.9973, the molar absorptivity was 1013.72 l.mol^{-1}.cm^{-1}, and the Sandel sensitivity was 0.4 µg.cm $^{-2}$.
The method was applied in the estimation of AMD in pharmaceutical preparations, and the results obtained from the composition of the resulting compound were consistent and accurate, as the Rec values ranged (96.6429 - 102.7166) while the RSD % values ranged (0.4973 -1.2813) , the detection limit (0.0184 µg.ml^{-1}) , the quantitative limit (0.125 µg.ml^{-1}) and the molar absorptivity was 654.2024 l.mol^{-1}.cm^{-1}. and the Sandel sensitivity was 0.625 µg.cm $^{-2}$.The method for the determination of (AMD) has also been successfully applied in its pharmaceutical preparations.

Keywords: spectrophotometric determination. Amlodipine, zinc oxide nanoparticle, complex composition.

Introduction

Amlodipine according to the IUPAC system 3-ethyl-5-methyl-2-(2-ethoxymethyl)-4-(2-Chlorophenyl)-4,1-dihydro-6-methyl-5,3-pyridine dicarboxylate. **(2,1)**
Its molecular formula is $C_{20}H_{25}ClN_2O_5$, its molecular mass is 408.879 g/mol, and its molecular structural is **(3.4)** as shown in Figure (1).

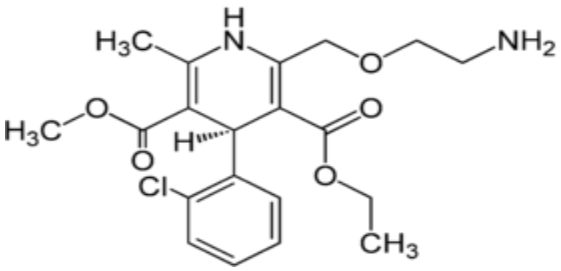

Figure (1) Molecular structural of Amlodipine

This class of drugs is mainly used in the treatment of hypertension, angina pectoris, peripheral arterial disease, coronary artery disease and myocardial infarction **(5-7)**, and its use in these cases is due to its effect on the heart muscle and blood vessels as it reduces heart rate and force of contraction. As a result of slowing the electrical current in the heart muscle and widening the arteries, there is research on the effectiveness of this type of medication in the treatment of Alzheimer's disease **(8)**. It is also used traditionally as a preventive treatment to prevent or reduce the occurrence of migraine attacks, ie migraines **(9, 10).**

A rapid, green, sensitive and accurate analytical method using high-performance liquid chromatography has been developed for the determination of amlodipine in the presence of celecoxib **(11)**

A robust and rapid UPLC-MS/MS method was developed, improved, and validated for the determination of amlodipine (AMD), indapamide (IND) and perindopril (PRN) in human plasma. **(12)**

Development of a method using liquid chromatography coupled with mass spectrometry (LC-MS/MS) for the simultaneous analysis of the mixture in human plasma. To estimate AML, OLM, and HCT. **(13).**

The aim of the research:

The current study aims to develop a new spectrophotometric method for the determination of amylodbin based on oxidation and coupling reactions.

Experimental

A double-beam device (SHIMADZU UV-Visible-1650 - Japan) was used in the analysis of these drugs. Measurements were made in the wavelength range 90-380 nm, aperture width of 2.0 nm, average scanning speed, and using quartz cells.

Amlodipine Standard Solutions (1000 µg ml^{-1})

Dissolve (0.1 gm) of (AMD) prepared from the Samarra Pharmaceutical Company (SDI) in 100 ml volumetric flask by distilled water to prepare (1000µgml^{-1}) solution than prepare solutions in the required concentrations as needed by dilution.

Standard Solutions

Zinc Oxide Nano particle (100µgml^{-1})

The precipitation method is one of the simplest and least expensive methods for preparing ZnO NPs. It is done by adding an amount of sodium hydroxide solution in

the form of drops to aqueous zinc sulfate (BDH) solution until reaching a basic pH, after which the precipitate is washed and filtered for several times and then dried for a period of time. The time until obtaining a white precipitate of oxide nanoparticle **(14)** dissolved (0.01 gm) of (zinc oxide nanoparticle) in 100 ml of distilled water in a volumetric flask, and the solutions were prepared in the required concentrations as needed by dilution.

sodium hydroxide solution 0.1M

This solution was prepared by weight (0.04) g of NaOH (Fluka) and dissolved in distilled water and completed to the mark in a volumetric flask 100 mL.

