Article

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Anew Merging-Zone Flow Injection System For Determination Of Zn(II) in Pharmaceutical Samples By New Reagent Derivative From 4- aminoantipyrine

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Abstract:

A straightforward, automatic, delicate fresh , high throughput sample and creative spectrophotometric design for a new Merging Zone Injection system The automated method is based on the formation of red complex between Zn(II) and new reagent (4(4-(1,5 dimethyl-2-phenyl-pyrazole-3-one azo)-2-methoxy -5- propenyl-phenol)(DMPPAMPP) at 490 nm to measure Zn(II). The impact of several system factors, including flow rate, reaction coil, and others, was examined. length, sample volume, reagent volume, reagent concentration, and medium pH. The determined linearity range , detection's limit LOD and limit of quantification was found to be 1 to 10 mg ml^{-1},0.323 mg.L^{-1}, and 1.076 mg.L^{-1}, correspondingly , 120 samples per hour is the sampling frequency. The technique was successfully used to determine the amount of Zn(II) in pharmaceutical formulations. The recovery average ranged between 90.35and 118.45%.

Keywords: Merging Zone , Zn(II) , pharmaceutical , DMPPAMPP.

Introduction

The injection approach is a simple, fast, and affordable method for chemical analysis due to its consistency and minimal detector requirements. Flow analysis offers low sample usage, high throughput, and automated monitoring systems, making it a powerful substitute for traditional methods [1-4].

For processes that are unstable or do not reach equilibrium, FIA is a quick and simple technique that can be used[5].The main goal of various FIA approaches is to analyze as many samples as possible with the least amount of the reagent, sample, and analysis time[6].

One of the most crucial trace nutrients for human health is zinc, which has a variety of biological uses from enzymatic catalysis to being essential for cellular neural systems, makes up around 2-3 g of the adult human body[7].

After copper, aluminum, and iron, zinc is the fourth most common metal in the world. With a global demand of 13.77 Mt, the global zinc supply grew to 13.4 Mt in 2018[8], After iron, zinc is the second most prevalent trace metal in the human body. It is necessary for many cellular processes, including immune system functioning [9-10].

Zn(II) ions have relatively low melting and boiling temperatures, at 419.5°C and 907°C, respectively. Among its many important applications, zinc is also utilized in a variety of coatings and as an alloying element in alloys made of magnesium, aluminum, bronze, and brass. Zinc is also utilized as an oxide in the chemical, pharmaceutical, cosmetic, paint, rubber, and agricultural sectors. Recent research has examined zinc as a new biodegradable metal that exhibits potential as an iron and magnesium replacement[11].

The Zn(II) ion's base reaction with the dithizone complex in the aqueous phase with SDS anionic is used in the first technique (sodium dodecyl sulfate) at pH=5 to measure trace levels of Zinc and other elements in various materials. The linear range of the FIA and SIA systems is 2–10 mg/l and 1–15 mg/l, respectively. The absorption maximum occurs at 565 nm(12], The calibration graph showed linearity in ideal conditions, with R^2 = 0.9989 and 0.9997. The methodology used measured absorbance of a Zn(II) complex with ANPDP at pH 9.0 for methods without and with CPE[13],The proposed approach complies with Beer's law across a ratios range of 1–10 mg/L and The Brown complex, which had the greatest absorbance at 420 nm, was created when Cupper(II) and the Azo derivative of the 8-hydroxy Quinoline reagent interacted in a neutral solution [14].
In an alkaline solution, the investigated medication is oxidized with potassium permanganate, and the green oxidation product's absorbance is measured at 610 nm. In the concentration ranges of 0.5–25 and 1–25 μg mL−1, respectively, the calibration graphs for the spectrophotometric and merging-zone flow injection techniques were linear[15],using a spectrophotometer set at 528 nm, the reaction between Fe (III), salicylamide, and 1,10-phenanthroline at 55°C was investigated. Thus, with correlation coefficients (R^2) of 0.9669 and 0.9781, respectively, the novel design demonstrated excellent linearity over the concentration range of (2-30 μg/mL) and (25-300 μg/mL)[16]

The current study outlined straightforward, quick, and affordable batch and merging-zone flow injection techniques for the spectrophotometric measurement of zinc(II) ion based on the reaction using DMPPAMPP as an areagent in a room temperature acidic medium. By decreasing the amount of solutions utilized, cutting down on analysis time, and removing the interfering effect, this method provides a

straightforward, quick, and efficient way to optimize a variety of basic and complex classical reactions with excellent repeatability and throughput.

EXPERIMENTAL

APPARATUS

To test all absorbance data, the manual merging zone flow injection system was used. Using the pH/mV/Ion/0C/0F Oakton 2100 Series meter, the pH value was determined. A Pioneer Weighing was done using an analytical balance (Ohaus PA214) each sample. To choose the maximum wavelength, a Shimadzu UV-1700 spectrophotometer was used. The zinc samples' true value was ascertained using flame atomic absorption spectrometry. The reagent and Zn(II) complex were diagnosed using Spectroscopy in the infrared Proton nuclear magnetic resonance spectroscopy (^{1}HNMR), (^{13}CNMR), energy dispersive X-ray spectroscopy (EDX), ultraviolet-visual radiation, and I.R. and mass spectrometry (LCMS 2010 A Shimadzu).

RESOURCES

All of the compounds were analytical grade and utilized without further purification, with the exception of the DMPPAMPP reagent. Distilled water was used to create the solutions used in this investigation.

Preparation of standard stock solutions

1) **Zn(II) ion Solution 100 mg.L^{-1}**: To make a stock solution, 0.0208 g of zinc chloride were dissolved in 100 mL of D. water. The stock solution was then further diluted to produce working solutions.

2) **New organic reagent solution (DMPPAMPP) 2 ×10^{-3} mol/l**: To make a stock solution, 0.0756g of the organic reagent was dissolved in 100 mL of ethanol. More diluted reagent solutions were prepared as necessary.

3**) 4-Aminoantipyrine(4-AAP) Solution (0.01 mol)(0.5mol/l):**
(0.01mol) of 4-Aminoantipyrine is prepared by dissolving 2.0325 g in 20ml of D.water.

4) **Eugenol Solution (0.01 mol)**:(0.01 mol) of Eugenol is prepared by dissolving 1.6421 g in 20ml of basic alcohol solution.

Preparation of New Azo Reagent derivative:

2.0325 g, 0.01 mol of diazonium chloride was dissolved to create a solution.from 4 amino Antipyrine in 3 mL of rated HCl and 20 mL of water, then mix with 5ml of aqueous 0.7 g, 0.01 mole of $NaNO_2$ drop by drop and stirred for thirty minutes at(0 – 5)°C and formed diazonium salt[17]Fig. (1) illustrates the diazonium salt production stage.At the same time and same tempreture dissolving 1.6420 g, 0.01 mole from eugenol in 20 ml of basic alcohol solution.

