University of Kerbala
College of Science
Department of Chemistry

Flow Injection System and Spectrophotometry for The Determination of Mefenamic Acid Using Charge Transfer Complexes Between Copper and Chromium with Neocuproine Reagent

A Dissertation

Submitted to the Council of The Faculty of Science University of Kerbala

in Partial Fulfilment of The Requirements for the degree of Philosophy

Doctor of Science in Chemistry

By

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September 2024 A.D

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

﴿ يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ ﴾

صدق الله العلي العظيم

(المجادلة 11)

Dedication

To the one who drank the cup empty to give me a drop of love...
To the one whose fingers are exhausted to give us a moment of happiness....
To the one who harvested the thorns in my path to pave the path of knowledge for me.
To the big heart..... (Dear father)

To the one who breastfed me with love and tenderness.
To symbol of love and healing balm.
To the bright white heart.
To the one whose prayers were the secret of my success and whose tenderness was the balm for my wounds..... (My beloved mother)

To my angel in life.
To my soul mate and my soul mate.
To the meaning of love and tenderness.

To the smile of life..... (My wife and my lover)

To my beautiful daughter (Jwan) and wonderful son (Haider)

My sisters and brothers whose love motivated me to challenge myself and to work harder.

I dedicate this humble effort to the Imam of our time and the intercessor of our hearts, Sahib al Asir Wal zaman, the Awaited Imam Mahdi (may God bless him and grant him peace).

ALI NASER

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Acknowledgement

First and foremost, thanks and praise be to Allah, the source of honors for the completion of this task.

My deepest gratitude and appreciation to my worthy supervisors, Prof. Dr. Alaa Frak Hussain and Prof. Dr. Dakhil Nassir Taha, for their suggested study topic and positive thinking, endless encouragement, patience, and, most importantly, guidance throughout my research, as well as for suggesting the project of this research, continuous encouragement, and providing all necessary laboratory facilities.

I am immensely thankful to Prof. Dr. Hassan Jamil Jawad Al-Fatlawi, Dean of the college of science and grateful thanks to the Head of chemistry department Prof. Dr. Thaer Mahdi Madlool Al-Rammahi for all the facilities that they offered me during my research.

I offer my genuine thanks and appreciation to my most treasured family who supported me with all assistance and patience along the study period.

Finally, yet importantly, I bow my head in utter humility and complete dedication from within my heart before the Almighty for facilitation at every stage to accomplish this task.

2024 *ALI NASER*

SUMMARY

The first part involves developing indirect, simple, accurate, fast, selective, and highly sensitive spectrophotometric method using the copper-neocuproine complex for the determination of trace amounts of mefenamic acid in aqueous solutions and pharmaceuticals. The proposed method relies on forming complexes using the redox reaction, where the suggested approach involves reducing the Cu(II)-Neocuproine complex to the yellow-orange Cu(I)-Neocuproine complex. Optimal experimental conditions such as acidity, stability time of the formed complex, buffer solutions, volume and concentration of copper (II), volume and concentration of the reagent (Neocuproine), temperature, sequence of addition, and the impact of interferences on complex estimation were chosen. The calibration curves exhibited linearity in the concentration range of 5.0-60.0 μg.2mL^{-1}, with a molar absorptivity (ε) value of 0.238 L. moL^{-1}.cm at 454 nm. The linearity coefficient(R^2) was found to be 0.9999, and the equivalence of the complexes was studied by finding the metal ion to reagent ratio (M: L) using the continuous variations and molar ratio methods. The results indicated that this ratio was 1:2. The stability constant (K_{sta}) for the formed complex was calculated and found to be (3.5×10^8). The charge of the soluble solid complex in ethanol was determined by measuring the conductivity of the formed complex, revealing that the [Cu(I)-Neocuproine] complex is charged. The accuracy and precision of the spectrophotometric method were evaluated using five solutions of different concentrations, resulting in a relative standard deviation percentage between (0.037% to 0.500%) and a recovery percentage between (99.50% to 100.32%). Detection and quantification limits were determined to be 0.7142 μg.mL^{-1} and 2.3568 μg.mL^{-1}, respectively. These results indicate that the spectrophotometric method is highly sensitive, accurate, and was successfully applied to aqueous and pharmaceutical solutions.

The second part included the design of an advanced flow injection system for the determination of mefenamic acid in pharmaceutical preparations (Ponstidin, Ponstan, Mefril), and aqueous solutions. The proposed method for mefenamic acid determination using continuous flow injection relied on the spectrophotometric reagent reaction neocuproine with copper (II) in the presence of mefenamic acid in an acidic medium to form the copper(I)-neocuproine complex. The absorbance of the formed yellow-orange complex was measured at its maximum wavelength of 454 nm. The optimal chemical and physical conditions for the proposed system were achieved the highest sensitivity, stability, and the best design for optimal response. Calibration curves demonstrated linearity in the concentration range of 1.0-80.0 $\mu g.2mL^{-1}$, with a Linearity coefficient (R^2) of 0.9998. The accuracy and precision of the method (continuous flow injection) were assessed using five solutions of different concentrations, resulting in a relative standard deviation percentage between (0.0235% to 0.0115%) and a recovery percentage between (99.30% to 99.94%). Detection and quantification limits were found to be (0.1983 $\mu g.mL^{-1}$) and (0.6543 $\mu g.mL^{-1}$), respectively. The proposed method exhibited good characteristics such as speed, sensitivity, repeatability, and precision, making it suitable for quantitative determination of mefenamic acid in pharmaceutical preparations and aqueous solutions. The proposed method was successfully applied to aqueous and pharmaceutical solutions, demonstrating high sensitivity and accuracy in determination.

The third part involved the development of a fast, direct, sensitive, accurate, and efficient spectrophotometric method for the determination of mefenamic acid in aqueous solutions and pharmaceutical drugs. The method relied on the formation of complexes using the redox reaction, converting the Cr(VI)-2,9DMP complex to the yellow-green Cr(III)-2,9DMP complex at 430nm. The favorable experimental conditions for the reaction were

selected, including acidity, stability time of the formed complex, regulated solutions, volume, and concentration of chromium (VI), volume and concentration of the reagent (neocuproine), temperature, and the order of addition. Calibration curves demonstrated linearity in the concentration range of 4.0-70.0 μg.2mL^{-1}, with a linearity coefficient (R^2) of 0.9998. The molar ratio of metal ion to the reagent (M: L) was determined using continuous variation method, indicating a ratio of (1:3). The stability constant (K_{sta}) for the formed complex was calculated and found to be (1.369×10^{10}). The charge of the soluble solid complex in ethanol was determined by measuring the conductivity of the formed complex, indicating that the complex [Cr(III)-Neocuproine] was charged. The accuracy and precision of the spectrophotometric method were evaluated using five solutions of different concentrations, resulting in a relative standard deviation percentage between (0.433% to 0.909%) and a recovery percentage between (99.90% to 101.10%). Detection and quantification limits were found to be (0.6111 μg.mL^{-1}) and (1.8333 μg.mL^{-1}), respectively. The proposed spectrophotometric method demonstrated high sensitivity and accuracy and was successfully applied to aqueous solutions and pharmaceutical preparations.

The fourth part included the design of an advanced flow injection system for the determination of mefenamic acid in pharmaceutical preparations (Ponstidin, Ponstan, Mefril) and aqueous solutions. The proposed method for mefenamic acid determination using continuous flow injection relied on the reaction of the spectrophotometric reagent neocuproine with chromium (VI) in the presence of mefenamic acid in an acidic medium to form the colored chromium (III)-neocuproine complex (yellow-green). The absorbance of the formed colored complex was measured at the maximum wavelength of 430 nm. The favorable chemical and physical conditions for the proposed system were studied to achieve

higher sensitivity, stability, and optimal design for the best response. Calibration curves exhibited linearity in the concentration range of 0.1-60.0 $\mu g.2mL^{-1}$, with a linearity coefficient (R^2) of 0.9998. The precision and accuracy of the continuous flow injection method were evaluated using five solutions of different concentrations, resulting in a relative standard deviation percentage between (0.4838% to 3.2680%) and a recovery percentage between (97.50% to 102.40%). Detection and quantification limits were found to be (0.1424 $\mu g.mL^{-1}$) and (0.4274 $\mu g.mL^{-1}$), respectively. The proposed method demonstrated good characteristics such as speed, sensitivity, and reliability, and it was successfully applied to aqueous solutions and pharmaceutical preparations, showing high sensitivity and accuracy in the determination process.

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List of Abbreviations

Meaning	Abbreviation
4-AAP	4-Aminoantipyrine
Abs	Absorption
Am	Maximum Absorption
As	Absorption at the equivalence point
C	Molar Concentration
CFA	Continuous Flow Analysis
D	Dispersion Coefficient
E%	Relative Error
ε	Molar Absorptivity
FDA	Food and Drug Administration
FIA	Flow Injection Analysis
K	Stability Constant
L.O. D	Detection Limit
L.O. Q	Limit Of Quantification
MEF	Mefenamic Acid
MSFA	Mono-Segmented Continuous Flow Injection Analysis
µg	Microgram
µL	Microliter
NC	Neocuproine
NQS	1,2-Naphthoquinone-4-Sulfonic Sodium
r	Correlation Coefficient
R.C	Reaction Coil
R.S.D%	Relative Standard Deviation
R^2	Linearity Coefficient
Rec%	Recovery%
S.D	Standard Deviation
SIA	Sequential Injection Analysis
UV-Vis	Ultra Violet-Visible
VL	Volume Of the Reagent
Vm	Volume Of the Metal
$\bar{X}$	Mean
α	Degree Of Dissociation
λmax	Maximum Wavelength

CHAPTER ONE

Chapter One

Introduction

First Part: Spectrophotometric Method

1.1 Metal Complex Analysis

The science of analytical chemistry provides methods for identifying the chemicals present in a sample and calculating their precise concentrations, Analytical chemistry plays a significant part in many different scientific domains, similarly topics as many as chemistry for the environment, chemistry for forensic archaeology and molecular biology. The importance of ion determinations at the trace level has been demonstrated in sectors like environmental and biological research and industries [1].

Numerous metallic ions, including cobalt (II), manganese (II), iron (II), zinc (II), and copper (II)and chromium (VI) are essential to the biological system and serve important roles in the lives of organisms, At specific levels of metal ion concentration, such as arsenic, cadmium, lead, and mercury, living biological systems become poisonous [2].

While some critical metal ions are hazardous when present in high concentrations, Small quantities of some hazardous metal ions have been shown by chemists to have detrimental effects on biological systems [3].

Whenever a metal ion forms a central bond with nearby molecules, ions, or atoms known as ligands, a complex is created. The complex has a minor metal dissociation in the solution, but it usually retains its chemical makeup, In his theory Werner outlined how the ligand meets the primary and secondary valences of the core metal ions in the complex, Werner observed that in metallic complexes, the connected ligands in the square bracket sphere counter the charge of the metal ion as primary valency and secondary

valency as coordination number. This ligand satisfies the primary valency known as the oxidation state equal to the charge on metal ion and the secondary valency called coordinated number by the attached ligand, By attaching a counterion, the charge of the metal complex ion is balanced [4].

In metal chelates, some kind of coordinate bonding was described by Neil Sidgwick in 1927, Lewis bases and acids are terms used to describe electron pair donors and acceptors, respectively, in ligands and metal ions[5].

1.2 Chemistry of Copper

Copper is a d-block substance or transition metal. Along with Silver (Ag) and Gold (Au), it is a member of Group-1B in the periodic table. $3d^{10}4s^{1}$ is the electronic arrangement, Major locations where it can be found are Superior, the Ural Mountains in Siberia, Assam, and (Singhbhum), a state in India. For copper (Cu), the three most significant oxidation states are zero (0) (pure metal), compound additions of cuprous (1^{+}) and cupric (2^{+}), produces Complexes with the appropriate radicals and ions[6].

1.3 Chemistry of Chromium

Chromium (Cr) is the 7^{th} most plentiful element in the mantle of the earth and core while being the twenty-first most abundant element in the crust, It belongs to group (VI)B of the periodic table and currently has the atomic number 24[7].

Chromium's electrical ground state arrangement is $1s^{2}$, $2s^{2}$, $2p^{6}$, $3s^{2}$, $3p^{6}$, $3d^{5}$, $4s^{1}$, the resulting arrangement of the outermost electrons is favored over $3d^{4}/4S^{2}$ due to the improved stability of the half-filled 3d shell with one electron in each orbital, The "S" state (L = 0), which is thought to be particularly stable because of the significant quantity of exchange energy, is reached by the half-filled shell[8].However, The electrons in the (d orbitals) can interact with the chemical environment because they are projected close to the ion places. Numerous colorful and paramagnetic compounds contain chromium, a frequent transition metal [9].

The following oxidation states exist for chromium 2^-, 1^-, 0, 1^+, 2^+, 3^+, 4^+, 5^+, 6^+; the most excellent oxidation state 6^+, is determined by summation of the 3d and 4s electrons. chromium can be found at the lowest oxidation states of 2^-, 1^-, 0, and 1^+ at substances like carbonyls, nitrosyls, and organometallic complexes. The element's most stable and significant oxidation state is chromium (3^+)[10].

When the oxidation state of a Cr complex is less than 3^+, it is said to be reducing, and when it is more than 3^+, it is oxidizing. One known human and animal carcinogen is chromium with an oxidation number of 6^+. Their potent oxidizing abilities are responsible for this [11].

1.4 Chemistry of Mefenamic Acid

Mefenamic Acid (MFA) it's a derivative of 2- [(2,3-dimethyl phenyl)] amino benzoic acid ($C_{15}H_{15}NO_2$) as shown in Figure (1-1) it's non-steroidal anti-inflammatory medication belonging to the fenamate class and a derivative of anthracitic acid (NSAIDs).[12] it is a strong inhibitor of prostaglandin formation, which is intimately associated with conditions associated with inflammation.[13] On the other hand, It is recommended, for instance, in situations of headaches, premenstrual syndrome, tooth pain, muscular pain and trauma discomfort. Additionally, mefenamic acid has demonstrated therapeutic results as an anticancer agent in the treatment of cancer cells, particularly colon and cancer of the liver cell lines, and neurodegenerative diseases like Alzheimer's[14].

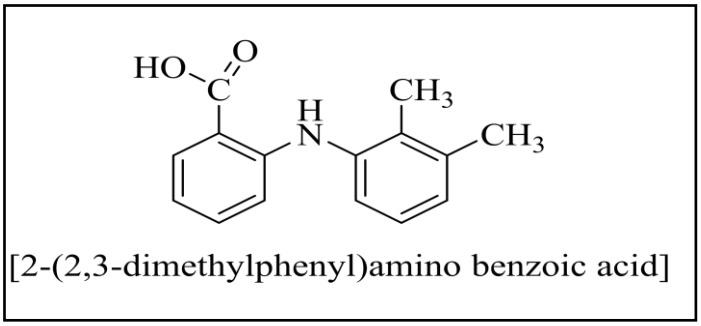


Figure (1-1) Chemical structure of MFA

There have been reports of certain complexes of metals of tolfenamic acid and mefenamic acid having considerable anticancer, antioxidant, antibacterial, and anti-fungal properties [15-16]

Mefenamic acid's molecular design shows coplanarity between its carboxylic group (COOH) and an aromatic ring's nitrogen atom. The three links around the nitrogen atom's angles add up to almost 360°, showing that the nitrogen atom's hybridization can be classified as sp^2 type. Calculations based on molecular mechanics revealed that the aromatic rings and the nitrogen atom's lone pair are in resonance[17].

1.5 Chemistry of Neocuproine

Neocuproine is a chelating agent substance and heterocyclic chemical molecule $C_{14}H_{12}N_2$. The derivatives substituted at the location 2 and 9 positions are among the most researched of the substituted phenanthrolines, having been initially published in the late 19th century[18].Similarly, In chemistry of copper(I), Neocuproine as a (NN ligands) with somewhat large substituents crucial. Because of its selectivity for copper(I) and potent visual absorbance of the $Cu(DMP)^{2+}$ adduct, 2,9-dimethyl-1,10-phenanthroline (NC) is typically the reagent chosen for the colorimetric measurement of copper(I) as shown in Figure (1-2).

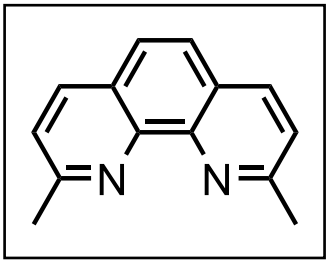


Figure (1-2) Chemical Structure of Neocuproine

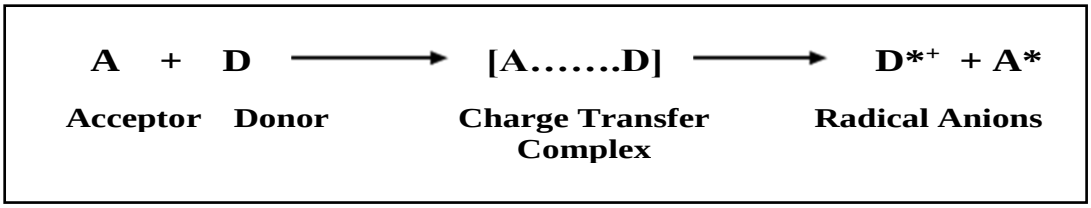
For transition-metal reactivity, Neocuproine based ligands have been employed extensively. Because of its unique absorbance in the UV–vis range after metalation, neocuproine has frequently been employed for biological uses as a copper marker[19].

In a neutral or somewhat acidic medium, the cuprous ion Cu(I) interacts with the compound 2,9-dimethyl-1,10-phenanthroline to create a complex. This complex is subsequently extracted using a variety of organic solvents that include a CCl_4-CH_3OH mixture and ethanol, to produce a yellow solution with a molar absorptivity of about 8000 at 454 nm [20].Whenever, the pH of the aqueous solution falls somewhere between 3 and 9, yellow-orange colour formation can be achieved; the colour is persistent in $CHCl_3$-CH_3OH for a couple of days. Hydroxylamine-hydrochloride is used to convert cupric ions into cuprous ions in the sample. To prevent metallic ions from precipitating though the pH is elevated, sodium citrate is utilized. Neocuproine in methanol is added when the pH is brought down to between 4 and 6. The complex that results is then extracted into $CHCl_3$. The absorbance of the solution is determined at 454 nm following the dilution of the $CHCl_3$ to an exact volume with CH_3OH [21].

1.6 Charge Transfers in Transition Metal Complex

Charge transfer complexes consist of interaction between electron donor compounds that have Low ionization potential and other electron-accepting compounds that have a high electronic affinity. According to research, in this particular type of complex, electrons move from the donor to the receiver[22].

The concept of "molecular bond" has been important in almost all areas of chemistry. The term (A) is a relatively electron poor molecule, interacts in one way or another with an electron rich molecule called a donor (D). The bond between components is weaker than the covalent bond. The literature also abounds with terms such as molecular complexes "π complexes" and charge transport complexes (CTCs), which all refer to some type of interaction between the donor and acceptor of an electron [23] as shown in Scheme (1-1).



Scheme (1-1) interaction between the donor and acceptor

Typically, in a straightforward ion-radical pair interaction, the charge transfer complexation manifests as an ionic band [24].

The “Mullikan” introduced the term "charge transport complexes" According to his hypothesis, which states that a charged transferred electron produces a resonant hybrid between a nonpolar and polar molecule [25 -26].

1.7 Previous Studies for Determination of Mefenamic Acid

A literature survey reveals that various analytical techniques were used for the determination of MFA such as UV spectrophotometry. [27-29] Thin layer chromatography (TLC) technique[30]. High-performance liquid chromatography (HPLC) technique[31]. Hydrotropic solubilization technique[32] Potentiometric method[33]. Merging zone-continuous flow injection.[34] Electrochemical sensors[35]. Gas chromatography[36] Flow injection[37]. However, a quick, inexpensive, and selective approach is clearly required, particularly for regular quality improvement screening of pharmacological products which have MFA, so we will illustrate the spectrophotometric method for mefenamic acid estimation as shown in Table (1-1).

Table (1-1) Some of Spectrophotometric Methods for Determination of Mefenamic Acid.

No	Method	Reagent	λ_{max} nm	Linear Range	L.O. D	L.O. Q	R^2	Ref.
1	The method is based on a tris(2,2′-bipyridyl)ruthenium(III)chemilumine-scence reaction. $Ru(bipy)_3^{3+}$ is chemically generated by mixing two streams containing solutions of tris(2,2′-bipyridyl) ruthenium(II) and acidic cerium(IV) sulphate.	tris(2,2-bipyridyl) ruthenium(I)	450	0.05–6.0 $\mu g.mL^{-1}$	0.05 $\mu g.mL^{-1}$	0.152 $\mu g.mL^{-1}$	0.9996	[38]
2	This method based on the reaction of cited drug with 1,2-Naphthoquinone-4-Sulfonic sodium (NQS)	NQS	450	0.5-10.0 $\mu g.mL^{-1}$	0.189 $\mu g.mL^{-1}$	0.567 $\mu g.mL^{-1}$	0.9950	[39]
3	This method based on ratio spectra derivative spectrophotometry	NaOH:Methanol (1:9)	363.5	2.0–10.0 $\mu g.mL^{-1}$	1.10 $\mu g.mL^{-1}$	3.30 $\mu g.mL^{-1}$	.....	[40]
4	This method based on a diazo coupling reaction using diazotized 4-amino-3,5-dinitrobenzoic acid (ADBA) as a chromogenic derivatizing reagent	(ADBA)	490	1.00-6.0 $\mu g.mL^{-1}$	0.333 $\mu g.mL^{-1}$	1.000 $\mu g.mL^{-1}$	0.9875	[41]
5	This method based on Prussian blue formation	potassium ferricyanide	500	3.0-14.0 $\mu g.mL^{-1}$	0.31 $\mu g.mL^{-1}$	1.04 $\mu g.mL^{-1}$	0.9900	[27]
6	This method based on the oxidation of mefenamic acid with cerium (IV) to produce cerium (III)	cerium (IV) in sulphuric acid medium	354	0.03-1.5 $\mu g.mL^{-1}$	0.009 $\mu g.mL^{-1}$	0.027 $\mu g.mL^{-1}$	0.9979	[42]
7	This method based on the coupling of mefenamic acid with diazotized 4-aminohippuric acid in alkaline medium	D-4AHA	432	0.5-20 $\mu g.mL^{-1}$	0.11 $\mu g.mL^{-1}$	0.32 $\mu g.mL^{-1}$	0.9982	[43]
8	This method based on the reaction of mefenamic acid as N-donor with p-chloranilic acid as a π-acceptor to give A red colour product	P-chloranilic acid	520	10-300 $\mu g.mL^{-1}$	2.50 $\mu g.mL^{-1}$	8.43 $\mu g.mL^{-1}$	0.9997	[44]
9	The method is based on oxidative coupling reaction of these compounds with 4-aminoantipyrine (4-AAP) in the presence of copper sulphate as	4-AAP	440	1-20 $\mu g.mL^{-1}$	0.03 $\mu g.mL^{-1}$	0.099 $\mu g.mL^{-1}$	0.9991	[45]

	oxidizing agent in alkaine medium forming a reddish brown colour.							
10	The method is based on the charge-transfer complexation between mefenamic acid as an n-electron donor and chloranil as a π-acceptor to form a violet chromogen	Chloranil	540	10–60 $\mu g.mL^{-1}$	2.16 $\mu g.mL^{-1}$	7.15 $\mu g.mL^{-1}$	0.9996	[46]
11	The method is based on the oxidation of mefenamic acid by iron(III), and subsequent complexation of iron(II) with o-phenanthroline, forming a red-colored complex (ferroin)	o-phenanthroline	510	0.4-2.0 $\mu g.mL^{-1}$	0.065 $\mu g.mL^{-1}$	0.195 $\mu g.mL^{-1}$	0.9993	[47]
12	The method is based on oxidation-reduction reaction between mefenamic acid and cerium (IV) ion, and subsequent Ce (III) reaction with arsenazo (III) reagent in acidic medium to produce a greenish-blue complex	Arsenazo (III) reagent in acidic medium	654	1-10 $\mu g.mL^{-1}$	0.92 $\mu g.mL^{-1}$	2.76 $\mu g.mL^{-1}$	0.9972	[48]
13	A Spectrophotometric Approach for Estimation of Non-steroidal anti-inflammatory drug (NSAIDs) in Pharmaceutical Drug formulations by Neocuproine	Neocuproine	454	5.0-60 $\mu g.mL^{-1}$	0.7142 $\mu g.mL^{-1}$	2.3568 $\mu g.mL^{-1}$	0.9999	Our Study
	New Spectrophotometric Method for Determination of Mefenamic Acid in Pharmaceutical Formulation using Cr(VI)	Neocuproine	430	4-70 $\mu g.mL^{-1}$	0.8433 $\mu g.mL^{-1}$	2.5299 $\mu g.mL^{-1}$	0.9998	Our Study

Second Part: Flow Injection Analysis Technique

1.8 Introduction

One of the most significant advancements in analytical chemistry over the past forty years has been the introduction of commercial automated analytical systems, which deliver analytical data with little to no operator involvement. These systems were first developed to meet the requirements of medical laboratories, where the presence of 30 or more species is typically detected in samples of blood and urine for diagnostic and screening reasons. Hundreds of millions of clinical analyses are done domestically every year, it is evident that their cost should be kept at an acceptable level.[49]

According to Ruzicka and Hansen, the FIA technique is "based on injection of a fluid sample into a moving not segmented continuous flow of appropriate liquid." The injected sample generates a zone that is subsequently moved toward a detector that continually records the absorbance, electrode potential, or another physical characteristic as it continuously changes due to the transit of sample material through the flow cell. The wording has been changed to read, "Information-gathering from a concentration gradient formed from an injected, well-defined zone of fluid, dispersed into a continuous unsegmented stream of the carrier."[50]

A typical definition describes FIA as “A simple and versatile analytical technology for automating wet chemical analysis, based on the physical and chemical manipulation of a dispersed sample zone formed from the injection of the sample into a flowing carrier stream and detection downstream”[51].

1.9 Flow Analysis

It is non-exclusive name proposed for all analytical techniques that depend on the preparation and delivery of test samples in streaming media. Two things can serve as the foundation for an essential order:

1. How the test portion is delivered, such as continuously or sporadically.
2. The fundamental characteristics of the streaming media, such as whether it is segmented, unsegmented, or mono segmented, where segmentation is primarily associated with the intention of preventing the mixing of progressive analyte zones[52].