Hydrochloric acid solution 0.1M

This solution was prepared by diluting (1) ml of concentrated acid (Fluka) to 100 ml in a volumetric flask using distilled water.

Analysis of drug samples for AMD.

For the purpose of analyzing medicinal samples for AMD, the pharmaceutical preparation AML-5 was used by Shreya Life Science Pvt, where 20 tablets containing AMD at a concentration of 5 mg were weighed and ground well using a ceramic mortar and mixed well. Their weight was 3.7255 g, and the equivalent of one tablet was taken 0.1862 g, and dissolved in water. The solution was filtered by Whatman No.42 filter paper and the volume was completed up to the mark with the same solvent in a 100ml volumetric flask. The filtrate containing 50 $\mu.mL^{-1}$ of AMD was kept. The required concentrations were prepared from it.

Results and discussion

1- Study of the optimum conditions

The various factors that affect the absorbance of the prepared compound that was formed as a result of AMD with ZnO NPs reaction at the wavelength 364 nm were studied, then the optimum conditions were selected and fixed as shown.

1-1- Effect of the type of base on the AMD oxidation

Many types of bases were used, but it was found that the best base added to AMD after measuring the absorption of the product of the oxidation reaction, and it gave the highest absorbance is NaOH, as shown in Table (1)

Table (1) effect of the type of base on AMD oxidation

Type of base	Volume	λ)nm)	Absorbance
NaOH	0.2	364	0.354
KOH	0.2	364	0.274
NH_4OH	0.2	364	0.282
Na_2CO_3	0.2	364	0.244

1-2- Effect of HCl volume on AMD oxidation

The effect of the volume of hydrochloric acid on the drug was studied, as volumes of 0.05-0.35 ml of 0.1M HCl were added to a group of volumetric bottles containing the reaction solution. Compared to the base NaOH and as shown in Figure (2), the acidic medium was excluded and the basic medium was kept to complete the reaction

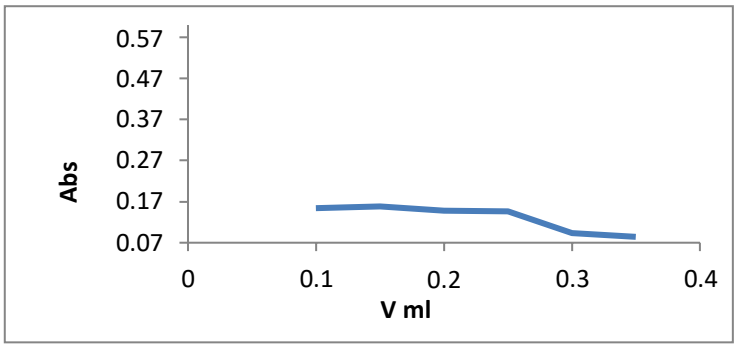

Figure (2) effect volume of acid on AMD oxidation

1-3- Effect of base volume after adding ZnO NPs

From the study of the effect of the volume of the added base (0.1 - 0.6) ml, it was found that 0.2 ml of 0.1M base concentration is the best volume of the base because it gave the highest absorption, as shown in Figure (3).