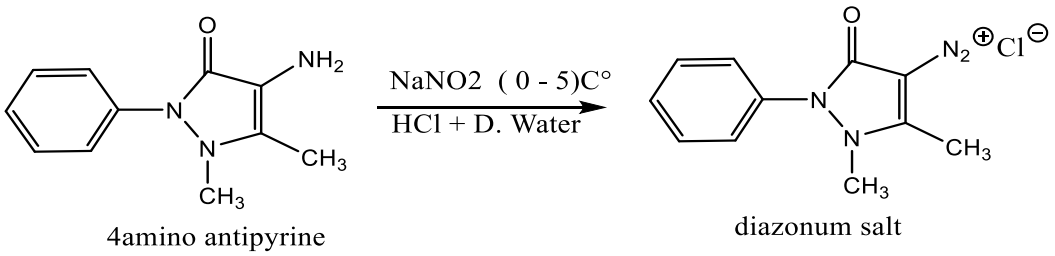
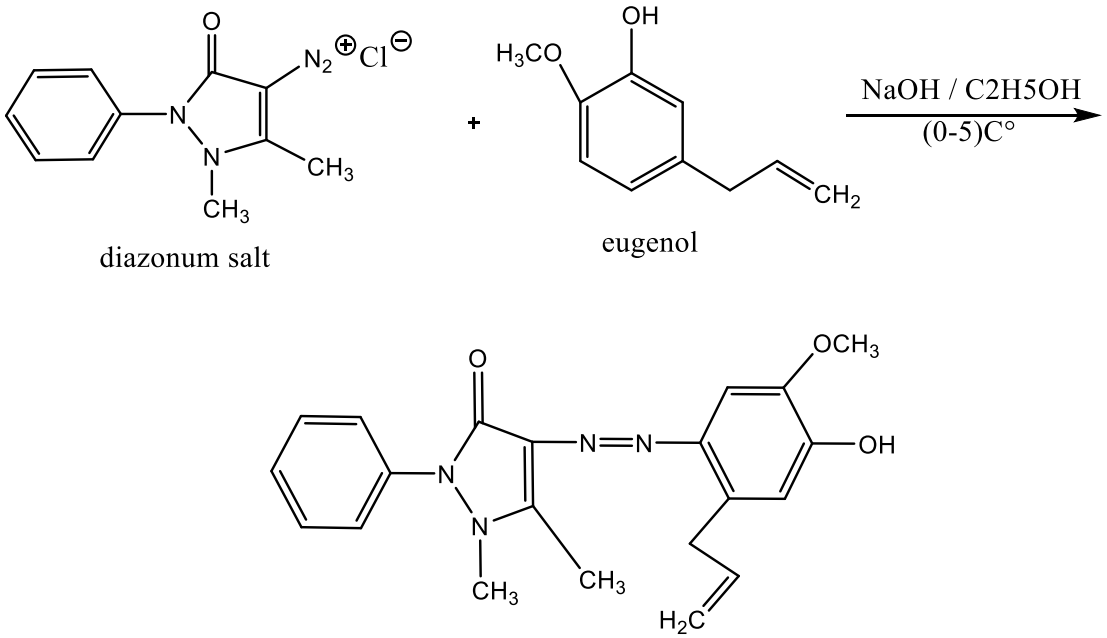


Fig.1: Formation stage of diazonium salt

1) Slowly adding Eugenol solution to diazonium salt , the colour of solution is became deep orange or red, Left the solution for whole day , covered to prevent dust.
2) Filtered and dry , complete washing the precpetite by disteld water after that dry and dissolve by using ethanol and dry to obtain pure crysta, then re crystalline with ethanol and dry the precipitate and measure that melting point of it.



(4(4-(1,5 dimethyl-2-phenyl-pyrazole-3-one azo)-2-methoxy -5- propenyl phenol)
Fig. 2: Formation stage of Reagent(DMPPAMPP)

The Stoichiometry of Zn(II) – DMPPAMPP Complex

To found the reagent: complex ratio , a series of solutions were made, in these solutions, the Zn(II) concentration and volume are held constant at 1 x 10^{-4} mol/l and 1.00 mL respectively, while the reagent (DMPPAMPP) volume is regularly varied from 0.5 to 3 mL at 1 x 10^{-4} mol/l. The absorbance of these solutions is measured at 490 nm with all optimum conditions. The metal-reagent mole ratio is confirmed to be 1:2 as shown in fig. 3.

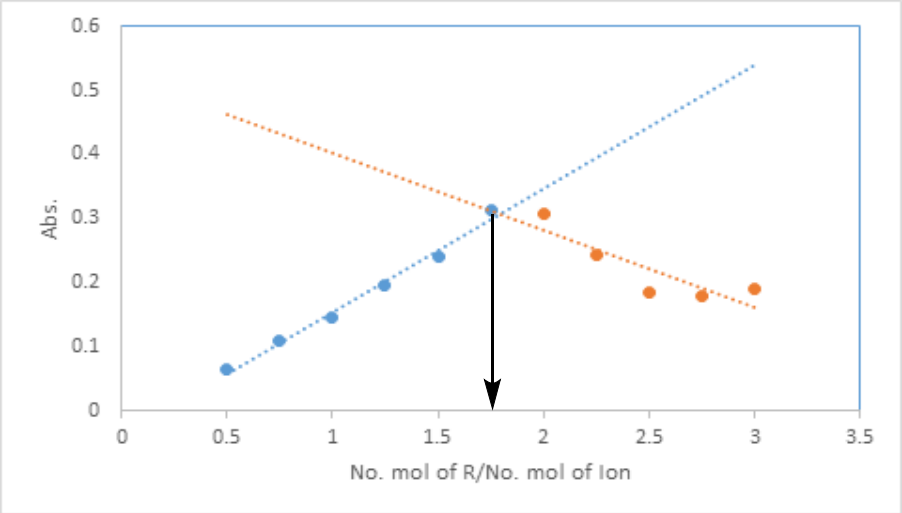


Fig. 3: Studying stoichiometry for the Zn(II) complex using the mole ratio approach

Mechanism of the Reaction

The mechanism of this reaction Zn(II) with DMPPAMPP to form a red complex immediately at λmax 490nm as shown in Fig. 4. The stoichiometry of this reaction was investigated, the number of moles of Zn(II) with DMPPAMPP reagent 1:2 (M:L), as shown in Fig. 3.