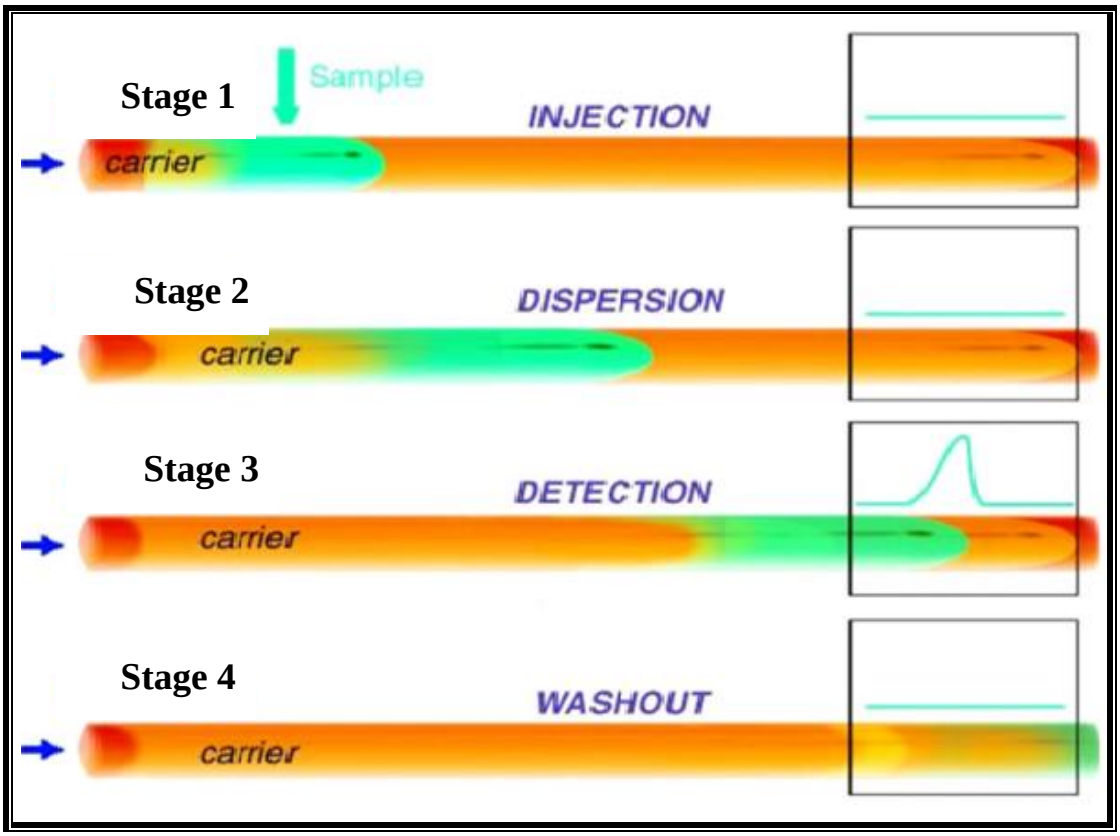
The flow analytical systems should be represented by the form of a stream, the sample presentation method (aspiration or injection), and whether the reagent or sample is injected when using injection. In the case of segmented stream approaches, a facilitation explanation should be provided when, for example, the type of measuring medium is not air[53].

It appears that the separation of mixtures of chemical compounds on a flow-through column packed with a solid sorbent, which launched the growth of chromatographic procedures, was the first chemical occurrence to be seen during flow conditions and used for the purpose of analysis. This is frequently credited to research done by Cwett at the University of Warsaw at the start of the 20th century[54].

1.9.1 Principles of FIA

The flow injection analysis technique (FIA) is based on the injections of liquids through a moving transport stream.in the same manor ,the injected substance is transmitted by the non-segmented uninterrupted carrier medium, the injected sample forms a zone, which is then transported toward detector, which subsequently calculates the change in absorbance, pole effort, or any additional parameter as an effect of a physical change

moving through the flow cell [55]. The four stages of flow injection analysis have been shown in Scheme (1-2).

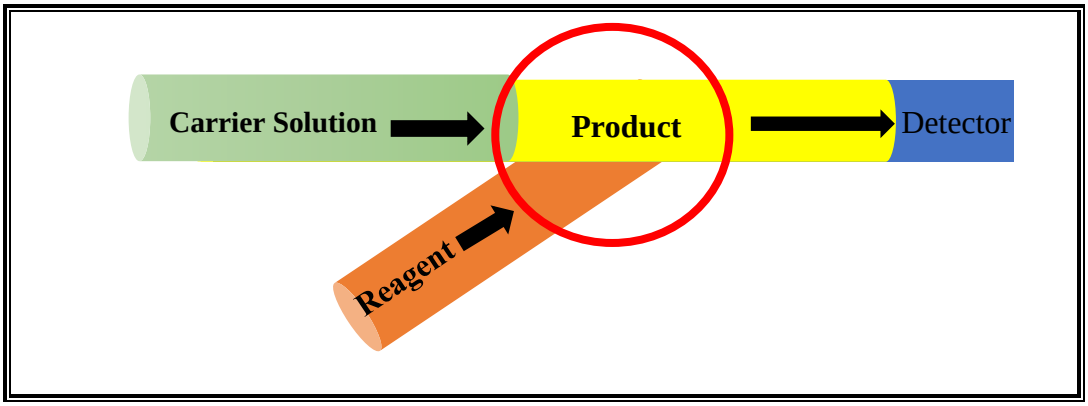


Scheme (1-2) The Four Stages of Flow Injection Analysis [56]

1.10 Technique Mode of Flow Injection Analysis

1.10.1 Merging Zone Analytical Technique

Bergamin first used the merging zone technique in 1978[57].This method circumvented the drawback of continuous flow injection(CFIA), which continued to consume the reagent even in the absence of the sample. Even though the continuous flow injection volume is only a few hundred microliters, further economies were made using management zone approaches, particularly when using pricey reagents. The sample and reagent are each independently injected into the carrier stream via a multi-injection valve in this method, forming the sample agent zone before it reaches the detector as indicated in the Scheme (1-3)[58].



Scheme (1-3) Merging Zone Technique [58]

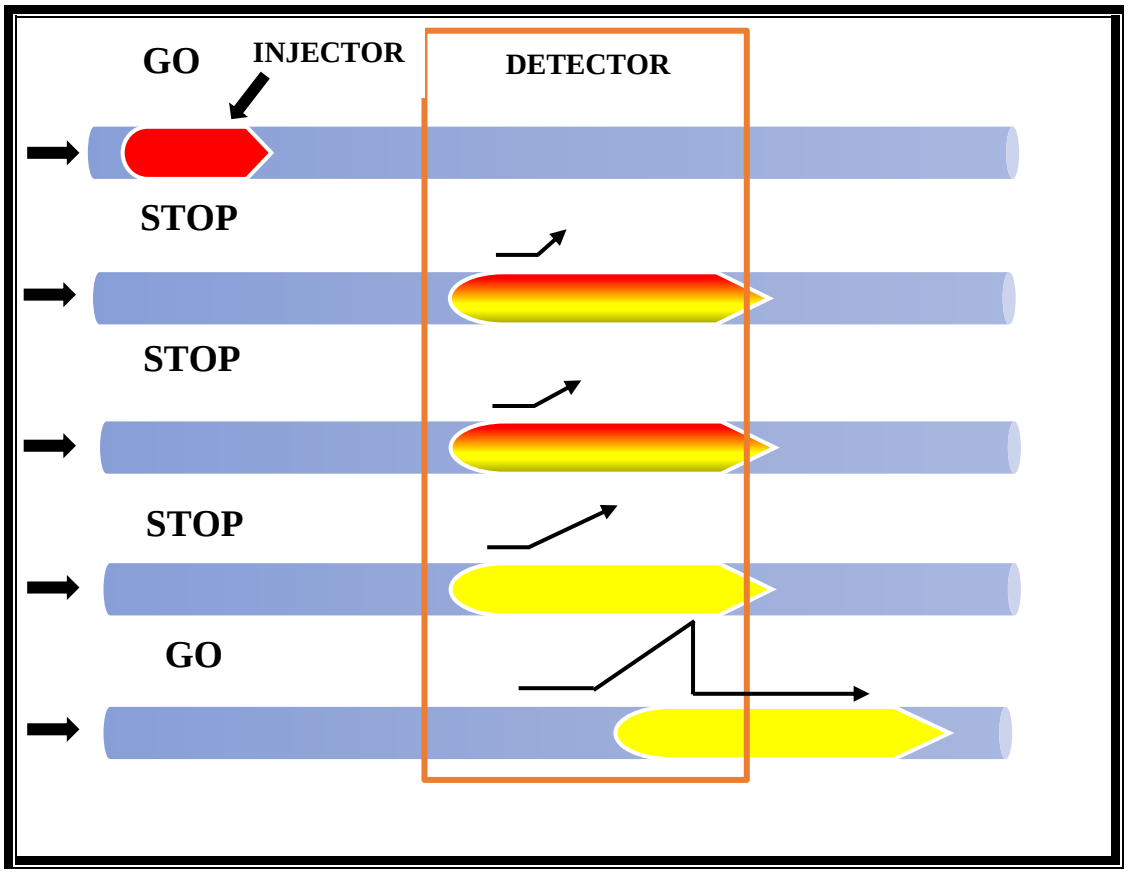
In the FIA technique, the sample is injected into a constant flow of chemicals (carrier solution) that fill the entire system while no sample is present. One method for reducing reagent consumption is to inject the substance (sample drug or other fluid) and reagent into an inert flow and combine these segments in the manifold where the carrier is washing solution or water. This technique, known as the merging zone technique, significantly decreases reagent use to a few microliters per sample. This is significant if an expensive reagent is employed[59].

The merging zones method is utilized in a single flow injection system for simultaneous spectrophotometric evaluation of pharmaceuticals and other fluids in plant material as well as blood, serum, plasma, organic and inorganic samples using chemical reagents with diverse responses.[60]

The merging zones approach to flow injection systems involves integrating the entry of samples and reagents into chemically inert unsegmented carrier flows. The injected species begin as distinct zones that mix to allow chemical reactions to occur throughout delivery to the detector. The most appealing analytic feature of such approaches is their extremely low reagent use [61].

1.10.2 Stop Flow Injection Analysis

For the purpose of determining the levels of glucose and urea in blood serum, Ruzicka and Hansen originally proposed the use of stop flow injection analysis in 1979 [62]. As shown in Scheme (1-4).

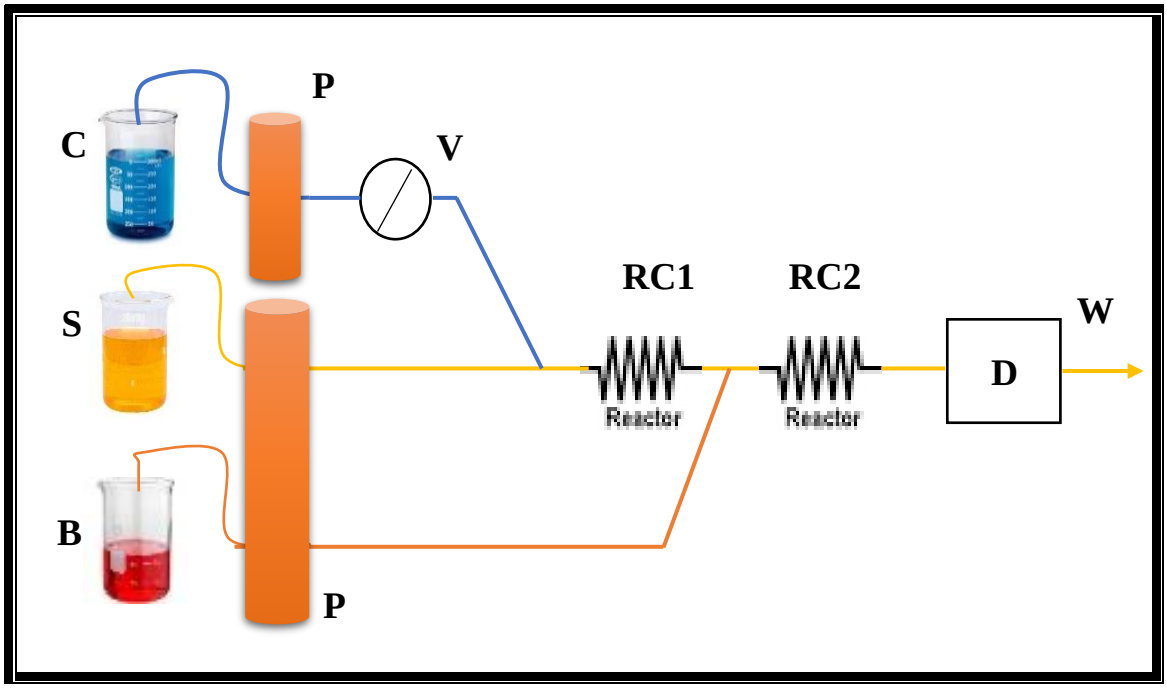


Scheme (1-4) Signals In (SFIA)[63]

The sample is injected similarly to flow injection analysis. However, the flowing stream is interrupted when the sample and reagent zone enter the next cell. By choosing and adjusting the stopping time, this method allows kinetic studies to be carried out at various concentrations and wavelengths while minimizing reagent use and waste formation[63].

1.10.3 Reversed Flow Injection Analysis

Johnson and Petty first presented this method in 1982 as an early attempt to measure phosphate in seawater using an injection of the reagent Instead of using a carrier solution on the premixed reagent. similarly, a reverse flow injection analysis depending on the continuous pumping of the sample was used [64]. The reversed flow injection system used for the determination of EG in antifreeze samples was shown in Scheme (1-5). It was equipped with a Desagapl Heidelberg, England peristaltic pump (6 channels, variable speed) to drive the carrier (D.W.) or sample and the periodate streams and an actuated rotary six-port injection valve (Rheodyne-USA) supplied with variable loops[65].



Scheme (1-5) Reversed FIA System, C=carrier stream, S= Sample stream, B=Buffer solution, P=pump, V=valve, RC=reaction coil, D=detector, W=west.[60]

1.10.4 Monosegmented Flow Analysis

Monosegmented flow analysis (MSFA) can be used, which involves the injection of a discrete volume of sample solution into a pocket of air within the carrier. This not only minimizes the dispersion experienced by the sample on the way to the nebulizer compared to when introducing the sample using flow injection analysis [66]. This approach offers the opportunity to perform the analysis either in the absence of a chemical reaction or increased Residence time can be, allowing the reaction to reach equilibrium without axial dispersion in between the sample/reagent zone and the carrier solution. The sample is inserted via two air bubbles or (inert gas) to prevent the dispersion of the sample zone with carrier solution [67].

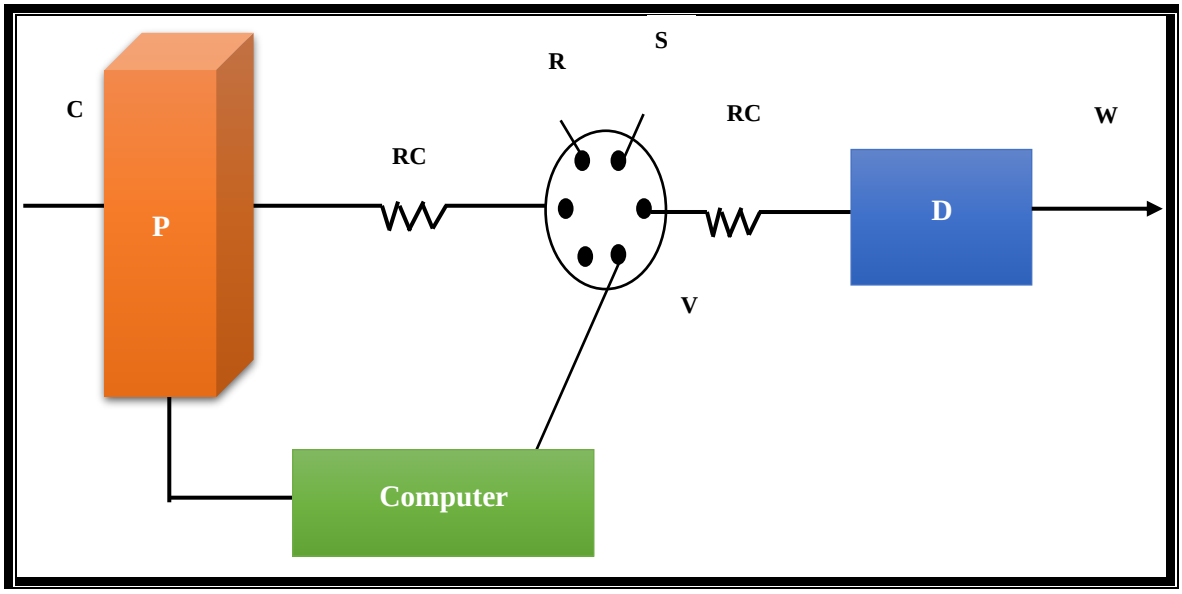
1.10.5 Sequential Injection Analysis

Ruzicka and Marshall (1990) suggested a significant methodological advancement in the field of continuous flow analysis methods in the 1990s that preserved the benefits of flow injection analysis while minimizing the drawbacks that prevented its use as a standard tool [68].

Although the aspiration and propulsion systems used in SIA and FIA are different, their essentials and basic principles the sequential injection of well-defined segments of samples and reagents, which dispersed and penetrate, simultaneously allowing the formation of a reproducible overlapping zone are substantially similar .However, Due to the well-defined zones of concentration gradient where the reaction products develop, repeatable analytical data can be obtained from the transitory signals produced [69].

In SIA, much like FIA, the systems can be more complicated, particularly when operations like dilution of concentrated samples, automated system calibration, use of the standard addition method as an analytical measurement methodology, or performing online titrations are

being performed. It is also possible in these systems to apply the samples to special processing in the manifolds as showing in Scheme (1-6) which may entail using, for instance, gas-liquid separation cells, gas- diffusion, and dialysis units, digestion minipumps made of Teflon in microwave ovens, and mini-columns packed with ion exchange resins, polymers, or metal reductants [70] .

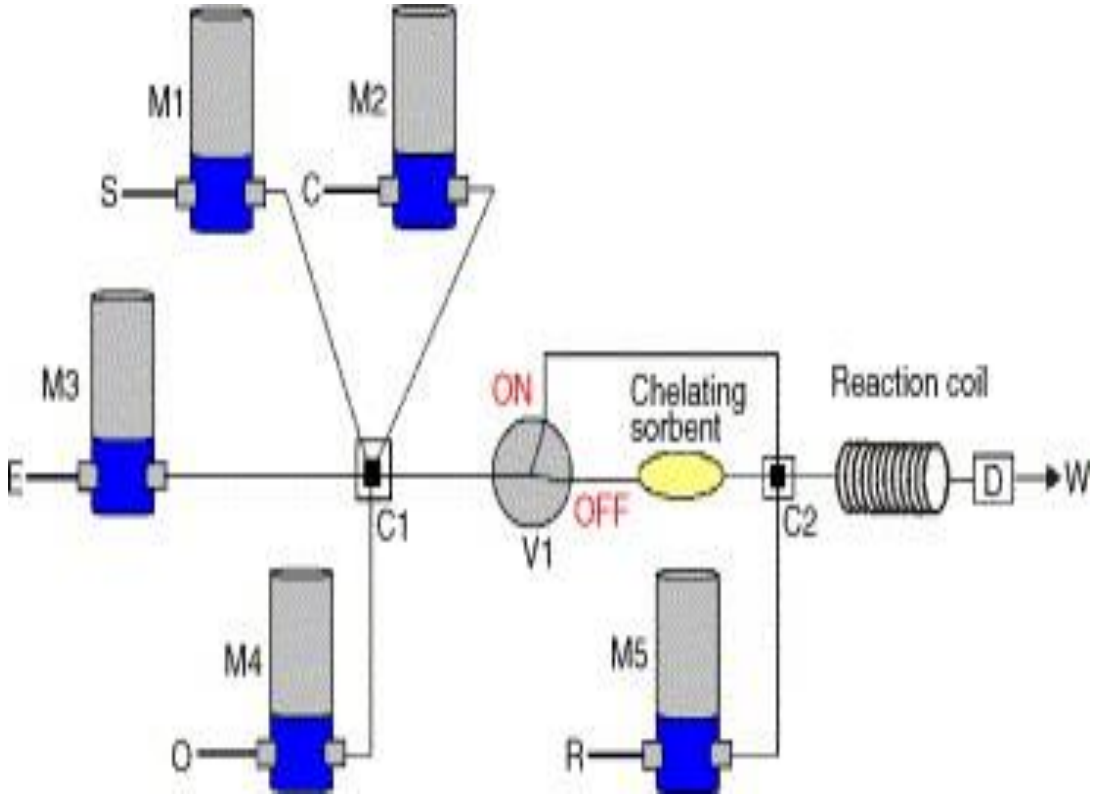


Scheme (1-6) Sequential Injection Analysis manifold: C=carrier; P=pump, RC= reaction coil, V=selection valve. R=reagent ,S=sample, RC=reaction coil ,D=detector, W=waste [71]

1.10.6 Multi-Pumping Flow System

A multi-pumping flow system (MPFS) was reported in 2002 that employs solenoid micropumps (SMP) as the liquid drivers. The SMPs provide highly pulsed semi-continuous turbulent flow providing good mixing between the analyte and reagents. That is generally superior to other methods because a laminar flow is pro- vided. The MPFS is very compact and has great versatility. In addition, this system utilizes low consumption of reagents and sample, and therefore is a good alternative to the other systems that employ peristaltic pumps or other liquid drivers [72].

Multi-pumping flow systems (MPFS) have recently been proposed and their potentialities are discussed herein. In these systems, multiple micro-pumps are operated individually for the propulsion of liquids, introduction of sample and mono-commutation of reagents. The micro-pumps are characterised by a fixed stroke volume in such a way that a precise and effective control of the volume of sample and reagents, at a given flow rate, is accomplished by appropriate dimensioning of the frequency and number of strokes (pulses). Through individual control of each micro-pump, a selective and versatile introduction of reagents and samples can be explored as shown in Scheme (1-7) [73].

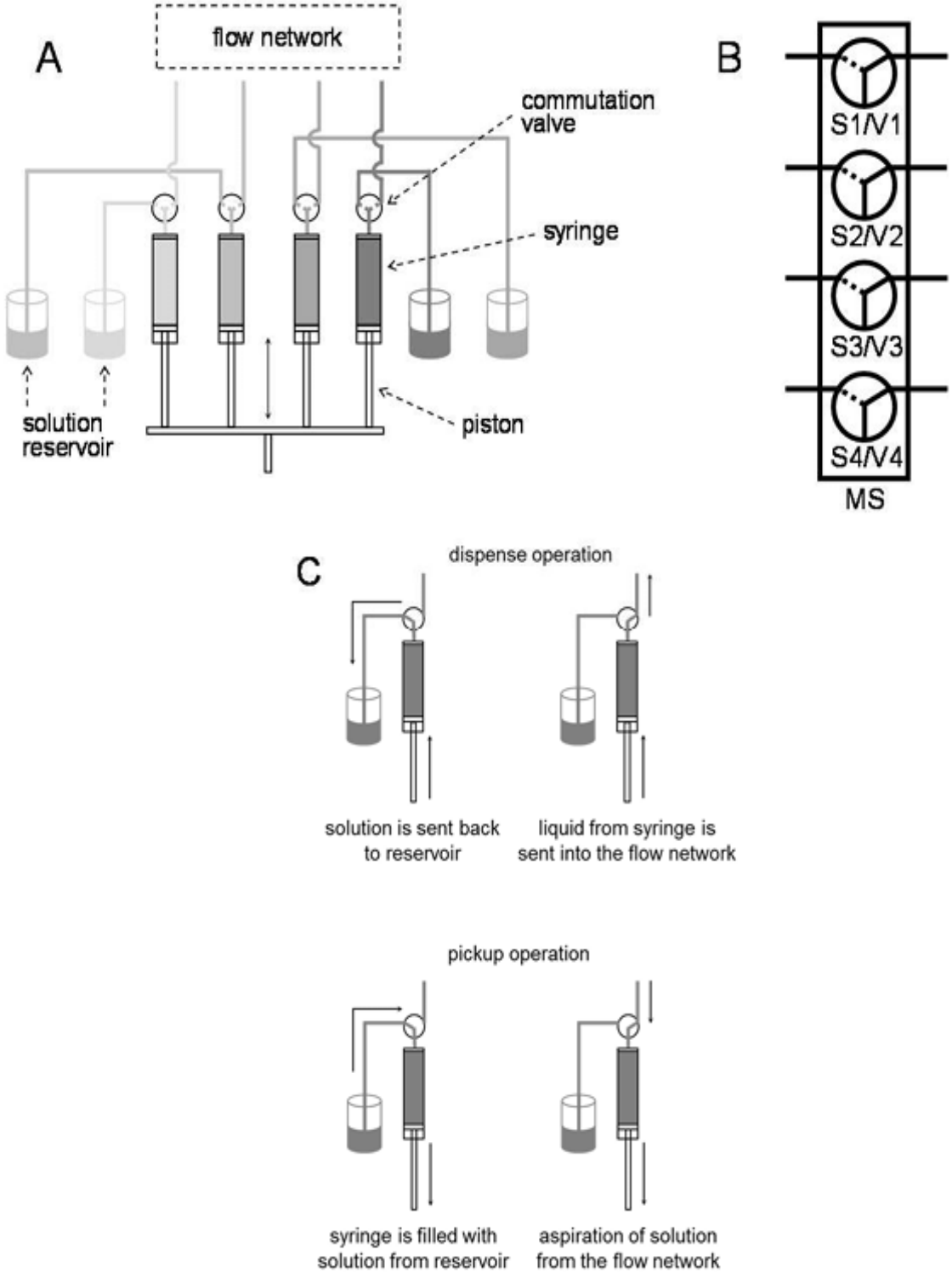


Scheme (1-7) Experimental MPFS set-up proposed for the determination of iron. S: sample; C: carrier; E: eluent; O: oxidizing agent; R: chromogenic reagent; W: waste; D: detector; M1-M5: micro-pumps V1: commutation valve; C1 and C2: cross-junctions; Reaction coil: 32 cm [74]

1.10.7 Multisyringe Flow Injection Analysis [75]

Multisyringe flow injection analysis (MSFIA) was introduced by Víctor Cerdà and co-workers in 1999 as a robust alternative to its predecessor flow injection techniques, combining the multi-channel operation of flow injection analysis with the possibility of flow reversal and selection of the exact volume of sample and reagent required for analysis as presented in sequential injection analysis (Ruzicka & Marshall, 1990).

This type of automatic flow injection systems is based on the utilization of a Multisyringe burette, depicted schematically in Scheme (1-8A) and(1-8B). It is a multiple channel piston pump, containing up to four syringes, driven by a single motor of a usual automatic burette and controlled by computer software through a serial port. A two-way commutation valve is connected to the head of each syringe, allowing optional coupling to the manifold lines or to the solution reservoir. Because the four syringes are driven by the same motor, all pistons move at once in the same direction either delivering (dispense operation) or loading the syringes (pickup operation) with liquids. Considering that the commutation valves can be placed in two positions, there are four possibilities for flow management as depicted in Scheme (1-8C). Hence, when the pistons are moving upwards, it is possible to dispense liquid into the flow system or send it back to its reservoir. This feature enables that only the necessary amount of reagent solution is introduced into the flow system. Furthermore, when the pistons are moving downwards, it is possible to refill the syringes with solutions present in the respective vessel or to aspirate solutions from the system in order to perform the sampling operation.