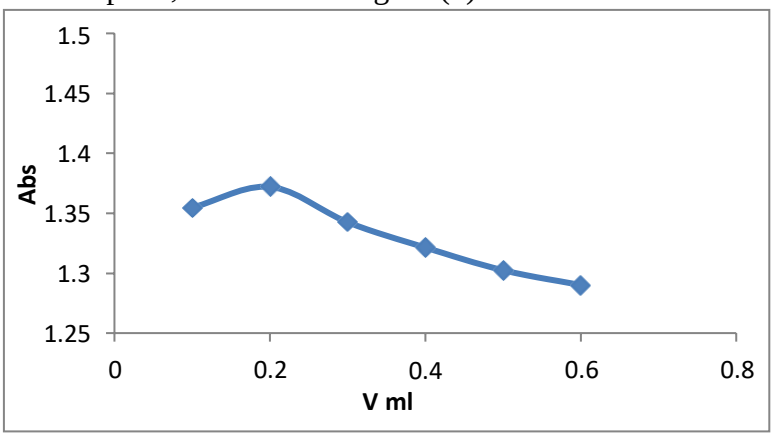

Figure (3) Effect of base s volume on complex adsorption

1-4- Effect of ZnO NPs volume

The effect of ZnO NPs volume (0.5 – 3.0 ml with a concentration of 20 µg.mL^{-1} added to AMD drug with a concentration of 100 µg.mL^{-1}) was studied. The results indicated that 1 ml of ZnO NPs used was the best volume for the reagent because it gave a higher absorption as shown in Figure (4).

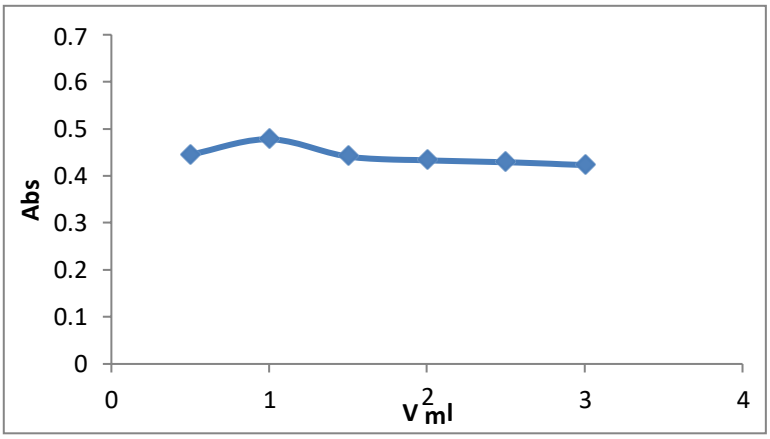

Figure (4) Effect of ZnO NPs volume on complex absorption

1 -5- Effect of temperature

The effect of temperature between 25-95 C̊ on the reaction product was studied, and the results indicated that the best temperature was between 85-95 C, which gives the maximum absorbance as shown in Figure 5.

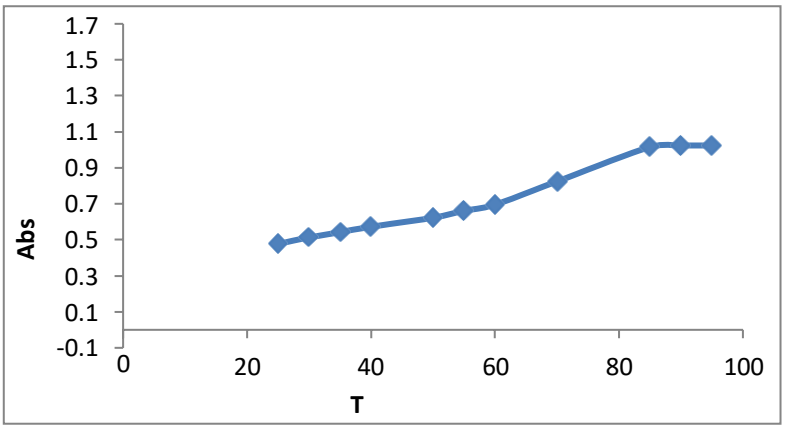

Figure (5) shows the effect of temperature on the reaction product

1-6- Effect of time after heating

The effect of time on the formation of the blue complex has been studied by waiting for different times that ranged between (1-9 minutes) and then after heating in a water bath at 95° C. The results showed that time has no effect on the reaction and that the product is formed upon reaching a temperature 95 °C directly and the intensity of absorption decreases slightly over time as shown in Figure 6, so it is preferable to measure immediately after the color is formed at 95 °C