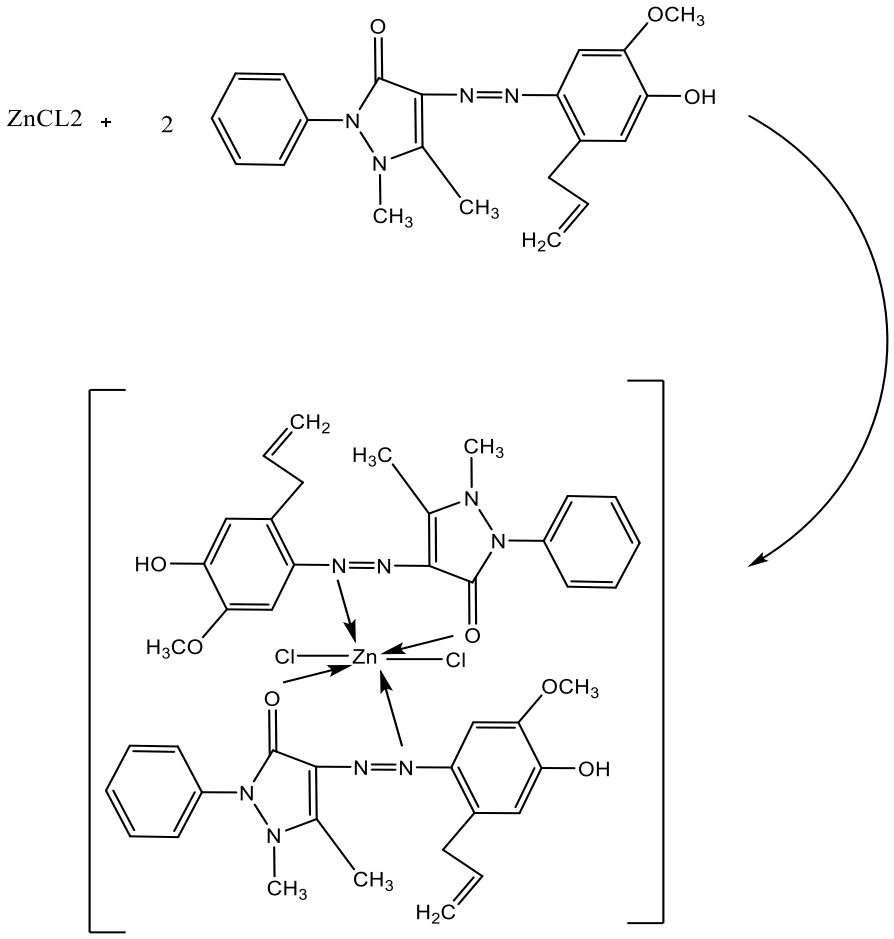


Fig. 4: Proposal mechanism between Zn(II) and DMPPAMPP reagent.

The collection of Samples

Various samples were subjected to the proposed approach. samples included medicine sample. All samples are prepared by performing calculations for each sample, dissolving the calculated weight in 100 ml of distilled water. For liquid samples, a specific volume is taken after performing the calculations and the volume is completed to 100 ml. ... Atomic Absorption Spectrometry in Flame (FAAS), a standard method, was used to quantify the zinc content on these samples. This number was taken as the actual zinc concentration in the comparative samples with the recommended approach.

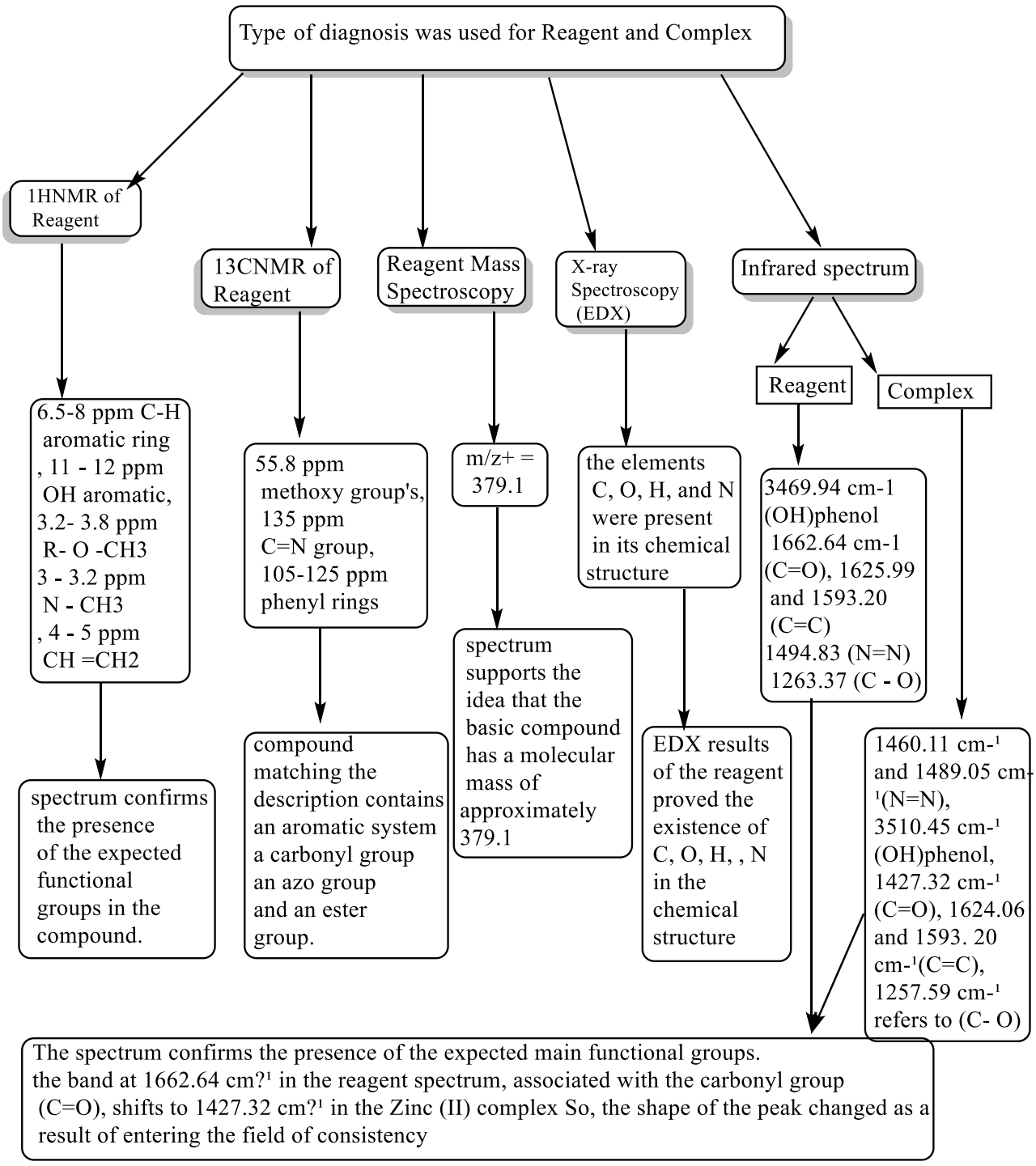
Preparation Solutions of Pharmaceutical[18]