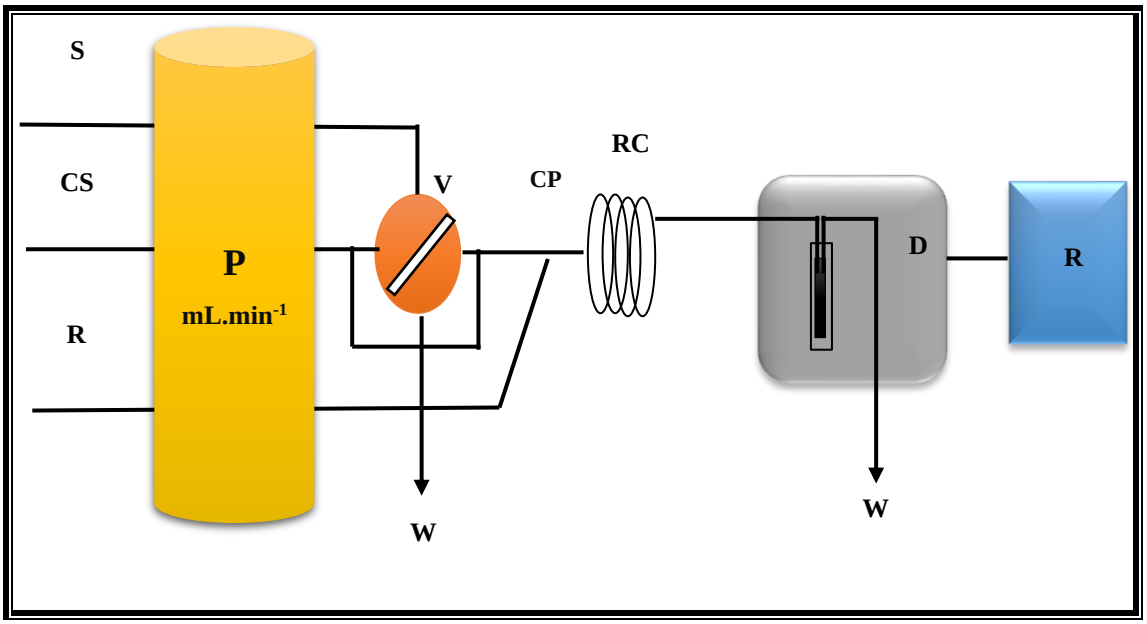
Scheme (1-8) Multisyringe apparatus, with indication of the different components (A) or simplified (B). Flow management possibilities for one syringe during operation of Multisyringe apparatus are also given (C). MS = Multisyringe, S = syringe, V = commutation valve.

1.10.8 Continuous Flow Injection Analysis (CFIA)

Flow injection analysis (FIA) it is a chemical sample is injected into a large stream of a different reagent at a low rate of flow of a few millilitres or microliters per minute, When the sample passes through a coiled reactor, products are analyzed using a variety of methods, including UV spectroscopy, chromatography, ion-selective electrodes, and biosensors[76].

The earliest descriptions of flow injection techniques were made in the middle of the 1970s by Ruzicka and Hansen in Denmark and Stewart and colleagues in the United States[77-78].

The typical three-line systematic diagram of flow injection system will be illustrated below in Scheme (1-9).



Scheme (1-9) Flow Injection System [79]

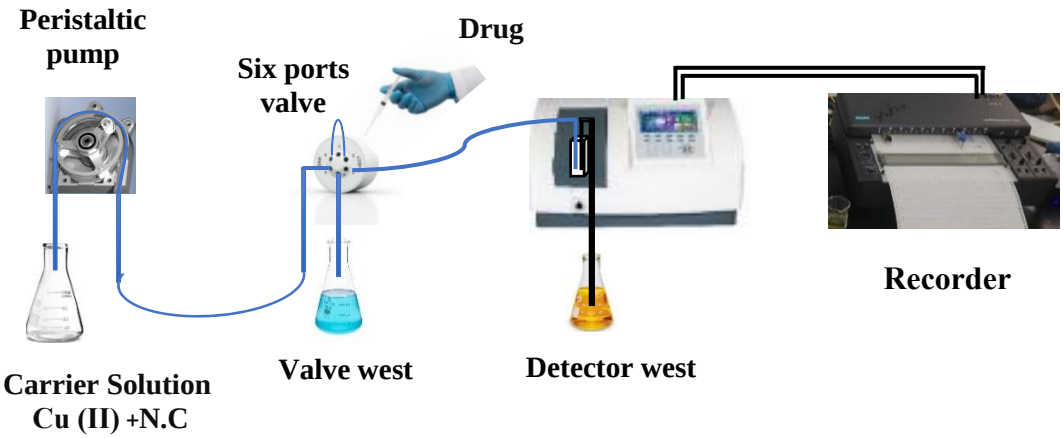
P= peristaltic pump, S=sample, CS= carrier solution, R=reagent, V= six-way injector valve, CP=confluence point, RC=reaction coil, D= detector, R=recorder, W=waste.

1.11 Flow Injection System Instrumentation

The FIA apparatus is supposed to be designed so that:

1. The transporter fluid passes via a thin tube of equal inner diameter, containing the injector and detector sections.
2. The solution that is being tested is injected as an immediate pulse of the correct amount with a short duration so that the carrier stream's movements are unaffected.
3. Side streams are introduced to the mainstream in a repeatable fashion.
4. All streams have a pulse-free flow, and their movements can be initiated and halted instantly.
5. Detector responds to analyte concentration promptly and selectively with the highest signal yield

The basic FIA system comprises a pump, an injection valve, an analytical manifold, a detector, and a recorder. Furthermore, an automatic sampling device can be applied to automate sample injection and data-processed systems.as shown in Scheme (1-10) [80].



Scheme (1-10) Flow Injection Analysis System That Made in Our Laboratory.

In order to achieve great precision, two parameters must be managed. First and foremost, repeatable timing is required. Because the system is not in equilibrium and the degree of dispersion depends on the sample residence time, the system flow rate needs to be accurately managed.

Second, the amount of sample and timing of injection should be accurately regulated to inject a highly reproducible sample bolus into the carrier stream[81]. However, these parts will explain in details:

1.11.1 Pumps

An ideal pump would deliver a continuous, pulse-free flow of carriers and chemical reagents. FIA systems have been built using syringes, pressure bottles, and rotary and peristaltic pumps, The specific kind of pump chosen is determined by the system's application and goal[82].

The peristaltic pump is particularly the most common. It is a multichannel pump with variable flow rates obtained by altering the internal diameter of the pump tubes utilized in every single channel[83].

Typical pumps feature 8-10 rollers organized in a circular pattern, with half of the rollers compressing the tube at any given moment.

Peristaltic pump - device in which fluid is squeezed through plastic tubing by rollers. similarly, the flow rate is controlled by the speed of the motor (greater than 30 rpm) and the inner diameter of the microbore tubing (0.2 - 3 mm).[84]

The peristaltic pump sweeps up liquids by creating a condition known as a vacuum by employing cyclic compression motions and its flexible tubing. and when the rotation rate of the peristaltic pump motor is Changed that will be affected on the flow rate as shown in Image (1-1).

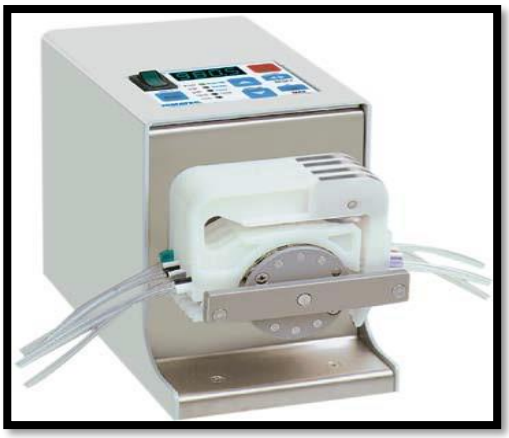


Image (1- 1) peristaltic pump [85]

1.11.2 Injectors Valve

The injection valve must be constructed to insert a highly reproducible wave infusion of sample into the carrier stream in such a way that an injection occurrence does not modify the flow of the stream. The sample size for flow injection analysis ranges from less than 1µL to 200 µL, with the majority of applications requiring 10µL to 30 µL.[86]

1.11.2.1 Important Characteristics of FIA-Compatible Valves[87]

- Precision.
- Fast changing.
- Pressure limitations of roughly 100 psi.
- Capability to inject sample amounts ranging from a few microliters to hundreds of microliters

Rolling and moving injection valves are the most commonly used types of injection valves. For sample injection, rotary valves, such as six-port HPLC rotary valves, can be employed. The sample is injected by rotating the valve and connecting the sample loop to the transporter flow. Slider valves, such as a four-part slider valve, can be used. Both valves are required. After loading the sample loop, the valve state is altered so that the carrier flow is directed via the sample loop. As showing blow in Image (1-2)

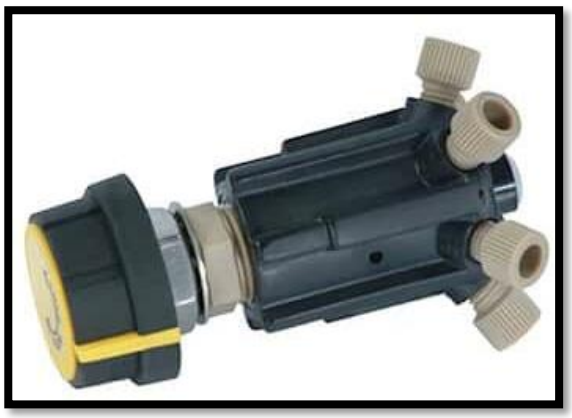


Image (1-2) Six- Port Valve

1.11.3 Manifold

The manifold is the FIA system's heart. The design of it is determined by the application. Tubing can be used to build it for simple chemical reactions. Specialized modules are required for applications such as solvent extraction, dialysis, and others, Polypropylene, polyethylene, or Teflon piping is commonly used to make manifold coils. Teflon is the best material for tubes. Standard chromatographic plastic ferrules and washers are used to connect tubing to one another and to other system components. Dead volume must be reduced when building a manifold since it increases dispersion and forms a tailing peak[88].

A wide range of tube sizes are commercially available, A 50 cm long reactor coil that has been tightly bound to increase mixing with flow rates ranging from 0.0005 mL/min to 40 mL/min. A coiled length of tubing (diameter 1 cm or smaller) is frequently used in flow injection systems to improve axial dispersion and promote radial mixing of the sample and reagent, both of which result in more symmetrical peaks.[89]

1.11.4 Detectors

Almost any detector specified for use with HPLC can be employed in an FIA system. However, the essential parameters for an FIA detector are that the detector has quick response times and a small volume. Because most FIA has a few seconds of peak width, the detector must have a response time of less than one second. Peak form might be affected by a slower response time[90].

Detectors employed in FIA systems include: amperometric, atomic absorption, chemiluminescence, coulometric, fluorometry, potentiometric, ion selective electrode, spectrophotometric, nephelometry and flame[91].

1.12 The Advantages of FIA [92]

When contrasted to manual analyses, it is clear that the tubing lines act as solution containers and transfer vessels, the injection valve serves as a micropipette, and the pump replaces the lab operator.

The FIA has had a lot of success in simplifying chemical assays. The following are the most significant explanations for FIA's effectiveness versus traditional manual methods:

1. Sample preparation and detection automation.
2. FIA design simplification.
3. Extensive sampling (usually 100-300 samples per hour).
4. Quick response time (typically less than one minute) with high repeatability.
5. Quick startup and shutdown.
6. Less sample and reagent usage, resulting in less waste formation.
7. Simple, adaptable, and inexpensive.
8. Reduced analysis and labor costs when a large number of samples must be evaluated.
9. Greater precision as compared with spectrophotometric method methods.

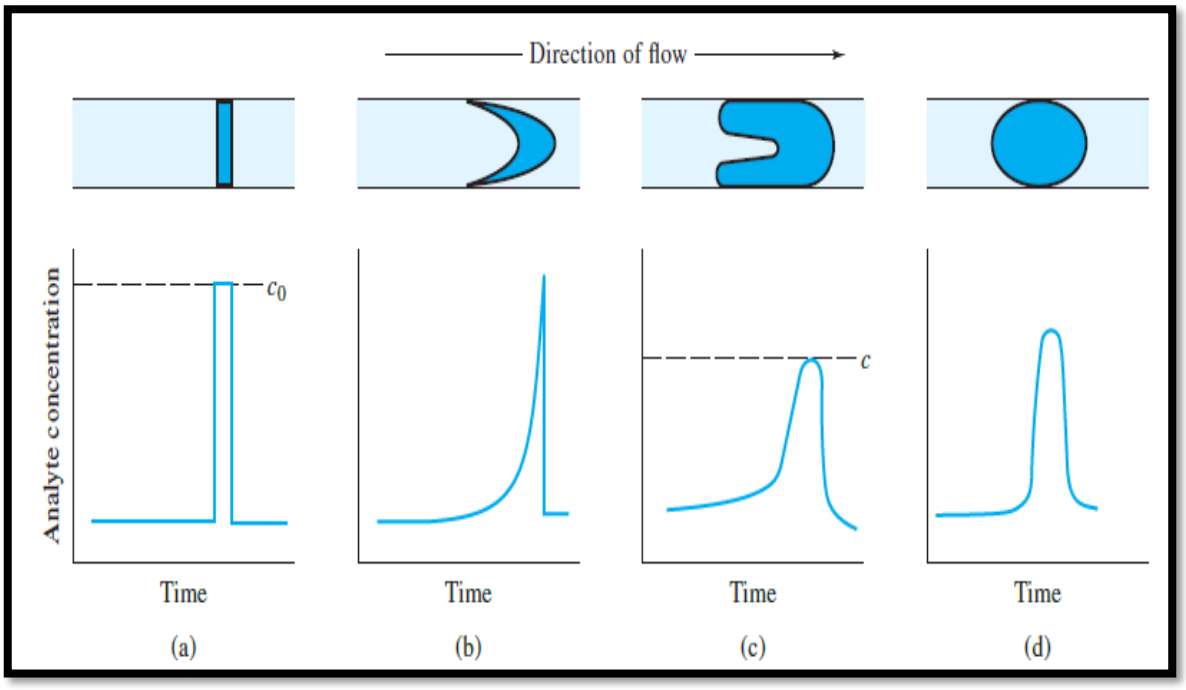
1.13 Dispersion and Dispersion Coefficient[93]

Instantly after injection the sample through a sampling valve, the sample zone in a flow-injection device has a rectangular concentration pattern which is illustrated in Scheme (1-11a).

Band broadening (dispersion) occurs as it passes through the tubing. Two phenomena determine the form of the resulting zone. The parabolic front and skewed zone profile seen in Scheme (1-11b) are the results of convection resulting from laminar flow, in which the fluid's center flows more quickly than the liquid next to the walls.

Broadening can also be a result of diffusion. In theory, two forms of diffusion can occur radial diffusion, which is perpendicular to the flow path, and longitudinal diffusion, which is identical to the flow. In narrow tubing, longitudinal diffusion is minor, whereas radial diffusion is far more essential. In reality, radial diffusion is the primary driver of dispersion at low flow rates. Under these situations, the symmetrical distribution illustrated in Scheme (1-11d) is approximated[94].

In reality, flow injection analyses are typically done at circumstances that allow for dispersion by both convection and radial diffusion, yielding peaks similar to those seen in Scheme (1-11c).



Scheme (1-11) Effect of both type of diffusion's on the concentration of analyte levels: (a) no dispersion, (b) convectional dispersion, (c) convectional dispersion and radial diffusion, and (d) diffusional dispersion.[95]

The dispersion coefficient (D), which has been defined as the percentage of the amounts of the sample substance before (C_o) and after (C) the dispersion has occurred in that component of the fluid that gives the analytical reading out, is used to quantify the dispersion in an FI-system. In other words: $D = C_o/C$[96].

The dispersion coefficient (D) is influenced by the following factors [97-102]:

- Sample volume
- Tubing length
- Flow rate
- Tubing internal diameter
- Degree of mixing of solutions
- Concentration of reactants

The physical parameters of the employed FI-manifold, such as the length and (i.d) of the tube, the flow rate of pumping, and the total number of merging points, may be employed to control the dispersion, depending on the required analytical work. Therefore, they found four types of dispersion[103]:

1. **Limited Dispersion (D = 1-3)** is employed when the injected sample is to be transported to a detector in undiluted form, meaning that the FIA system is used to ensure strict and accurate sample transfer to the detection device (such as an ion-selective electrode or an atomic absorption spectrometer) [104].
2. **Medium Dispersion (D = 3-10)** is used When the substance is measured and the carrier/reagent stream must combine and react to produce a product that can be identified [104].
3. **Large Dispersion (D > 10)** is only employed when sample dilution is necessary to put the sample into the dynamic reading range for the detection sensor [104].
4. **Reduced Dispersion (D < 1)** in which the concentration of the sample measurement is larger than the concentration of the substance to be injected, means preconcentration (e.g., via liquid, solid-phase, or coprecipitation) [105].

1.14 Literature Review of Flow Injection Analysis for Determination of Mefenamic Acid

Flow injection analysis have been used effectively in determination of mefenamic acid. However, below in table some method for determination of mefenamic acid by different chemical method used flow injection technique compare with proposed method as we shown in Table (1-2)

Table (1-2) Methods of Analysis for Mefenamic Acid Measurement by Flow Injection

Method	λ max nm	Linearity Range	Reagent	RSD%	L.O. D	Ref.
Flow-injection spectrometric determination of sodium diclofenac or mefenamic acid in pharmaceuticals	465	1.00-100 $\mu g.mL^{-1}$	ferricyanide	4.3	0.18 $\mu g.mL^{-1}$	[106]
Flow-injection spectrofluorimetric determination of flufenamic and mefenamic acid in pharmaceuticals	440	0.30 - 16.1 $\mu g.mL^{-1}$	Aluminum chloride solution	1.30	0.12 $\mu g.mL^{-1}$	[37]
determination of mefenamic acid using ce(iv)sulfate as an oxidant reagent via the use of the new mode of irradiation (array of six identical leds) and detection (twin solar cells) through turbidity measurement by CFIA	454	0.3-7.0 $\mu g.mL^{-1}$	Ce (IV)Sulfate	0.2	0.3 $\mu g.mL^{-1}$	[107]
Determination of Mefenamic Acid Using a New Mode of Irradiation (Array of Six Identical LEDs) and Detection (Twin Solar Cells) Through Turbidity Measurement by CFIA	222	0.3-7.0 $\mu g.mL^{-1}$	0.5 phosphomolybdic acid	<0.3	0.3 $\mu g.mL^{-1}$	[108]

Determination of Mefenamic Acid in Aqueous Solutions Using Reverse - Continuous Flow Injection Analysis	477	1.0-30 $\mu g.mL^{-1}$	sodium 1,2-Naphthoquinone-4-Sulfonic (NQS)	0.3	0.021 $\mu g.mL^{-1}$	[109]
Simple, Rapid and Sensitive Method for the Determination of Mefenamic Acid by Continuous flow and stopped-flow injection in Pharmaceutical Preparations	647	10.0–500 $\mu g.mL^{-1}$	(SNP) in the presence of hydroxylamine hydrochloride (HAH)	1.8	1.2 $\mu g.mL^{-1}$	[110]
Determination of mefenamic acid in aqueous solutions using merging zone – continuous flow injection	477	2.0–20 $\mu g.mL^{-1}$	sodium 1,2-Naphthoquinone-4-Sulfonic (NQS)	….	0.02 $\mu g.mL^{-1}$	[111]
New Mode Semi-Automated Turbidimetric Determination of Mefenamic Acid by Ayah 6SX1-ST-2D Solar cell -CFI	450	2.0-50 $\mu g.mL^{-1}$	Barium chloride	0.14	0.2 $\mu g.mL^{-1}$	[112]
Simultaneous Determination of Trace Mefenamic Acid in Pharmaceutical Samples via Flow Injection	405	0.5-2.3 $\mu g.mL^{-1}$	calcein blue (CLB)	1.48	0.760 $\mu g.mL^{-1}$	[113]
Flow Injection Determination of mefenamic acid using Silver with Tris(2,2-Bipyridyl) Ruthenium (III)-Ce(IV) Chemiluminescence Detection	……	5.6×10^{-9} - 2.2×10^{-6} M	Tris(2,2-Bipyridyl) Ruthenium (III)-Ce(IV)	2.1	$1.5x10^{-9}$ M	[114]
Novel sensitive flow injection Method for the Determination of Mefenamic Acid by using Cu(II)-Neocuproine complex	454	1.0-80 $\mu g.mL^{-1}$	Neocuproine	0.0115-0.0235	0.1983 $\mu g.mL^{-1}$	Our Study
New flow injection Method for the Determination of Mefenamic Acid by using Cr(VI)-Neocuproine complex	430	0.1-60 $\mu g.mL^{-1}$	Neocuproine	0.4838-3.2680	0.1 $\mu g.mL^{-1}$	Our Study

1.15 The Aims of This Study

The aims of this project are to:

1- Designing new flow injection system for determination of mefenamic acid in aqueous and pharmaceutical dosage form by using Cu(I)-Neocuproine.

2- Designing new flow injection system for determination of mefenamic acid in aqueous and pharmaceutical dosage form by using Cr(VI)-Neocuproine.

3- proposing new spectrophotometric method for Determination of mefenamic acid drug that has biological importance by using Cu(I)-Neocuproine complex.

4- proposing new spectrophotometric method for Determination of mefenamic acid drug by using Cr(VI)-Neocuproine complex.

5- Study the optimum conditions for drug determination such as the acidity function, concentration of reaction substances, reaction time, sequence of addition. to obtain a method with high sensitivity and accuracy for the determination of the drug in batches.

6- Applying the result of the four methods for drug determination in some pharmaceutical preparation.

CHAPTER TWO
EXPERIMENTAL

Chapter Two
Experimental

2 - Apparatus and Chemicals

2.1 Apparatus

The main instruments and the information that are employed in this study illustrated in Table (2-1).

Table (2-1) The Main Equipment Used in this study

No	Name of apparatus	Manufacturer's company and place
1	spectrophotometer	[Double Beam UV-visible Spectrophotometer –1800, Shimadzu, (Japan)] At the University of Kerbala/college of science/chemistry department
2	spectrophotometer	[Single Beam - visible Spectrophotometer -721, FAITHFUL (China)] At the University of Kerbala/college of science/chemistry department
3	pH meter	pH-meter-HI1271, HANNA (ROMANIA) At the University of Kerbala/college of science/chemistry department
4	Recorder	Siemens C 1032, Germany At the University of Kerbala/college of science/chemistry department
5	Sensitive analytical balance (Four decimal places)	Denver Instrument, Germany At the University of Kerbala/college of science/chemistry department
6	Heater	Labtech Korea Magnetic Stirrer At the University of Kerbala/college of science/chemistry department
7	Peristaltic pump	Ismatic, Germany At the University of Kerbala/college of science/chemistry department

8	The port injection, plastic 6 port valve	Germany At the University of Kerbala/college of science/chemistry department
9	Ultrasonic Bath Cleaners	Germany At the University of Kerbala/college of science/chemistry department
10	Water bath	BS-11 JEIO TECH (Korea). At the University of Kerbala/college of science/chemistry department
11	Conductivity meter	Digital conductivity Meter- WTW - 720 –inoLab (Germany) At the University of Kerbala/college of science/chemistry department

2.2 Chemical Materials

Unless otherwise specified, all chemicals used in this study were of analytical reagent quality, Table (2-2) summarizes the main compounds utilized as standard stock solutions in the current study. Following a dilution of the stock solution, several standard solutions were used.

Table (2-2) The Main Chemicals Employed in This Study

No	Subject Name	Chemical formula	Molecular weight g/mol	Company	Purity
1	Sulphuric Acid	H_2SO_4	98.080	BDH	99%
2	Cupper (II)nitrate.3-hydrate	$Cu(NO_3)_2.3H_2O$	241.600	BDH	99%
3	Chromium (III) Nitrate	$Cr(NO_3)_3$	238.011	BDH	99 %
4	Cobalt (II) nitrate -6-hydrate	$Co(NO_3)_2.6H_2O$	290.930	BDH	99%
5	Iron (III) nitrate -9-hydrate	$Fe(NO_3)_3.9H_2O$	403.997	BDH	99%
6	Ascorbic Acid	$C_6H_8O_6$	176.120	BDH	99%
7	Gelatine	$C_{31}H_{27}NO_4$	477.550	BDH	99%

8	Neocuproine	$C_{14}H_{12}N_2.3H_2O$	208.260	Sigma-Aldrich	99%
9	Potassium Dichromate	$K_2Cr_2O_7$	294,180	Sigma-Aldrich	99.98%
10	Acetic Acid	CH_3CO_2H	60.052	Sigma-Aldrich	99%
11	Mercury (II) nitrate-1-hydrate	$Hg(NO_3)_{2.}H_2O$	342.620	Sigma-Aldrich	99.99%
12	Sodium Hydroxide	NaOH	40.000	Merck, Germany	annular
13	Hydrochloric Acid	HCl	36.460	Merck, Germany	annular
14	Potassium Dihydrogen Phosphate	KH_2PO_4	136.090	MEREK	99%
15	Potassium Hydroxide	KOH	56.110	MEREK	99%
16	Ammonium Chloride	NH_4Cl	53.490	SRL chemicals	99.5%
17	Sodium Acetate	$C_2H_3NaO_2$	82.03	SRL chemicals	99%
18	Potassium Hydrogen Phthalate	$C_8H_5KO_4$	204.22	SRL chemicals	99.5%
19	Phosphoric Acid	H_3PO_4	97.994	SRL chemicals	99%
20	Diethyl Amine	$(CH_3CH_2)_2NH$	73.14	SRL chemicals	99%
21	Glucose	$C_6H_{12}O_6$	180.156	Xilong Scientific Co., Ltd.india	99%
22	Lactose	$C_{12}H_{22}O_{11}$	342.300	Xilong Scientific Co., Ltd.india	99%
23	Dextrose	$C_6H_{14}O_7$	198.170	Xilong Scientific Co. Ltd.india	99%

24	Potassium Chloride	KCl	74.550	Cynor, India	99.5%
25	Citric Acid	$C_6H_8O_7$	192.124	Cynor, India	99.8%
26	Ammonium Acetate	$C_2H_7NO_2$	77.080	SRL chemicals	98%
27	Disodium Hydrogen Phosphate	Na_2HPO_4	119.980	SRL chemicals	99%
28	Phosphoric Acid	H_3PO_4	98.000	Pure Chemicals Co. India	99%
29	Nitric Acid	HNO_3	63.012	Reagent, UK	99%
30	Starch	$C_6H_{10}O_5$	162.140	Cynor, India	99%
31	Ethanol	C_2H_5OH	46.068	Fluka	99%
32	Sodium Alginate	$C_6H_9NaO_7$	216.121	AUS Chem Source	98%
33	Sodium Lauryl Sulfate	$NaC_{12}H_{25}SO_4$	288.380	Finar, India	98%
34	Copper Sulphate	$CuSO_4$	158.600	ACS chemicals	99.8%

2.3 Preparation of standard Solutions

2.3.1 2,9-dimethyl 1-10 phenanthroline solution 5.0×10^{-3} M

A stock solution prepared by dissolvation accurate quantity of 0.0520 g Neocuproine in 50 mL of utilized solvent ethanol to and then dilute to various concentrations.