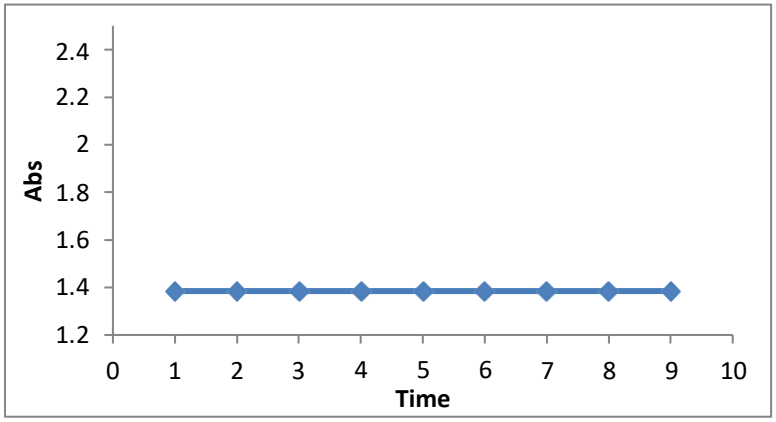

Figure (6) Effect of time on AMD oxidation

2-Effect of Order of additions.

The sequence of addition of ZnO NPs reagent, AMD and NaOH reagent was studied. Despite the absence of a significant difference in the absorption values obtained when following the different sequences of mixing, it is noted that the highest absorption value was when mixing the drug with the reagent and the base in the following order:

Drug solution (A) + ZnO NPs solution(B) + NaOH solution (C) as shown in Table 2.

Table (2) effect of the addition sequence

Order of addition	Absorbance
A+ B +C	**1. 0783**
B +C+A	1. 0537
C +A +B	1. 0613

3-Final absorption spectrum

The figure shows the absorption spectrum after optimum conditions are stabilized

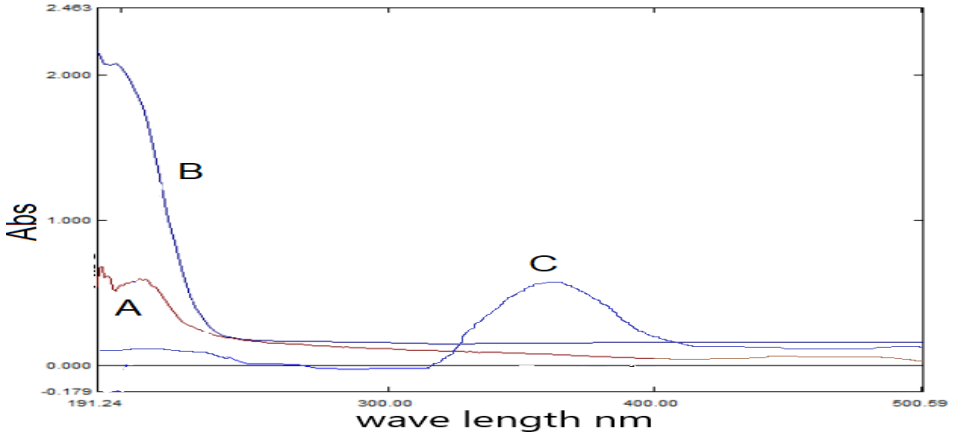

Figure (7) spectrum of amlodipine A , zinc oxide nanoparticles B, and product C

4 - Calibration graph

After fixing the optimal conditions to complete the reaction, a calibration curve was drawn as in Figure 8 and it shows that the Beer-Lambert law was followed to estimate AMD for concentrations of 10-100 µg.mL^{-1} with a final volume of 10mL and with a correlation coefficient (r = 0.9973), then a deviation occurs at the higher concentrations. The molar absorptivity was calculated and it was 1013.72 liters.mol^{-1}cm^{-1} and Sandel's index was 0.4 µg.cm^{-2}