Tablet solution: A concentration of 100 $mg.L^{-1}$ was prepared by weighing ten pharmaceutical tablets for each drug, and the tablets were crushed into a fine powder in a mortar, they were then dissolved in water, after diluting the solution to the volume with diluent, thoroughly mix it, and the solution was filtered through Whatman filter paper, and the remaining concentrations were made by diluting the original solution of each drug

1- Suspended syrup solutions

A specific volume of drug was measured and dissolved in the aqueous solution. Then the solution was filtered with Atman-type filter paper, and a concentration of 100 $mg.L^{-1}$ was prepared by dilution of drug.

Characterization of Azo Reagent(DMPPAMPP) and Its Complex with Zn(II)


Type of diagnosis was used for Reagent and Complex
1HNMR of Reagent
13CNMR of Reagent
Reagent Mass Spectroscopy
X-ray Spectroscopy (EDX)
Infrared spectrum
Reagent
Complex
6.5-8 ppm C-H aromatic ring , 11 - 12 ppm OH aromatic, 3.2- 3.8 ppm R- O -CH3 3 - 3.2 ppm N - CH3 , 4 - 5 ppm CH =CH2
55.8 ppm methoxy group's, 135 ppm C=N group, 105-125 ppm phenyl rings
m/z+ = 379.1
the elements C, O, H, and N were present in its chemical structure
3469.94 cm-1 (OH)phenol 1662.64 cm-1 (C=O), 1625.99 and 1593.20 (C=C) 1494.83 (N=N) 1263.37 (C - O)
spectrum confirms the presence of the expected functional groups in the compound.
compound matching the description contains an aromatic system a carbonyl group an azo group and an ester group.
spectrum supports the idea that the basic compound has a molecular mass of approximately 379.1
EDX results of the reagent proved the existence of C, O, H, , N in the chemical structure
1460.11 cm-1 and 1489.05 cm-1(N=N), 3510.45 cm-1 (OH)phenol, 1427.32 cm-1 (C=O), 1624.06 and 1593. 20 cm-1(C=C), 1257.59 cm-1 refers to (C- O)
The spectrum confirms the presence of the expected main functional groups.
the band at 1662.64 cm?1 in the reagent spectrum, associated with the carbonyl group (C=O), shifts to 1427.32 cm?1 in the Zinc (II) complex So, the shape of the peak changed as a result of entering the field of consistency