2.3.2 Sodium Hydroxide ≈ 0.1 M solution

The sodium hydroxide solution with a concentration of ≈ 0.1 M was made by dissolving 0.400 g into the volume of deionized water that was necessary, and then further diluting the solution with deionized water until it reached the desired volume (100 mL).

2.3.3 Cupper (II) solution

Dissolve 0.9758 g of Cupper Nitrate Cu $(NO_3)_2.3H_2O$ in 20 mL of deionized water and dilute it in a volumetric flask with 100 mL deionized water.

2.3.4 Hydrochloric Acid ≈ 0.1 M Solution

After adding (0.833 mL) of concentrated acid (37%) to a specific volume of deionized water, the resulting solution was diluted to (100 mL) deionized water.

2.3.5 Buffer Solutions Preparations[115-116]

2.3.5.1 Copper sulphate solution pH 4.0

Dissolve 0.25 g of copper sulphate and 4.5 g of ammonium acetate in dilute acetic acid and dilute to 100.0 mL with dilute acetic acid.

2.3.5.2 Acetate Buffer Solution pH 4.0

Dissolve 136 g of sodium acetate and 77 g of ammonium acetate in deionized water then add 250.0 mL of glacial acetic acid and dilute to 1000.0 mL with deionized water.

2.3.5.3 Phthalate Buffer Solution pH 4.0

Dissolve 2.042 g of potassium hydrogen phthalate in 50.0 mL of deionized water, add 7.5 mL of 0.2 M sodium hydroxide and dilute to 200.0 mL with deionized water.

2.3.5.4 Acetate Buffer Solution pH 4.0

Dissolve 77.1 g of ammonium acetate in deionized water. Add 70 mL of glacial acetic acid and dilute to 1000.0 mL with deionized water.

2.3.5.5 Phosphate Buffer Solution pH 4.0

Dissolve 6.80 g of potassium dihydrogen phosphate in 1000.0 mL of deionized water.

2.3.5.6 Sodium Acetate Buffer Solution pH 4.0

Dissolve 63 g of anhydrous sodium acetate in deionized water, add 90 mL acetic acid and dilute to 1000 mL with deionized water then adjust to pH 4.0

2.3.5.7 Acetate Buffer Solution pH 6.0

Dissolve 100 g of ammonium acetate in 300 mL of deionized water, add 4.1 mL of glacial acetic acid, and dilute to 500.0 mL with deionized water.

2.3.5.8 Dimethylammonium Phosphate Buffer Solution pH 6.0

Dilute 68 mL of phosphoric acid to 500 mL with deionized water. To 25 mL of this solution add 450 mL of deionized water and 6.0 ml of dimethylamine and dilute to 500.0 mL with deionized water.

2.3.5.9 Phosphate Buffer Solution pH 6.0

Mix 63.2 mL of a 71.5 g/L solution of disodium hydrogen phosphate and 36.8 mL of a 21 g/L solution of citric acid.

2.3.5.10 Phosphate Buffer Solution pH 6.0

Dissolve 6.8 g of sodium dihydrogen phosphate in deionized water and dilute to 1000.0 mL with deionized water.

2.3.5.11 Phosphate Buffer Solution pH 6.0

To 250.0 mL of 0.2 M potassium dihydrogen phosphate then add 28.5 mL of 0.2 M sodium hydroxide and dilute to 1000.0 mL with deionized water.

2.3.5.12 Phosphate buffer solution pH 6.0

Dissolve 2.5 g of disodium hydrogen phosphate, 2.5 g of sodium dihydrogen phosphate and 8.2 g of sodium chloride in 950 mL of deionized water.

2.3.6 Chromium Ion Cr (VI) 0.02 M

The chromium (VI) standard solution was made at a concentration of 0.02M, by dissolving 0.5883 g of Potassium dichromate in 100 mL deionized water. This standard solution was used as a starting point for the preparation of other standard solutions, which involved step by step dilution with deionized water.

2.4 Interferences Solutions (500 µg.mL^{-1})

These were made by dissolving various interference as shown in Table (2-3) in deionized water (glucose, lactose, gelatine, starch, sodium alginate, sodium lauryl sulfate). They were transferred to a 100 mL volumetric flask and diluted to the concentration that was needed with deionized water.

Table (2-3) Interference Solutions

Interfering Solution	Weight (g)
Glucose	0.0486
Lactose	0.0479
Starch	0.0487
gelatine	0.0477
Sodium Alyinate	0.0497
sodium lauryl sulfate	0.0490

2.5 Application Solutions

2.5.1 Standard Mefenamic Acid (100 µg.mL^{-1})[39]

in order to prepare standard MFA solution, we can dissolve 0.0100 g MFA in 25 mL of standardized 0.03 M NaOH and complete volume up to 100 mL with deionized water, working solutions were daily and freshly prepared by various subsequent dilutions.

2.5.2 Pharmaceutical Preparations of Mefenamic Acid Solutions [40]

Ten MEF capsules from each type (Ponstane Capsule N.D.I-IRAQ 250mg),(Ponstidin Capsule GMBH, Germany 250 mg) and (Mefril Bangalore-India250 mg) were crushed and combined with each other, and an exact weighted quantity of powder was dissolved in 25 mL of 0.03 M NaOH, then stirred and let to stand for 7 minutes in Ultrasonic Bath and transferred to 100 mL volumetric flask, which was completed with deionized water to the mark, the resulting solution was filtered using Whatman filler paper no. 41 to remove any undissolved or suspended contaminants. Working solutions were created every day by a series of dilutions with deionized water, and they were then evaluated using the recommended procedure.

1.0 mL of copper(II) or chromium(VI) solutions were taken and putting in 10.0 mL volumetric flask and added to it 2.0 mL of the reagent solution at a concentration in optimum conditions reached in this study and the pH was adjusted (pH=4.0) when used copper(II) and (pH = 6.0) when used for chromium(VI) then 2.0 mL of drug solution was added and the volume was completed with deionized water to the mark, prepared using the same complex process, but substituting an equivalent volume of deionized water for the volume of a drug solution.

2.6 The Stoichiometry for The Metal Complexes

Under ideal circumstances, the metal-to-ligand ratio (M:L) is examined using the Job method (continuous variations) and the mole ratio approach.

2.6.1 Job's (continuous variation) method [117]

2.6.1.1 Determination of Mefenamic Acid by Using Copper (II) Ion Complex.

This method entails preparing two solutions with the same concentration of metal ion and reagent for copper (II) complex at concentration (1.0×10^{-2} M) , and modifying the acidity function at (pH =4.0) with equal final volume every time , and finally drawing a curve between the absorbance and the volumetric ratio at the appropriate maximum wavelength (λ max =454 nm) for copper(II) complex .

2.6.1.2 Determination of Mefenamic Acid by Using Chromium (III) Ion Complex.

This method entails preparing two solutions with the same concentration of metal ion and reagent for chromium (III) complex at concentration (3.0×10^{-3} M), and modifying the acidity function at (pH =6.0) with equal final volume every time, and finally drawing a curve between the absorbance and the volumetric ratio at the appropriate maximum wavelength (λ max =430 nm) for chromium (III) complex.

2.6.2 Mole Ratio Method [118]

2.6.2.1 Determination of Mefenamic Acid by Using Copper (II) Ion Complex.

Using this procedure, a set of volumetric flasks (10.0 mL) were taken and filled with rising and proportionate amounts of the reagent ($0.5\text{-}4.5 \times 10^{-2}$ M) (2.0 mL) in addition to a fixed and known concentration of the copper (II) ion (1.0×10^{-2} M) (1.0 mL) and the Cu(II) complex optimal acidic function was adjusted at (pH=4.0). The comparison solutions were

made using the same methodology as the complex preparation, with the exception that an equal volume of deionized water was used in place of the copper (II) ion solution. At (λ max=454 nm), the molecule absorbs all solutions.

2.6.2.2 Determination of Mefenamic Acid by Using Chromium (III) Ion Complex.

The procedure involved taking a set of volumetric flasks (10 mL) and filling each one with a fixed and known concentration of the chromium (III) ion ($3.0x10^{-3}$ M) (1.0 mL) with increasing and proportionate amounts of the reagent (0.5-4.5 $x10^{-3}$ M) (3.0 mL) and adjusting the acidity function at (pH = 6.0), The comparison solutions were made using the same method as the complex preparation, but an equal volume of deionized water was used in place of the chromium (III) ion solution. At (λ max=430 nm), the molecule absorbs all of the solutions.

2.7 The Flow Rate and Volume in Peristaltic Pump

A certain volume of liquid will be transferred by peristaltic movement through the flow injection system Table (2-4) indicate that the volume of liquid is estimated by collecting it in a graduating cylinder with a constant of time (one minute).

Table (2-4) Flow rate of the peristaltic pump

Pump Speed	Flow Rate ($mL.min^{-1}$)	Pump Speed	Flow Rate ($mL.min^{-1}$)
10	1.50	60	7.20
20	2.30	70	8.40
30	3.00	80	9.30
40	4.90	90	10.50
50	6.20	100	12.40

2.8 Lengths and Volumes of The Loops

Equation (2-1) was used to determine the volumes of Teflon loops based on their lengths:

$$V=\pi r^2 L \quad \text{(2-1)}$$

Where:

V: the volume of loop per μL.

r: the radius of loop (mm).

L: length of the loop (cm) .

Consider that the loop has a diameter of 1.00 mm.

CHAPTER THREE
RESULTS & DISCUSSION

Chapter Three

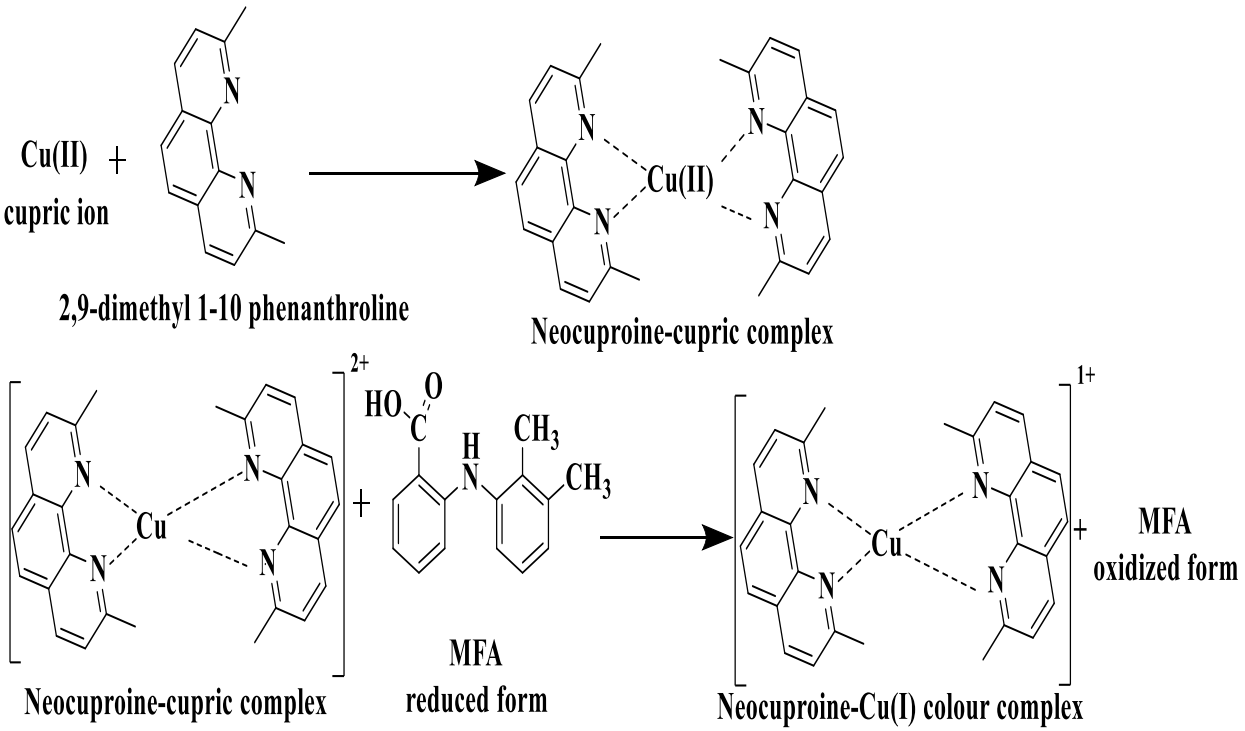
Results and Discussion

Part One: Spectrophotometric Method for Determination of Mefenamic Acid by using copper (II)

3- Results and Discussion

3.1 Reaction principle Concept [119]

The type of chemical system employed and the designed system are the key for visible spectrophotometric method for determination of mefenamic acid by using copper(II). In order to identify mefenamic acid will use the following reaction which describe below in scheme (3-1).



Scheme (3-1) Schematic Diagram for Proposed Method

In the previously mentioned process, it is an indirect method with a two-step reaction designed to determine mefenamic acid, in first step

Neocuproin reagent interact with Cu (II)to form Cu(II)-neocuproin colourless complex,in the second step mfenamic acid (reducing agent) give the colourless complex two electron to produce Cu(I)-Neocuproin complex, The resultant yellow-orange coloured complex shows a maximum of absorbance at 454 nm.

3.2 Determination of Maximum Wavelength (λ_{max})

To determine the appropriate λ_{max}, an aliquot of (2.0 mL) 2,9-DMP solution 1×10^{-3} M was added to (1.0mL) of copper (II) solution $1x10^{-2}$ M then the pH was adjusted in (pH=4), then (2.0 mL) of standard solution 50 µg of MFA was transferred to (10.0 mL) volumetric flask, The contents diluted to (10.0 mL) with deionized water, subsequently; absorbance of colored product's was measured against to a reagent blank in the 190–1100 nm range. The maximum wavelength of MFA's absorption was observed to be 454 nm and colour of complex was yellow-orange as in Figure (3-1) Each reagent blank displayed a negligible absorbance at the relevant λ_{max} under

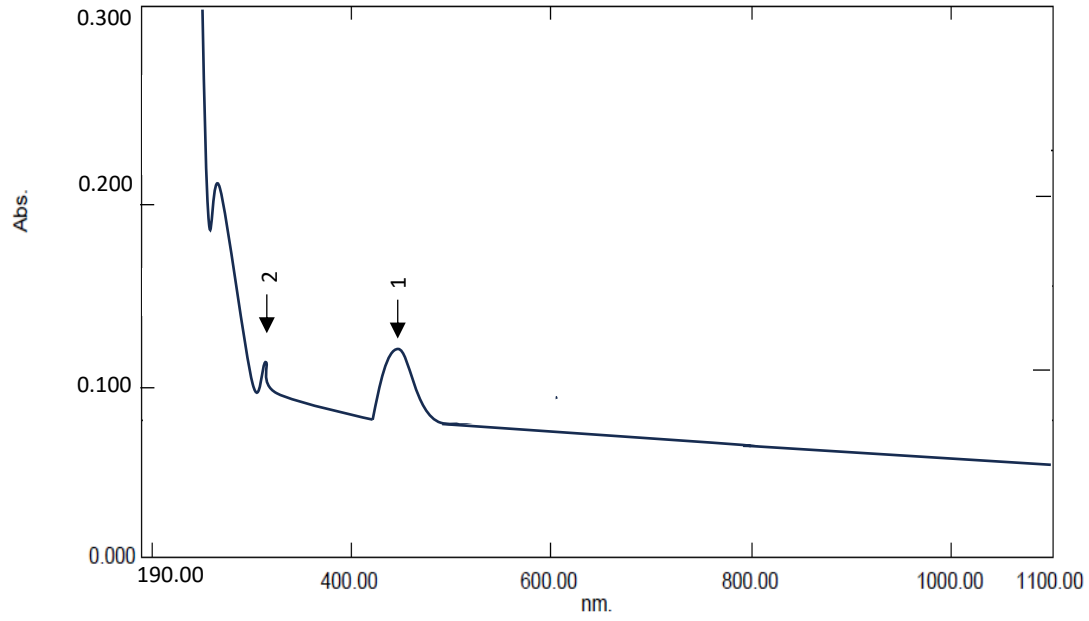


Figure (3-1) Absorption Spectra of Reaction Product complex against blank.

3.3. Study of Optimum Parameters

3.3.1 Effect of pH value

Different volumes of hydrochloric acid and sodium hydroxide solutions was added to an aliquot of solution containing 50 µg .2mL^{-1} of MFA to examine the effect of pH on formed complex. Absorption intensity for colored complex compounds was measured at 454 nm. However, the system conditions were as follows:

1) 2.0 mL of neocuproine (1.0×10^{-3}) M
2) 1.0 mL of Cu (II) (1.0×10^{-2}) M
3) MEF Conc.= 50 µg.2mL^{-1}
4) pH range (1.0-10.0)
5) Temperature (25 C^{o})

Table (3-1) pH Effect on Absorption of Complex Formation

pH	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0
Absorbance	0.111	0.120	0.181	0.196	0.160	0.151	0.139	0.096	0.082	0.061

n=3

The results show that the absorbance increases as the acidic functions increase until reach to pH =4.0 due to good intensity On the other hand, the absorbance value of the complex decreases when the acidic function increases, which can be attributed to either the precipitation of ion or the formation of unstable complex ion [120-121] as shown in Table (3-1).

3.3.2 Effect of Types of buffer solution

Six buffer solutions of pH 4.0 with different composition have been tested, copper sulphate-ammonium acetate, sodium acetate- ammonium acetate , potassium hydrogen phthalate - sodium hydroxide, ammonium acetate - glacial acetic acid , potassium dihydrogen phosphate - water and anhydrous sodium acetate - acetic acid as shown in (Table 3-2) [122] ·However, the system conditions were as follows:

1) 2.0 mL of neocuproine (1.0×10^{-3}) M
2) 1.0 mL of Cu (II) (1.0×10^{-2}) M
3) pH value (4.0)
4) Temperature (25 C^{o})

Table (3-2) The Effect of Buffer Solution at pH 4.0 on Absorption of Complex Formation

Buffer Solutions	Absorbance
Copper sulphate-ammonium acetate	0.149
Sodium acetate	0.170
Potassium hydrogen phthalate	0.182
Ammonium acetate	0.106
Potassium dihydrogen phosphate	0.184
Anhydrous sodium acetate	0.136

The results shown in Table (3-2) indicate that all types of buffer solutions decrease the intensity of the coloured complex.

3.3.3 Effect of time

Under the ideal experimental conditions established, the influence of time on stability of colored compound for concentration of MFA was examined. the system conditions were as follows:

1) 2.0 mL of neocuproine (1.0×10^{-3}) M
2) 1.0 mL of Cu (II) (1.0×10^{-2}) M
3) pH value (4.0)
4) Temperature (25 C^{o})
5) Time (01-90 min)
6) MEF Conc.=50 μg.2mL^{-1}

Table (3-3) Time Influence on Absorption of Complex Formation

Time/min	Absorbance
01	0.196
05	0.196
10	0.197
20	0.197
30	0.197
40	0.197
50	0.195
60	0.195
70	0.195
80	0.186
90	0.179

From the result, the complex's absorbance intensity remained steady for at least (90 min) as shown below in Table (3-3).

The stability period is long enough from above to allow for the sequential performance of many measurements.

3.3.4 Effect of Temperature

The color intensity in the proposed approach was evaluated at various temperatures and the system conditions were as follows:

1) 2.0 mL of neocuproine (1.0×10^{-3}) M
2) 1.0 mL of Cu (II) (1.0×10^{-2}) M
3) pH value (4.0)
4) Time (05 min)
5) Temperature (10-60 C^{o})
6) MEF Conc.=50 $\mu g.2mL^{-1}$

Table (3-4) Temperature Effect on Absorption of Complex Formation

Temp C^{o}	10	20	25	30	40	50	60
Absorbance	0.110	130	0.197	0.190	0.178	0.163	0.142

The findings demonstrate that maximum absorbance values at 25C^{o} then decreased This may have been caused by the decrease in stability or dissolution at high temperatures as shown in Table (3-4).

3.3.5 Effect of Concentration of Cu (II)

The effect of Cu (II) concentration on the absorbance was investigated in the range between 0.5×10^{-2} and 4×10^{-2} M, by employing the principle of any single change to one variable and the remaining all other variables as constants throughout this investigation, the system conditions were as follows:

1) 2.0 mL of neocuproine (1.0×10^{-3}) M
2) 1.0 mL of Cu (II) (0.5×10^{-2} - 4×10^{-2}) M
3) pH value (4.0)
4) Temperature (25 C^{o})
5) Time (05 min)
6) MEF Conc.=50 μg.2mL^{-1}

Table (3-5) Concentration effects of Cu (II) On absorption of complex formation

Cu (II) concentration (M)	Absorbance
$0.5x10^{-2}$	0.175
$1.0x10^{-2}$	0.197
$1.5x10^{-2}$	0.170
$2.0x10^{-2}$	0.159
$2.5x10^{-2}$	0.140
$3.0x10^{-2}$	0.131
$3.5x10^{-2}$	0.128
$4.0x10^{-2}$	0.118

The results show revealed that the absorbance increases as Cu (II) concentration increases until it reaches $1x10^{-2}$ M, then it starts to decrease. This may be due to the formation of new varieties in the solution that are absorbed at Hight concentrations and possibly to the condensation of the ions with each other and cause's cloud of ions so this led to lowering the absorbance or may be the completeness or sufficiency of the coordination failed of the ion with the reagent. As a result, the optimum concentration of Cu (II) was $1x10^{-2}$ M, as shown in Table (3-5)[123].

3.3.6 Effect of Concentration of Neocuproine

The effect of Neocuproine concentration on the absorbance was investigated in the range between 0.5×10^{-3} and 4×10^{-3} M, by employing the principle of any single change to one variable and the remaining all other variables as constants throughout this investigation, the system conditions were as follows:

1) 2.0 mL of neocuproine (0.5×10^{-3} - 4×10^{-3}) M
2) 1.0 mL of Cu (II) (1.0×10^{-2}) M
3) pH value (4.0)
4) Temperature (25 C^{o})
5) Time (05 min)
6) MEF Conc.=50 $\mu g.2mL^{-1}$

Table (3-6) Effects of Neocuproine Concentration on Absorption of Complex Formation

Neocuproine concentration (M)	$0.5x10^{-3}$	$1.0x10^{-3}$	$1.5x10^{-3}$	$2.0x10^{-3}$	$2.5x10^{-3}$	$3.0x10^{-3}$	$3.5x10^{-3}$	$4.0x10^{-3}$
Absorbance	0.165	0.197	0.181	0.162	0.152	0.131	0.122	0.111

The results of the study show revealed that the absorbance increases as Neocuproine concentration increases until it reaches 1×10^{-3} M. However, as the conc. of the reagent increase, the absorption values begin to decrease. This could occur because the reagent may be not completely soluble in the solvent, or it might be because the completeness or sufficiency of the coordination failed of the ion in the reagent. As a result, the optimum concentration of Neocuproine was 1×10^{-3} M, as shown in Table (3- 6)[123].

3.3.7 Effect of Volume of Cu (II)

The effect of volume of Cu (II) on the absorbance was investigated in the range between (0.5 -3.0) mL, by employing the principle of any single change to one variable and the remaining all other variables as constants throughout this investigation, the system conditions were as follows:

1) 2.0 mL of neocuproine (1.0×10^{-3}) M
2) (0.5 - 4.0) mL of Cu (II) (1.0×10^{-2}) M
3) pH value (4.0)
4) Temperature (25 C^{o})
5) Time (05 min)
6) MEF Conc.=50 μg.$2mL^{-1}$

Table (3-7) Volume Effects of Cu (II) on Absorption of Complex Formation

Volume of Cu (II) mL	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
Absorbance	0.185	0.197	0.186	0.182	0.176	0.161	0.153	0.141

The results of the study show revealed that the absorbance increases as volume of Cu (II) increases until it reaches 1.0 mL, at then it starts to decrease. As a result, the optimum of volume of Cu (II) was 1.0 mL, as shown in Table (3- 7).

3.3.8 Effect of Volume of Neocuproine Reagent

The influence of reagent volume on resultant-colored product was studied. Varying volumes of standard reagent solutions in the range (0.5-4.0 mL) were added and measuring the absorbances of the solutions, the system conditions were as follows:

1) 0.5 -4.0 mL of neocuproine (1.0×10^{-3}) M
2) 1.0 mL of Cu (II) (1.0×10^{-2}) M
3) pH value (4.0)
4) Temperature (25 C^{o})
5) Time (05 min)
6)MEF Conc.=50 μg.2mL^{-1}

Table (3-8) Effect of Volume of 2, 9.DMP Reagent on Absorption of Complex Formation

Volume of Neocuproine (mL)	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
Absorbance	0.170	0.184	0.188	0.196	0.186	0.172	0.166	0.152

The results showed that 2.0 mL of 2,9.DMP solution gave maximum absorbance due to its full intensity and further volume additions of reagent would produce in a systematic decrease in absorbance of colored product, this is may be due to formation of new species .Similarly, may be attributed to the fact that high concentrations of neocuproine would result in interference from Cu(II) which could have arisen from incomplete conversion of Cu (II) into the Cu (I)–neocuproine complex as shown in Table (3- 8).

3.3.9 Effect of Sequence of Addition

The reagent added sequence should really be accompanied to obtain good color intensity which lead to best results. Otherwise, a loss in color intensity was seen, the system conditions were as follows:

1) 2.0 mL of neocuproine (1.0×10^{-3}) M
2) 1.0 mL of Cu (II) (1.0×10^{-2}) M
3) pH value (4.0)
4) Temperature (25 C^{o})
5) Time (05 min)
6)MEF Conc.=50 μg.2mL^{-1}

Table (3-9) Effect of Sequence of Addition on Absorption of Complex Formation

Sequence of addition	Absorbance
Cu (II)+R+ pH4.0+D	0.197
R+Cu(II)+ pH4.0 +D	0.197
Cu(II)+D+ pH4.0 +R	0.195
R+D+ pH4.0 +Cu(II)	0.060

In Which: R: Neocuproine Reagent D: Mefenamic Acid Drug

The order of addition in all method gives good color intensity of formed complex which gives absorbance 0.197 except, R+D+ pH4.0 +Cu(II) due to there is no formed complex or might be due to the competition of ions negative acid or base in the bond with the metal, which leads to lower absorption values as illustrated in Table (3-9) .