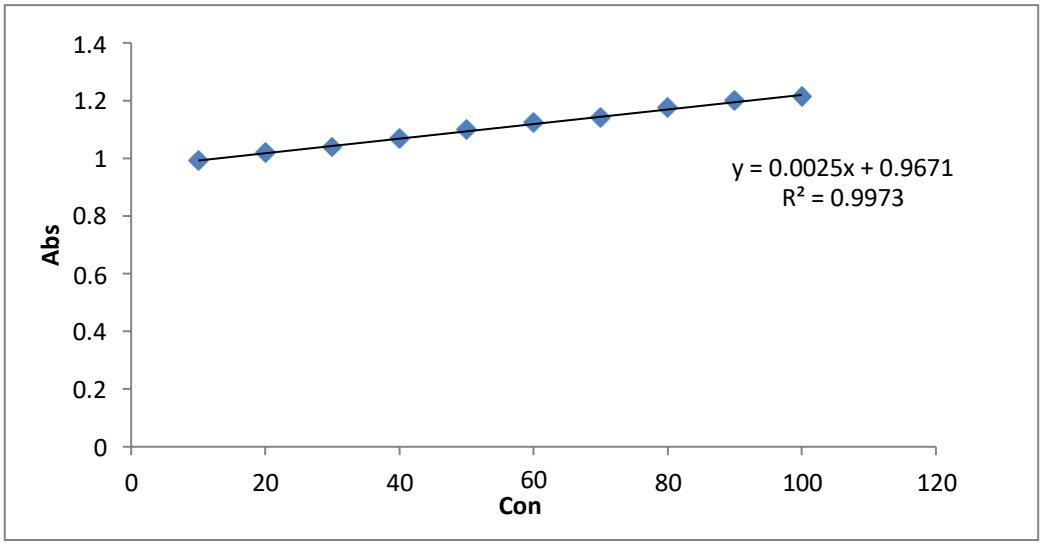

Figure (8) The AMD calibration curve of the proposed method

5- Accuracy and Precision

The obtained results show that the proposed method has good precision and accuracy. The concordance is expressed in terms of the relative standard deviation percentage RSD%, which values ranged from 0.4973-1.2813%, as well as for accuracy, which was expressed in percentage Rec%, which ranged from 96.6429 - 102.7166%. times n=7 as in Table 3.

Table (3) Accuracy and precision of the proposed method

Concentration of AMD µg/ml	Concentration found µg/ml	RSD%	Rec %
10	9.664286	1.1365	96.6429
20	19.71984	0.5668	98.5992
30	30.7254	0.5595	102.4180
40	40.36508	0.6384	100.9127
50	49.17063	0.5758	98.3413
60	60.43016	0.9373	100.7169
70	71.90159	0.7269	102.7166
80	79.20317	0.7193	99.0040
90	90.11508	0.4973	100.1279
100	100.8437	1.2813	100.8437

6 - Application

6-1- Direct Method

In it, the concentrations of AMD in the pharmaceutical preparation are obtained through the straight line equation after measuring the absorption of the prepared concentrations, which are 10,20,30 µg.mL^{-1} as shown in Table 4.

Table (4) The direct method

Concentration µg/ml	Found	Rec%	RSD%
10	10.08639	100.8639	1.2381
20	20.95288	104.7644	2.423
30	30.89529	102.9843	1.1294

6 -2- Standard Addition Method

The standard addition method was applied to the AMD pharmaceutical preparation with a concentration of 30 µg. ml^{-1}, as the results showed the success of the used method, as the Rec value was 96.9350%, while the RSD value was 1.462%, which is as shown in Table (5) and Figure (9).

Table (5) standard addition

Concentration µg/ml	Abs
0	0.622
5	0.810
10	0.919
15	1.033
20	1.180
25	1.254
30	1.390
35	1.525