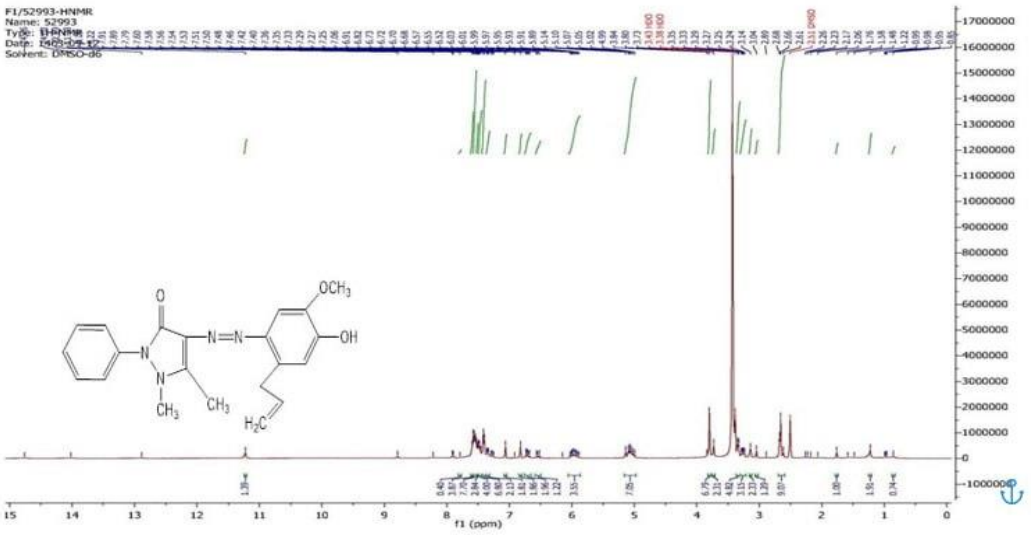


Fig.5 : 1HNMR of Reagent

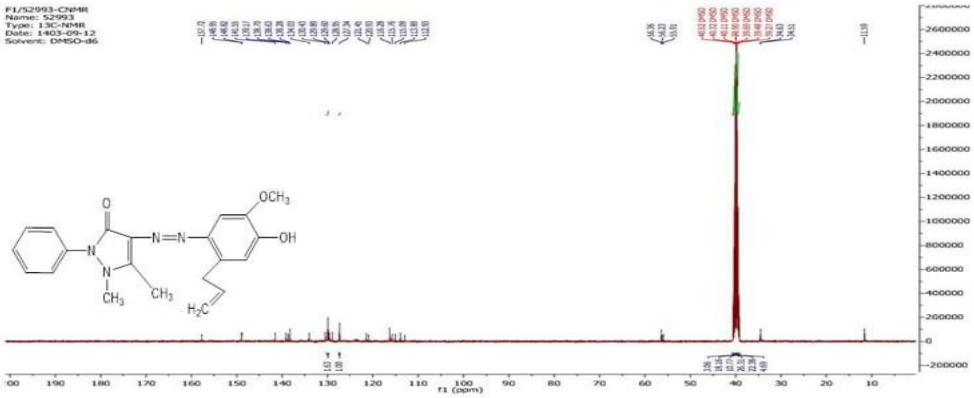


Fig. 6 : ^{13}CNMR of reagent

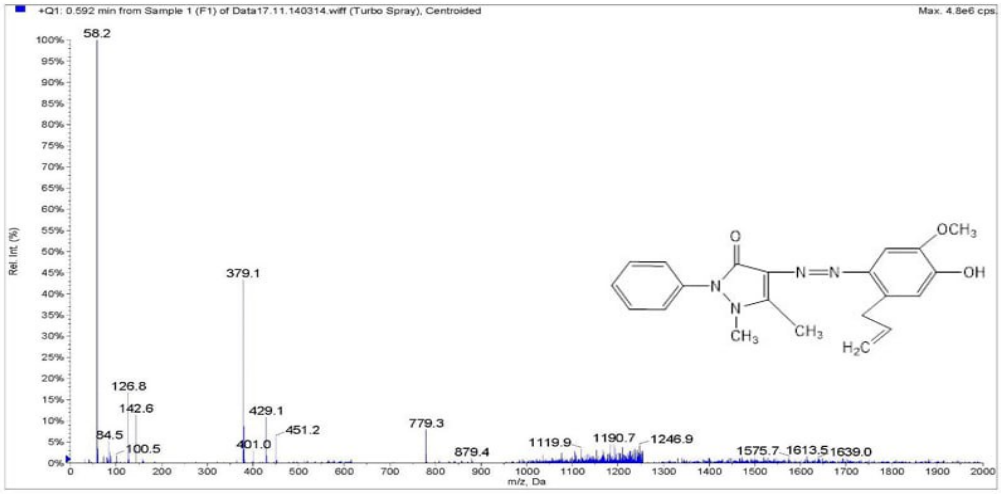


Fig.7 : Mass Spectroscopy for Reagent

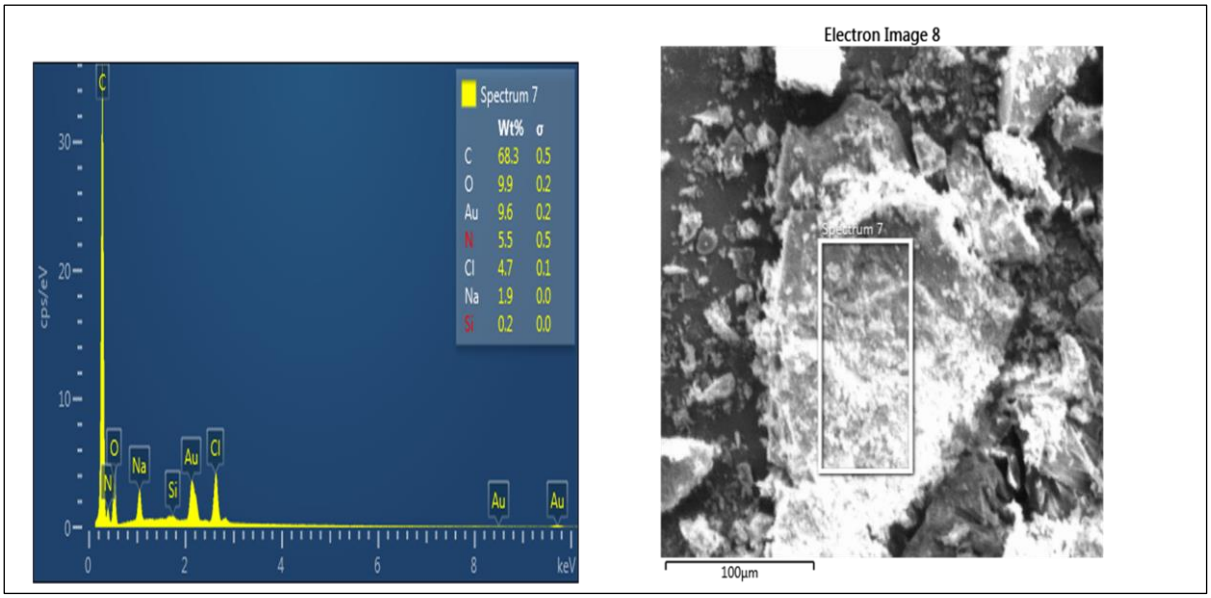


Fig.8 : Energy dispersive X-ray spectrum (EDX) for reagent(DMPPAMPP)