3.3.10 Effect of Acid type

Various type of acids has been investigated, including (HCl, HCOOH, CH_3COOH, H_3PO_4, HNO_3, H_2SO_4), The mixture of 50 μg.2mL^{-1} drugs was added to 1.0 mL of 0.05 M standardized acids in order to determine the most efficient type of acid used at the suitable pH level. Then, 2.0 mL of 1.0×10^{-3} M 2, 9.DMP solution, 1.0 mL of copper metal ion 1.0×10^{-2} M.

Table (3-10) Effect of Acid type on Absorption of Complex Formation

Acid Type	Absorbance
HCl	0.197
HCOOH	0.150
CH_3COOH	0.120
H_3PO_4	0.111
HNO_3	0.095
H_2SO_4	0.035

However, the results in Table (3-10) shows that HCl was indeed the best type of acid due to highest absorbance and there is no any interference with complex.

3.3.11 Effect of interference

It is possible to study the effects of some foreign compounds as shown in Table (3-11) that frequently accompanied pharmaceutical preparations and the system conditions were as follows:

1) 2.0 mL of neocuproine (1.0×10^{-3}) M
2) 1.0 mL of Cu (II) (1.0×10^{-2}) M
3) pH value (4.0)
4) Temperature (25 C^{o})
5) Time (05 min)
6) MEF Conc.=50 $\mu g.2mL^{-1}$

Table (3-11) The effect of interference on absorption of complex formation

Type of excipients	Glucose	Lactose	gelatine	Starch	Sodium Alyinate	Sodium Lauryl Sulfate
Absorbance	0.197	0.198	0.196	0.195	0.195	0.198

The results from above Table (3-11) showed the applying suggested proposed procedure to determine MFA did not cause any interference from the examined foreign chemicals.

3.4 Standard Calibration Curve for Determination of Mefenamic Acid

When absorbance was taken at 454 nm for 10.0 mL series volumetric flasks containing an increasing amount of MFA 5.0-60 µg.2mL^{-1} and 2.0 mL of 2,9 DMP, 1.0 mL cupper nitrate 0.01 M solution then adjust the pH 4.0, The flasks were then diluted to the mark with de ionized distilled water, mixed thoroughly, and the absorbance at 454 nm was measured toward the blank sample of reagents. Beer's law was followed in the concentration range 5.0-60.0 µg.2mL^{-1} with a limit of detection of 0.7857 µg/mL, and the correlation coefficient obtained was 0.9999 at 454 nm as shown in Table (3-12) and Figure (3-2)

Table (3-12) The effect of the concentration of mefenamic acid on absorption of complex formation

Conc. of Mefenamic acid µg.2mL^{-1}	**Absorbance**
5.0	0.007
10.0	0.027
20.0	0.070
30.0	0.113
40.0	0.155
50.0	0.197
55.0	0.219
60.0	0.240

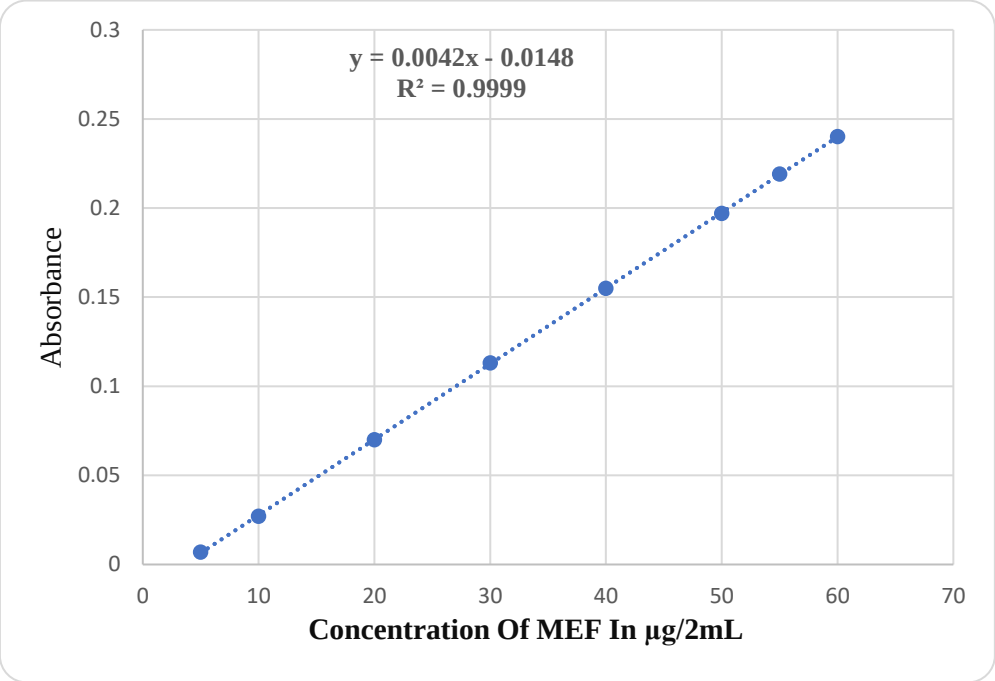


Figure (3-2) Calibration graph for determination of MFA.

3.5 Accuracy and precision [124]

By applying five different concentrations of standard solutions of mefenamic acid, the accuracy and precision of the suggested procedure for the determination of mefenamic acid have been investigated under ideal conditions. Table (3-13) provides the results of calculating the E%, Rec.%, and RSD% of Three readings of each of five distinct concentrations (10, 20 ,30 ,40 ,50 µg.2mL^{-1}) using equations (3-1), (3-2) and (3-3).

$$E\ error\% = \frac{measured\ value - actual\ value}{actual\ value}\ x\ 100 \quad \text{..... 3-1}$$

$$Rec\% = 100 \pm E_{error}\% \quad \text{..... 3-2}$$

$$R.S.D\% = \frac{SD}{X} \times 100\% \quad \text{..... 3-3}$$

Table (3-13) Accuracy and Precision for proposed method

Concentration of MFA µg.2mL^{-1}		RSD%	Eerror %	Recovery%
Present	**Found**			
10.0	9.95	0.037	-0.5	99.50
20.0	19.92	0.995	-0.40	99.60
30.0	30.16	0.618	0.53	100.53
40.0	40.16	0.650	0.40	100.40
50.0	50.16	0.500	0.32	100.32

n*= 3

The findings demonstrate good accuracy and precision for proposed method.

3.6 Limit of Detection and Limit of Quantification

The limits of detection (LOD) and quantitation (LOQ) were assessed as equation 3.4 and 3.5 and Table (3-14) below. [124-125]

LOD=3.0 SD/slope ……..3.4

LOQ=3.3 LOD ……..….3.5

Table (3-14) Limit of Detection and Limit of Quantification

Absorbance*	SD	LOD µg.mL^{-1}	LOQ µg.mL^{-1}
0.198	0.001	0.7142	2.3568

* n=3

3.7 Rate of Sample Analysis

After stabilizing the optimal conditions for the reaction, the number of analyzed samples per hour was calculated to determine the reaction time and the speed of the proposed method. This was done by calculating the time taken from mixing the input materials into the reaction (copper) until the absorbance appeared. It was found that the time required to reach the maximum absorbance was 5 minutes. Therefore, this method is capable of analyzing 12 samples per hour, The ideal circumstances and statistical analyses for the suggested approach are listed in Table (3-15).

Table (3-15) The Optical features and statistical information for suggested method

Analytical Data	Value
λmax	454 nm
Regression equation	y = 0.0042x - 0.0148
Cu (II) Concentration	1×10^{-2} M
Neocuproine Concentration	1×10^{-3} M
Mefenamic acid concentration	50 µg.2mL^{-1}
pH	4.0
Temperature	25 C^{O}
Slope	0.0042
Intercept	0.0148
Linearity Range	5-60 µg.2mL^{-1}
linearity coefficient (R^2)	0.9999
Colour	Yellow-orange

3.8 Stoichiometry of Formed Complex

3.8.1 Job's method of Continuous variation

The donor and acceptor were utilized in equivalent concentrations, and a number of solutions were prepared after that. Under constant pH =4.0 circumstances, the volume of the donor and acceptor is equivalent to (10) mL, then leave the solution in the solution for 5 minutes at 25 C°. The absorbance is then calculated at maximum λ_{max} = 454 nm.as shown in Figure (3-3)

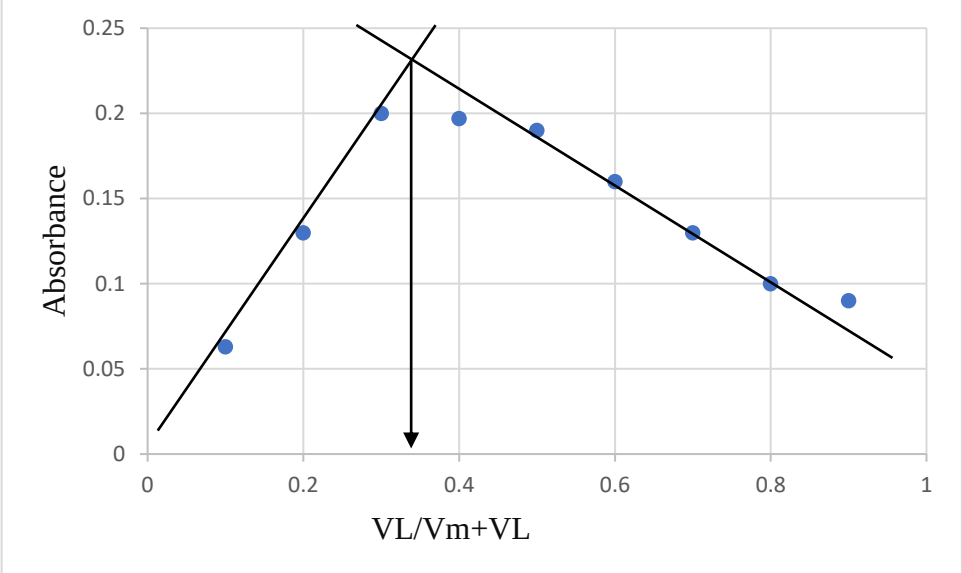


Figure (3-3) Jobs Method

we observed that the donor-acceptor ratio is (1:2) , in which one mole from metal Cu(I) to two mole from the ligand (neocuproine) ,This indicates that the resulting complex has possible empirical formula is (ML_2), as indicated above [125].

3.8.2 Mole ratio method

a fixed concentration of metal ion (copper) 1×10^{-2} M was taken with increasing concentrations of the 2, 9.DMP reagent 0.5-4.0×10^{-2} M under optimum conditions and the reaction ratio between CL/CM was calculated as shown in Figure (3-4).

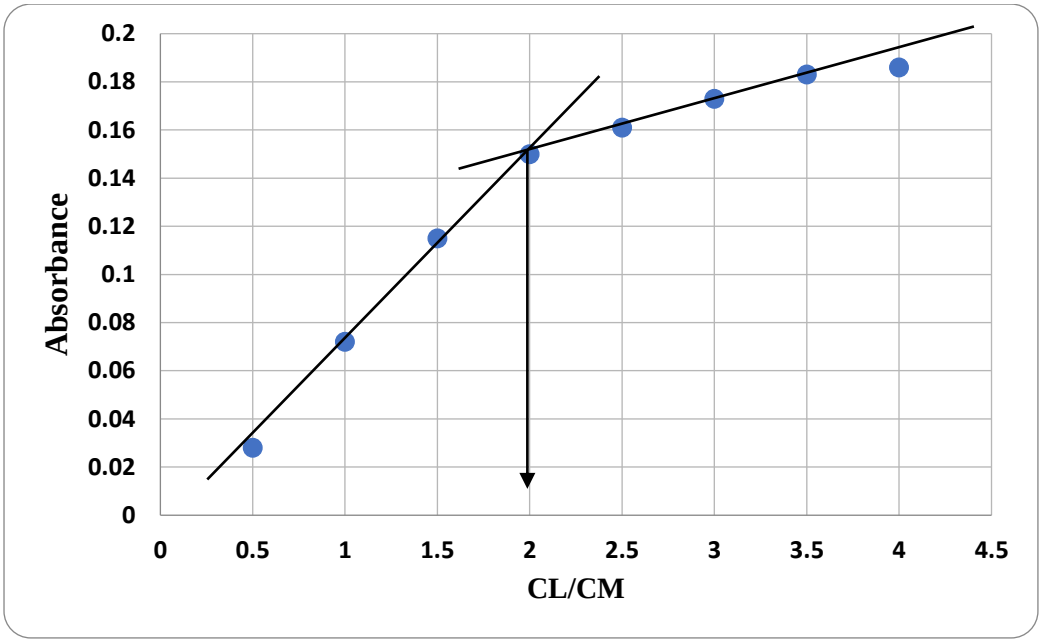


Figure (3-4) Mole Ratio Method

we observed that the donor-acceptor ratio is (1:2), in which one mole from metal Cu(I) to two moles from the ligand (Neocuproine), This indicates that the resulting complex has possible empirical formula is (ML_2) as indicated above [126].

3.9 Stability Constant Calculations for The Produced Complex

The stability constant for the formed complex is calculated by writing the following equilibrium reaction for complex formation with an (α degree) as shown in equations (3-6) (3-7) and table (3-16)

$$M^{+2} + 2L^{-} \rightleftharpoons ML_2$$

αC 2αC (1-α) C

$$K = \frac{(1-\alpha)C}{\alpha C(2\alpha C)2} \quad \text{..... 3-6}$$

$$\alpha = \frac{Am-As}{Am} \quad \text{.....3-7}$$

In which:

α= degree of dissociation
K= stability constant
C= concentration of metal ion
A_m= maximum absorbance
A_s= absorption at the equivalence point

Table (3-16) Stability Constant Values for complex

Complex	Am	As	α	K
[Cu (Nc.)$_2$]	0.197	0.150	0.238	3.5×10^8

3.10 Application of the suggested method to MFA analyses in pharmaceutical formulation

The method could be used with pharmaceutical formulations including MFA, such as Ponstidin Capsule (250 mg) GMBH, Germany , Ponstidin Capsule (250 mg) N.D.I-IRAQ, and mefril 250 mg India, MFA determination in pharmaceutical samples was accomplished successfully and with good recovery rates using the proposed method. [127] as we describe below in Table (3-17).

Table (3-17*)* Application of the novel framework for analysis of commercial MFA formulations in tablet dosage form

Pharmaceutical preparation	MFA present µg.mL^{-1}	MFA measured µg.mL^{-1}	RSD%	Error%	%Recovery
Ponstidin Capsule N.D.I-IRAQ 250 mg	5.0	4.92	0.8247	-1.60	98.40
	20.0	19.92	0.7246	-0.40	99.60
	40.0	40.16	0.6451	0.40	100.40
	60.0	60.40	0.2405	0.66	100.66
Ponstidin Capsule GMBH, Germany 250 mg	5.0	5.11	0.9467	2.20	102.20
	20.0	20.32	0.9254	1.60	101.60
	40.0	40.25	0.4184	0.62	100.62
	60.0	60.76	0.3219	1.26	101.26
Mefril Bangalore-India 250 mg	5.0	4.59	1.7543	-8.17	91.83
	20.0	19.95	1.3761	-0.25	99.75
	40.0	40.58	0.6024	1.45	101.45
	60.0	60.10	0.6911	0.16	100.16

Above clearly shows that the developed UV technique provided good recovery beliefs in accordance with the marked quantities for every one of the analyzed samples collected from different pharmaceutical industry. Furthermore, quantities analyzed within range of USP-specified permissible 90–110% of the MFA designated quantity.[128]

Part Two : Flow Injection Analysis For Determination of Mefenamic Acid by Using Copper (II)

This part of result shows choseen of optimum design of the flow injection system from eight flow injection system model designed in our laboratory. However, At the first we should choose the optimum flow injection system model designed from (8 designed FIAs) system model as shown below .

3.11 The Design of the System Applied for Reaction and the Fundamental Concept in its Work

The type of chemical system employed and the designed system for it are the key determinants of the flow injection system's design. In order to identify mefenamic acid we will use the following reaction which describe in scheme (3-1) ,in this part, we aimed to design and build an integrated flow injection system.

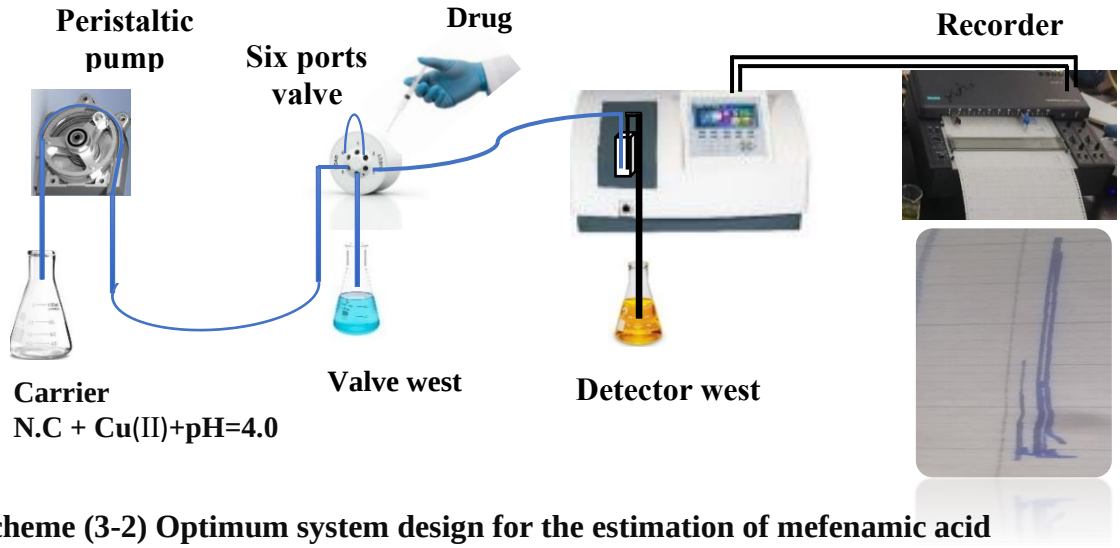
In the previously mentioned process, it is an indirect method with a two-step reaction designed to determine mefenamic acid, The resultant yellow-orange coloured complex shows a maximum of absorbance at 454 nm.On the other hand ,The system is built in accordance with the chemical reaction mentioned and as given by both equation .

3.12 Flow Injection System Model Designed

3.12.1 Model Designed (1)

In the first model designed, In step (1), the metal ions Cu(II) and neocuproine react in an acidic solution to generate a Cu(II)-Neocuproine Colorless complex . The created Cu(II)-Neocuproine Colorless complex then reacted with mefenamic acid in step (2) to yield Cu(I)-Neocuproine Color Complex, whose absorbance is measured at the maximum wavelength. As a result, a flow injection system with a single stream manifold was designed, which operates on both concepts:

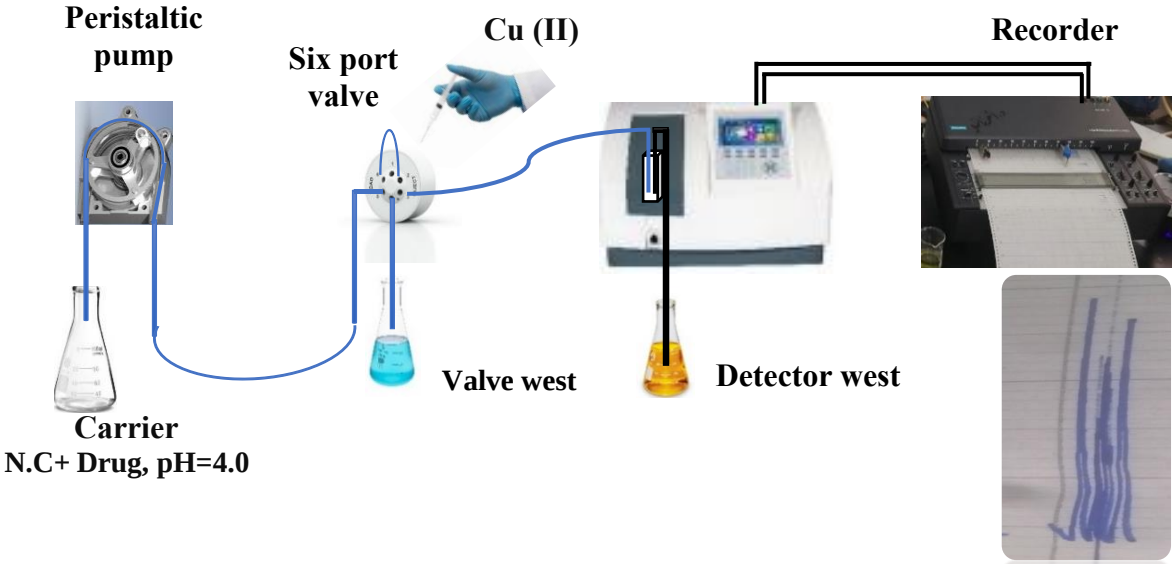
1. introducing a carrier into the system.
2. Sample injection into the sample loop, followed by carrier solution to the unit. The flow injection system design for this work is depicted in Scheme (3-2).



Scheme (3-2) Optimum system design for the estimation of mefenamic acid Model (1) Cu (II)=1×10^{-2} M, NC=1×10^{-3} M, MEF=50 µg.2mL^{-1}.

3.12.2 Model Designed (2)

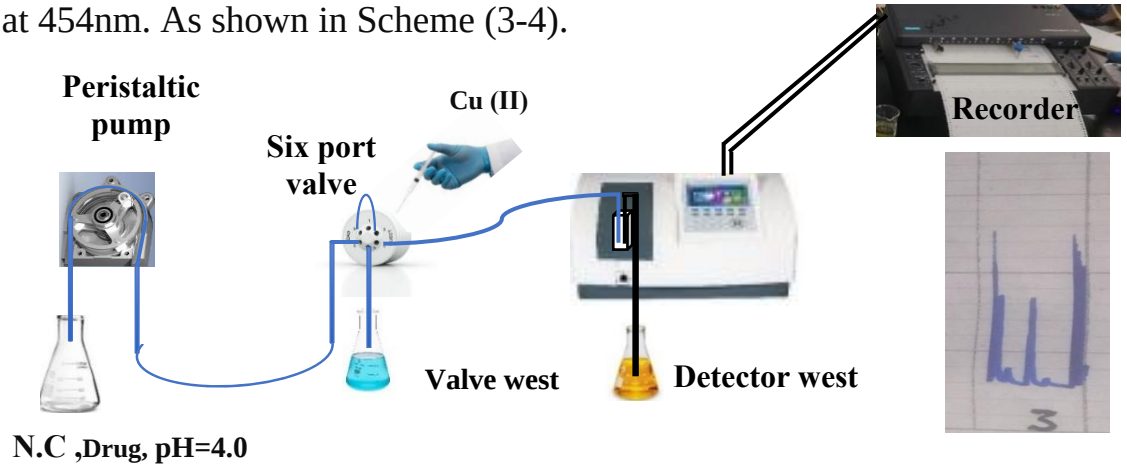
In the second model designed, In the step (1), the neocuproine react with mefenamic acid in an acidic solution. Then Cu(II) injected in loop of valve in step (2), whose absorbance is measured at the maximum wavelength at 454 nm. As shown in Scheme (3-3).



Scheme (3-3) Optimum system design for the estimation of mefenamic acid Model (2) Cu (II)=1×10^{-2} M, NC=1×10^{-3} M, MEF=50 µg/2mL.

3.12.3 Model Designed (3)

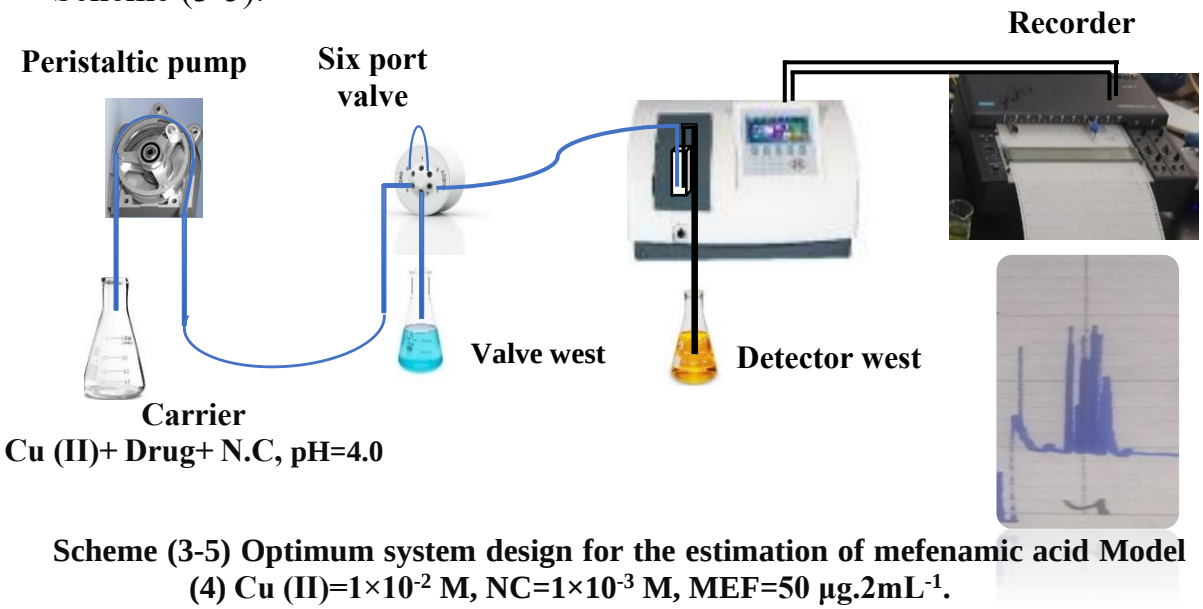
In the third model designed, In the step (1), the neocuproine react with mefenamic acid in an acidic solution. Then Cu(II) injected in loop of valve in step (2), whose absorbance is measured at the maximum wavelength at 454nm. As shown in Scheme (3-4).



Scheme (3-4) Optimum system design for the estimation of mefenamic acid Model (3) Cu (II)=1×10^{-2} M, N.C=1×10^{-3} M, MEF=50µg.2mL^{-1}.