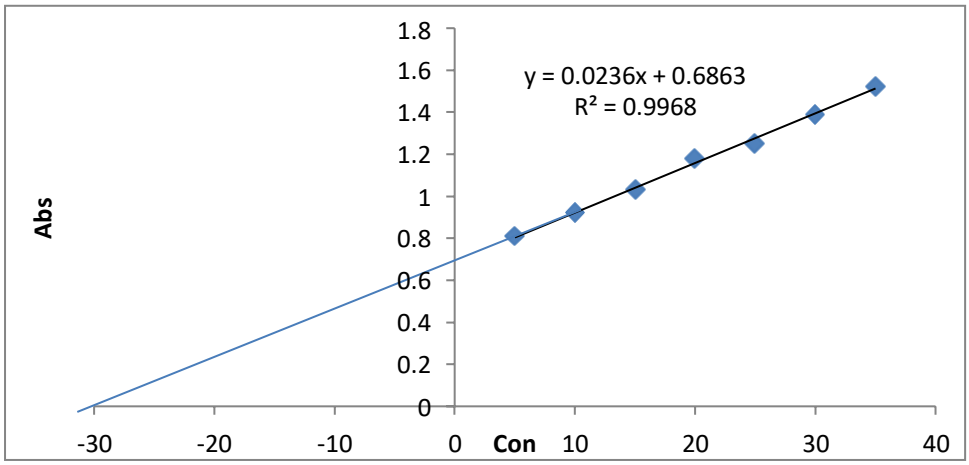

Figure (9) The standard addition

6-3- Job's method for variable ratios

Job's method was used for variable ratios and it was found that the correlation ratios between AMD and ZnO NPs is 1 AMD: 2 ZnO NPs as in Figure 10, by taking opposite volumes of each of AMD and ZnO NPs. These volumes ranged between 0.9-0.1 ml of each of AMD and ZnO NPs and for the concentration 1×10^{-3} M.

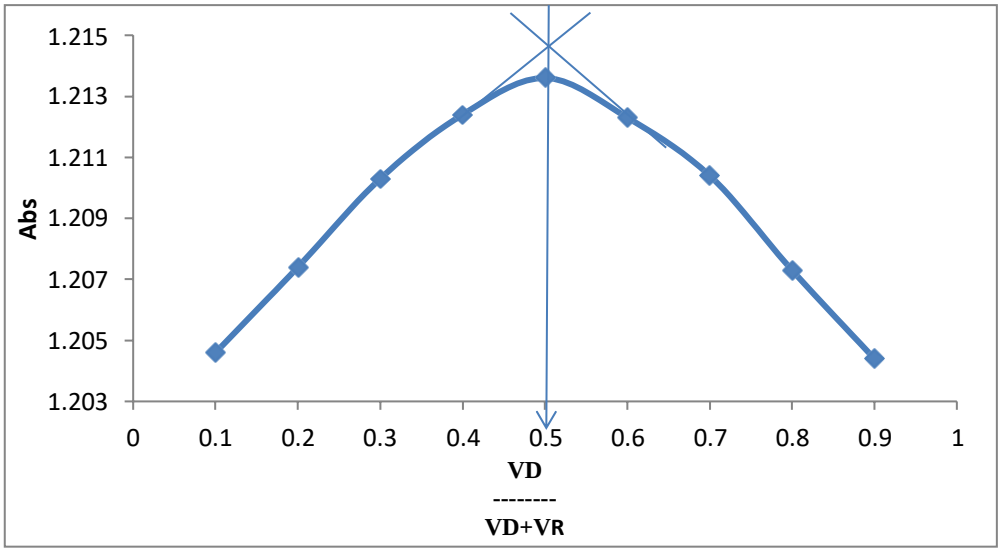

Figure (10) Job's curve for the variable ratios of AMD and ZnO NPs

7- Effect of interferences

The effect of drug additives on AMD was studied. The results are summarized in Table (6). The additives have no effect when they are ten times the drug concentration 100 $\mu g.ml^{-1}$, as the percentage reversion value ranged between 99.793-99.233.

Table (6) The effect of interferences on AMD at a concentration of 100 $\mu g.mL^{-1}$

interferences	Concentration, $\mu g.mL^{-1}$	RSD%	Rec%
Lactoes	1000	1.432	99.793
Talc	1000	1.296	99.626
PHB	1000	1.019	99.531
Mg.stearate	1000	1.864	99.793
MHB	1000	1.187	99.233

8 - IR technical study of the complex

The molar conductivity ratio showed a complex formation between AMD and ZnO NPs, and this was consistent with the study of the IR spectrum, as the IR spectra indicated the appearance of the peak that belongs to the C=N bond. As shown in Figures IR 11,12,13