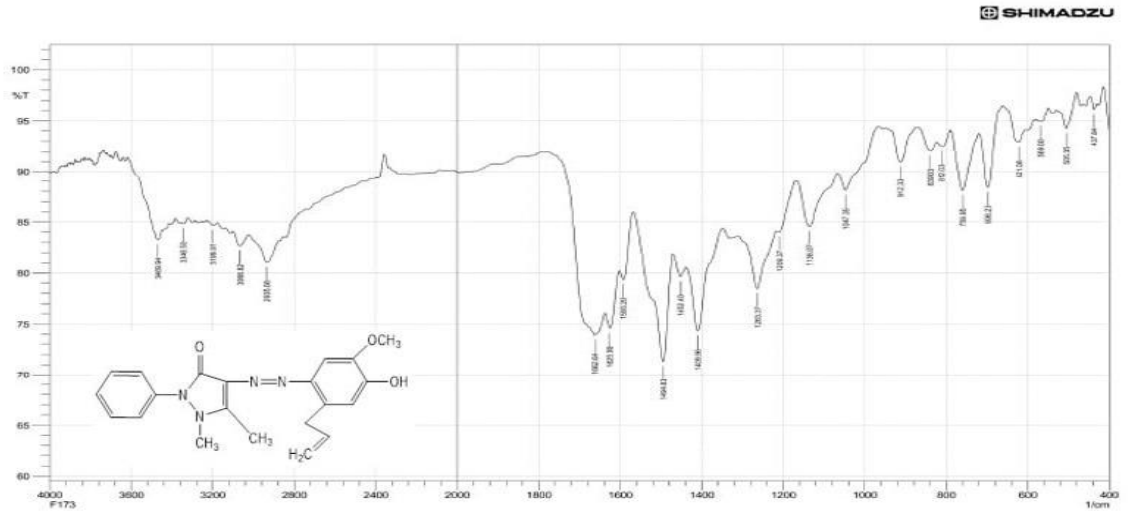


Fig. 9 : FTIR spectrum for reagent

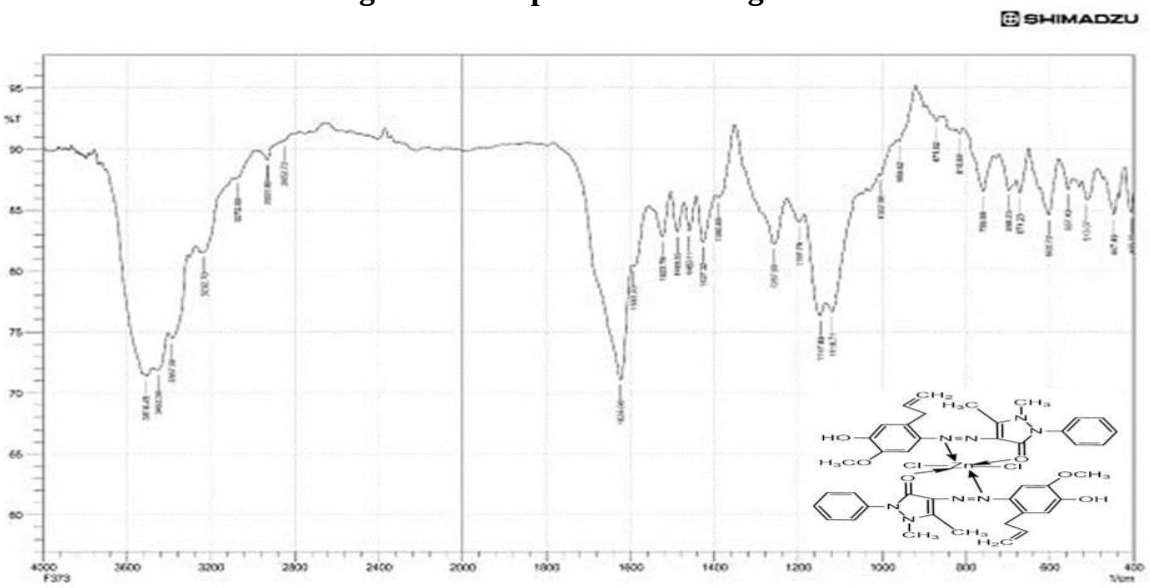


Fig.10: FTIR spectrum for Zn(II) complex

The azo reagent's maximum absorbance wavelength and its complex

A UV-1700 spectrophotometer Shimadzu's was used to determine the maximum absorbance of the reagent at 368 nm and the maximum absorbance of the Zn(II) complex at 490 nm. To attain the highest sensitivity In a variety of mediums, the maximum absorbance of DMPPAMPP and its combination with Zn(II) was investigated (acid, basic, and neutral), as shown in Fig.11, The results indicated that the neutral medium produced the best spectra. These findings led to the selection of 490 nm as the study's maximum wavelength (λ).

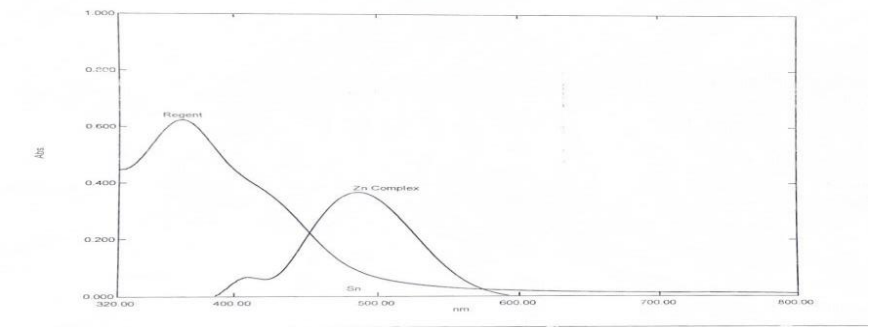


Fig 11: absorption spectra of Zn(II) complex and the reagent in acidic medium And ethanol (99%) as solvent of reagent.

Determination of Zn(II) by Using a New Merging Zone – Flow Injection System

The New Designed Valve: A lab-designed valve with an easy-to-follow replacement and maintenance procedure has been developed, featuring four manually operated secondary valves with three ports each. It serves as the connection between the reaction coil and peristaltic pump in a flow injection system for zinc measurement.

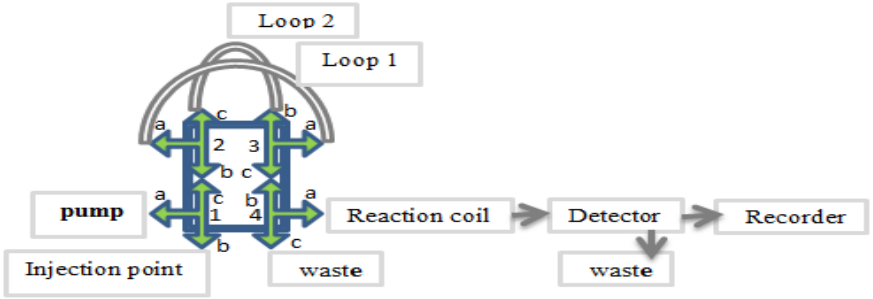


Fig.12: The structure of the valve

Optimization of the Reaction Conditions

1- For the Batch Spectrophotometric Method

The concentration and the volume of Zn(II) was 20mg.L^{-1} , 2ml, the concentration and the volume of DMPPAMPP reagent 2x10^{-3}mol.L^{-1},1.25ml , acidic medium PH=6, LOD= 0.312, LOQ=1.039, Linearity range 1 – 20.

2- Examining the Ideal Situations Impacting the Injection of Flow

The Effect of the Flow Rate for Zn(II) Determination:

Zn(II) conc. = 10mg.L^{-1}, Zn(II) volume= 157 µL,reagent conc.= 1x10^{-4}mol/l, reagent volume = 157 µL, reaction coil length = 20 cm, pH value = 6. The absorbance was affected by the flow rate in the 3–11 ml/min range as shown in fig.13, which indicates that the rate of flow was 7.5 ml/min as the ideal pace, following which the absorption dropped.

The Effect of the Reaction Coil Length on Zn(II) Determination:

Zn (II) concentration = 10mg/L, Zn(II) volume =157.00 µL, reagent conc.= 1x10^{-4}mol.L^{-1} reagent volume =157.00 µL, cm, flow rate 7.5 mL min.-1, pH value = 6. A result of various coil lengths of the reaction (20, 30 and 40) cm was 20 cm as shown in fig.14 as the optimal lengths after which the absorbance decreased.

The Effect of pH Value on Zn(II) Determination Zn(II) conc.= 10 mg.L^{-1},Zn(II) volume= 157.00 µL,reagent conc=1x10^{-4}mol.L^{-1}, reagent volume = 157.00 µL, reaction coil length = 20 centimeters, rate of flow 7.5 ml/ min., at room temperature. The effect of pH was studied in the range of 3–8. The Optimum was 6 this means for the high stability of complex component at this value of pH, as shown in fig.15.

The Effect of Reagent Concentration Value on Zn(II) Determination:

Zn(II) concentration =10mg.L^{-1}, The ion's volume =157.00 µl(20 cm), Volume of the Reagent = 157.00 µl (20cm), Rate of flow = 7.5 ml/min, pH= 6, Length of reaction coil= 20 cm. The range of the reagent's concentration varied (0.00001 - 0.0005) mol.l^{-1} in merging zone system in an effort to increase the absorption. As shown in fig.16, which indicates that the best conc. Was 1×10^{-4} as the optimal conc. after which the absorbance decreased.