3.12.4 Model Designed (4)

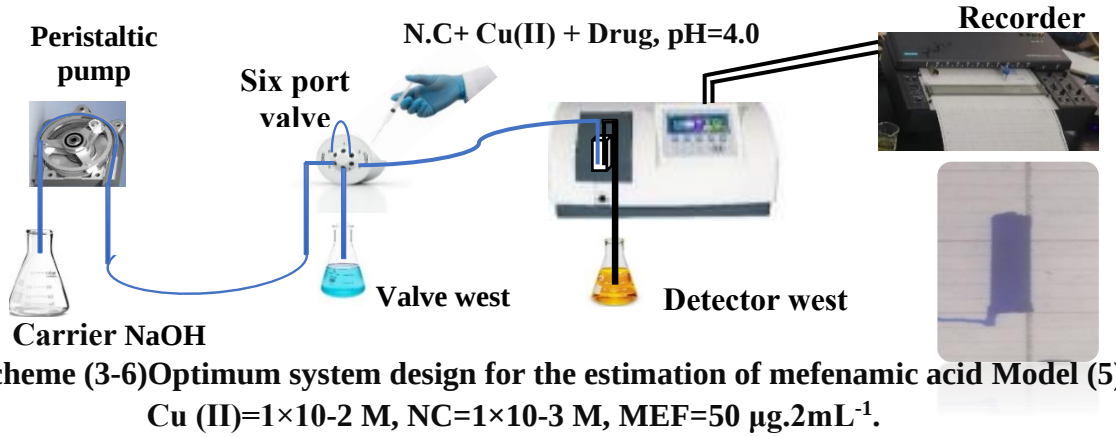
In forth flow injection system model designed, it is a one-step reaction in which the oxidation and reduction reaction is occurred between mefenamic acid and Cu (II) to form Cu(I) then the resultant reacted with Neocuproine reagent to form a yellow-orange color complex of Cu (I)-Neocuproine which absorb the light at 454 nm. As shown in Scheme (3-5).



Scheme (3-5) Optimum system design for the estimation of mefenamic acid Model (4) Cu (II)=1×10^{-2} M, NC=1×10^{-3} M, MEF=50 µg.2mL^{-1}.

3.12.5 Model Designed (5)

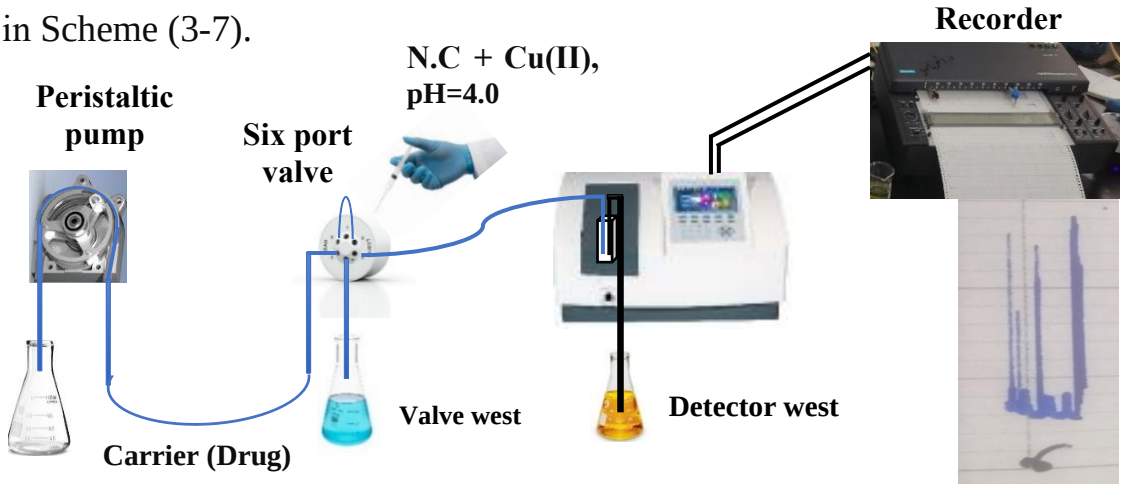
In fifth flow injection system model designed, the reaction is occurred between mefenamic acid and copper (II) and Neocuproine reagent, these three-reactant mixed together and injected in loop of valve, and NaOH consider as Carrier solution, the resultant is a yellow-orange color complex which absorb the light at 454 nm. As shown in Scheme (3-6).



Scheme (3-6)Optimum system design for the estimation of mefenamic acid Model (5) Cu (II)=1×10-2 M, NC=1×10-3 M, MEF=50 µg.2mL^{-1}.

3.12.6 Model Designed (6)

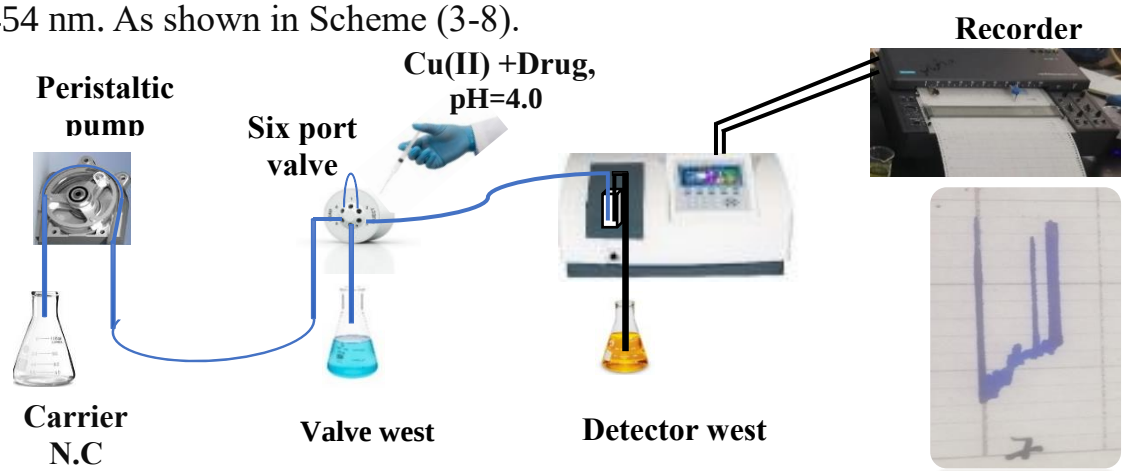
the sixth flow injection system model designed are two step reaction in which mefenamic acid consider as carrier solution, in first step the mefenamic acid is pumped through peristaltic pump as carrier solution, in the second step the Neocuproine reagent and copper (II) is mixed in an acidic solution and injected in loop of valve, the resultant is a yellow-orange colour of Cu(I)-Neocuproine complex which absorb the light at 454 nm. As shown in Scheme (3-7).



Scheme (3-7) Optimum system design for the estimation of mefenamic acid Model (6) Cu (II)=1×10^{-2} M, NC=1×10^{-3} M, MEF=50µg.2mL^{-1}.

3.12.7 Model Designed (7)

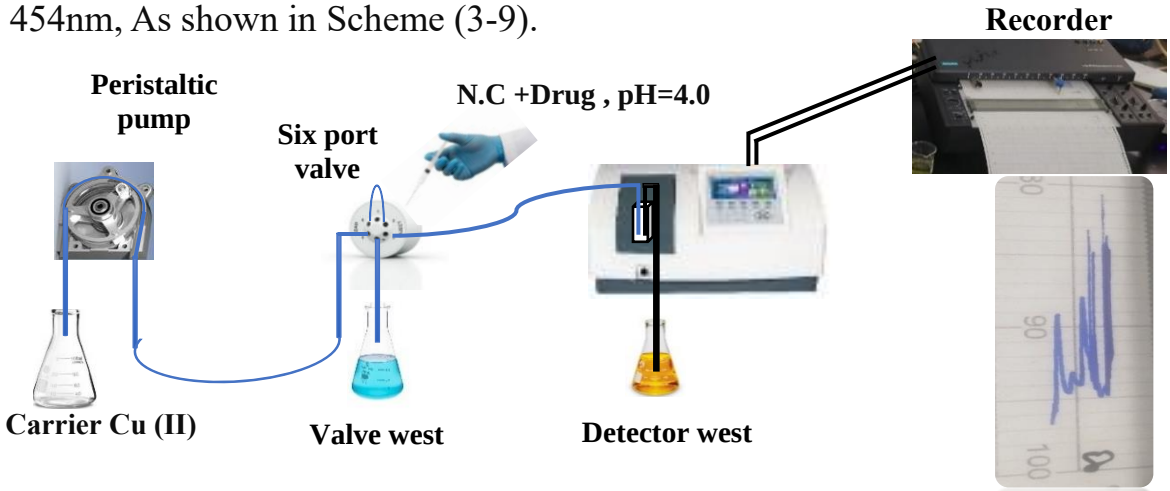
In step (1), the neocuproine reagent consider as carrier solution which is pumped through peristaltic pump, then in second step the metal ions Cu (II) is reduced by the action of mefenamic acid to Cu(I) which is act as reducing agent and then injected into loop of valve. However, the resultant complex Cu(I)-Neocuproine Color Complex, which absorb the light at 454 nm. As shown in Scheme (3-8).



Scheme (3-8) Optimum system design for the estimation of mefenamic acid Model(7) Cu (II)=1×10^{-2} M, NC=1×10^{-3} M, MEF=50 µg.2mL^{-1}.

3.12.8 Model Designed (8)

In the third model designed, In the step (1), the Cu(II) consider as carrier solution which is pumped through peristaltic pump, then in the second step the reaction is accrue between neocuproine and mefenamic acid in an acidic solution then injected in loop of valve then the absorbance read at 454nm, As shown in Scheme (3-9).



Scheme (3-9) Optimum system design for the estimation of mefenamic acid Model (8) Cu (II)=1×10^{-2} M, NC=1×10^{-3} M, MEF=50 µg.2mL^{-1}.

As shown above in Scheme (3-2 to 3-9) and according to resultant peaks height, the optimum system's design for the estimation of mefenamic acid Model Designed (1) in Scheme (3-2) which give best peak height (3.4 cm).

3.13 Stages of the system's operation for determining mefenamic acid

3.13.1 The first phase

Start the first phase of the work of multiple peristaltic pump channels operating on the pumping carrier solution containing copper (II) and Neocuproine in which they're pumping them across their own channel to the system through the loop of the sample at the injection valve for creating copper (I)-Neocuproine complex, subsequently to a cell output and finally to the waste stream (Waste).

3.13.2 The Second Phase

The second phase typically consists of two phases of loading and injection, as seen below:

3.13.2.1 Loading phase

As the sample solution and other typical substances in the chemical reaction are loaded into the known loops of the valve (with known lengths and diameters), they are then emptied through the tube last linked to the valve gap. At the same time, another channel of the peristaltic pump attached to the inlet valve operates the recycling carrier stream continuously through the proper pipelines for paying the sample and other substances in the reaction to the exit port.

3.13.2.2 Injection phase

With the assistance of a peristaltic pump, the carrier solution [Cu(II) and Neocuproine] moves through the sample (Mefenamic acid) in a loop of the valve loaded as being pushed for the sample to the detector. As shown in image (3-1).

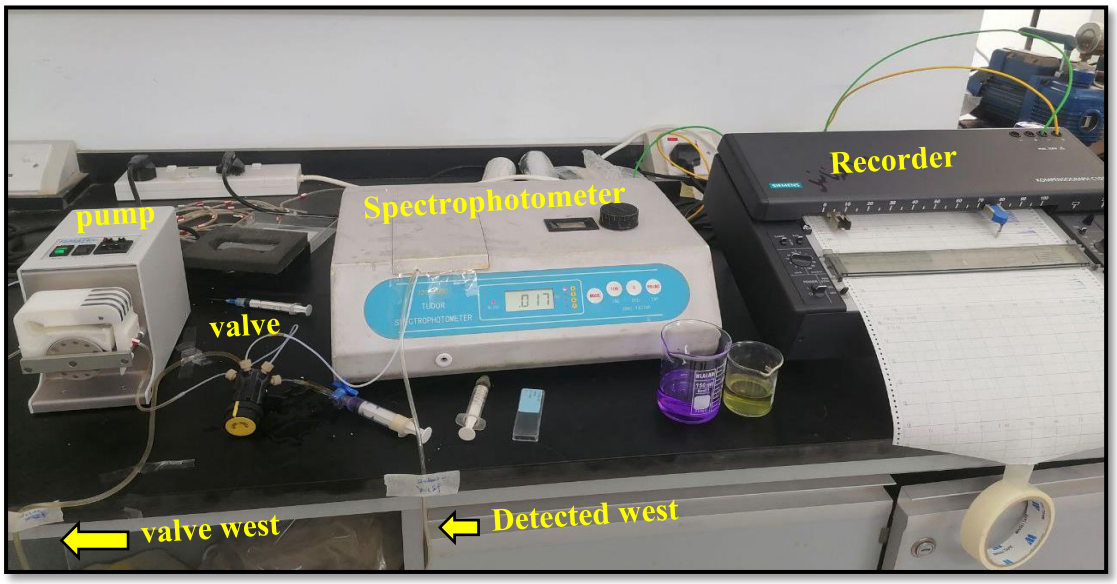


Image (3-1) Flow Injection Analysis System That Made in Our Laboratory.

3.14 Optimization of Experimental Conditions

To determine the best potential experimental conditions for the experiment, we monitored a single parameter and its influence on the absorbance of the colored species.

3.14.1 The Effect of Flow Rate

The effect of flow rate on the production of the colored product was investigated by changing the flow rate (1.5 - 4.0) mL/min and calculating the absorption of the colored product generated as it is shown in Table (3-18) and Figures (3-5,6).

Table (3-18) The effect of changing flow rate on formation of complex

Pump Speed	Flow Rate ($mL.min^{-1}$)	Peak height(cm)
10	1.5	3.0
20	2.3	4.3
30	2.5	2.8
40	2.6	2.7
50	3.1	2.5
60	3.9	2.4
70	4.0	2.3

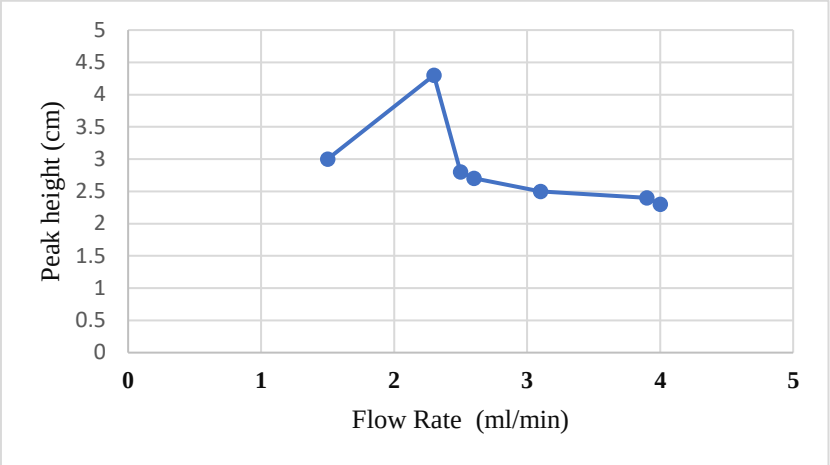


Figure (3-5) Effect of flow rate on the response, λmax= 454 nm, NC.Conc. = 50 µg.mL^{-1} pH=4.0, Cu(II)Conc. 350 µg.mL^{-1} Flow rate =1.5-4.0 mL/min, MEF Conc.=50 µg.2mL^{-1}

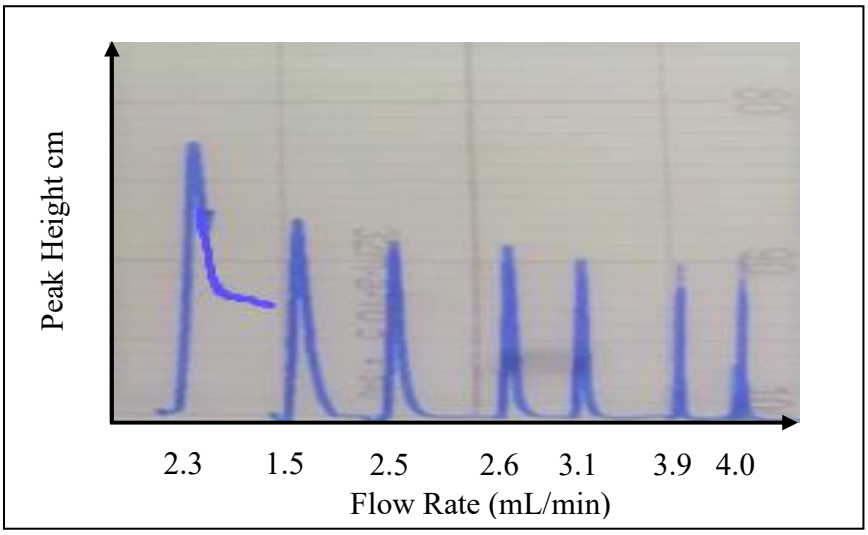


Figure (3-6) Effect of flow rate on the peak response

The study revealed that the first broad peack from the base have the highest peack but we discard here because incomplete mixing. However, the ideal flow rate was equal to 2.3 mL/min with peak height equal to 4.3 cm, which produces the best beak form and the most absorption, two were chosen above the other possibilities due to the superior time needed by speed.

3.14.2 The influence of pH changes

To investigate the effect of pH (1.0-9.0) on peak height for colored complex, various amounts of hydrochloric acid and sodium hydroxide solutions were added to an aliquot of solution containing 50 µg.2mL^{-1} of MFA as shown in Figures (3-7,8)

Table (3-19) The effect of pH changing on formation of complex

pH	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0
Peak height(cm)	2.0	2.5	3.9	5.6	4.1	3.0	2.2	1.50	0.5

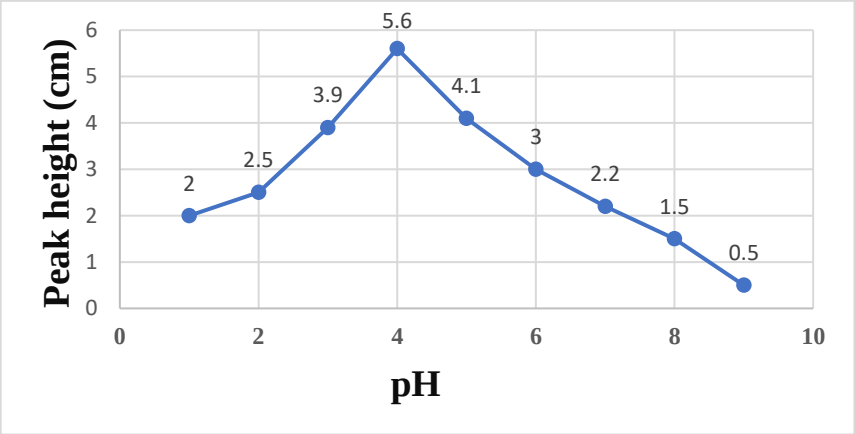


Figure (3-7) The Effect of pH changes, λmax= 454nm, NC.Conc. = 50 µg.mL^{-1} ,pH=1.0-9.0, Cu(II)Conc. =350 µg.mL^{-1} ,Flow rate =2.3 mL/min, MEF Conc.=50 µg.2mL^{-1}

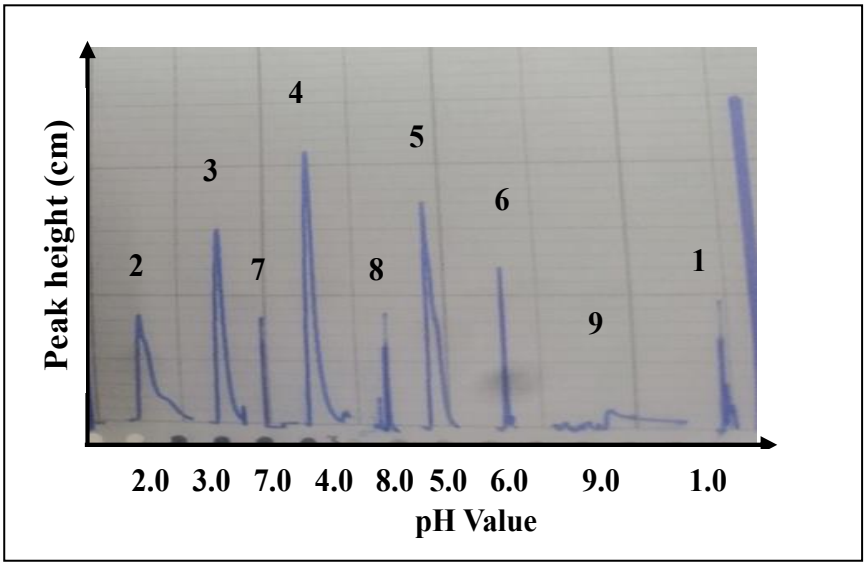


Figure (3-8) The Effect of pH changes

as shown in Table (3-19) and Figure (3-7,8), The optimum pH with higher absorption intensity at peak height 5.6 cm at pH 4.0 due to good intensity [121].

3.14.3 Effect of Cu (II) Concentration

The Effect of copper (II) concentration (50-500) µg.mL^{-1} on complex's absorbance was studied.

It was observed that 350 µg.mL^{-1} of Cu(II) gave the highest absorption with peak height 6 cm, which is strongly suggested for experimental procedures [79] as shown in Table (3-20) and Figures (3-9,10)

Table (3-20) Effect of Cu (II) Concentration On formation of complex

Cu(II) Conc. µg.mL^{-1}	50.0	100.0	150.0	200.0	250.0	300.0	350.0	400.0	450.0	500.0
Peak Height(cm)	1.8	2.5	3.0	3.8	4.5	5.2	6.0	2.3	2.2	0.8

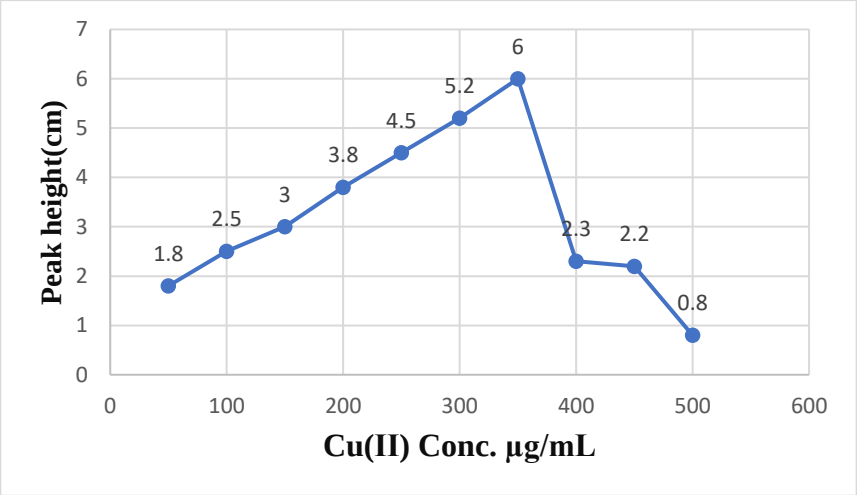


Figure (3-9) The Effect of Cu (II) concentration, λmax= 454nm, NC.Conc. = 50 µg.mL^{-1}, Cu(II)Conc. 50-500 µg.mL^{-1}, pH=4.0,Flow rate =2.3 mL/min, MEF Conc.=50 µg.2mL^{-1}

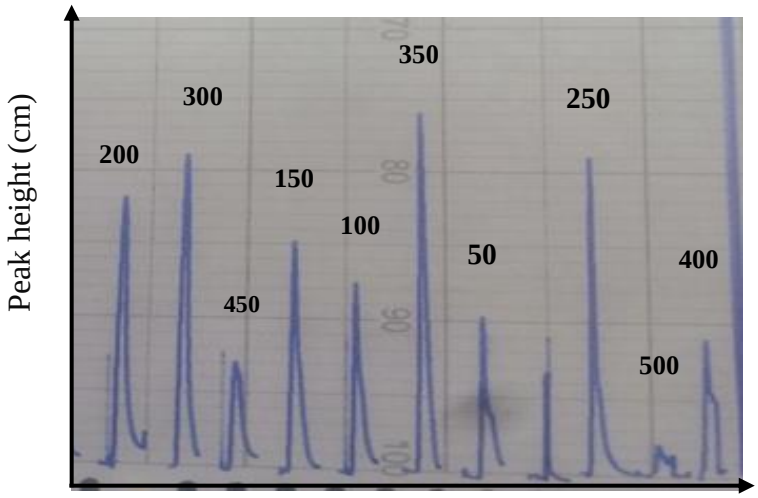


Figure (3-10) The Effect of Cu(II) concentration on peak Hight

as shown above the optimum Cu(II) Conc µg.mL^{-1} with higher absorption intensity at peak height 6.0 cm due to good intensity [123]

3.14.4 The Influence of Neocuproine Reagent Concentration

The impact of adjusting the reagent concentrations on the reaction was investigated in this study. Data was collected using a variety of Neocuproine reagent concentration (50-500) µg.mL^{-1}, However, it was also noted that high concentrations cause a decrease in response. However, this was possibly caused by an increase in particle density, which could have resulted in the buildup of precipitate particles in front of the detector as seen in Table (3-21) and Figures (3-11,12).