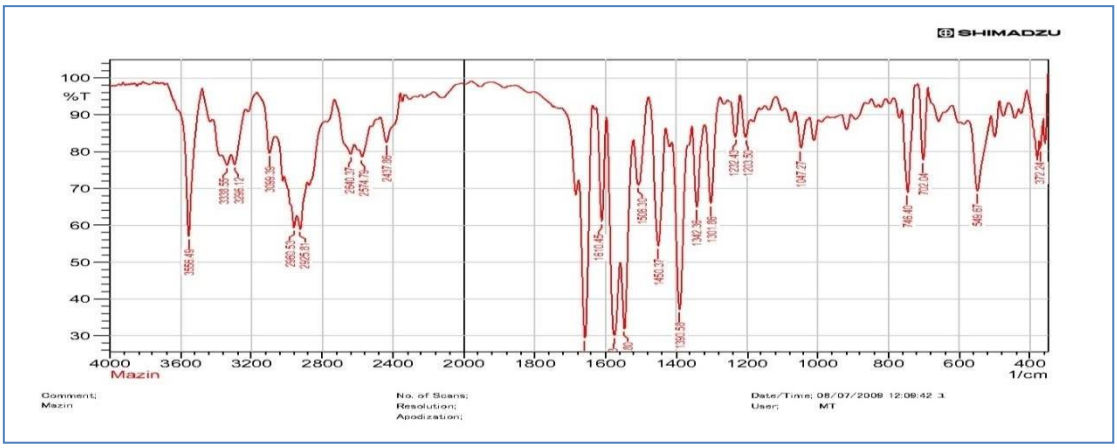

Figure (11) IR spectrum of AMD

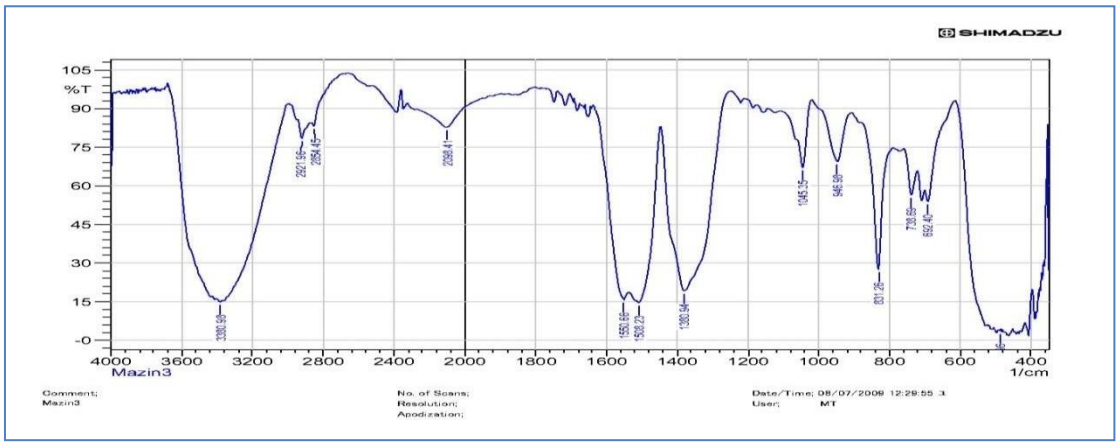

Figure (12) IR spectrum of ZnO NPs

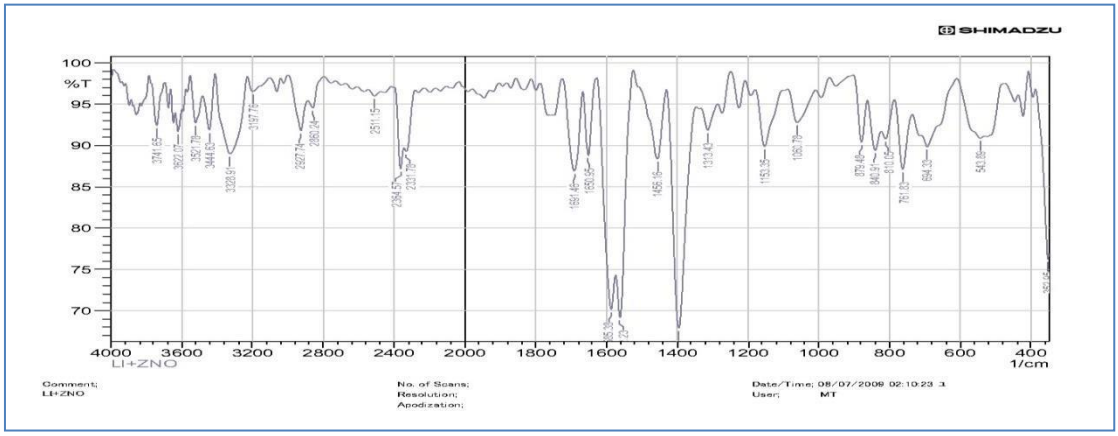

Figure (13) IR spectrum of AMD with ZnO NPs

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