The Effect of Ion Volume Value on Zn(II) Determination:

Concentration Zn(II) =10 mg.L^{-1}, Concentration of the reagent is=1×10^{-4}mol/l , Volume of reagent= 157.00 µl(20cm), Rate of flow = 7.5 ml/min, Length of Reaction coil= 20cm and pH=6. The impact of the various volumes Sample volumes of 78.5, 175.00, and 235.5 µl were observed; the sample volume that exhausted the highest absorbance was 78.5 µl, which accompanied by controlled dispersion and the value of sensitivity is better as possible as an optimal circumstance in subsequent experiments as shown in fig.17.

The Effect of Reagent Volume Value on Zn(II) Determination: Conc. of Zn(II) =10 mg.L^{-1}, The concentration of the reagent = 1×10^{-4} mol/l , The ion's volume = 78.5µl (10 cm), Rate of flow = 7.5 ml/min, Length of Reaction coil =20 cm and pH=6, The impact of the various volumes reagent volumes of 175.00, 235.5 and 314 µl were observed; the reagent volume that exhausted the highest absorbance was 157.00 µl, which was selected as the optimal value as shown in fig.18.

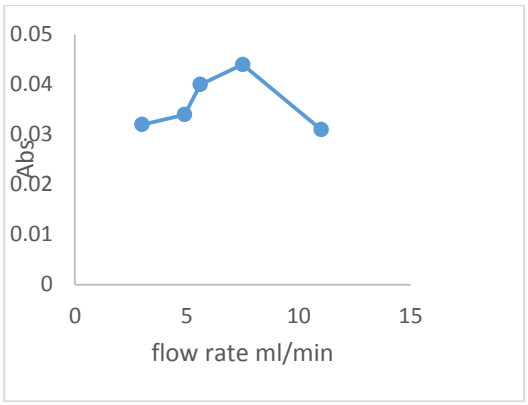


Fig.13: The effect of flow rate on the absorption value of Zn(II) complex

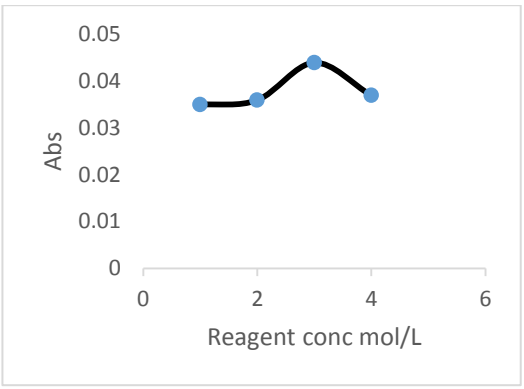


Fig.16 The effect of reagent concentration on the production of Zn(II) complex

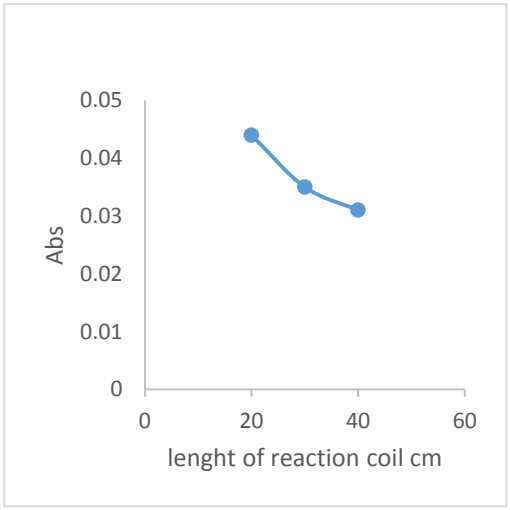


Fig.14: The effect of reaction coil length on the production of Zn(II) complex

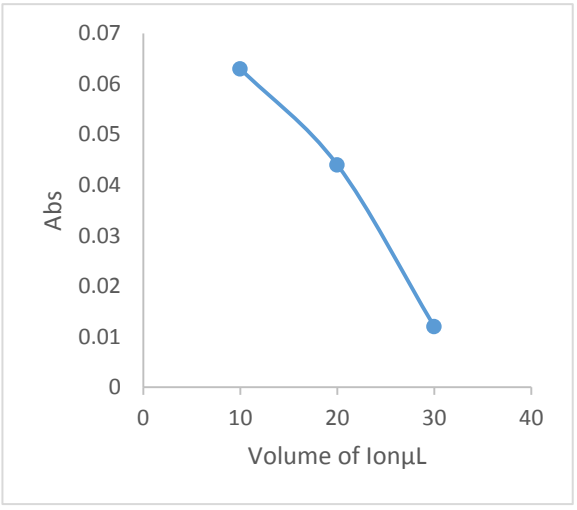


Fig.17: The effect of Zn ion volume on the formation of the Zn(II) complex

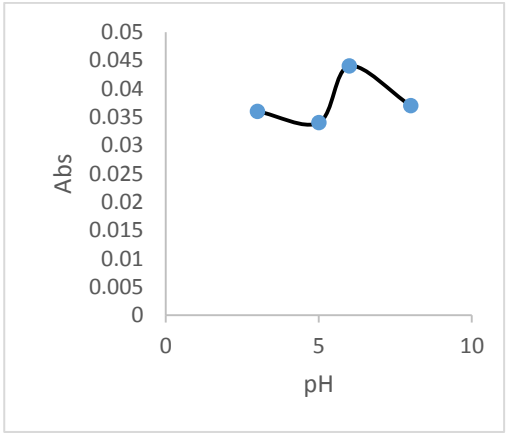


Fig.15: The effect of pH on the formation of the Zn(II) complex

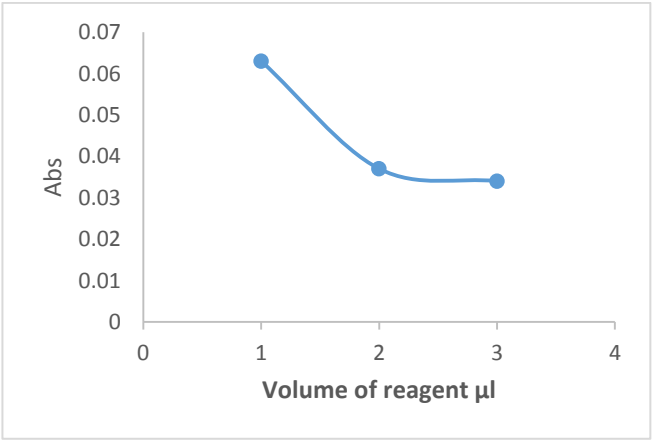


Fig.18: The effect of reagent volume on the formation of the Zn(II) complex

Calibration curve for Zn (II) complex

Under ideal circumstances, distilled water at pH 6 and a series of Zn(II) solutions ranging from 1 to 10 mg.L^{-1} are used to construct calibration curves of the zinc complex after analyzing the different variables and their effects on the absorbance value of Zn(II) complex.. Beer's law is observed by measuring the Zn(II) complex absorbance at 490 nm over the concentration range of 1–10 mg L^{-1} as illustrates in Fig.19 and Table1 The established Sandell's sensitivity, limit of quantification and detection's limit are 0.153 µg cm-2, 0.323 mg L^{-1}, and 1.076 mg L^{-1}, respectively.