Table (3-21) The Effect of Neocuproine Concentration Changing on Formation of Complex

Neocuproine. Conc. (µg.mL-1)	50.0	100.0	150.0	200.0	250.0	300.0	350.0	400.0	450.0	500.0
Peak height(cm)	6.00	1.40	1.50	2.30	2.50	2.45	3.60	3.30	2.50	4.00

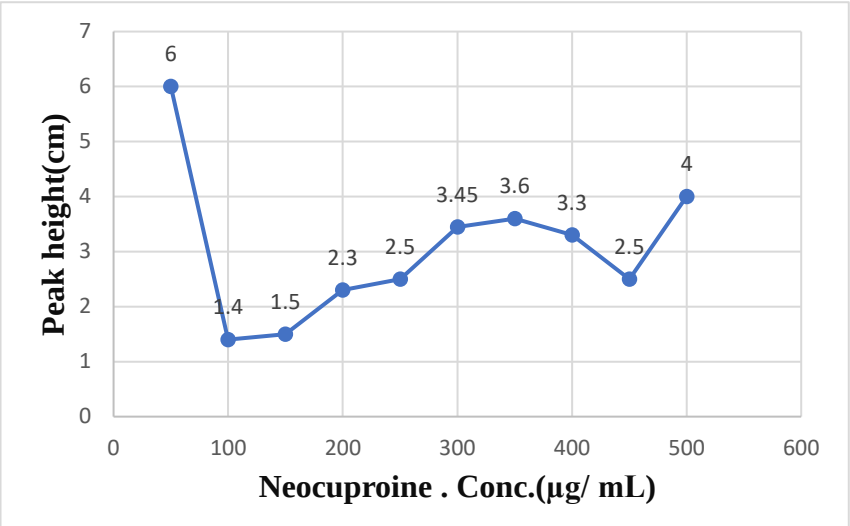


Figure (3-11) The Effect of Neocuproine reagent concentration, λmax= 454 nm, NC.Conc. = 50-500 µg.mL^{-1}, Cu(II)Conc. 350 µg.mL^{-1}, pH=4.0, Flow rate =2.3 mL/min, MEF Conc.=50 µg.2mL^{-1}

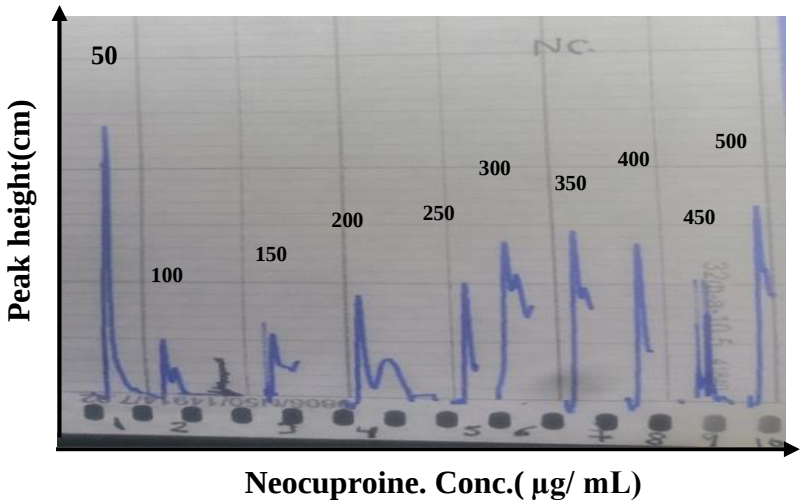


Figure (3-12) The Effect of Neocuproine reagent concentration (µg.mL^{-1}) on peak Hight

The findings indicated that the optimal absorption occurs when the reagent concentration is 50 µg.mL^{-1} ,This could occur at high concentration of neocuproine the absorbance decrease because the reagent may be not completely soluble in the solvent, or it might be because the completeness or sufficiency of the coordination failed of the ion in the reagent as shown above and at Hight concentration the dispersion will appeared [123].

3.15 Standard calibration curve for mefenamic acid in aqueous solution

This is done by preparation a series of solutions containing the concentrations in the range from (1.0 to 80.0) µg.2mL^{-1} and by using the optimum conditions which is used in this project. The results we are getting shown in Table (3-22) and Figures (3-13,14).

Table (3-22) Standard calibration curve for mefenamic acid

Drug Conc. (µg.2mL^{-1})	Peak height (cm)	Drug Conc. (µg.2mL^{-1})	Peak height (cm)
1.0	0.1	30.0	2.2
2.0	0.2	35.0	2.5
4.0	0.3	40.0	2.9
5.0	0.4	45.0	3.2
10.0	0.7	50.0	3.6
15.0	1.1	60.0	4.3
20.0	1.4	70.0	5.0
25.0	1.8	80.0	5.7

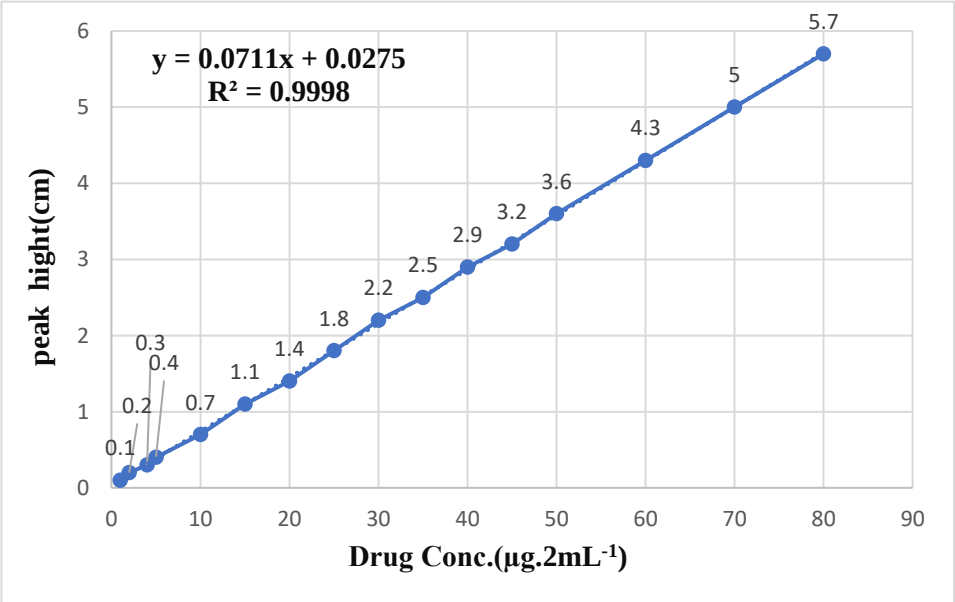


Figure (3-13) Standard Calibration curve of Mefenamic acid, λmax= 454 nm, NC.Conc. = 50 µg.mL^{-1} pH=4.0 , Cu(II)Conc. 350 µg.mL^{-1},Flow rate =2.3 mL/min, MEF Conc.=1.0-80.0 µg.2mL^{-1}

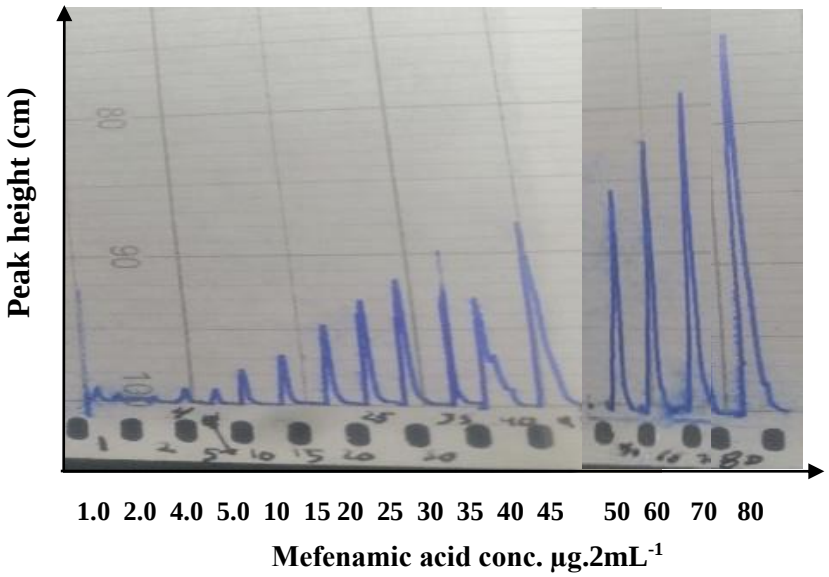


Figure (3-14) standard Calibration curve of Mefenamic acid

3.16 Rate of Sample Analysis

The number of analyzed samples per hour was calculated after establishing the optimal conditions for the reaction to determine the reaction time and the speed of the proposed method.[129] However, This was done by calculating the time elapsed from sample injection until the appearance of the peak. It was found that the time required to reach the maximum peak was 20 seconds. Therefore, this method is capable of analyzing 180 samples per hour. Table (3-23) lists the ideal circumstances and statistical analyses for the suggested approach.

Table (3-23) The Optimum conditions and statistical features for the proposed method

Analytical Data	Value
λ_{max}	454 nm
Flow Rate	2.3 mL/min
pH of carrier solution	4.0
Mefenamic acid Concentration	50 µg.2mL^{-1}
Neocuproine Concentration	50 µg.mL^{-1}
Cu(II) Concentration	350 µg.mL^{-1}
Linearity range	1.00-80.00 µg.2mL^{-1}
Regression equation	y = 0.0711x + 0.0275
Slope	0.0711
Intercept	0.0275
Colour	Yellow-orange
Linearity coefficient (R^2)	R^2 = 0.9998

3.17 Repeatability[130]

We discovered the repeatability of the suggested method by doing multiple tests on 6 sample solutions containing 50 µg.2mL^{-1} of mefenamic acid, the result which gotten was highly degree of repeatability and extremely excellent as shown in Table 3-24 and Figure (3-15).

Table (3-24) Repeatability of Mefenamic acid

Drug Conc. (µg.2mL^{-1})	Peak Height (Cm)						Mean $\overline{X}$	SD	RSD%
50	3.7	3.8	3.7	3.7	3.8	3.7	3.73	0.0471	1.2638

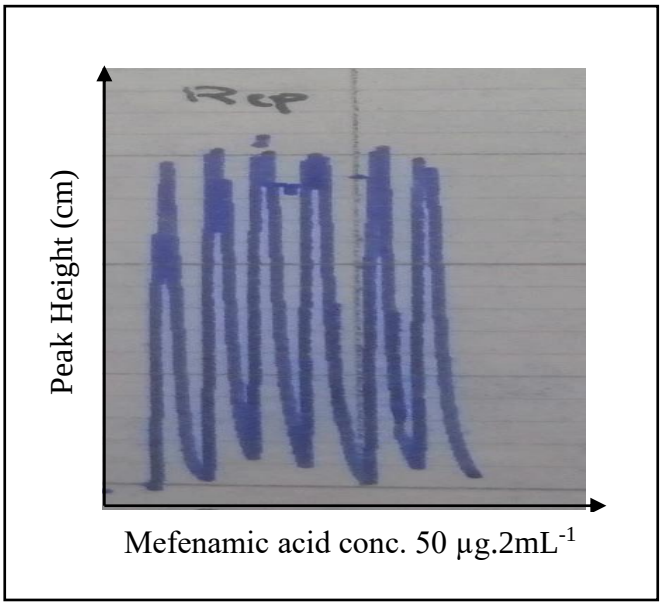


Figure (3-15) Depicted the repeatability of mefenamic acid , λmax= 454nm, NC.Conc. = 50 µg.mL^{-1} , pH=4.0 ,Cu(II)Conc. 350 µg.mL^{-1}, Flow rate =2.3 mL/min, MEF Conc.=50 µg.2mL^{-1}

3.18 Dispersion of Mefenamic Acid

dispersion of mefenamic acid is done by doing two experiments to estimate the dispersion value of MEF present in (50µg.2mL^{-1} and 70µg.2mL^{-1}). First, Mefenamic acid with concentration (50 µg.2mL^{-1}),The current experiment represents the sample's intensity response that passes into the investigation (H_{max})[131]. the two reactants (mefenamic acid and Neocuproine) have been mixed and then passed into a manifold unit, and the result shows no dispersion effect due to convection or diffusions.[133-134] This illustration depicts (H^0). It is possible to compute dispersion (D) by applying dispersions equation as shown: ($D^\circ = H^0/H_{max}$) ,D= 1.057 and by doing the same procedure for the second mefenamic acid concentration (70 µg.2mL^{-1}) we get dispersion value equal to D= 1.081 under optimum conditions[135],λmax=454nm,NC.Conc.=50µg.2mL^{-1},pH=4.0 ,Cu(II)Conc. 350 µg.mL^{-1}, Flow rate =2.3 mL/min, MEF Conc.=50,70 µg.2mL^{-1} as shown below in Table (3-25) and Figure (3-16).

Table (3-25) Dispersions of mefenamic acid at optimum conditions

Drug Conc. ($\mu g.2mL^{-1}$)	Peak Height (cm)		Dispersion(D)
	H^0	H_{max}	
70	5.3	4.9	1.081
50	3.7	3.5	1.057

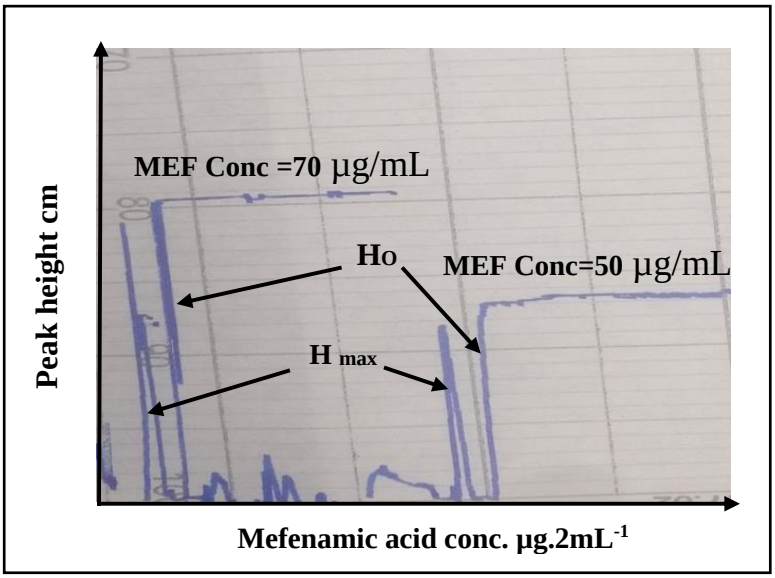


Figure (3-16) Dispersion of mefenamic acid, λmax= 454nm,NC.Conc. = 50 $\mu g.mL^{-1}$, pH=4.0 , Cu(II)Conc=350 $\mu g.mL^{-1}$, Flow rate =2.3 mL/min, MEF Conc.=50,70 $\mu g.2mL^{-1}$

3.19 Studying The Dead Volume

The dead volume define as the volume of substance held in the FIA system before the flow cell makes up. To guarantee that this unit produces reliable findings, the dead volume must be evaluated. Wherever the dead volume is low, the outcome will be better[136].However, Three tests have been conducted, In the first experiment, water was used in the loop instead of neocuproine as carrier solution, and no reaction occurred, In the second experiment, water was used as the carrier rather than the Cu(II), and no reaction occurred,in third experiment water was used in the loop instead of mefenamic acid, This demonstrates the system's effectiveness, as seen in Figure (3-17)

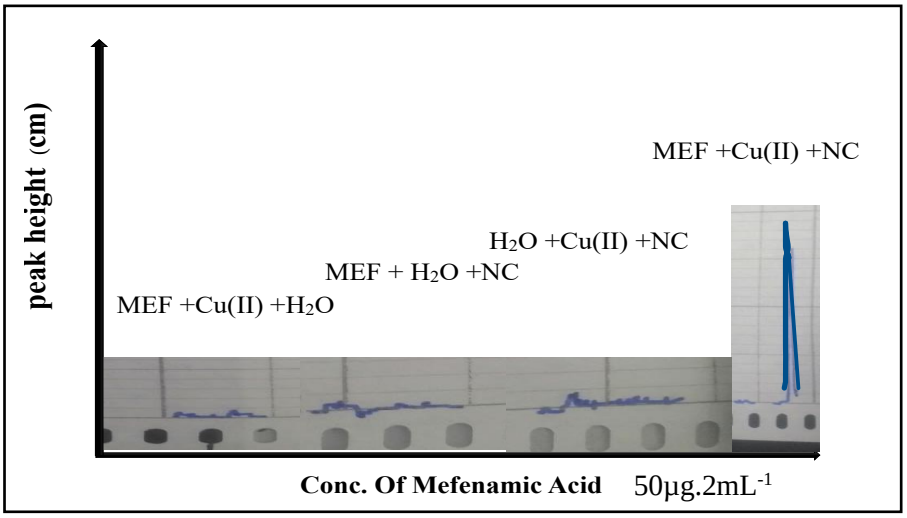


Figure (3-17) Dead Volume's Influence on response times, λmax= 454nm, NC.Conc.=50µg.mL^{-1},pH=4.0,Cu(II)Conc=350µg.mL^{-1},Flow rate =2.3 mL.min^{-1}, MEF Conc.=50,70 µg.2mL^{-1}

3.20 Accuracy and Precision

By applying five different concentrations of standard solutions of mefenamic acid, the accuracy and precision of the suggested procedure for the determination of mefenamic acid by flow injection technique have been investigated under ideal conditions. Table (3-26) provides the results of calculating the E%, Rec.%, and RSD% of five readings of each of five distinct concentrations (10, 20,30, 40, 50 µg.2mL^{-1}) using equations (3-1) (3-2) and (3-3). The findings demonstrate good accuracy and precision.[137]

Table (3-26) Accuracy and Precision for proposed method by flow injection technique

Concentration of MFA µg.2mL^{-1}		**RSD%**	**Eerror%**	**Rec%**
Present	**Found**			
10.0	9.93	0.0235	-0.70	99.30
20.0	19.91	0.0173	-0.54	99.46
30.0	29.95	0.0057	-0.16	99.83
40.0	39.98	0.0057	-0.05	99.95
50.0	49.97	0.0115	-0.06	99.94

3.21 Limit of Detection and Limit of Quantification

The limits of detection (LOD) and quantitation (LOQ) were assessed as equation (3-4) and (3-5)and Table (3-27) below.[138]

Table (3-27) Limit of Detection and Limit of Quantification

Peak height (cm)	SD	LOD µg.mL^{-1}	LOQ µg.mL^{-1}
3.60	0.0047	0.1983	0.6543

* n=3

3.22 Application of Mefenamic Acid in aqueous Solutions

The unknown concentrations of aqueous solutions of mefenamic acid can determined, three unknown aqueous solutions where prepared to determine concentrations. Then, measured the absorbance according to the optimum conditions that used in this study, as shown in Table (3-28) and Figure (3-18).

Table (3-28) Application of Mefenamic Acid in aqueous Solutions

Concentration of MFA µg.2mL^{-1}		Eerror%	Rec%	RSD%
present	found			
10.0	10.10	1.00	101.00	0.5716
20.0	19.94	-0.30	99.70	0.1808
30.0	29.95	-0.16	99.84	0.0578

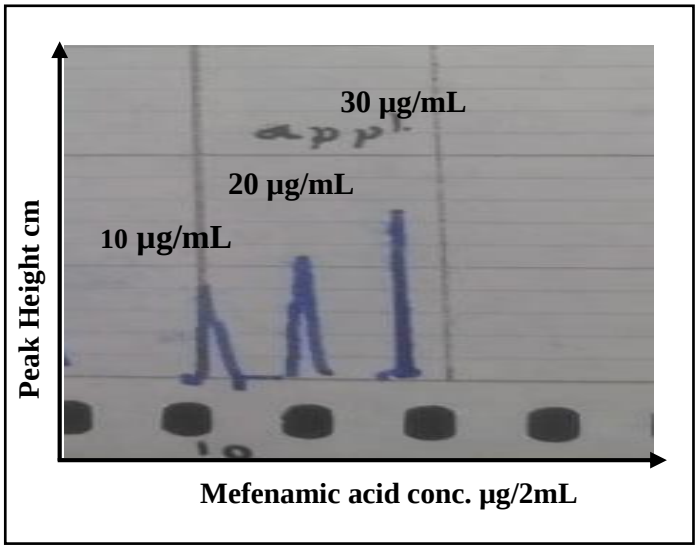


Figure (3-18) Application of MEF in unknown aqueous Solutions sample, λmax= 454 nm, NC.Conc. = 50 µg.mL^{-1}, pH=4.0 , Cu(II)Conc.=350 µg.mL^{-1}, Flow rate =2.3 mL.min^{-1},MEF Conc.=10,20,30 µg.2mL^{-1}

3.23 Application of Mefenamic Acid in pharmaceutical dosage form

The applicability of the proposed flow injection procedure was tested by commercial dosage form from different brands.in addition, the result shows that finding where to be constant as showed in label of pharmaceutical dosage form, as shown in Table (3-29) and Figures (3-19,20)

Table (3-29) Analysis of mefenamic acid in capsule formulations

Drug type	Conc. µg/2mL		Error %	Rec%	RSD%
	present	found			
Ponstidin Capsule 250mg	20.0	20.03	0.15	100.15	0.3036
	30.0	29.95	-0.16	99.84	0.0333
	40.0	39.97	-0.75	99.25	0.0382
	50.0	49.98	-0.04	99.96	0.0200
Mefril 250 mg	20.0	19.91	-0.45	99.55	0.1506
	30.0	29.94	-0.20	99.8	0.0192
	40.0	39.98	-0.05	99.95	0.0250
	50.0	49.97	-0.06	99.94	0.0400

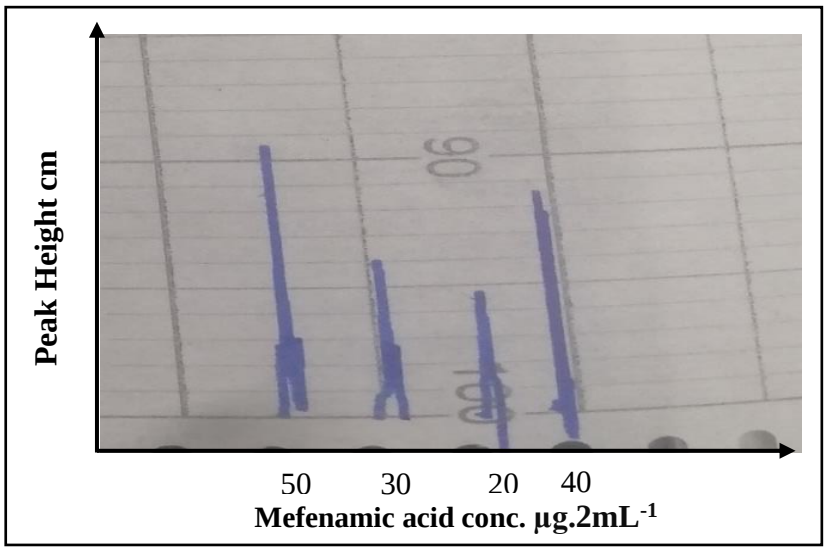


Figure (3-19) Analysis of mefenamic acid in capsule formulations Ponstidin Capsule (250 mg) , λmax= 454nm, NC.Conc. = 50 µg.mL^{-1}, pH=4.0, Cu(II)Conc. =350 µg.mL^{-1} Flow rate =2.3 mL/min, MEF Conc.=20,30,40,50 µg.2mL^{-1}

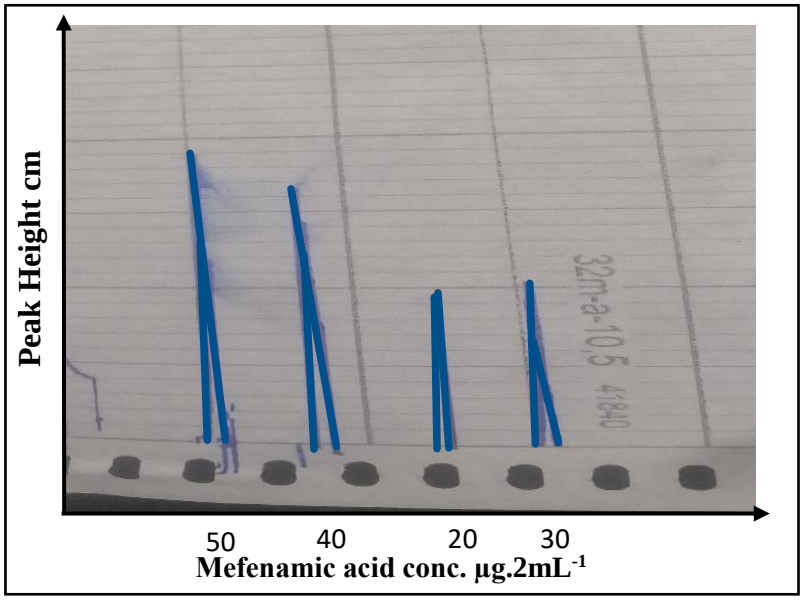


Figure (3-20) Analysis of mefenamic acid in capsule formulations mefril Capsule (250 mg), λmax= 454nm,NC.Conc.=50 µg.mL^{-1}, pH=4.0, Cu(II)Conc. =350 µg.mL^{-1} Flow rate =2.3 mL/min, MEF Conc.=20,30,40,50 µg.2mL^{-1}

3.24 Results Comparison of two Systems

The physical and chemical properties measured by using the new systems are summarized in Table 3-30.

Table (3-30) Results comparison of two systems

Analytical Data	Spectrophotometric Method for Cu (II)	Flow Injection Analysis Method for Cu(II)
λmax	454nm	454 nm
Flow Rate	.....	2.3 mL/min
Regression equation	y = 0.0042x - 0.0148	y = 0.0711x + 0.0275
Cu(II) Concentration	1×10^{-2} M	5.5×10^{-3} M
Neocuproine Concentration	1×10^{-3} M	2.4×10^{-4} M
Mefenamic acid concentration	50 µg.2mL^{-1}	50 µg.2mL^{-1}
pH	4.0	4.0
Temperature	25 C^{O}	25 C^{O}
Rate of Sample Analysis	12 sample.h^{-1}	180 sample.h^{-1}
Slope	0.0042	0.0711
Intercept	0.0148	0.0275
Linearity Range	5.0-60.0 µg.2mL^{-1}	1.00-80.00 µg.2mL^{-1}
Linearity coefficient (R^2)	0.9999	0.9998
RSD % at (10.0-50.0 µg/mL)	0.037-0.500	0.0235-0.0115
Error %	(-0.5)-(-0.32)	(-0.7)-(-0.06)
Recovery %	99.50-100.32	99.30-99.94
LOD	0.7142 µg.mL^{-1}	0.1983 µg.mL^{-1}
LOQ	2.3568 µg.mL^{-1}	0.6543 µg.mL^{-1}
colour	Yellow-Orange	Yellow-Orange

The flow injection analysis system for determination of mefenamic acid by using Cu (II) in aqueous solutions and pharmaceutical dosage form gave best value of Cu (II) Concentration($\mu g.mL^{-1}$), Neocuproine Concentration ($\mu g.mL^{-1}$), Sampling speed, Linearity Range, Recovery%, relative standard deviation RSD%, LOD, LOQ rather than Spectrophotometric innovate system.