Table1:calibration curve of Zn(II) complex when, Zn(II) conc.=10 mg.L^{-1}, Zn(II) volume= 78.5 µL, reagent volume = 157.00 µL, reagent conc. = 1x10^{-4}mol/l, reaction coil length = 20 cm, pH value = 6, flow rate 7.5 mLmin.$^{-1}$, at room temperature.

Zn (II) conc. mg/l	Absorption			Average	Standard devation	Relative SD%
1	0.004	0.004	0.004	0.004	0.0000	0.0000
3	0.019	0.019	0.019	0.019	0.0000	0.0000
5	0.032	0.032	0.032	0.032	0.0000	0.0000
8	0.051	0.051	0.052	0.051	0.0007	1.3725
10	0.062	0.062	0.064	0.063	0.0012	1.9047

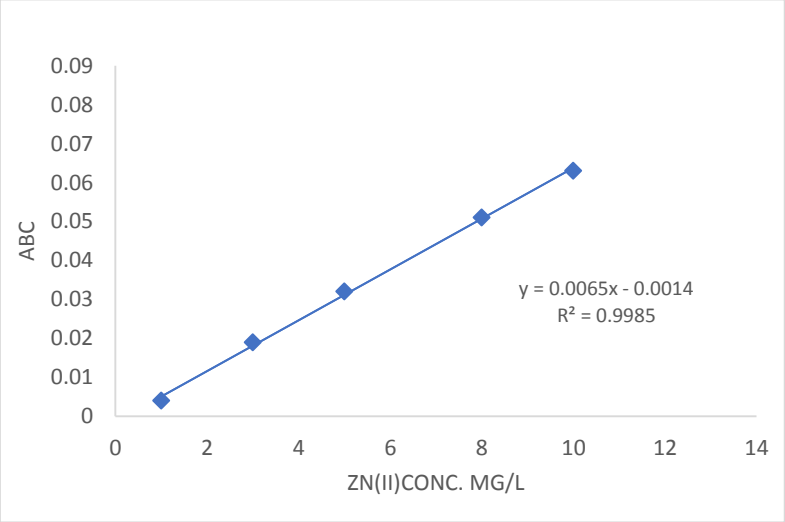


Fig.19 : Zn(II)complex calibration curve

Repeatability: Conc. of Zn(II) = 5 mgL^{-1}, Conc. of reagent= 1×10^{-4} mol/l, The ion's volume = 78.5 µl (10cm), Volume of reagent= 157.00 µl (20cm), Rate of flow = 7.5 ml/min ,pH=6 at λmax= 490nm Length of reaction coil = 20, In this experiment is carried out seven times at a dose of 5 mg.L^{-1} for Zn(II). in order to gauge the accuracy of the merging zone unit. Fig.20 displays the findings.

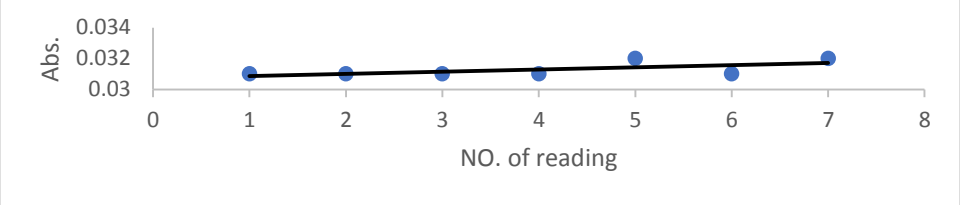


Fig.20: The Repeatability of response for 5 mgmL^{-1} of Zn(II) complex using merging zone system

Dispersion Coefficient

The dispersion coefficient (D) is defined as the ratio of the concentration of the sample material before and after dispersion and to found the dispersion process occur in FIA system by dispersion coefficient (D) determined as shown in Table 2 and from the result which indicated occur the limited dispersion in FIA system.

Table2: Values of the desperation coefficient

Zn^{+2}mg /L	Response		Dispersion D =A°/ Amax
10	A°	Amax	2.238
	0.141	0.063	

Sampling Rate

Sampling frequency calculated depending on the time for the highest absorption value and which was 30 seconds , resulting in a sampling rate of 120 samples per hour.

Applications

Recommended method is used to test a variety of samples to measure Zn(II). In accordance with FAAS, the pharmaceutical sample's Zn(II) concentration is determined directly. When compared to the FAAS approach, the suggested method provides good accuracy; Table 3 shows that recovery rates range from 84.1% to 118.48%.

Table3: : Determination of Zinc (II) in various drug samples using the FIA Merging Zone Injection Method

drug samples	Actual value **(FAAS) mg/L**	Value measured (proposed method) **SIA mg/L**	Error%	Recuperation percentage
Zinco Max (zinc sulphate)syrup Pioneer Iraqi	3.701	4.384	18.45	118.45
Prota Zinc (zinc gluconate) Protem pharma	2.243	2.522	12.43	112.43
Zinc (zinc as glucouate)	4.695	4,491	-4.34	95.65
Zinc(50 mg capsule)	2.644	2.389	-9.64	90.35

Awamedica				

CONCLUSION

Using the flow injection system and the merging-zone technique, a straightforward, quick, affordable, and practical unit was created to determine Zn(II). This innovative design's simplicity and speed were achieved via a direct redox reaction between DMPPAMPP and Zn(II). When compared to alternative methods, the unit's sample throughput was good. A merging zone in the homemade valve decreased the volume of the DMPPAMPP reagent and Zn(II) sample. The DIY valve was found to have numerous benefits, including low cost, great precision, ease of handling, and almost little maintenance. This system's measurement repeatability was precise with a very low relative standard deviation. As a result, Zn(II) in aqueous solution samples at a broad concentration range can be reliably determined using this novel unit.

Referance

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