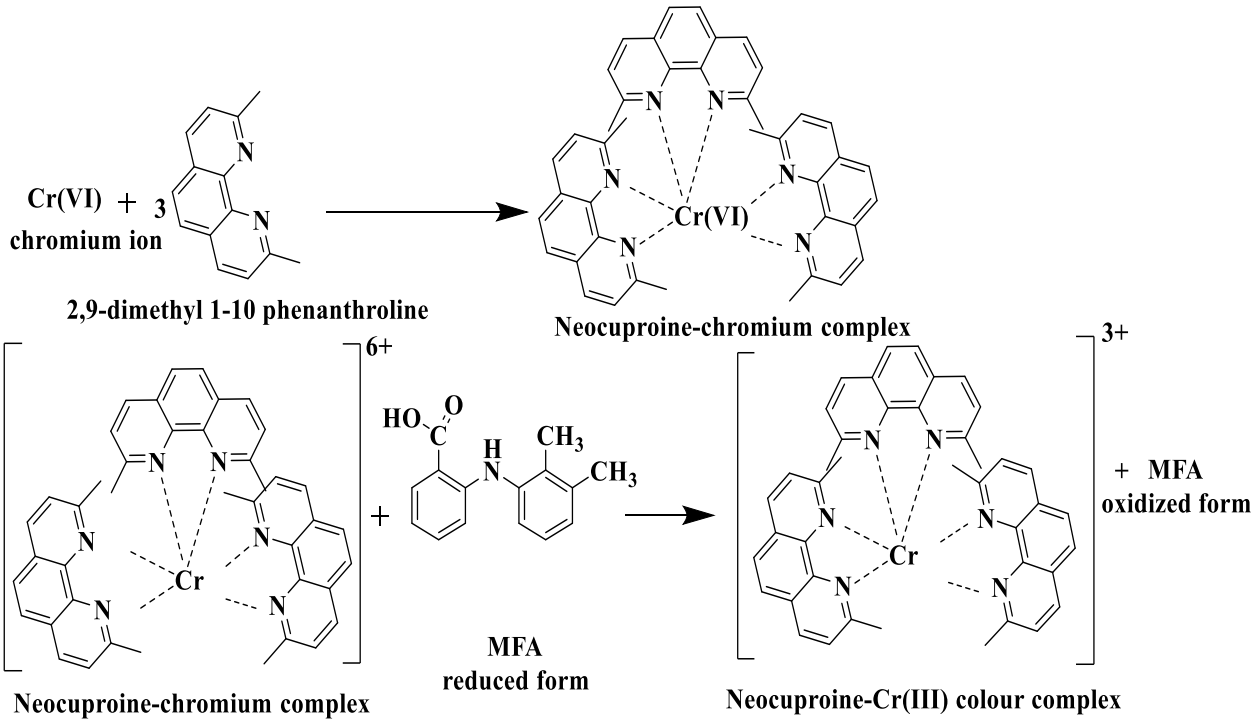
The Spectrophotometric system for determination of mefenamic acid by using Cu (II) in aqueous solutions and pharmaceutical dosage form gave best value of linearity coefficient (R^2), Error% rather than flow injection analysis innovate system.

The Spectrophotometric system for determination of mefenamic acid by using Cu (II) give limit of detection μg/mL, linear range μg/mL, Error%, SD, RSD% better than the results of literatures. [38-48]

Part Three: Spectrophotometric Method for Determination of Mefenamic Acid by using Chromium (VI)

3.25 Reaction principle Concept

The type of chemical system employed and the designed system for it are the key for visible spectrophotometric method for determination of mefenamic acid by using Chromium (VI). In order to identify mefenamic acid we will use the following reaction which describe below in Scheme (3-10)



Scheme (3-10) Schematic diagram for proposed method in presence of Cr(VI)

The current work employs as a novel method for determination of MEF. However, when the Cr(VI) reacted with Neocuproine reagent,to form Cr(VI)-Neocuproine, this formed complex will react with mefenamic acid (reduced form) to produce Cr (III)-Neocuproine complex at pH =6.0.

In the previously mentioned process, it is an indirect method with a two-step reaction designed to determine mefenamic acid.

3.26 Determination of Maximum Wavelength (λ_{max})

To determine the appropriate λ_{max}, an aliquot of (3.0 mL) 2,9-DMP solution 1×10^{-2} M was added to (1.0mL) of Chromium(VI) solution $1x10^{-3}$ M then the pH was adjusted in (pH=6.0), then (2ml) of standard solution 50 µg.2mL^{-1} of MFA was transferred to (10 mL) volumetric flask, The contents diluted to (10.0 mL) with deionized water, subsequently; absorbance of yellow-green product's was measured against to a reagent blank in the 380–1100 nm range. The maximum wavelength of MFA's absorption was observed to be 430 nm Figure (3-21). Each reagent blank displayed a negligible absorbance at the relevant λ_{max} under the testing conditions.

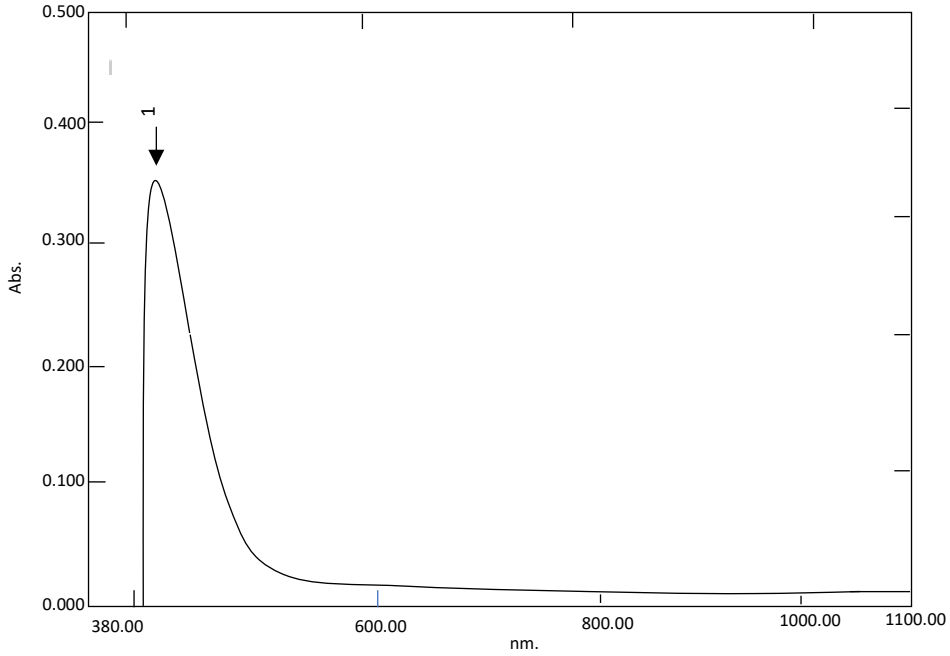


Figure (3-21) UV. Vis spectra of Reaction product (MFA=50 µg.2mL^{-1} complex) aganst blank.

3.27 Study of optimum Parameters

3.27.1 pH effect

Different volumes 0.0-2.0 mL of 0.001M standardized hydrochloric acid and 0.04 M standardized sodium hydroxide solution was added to an aliquot of solution containing 50 µg.2mL^{-1}of MFA to examine the effect of pH on colored complex. Absorption intensity for yellow-green complex compounds was measured at 430 nm. However, the system conditions were as follows:

1) 3.0 mL of Neocuproine (1x10^{-2}) M
2) 1.0 mL of Cr(VI) (3.0×10^{-3}) M
3) pH (1.0-10.0)
4) MEF Conc.=50 µg.2mL^{-1}
5) Temperature (25 C^{o})

Table (3-31) pH Effect on absorption of complex formation

pH	Absorbance
1.0	0.118
2.0	0.163
3.0	0.176
4.0	0.286
5.0	0.325
6.0	**0.345**
7.0	0.332
8.0	0.249
9.0	0.222
10.0	0.173

The results of the study show revealed that the absorbance increases up to pH of 6.0 then it begins to descend gradually, due to the formation of metal hydroxides as shown in Table (3-31).

3.27.2 Effect of Type of Buffer Solution

Six buffer solutions of pH 6.0 with different composition have been tested, ammonium acetate - glacial acetic acid, phosphoric acid - dimethylamine, disodium hydrogen phosphate - citric acid, sodium dihydrogen phosphate - water, potassium dihydrogen phosphate - sodium hydroxide and disodium hydrogen phosphate – sodium chloride as shown in Table (3-32). However, the system conditions were as follows:

1) 3.0 mL of Neocuproine ($1x10^{-2}$) M
2) 1.0 mL of Cr(VI) (3.0×10^{-3}) M
3) pH (6.0)
4) MEF Conc.=50 $\mu g.2mL^{-1}$
5) Temperature (25 C^{o})

Table (3-32) Effect of different types of solutions on absorption of complex formation

Buffer solutions	Absorbance
Ammonium Acetate- glacial acetic acid	0.194
Phosphoric Acid - Dimethylamine	0.242
Disodium Hydrogen Phosphate - Citric Acid	0.250
Sodium Dihydrogen Phosphate - Water	0.275
Potassium Dihydrogen Phosphate - Sodium Hydroxide	0.252
Disodium Hydrogen Phosphate- Sodium Chloride	0.244

The results shown in Table (3-32) indicate that all types of buffer solutions decrease the intensity of the coloured complex.

3.27.3 Effect of Time

Under the ideal experimental conditions established, the influence of time on stability of colored compound for various concentrations of MFA was examined. the system conditions were as follows:

1) 3.0 mL of Neocuproine ($1x10^{-2}$) M
2) 1.0 mL of Cr(VI) (3.0×10^{-3}) M
3) pH (6.0)
4) MEF Conc.=50 $\mu g.2mL^{-1}$
5) Time (05-90 min)
6) Temperature (25 C^{O})

Table (3-33) The influence of time on absorption of complex formation

Time /min	Absorbance
05	0.344
10	0.343
20	0.343
30	0.343
40	0.343
50	0.342
60	0.342
70	0.341
80	0.341
90	0.341

From the result, formation of colour immediately appeared after 5-minute (after addition all reaction ingredients components) and the complex's absorbance intensity hold steady for at least (90 min) as shown below in Table (3-33)

The stability period is long enough from above to allow for the sequential performance of many measurements.

3.27.4 Effect of Temperature

The color intensity in the proposed approach was evaluated at various temperatures and the system conditions were as follows:

1) 3.0 mL of Neocuproine ($1x10^{-2}$) M
2) 1.0 mL of Cr(VI) (3.0×10^{-3}) M
3) pH (6.0)
4) Time (05 min)
5) Temperature (10-50 C^{o})
6) MEF Conc.=50 $\mu g.2mL^{-1}$

Table (3-34) Effect of temperature on absorption of complex formation

Temp C^{o}	**Absorbance**
10	0.098
15	0.125
20	0.214
25	**0.344**
30	0.337
35	0.228
40	0.101
45	0.092
50	0.045

The findings demonstrate that such absorbance values reaction maximum value at $25C^{o}$ then start to decrease with rising temperature duo to

decline in its stability or decomposition of complex at Hight temperature as shown in the result in Table (3-34).

3.27.5 Effect of concentration of Cr (VI)

The effect of Cr(VI) concentration on the absorbance was investigated in the range between 0.5×10^{-3} and 4×10^{-3} M, by employing the principle of any single change to one variable and the remaining all other variables as constants throughout this investigation, the system conditions were as follows:

1) 3.0 mL of Neocuproine ($1x10^{-2}$) M
2) 1.0 mL of Cr(VI) ($0.5x10^{-3}$ - $4.0\ x10^{-3}$) M
3) pH (6.0)
4) Time (05 min)
5) Temperature (25 C^{o})
6) MEF Conc.=50 µg.2mL^{-1}

Table (3-35) Concentration Effects of Cr(VI) on absorption of complex formation

Cr(VI) concentration	Absorbance
$0.5x10^{-3}$	0.123
$1.0\ x10^{-3}$	0.228
$1.5x10^{-3}$	0.238
$2.0\ x10^{-3}$	0.247
$2.5x10^{-3}$	0.257
$3.0\ x10^{-3}$	0.347
$3.5x10^{-3}$	0.194
$4.0\ x10^{-3}$	0.122

The results of the study show revealed that the absorbance increases as Cr(VI) concentration increases until it reaches $3.0x10^{-3}$ M, at then it starts to decrease. As a result, the optimum concentration of Cr(VI) was $3.0x10^{-3}$ M, as shown in Table (3-35).

3.27.6 Effect of Neocuproine Concentration

The effect of Neocuproine concentration on the absorbance was investigated in the range between 0.5×10^{-2} and 4.0×10^{-2} M, by employing the principle of any single change to one variable and the remaining all other variables as constants throughout this investigation, the system conditions were as follows:

1) 3.0 mL of Neocuproine (0.5×10^{-2} - 4×10^{-2}) M
2) 1.0 mL of Cr(VI) (3.0×10^{-3}) M
3) pH (6.0)
4) Time (05 min)
5) Temperature (25 C^{o})
6) MEF Conc.=50 $\mu g.2mL^{-1}$

Table (3-36) Effects of Neocuproine Concentration on absorption of complex formation

Neocuproine concentration (M)	Absorbance
$0.5x10^{-2}$	0.123
$1.0\ x10^{-2}$	0.347
$1.5x10^{-2}$	0.337
$2.0\ x10^{-2}$	0.247
$2.5x10^{-2}$	0.244
$3.0\ x10^{-2}$	0.228
$3.5x10^{-2}$	0.194
$4.0\ x10^{-2}$	0.122

The results of the study show revealed that the absorbance increases as Neocuproine concentration increases until it reaches $1x10^{-2}$ M, at then it starts to decrease. This could occur because the reagent may be not completely soluble in the solvent, or it might be because the completeness or sufficiency of the coordination failed of the ion in the reagent, as a result the optimum concentration of Neocuproine was $1x10^{-2}$ M, as shown in Table (3-36)

3.27.7 Effect of volume of Cr(VI)

The effect of volume of Cr(VI) on the absorbance was investigated in the range between (0.5 -4.0) mL, the system conditions were as follows:

1) 3.0 mL of Neocuproine (1.0×10^{-3}) M
2) (0.5 – 4.0) mL of Cr(VI) (3.0×10^{-3}) M
3) pH (6.0)
4) Time (05 min)
5) Temperature (25 C^{o})
6) MEF Conc.=50 $\mu g.2mL^{-1}$

Table (3-37) Volume Effects of Cr (VI) on absorption of complex formation

Volume of Cr(VI) mL	Absorbance
0.5	0.246
1.0	0.347
1.5	0.306
2.0	0.266
2.5	0.236
3.0	0.180
3.5	0.134
4.0	0.115

The results of the study show revealed that the absorbance increases as volume of Cr(VI) increases until it reaches 1.0 mL, at then it starts to decrease. As a result, the optimum of volume of Cr(VI) was 1.0 mL, as shown in Table (3-37).

3.27.8 Effect of Volume of Neocuproine Reagent

The influence of reagent volume on resultant-colored product was studied. Varying volumes of standard reagent solutions in the range (0.5-4.5 mL) were added and measuring the absorbances of the solutions, the system conditions were as follows:

1) (0.5 – 4.5) mL of Neocuproine (1.0×10^{-3}) M
2) 1.0 mL of Cr(VI) (3.0×10^{-3}) M
3) pH (6.0)
4) Time (05 min)
5) Temperature (25 C^{o})
6) MEF Conc.=50 $\mu g.2mL^{-1}$

Table (3-38) Effect of volume of 2,9.DMP reagent on absorption of complex formation

Volume of Neocuproine (mL)	Absorbance
0.5	0.127
1.0	0.188
1.5	0.235
2.0	0.298
2.5	0.339
3.0	0.349
3.5	0.252
4.0	0.168
4.5	0.154

The investigation showed that 3.0 mL of 2,9.DMP solution gave maximum absorbance due to its full intensity and further volume additions of reagent would produce in a systematic decrease in absorbance of colored

product, this is may be due to formation of new species .Similarly, may be attributed to the fact that high concentrations of neocuproine would result in interference with Cr(VI) which could have arisen from incomplete conversion of Cr(VI) into the Cr(III) –Neocuproine complex, as shown in Table (3-38).

3.27.9 Effect of Order Addition of Reactants

The reagent added sequence should really be accompanied to obtain good color intensity which led to best results. Otherwise, a loss in color intensity was seen, the system conditions were as follows:

1) 3.0 mL of Neocuproine (1.0×10^{-3}) M
2) 1.0 mL of Cr(VI) (3.0×10^{-3}) M
3) pH (6.0)
4) Time (05 min)
5) Temperature (25 C^{o})
6) MEF Conc.=50 $\mu g.2mL^{-1}$

Table (3-39) Effect of order of addition on absorption of complex formation

Sequence of addition	Mean
Cr(VI) + R+ pH 6.0+D	0.342
R + Cr(VI) + pH 6.0 +D	0.341
Cr(VI) + D+ pH 6.0 +R	0.338
R+D+ Cr(VI) +pH 6.0	0.196

In Which:

R: Neocuproine Reagent D: Mefenamic Acid Drug

The order of addition in all method gives good color intensity of formed complex which gives good absorbance except method 4 due to there is no formed complex or could be brought on by the interaction of metal with

acid and basic ions as illustrated in Table (3-39), Hence the optimum order of addition was Cr(VI) + R+ pH 6.0+D.

3.28 Standard Calibration Curve for Determination of Mefenamic Acid

When absorbance was taken at 430 nm for 10.0 mL series volumetric flasks containing an increasing amount of MFA 4.0-70 µg/2mL, 3.0 mL of 2,9.DMP 1.0×10^{-2} M, 1.0 mL Cr(VI) 3.0×10^{-3} M solution then adjust the pH 6.0, The flasks were then diluted to the mark with de ionized distilled water then mixed thoroughly, and the absorbance at 430 nm was measured toward the blank sample of reagents. Beer's law was followed in the concentration range 4.0-70 µg.2 mL^{-1} and the correlation coefficient obtained was 0.9998 at 430 nm as shown in Figure (3-22).

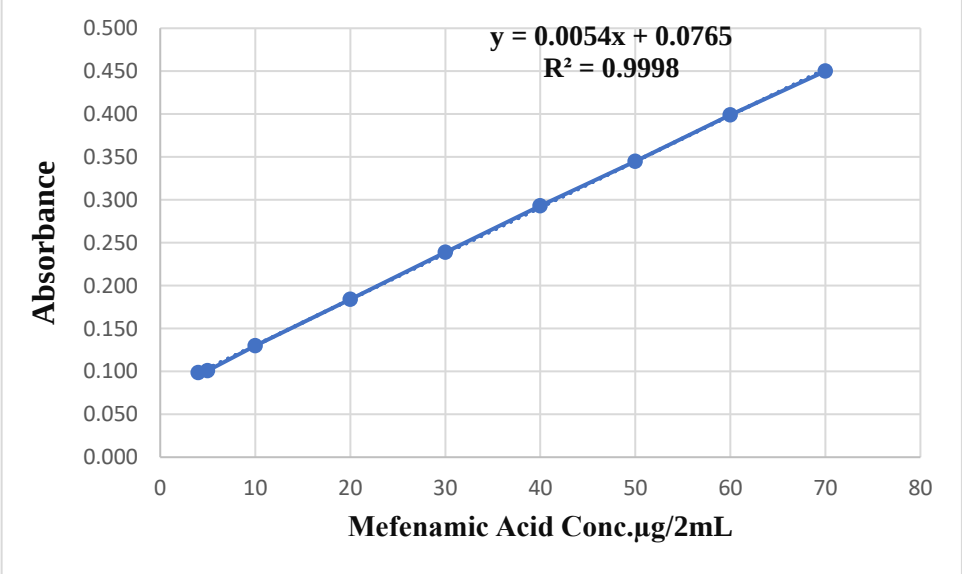


Figure (3-22) Calibration graph for determination of MFA

Table (3-40) lists the ideal circumstances and statistical analyses for the suggested approach.

Table (3-40) The Optical features and statistical information for suggested method's

Analytical Data	Value
λmax	430nm
Regression equation	y=0.0054x+0.0765
Cr(VI) concentration	3.0×10^{-3} M
Neocuproine concentration	1.0×10^{-3} M
Mefenamic acid concentration	50 µg.2mL^{-1}
pH	6.0
Temperature	25 C^{O}
Rate of Sample Analysis	12 sample.h^{-1}
Slope	0.0054
Intercept	0.0765
Linearity Range	4.0-70.0 µg.2mL^{-1}
Linearity coefficient (R^2)	0.9998
Correlation coefficient (r)	0.9999
Eerror % for (10.0-50.0) µg.2mL^{-1}	(1.00)-(-0.1)
Recovery %	99.90-101.10
Relative standard deviation R.S.D%	0.433-0.909
Colour	Yellow- green

3.29 Accuracy and precision [139]

By applying five different concentrations of standard solutions of mefenamic acid, the accuracy and precision of the suggested procedure for the determination of mefenamic acid have been investigated under ideal conditions. Table (3-41) provides the results of calculating the E%, Rec.%, and RSD% of five distinct concentrations (10, 20, 30, 40, 50 µg.2 mL^{-1}) using equations (3-1) (3-2) and (3-3).

Table (3-41) Accuracy and Precision for proposed method by using spectrophotometric technique

Concentration of MFA µg.2mL^{-1}		RSD %	Eerror %	Recovery %
Present	Found			
10	10.10	0.909	1.00	101.00
20	19.77	0.655	-1.15	98.85
30	30.07	0.418	0.23	100.23
40	40.44	0.678	1.10	101.10
50	49.95	0.433	-0.10	99.90

n*=3

The findings demonstrate good accuracy and precision for proposed method.

3.30 Sensitivity of spectrometric approach in mefenamic acid estimation

The sensitivity of the technique utilized in this method to identify mefenamic acid by the presentation limit of detection and limit of quantification were highlighted. However, as demonstrated by the subsequent equation formula (3-4) and (3-5) for measuring sensitivity (L.O.D) and (L.O.Q)

Table (3-42) Sensitivity of spectrometric of proposed method

Absorbance	S. D	L.O. D µg.mL^{-1}	L.O.Q µg.mL^{-1}
0.346	0.0015	0.9166	2.7498

As shown above in Table (3-42) the fact that this spectroscopic method have very low limit of detection and limit of quantification additionally supports the conclusion that the method is successful in identifying mefenamic acid with high sensitivity.

3.31 Rate of Sample Analysis

The time from the detection point of the maximum absorbance value was measured under the optimum physical and chemical conditions and used to calculate the Sampling speed. It was discovered that this absorbance only appears after (4) min, so the sampling speed rate was (15) sample.h^{-1}.

3.32 Stoichiometry of Cr (III)-Neocuproine Complex

3.32.1 Job's method of Continuous variation

The donor and acceptor were utilized in equivalent concentrations, and a number of solutions were created after that. Under constant pH= 6.0 circumstances, the volume of the donor and acceptor is equivalent to (10) mL, then leave the solution for 5 minutes at 25 C°. The absorbance is then calculated at λ_{max} = 430 nm.as shown in Figure (3-23)

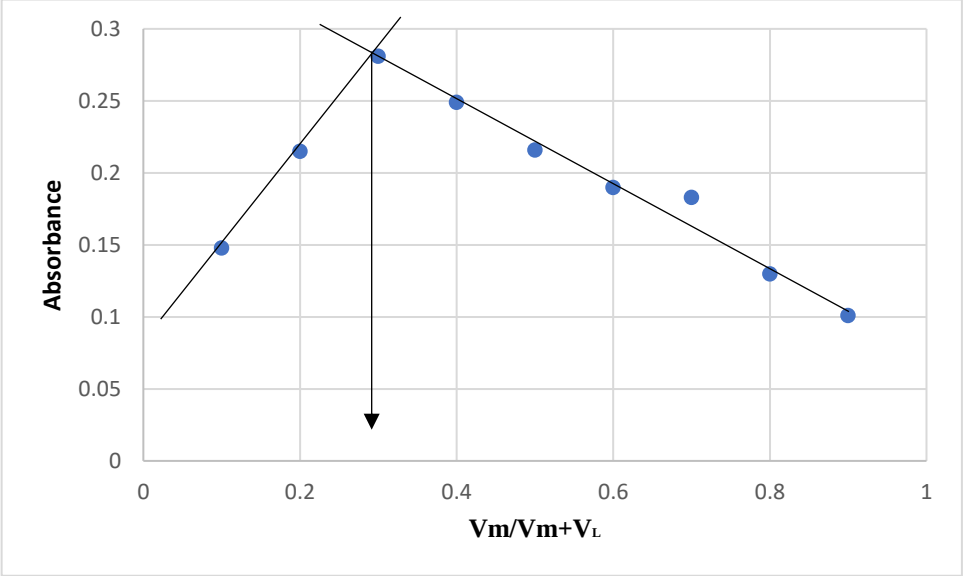


Figure (3-23) jobs Method for chromium (III) complex

we observed that the donor-acceptor ratio is (1:3) , in which one mole from metal Cr(III) to three mole from the ligand (neocuproine) ,This indicates that the resulting complex has possible empirical formula is (ML_3).

3.32.2 Mole Ratio Method[140].

Using the method of continual variations, a fixed concentration of metal ion Cr(VI) 1.0×10^{-3} M was taken with increasing concentrations of the 2, 9.DMP reagent 0.5-4.5×10^{-3} M under optimum conditions and the reaction ratio between Neocuproine and Cr(III), CL/CM was calculated as shown in Figure (3-24).

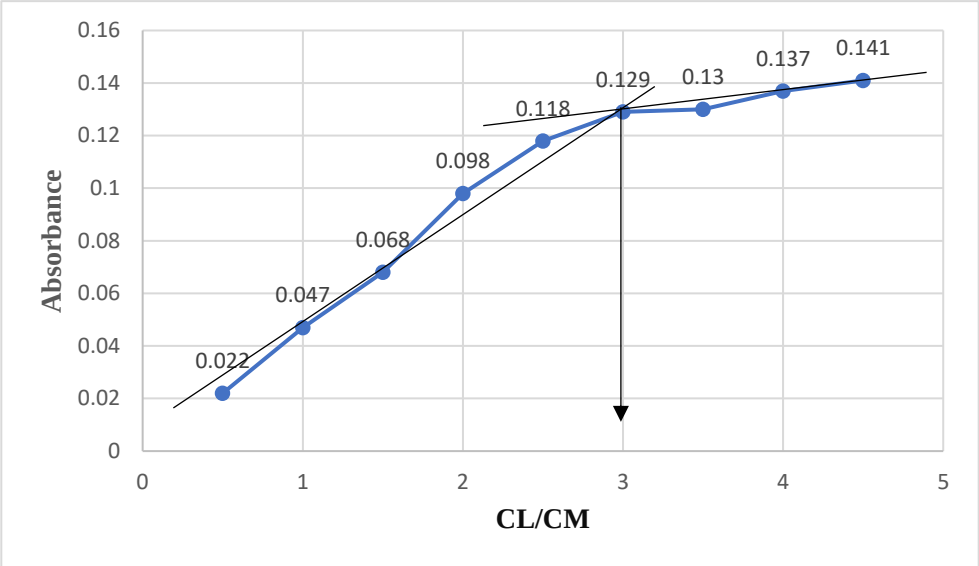


Figure (3-24) Mole Ratio for chromium (III) complex

we observed that the donor-acceptor ratio is (1:3) in which one mole from metal Cr(III) to three mole from the ligand (Neocuproine), This indicates that the resulting complex has possible empirical formula is (ML_3) as indicated above .

3.33 Stability Constant Calculations for The Produced Complex

The stability constant for the formed complex is calculated by writing the following equilibrium reaction for complex formation with an (α degree) as shown in Table (3-43).

Table (3-43) Stability Constant and Degree of Dissociation Values for Complex

Complex	Am	As	α	K
$[Cr(Nc.)_3]^{3+}$	0.141	0.129	0.078	1.369×10^{10}

Table (3-43) shows the high stability of the formed complex, which increases the process of this complexes , allowing the ligand to be used in the estimation of mefenamic acid using chromium (VI) ions.

3.34 Molar Conductivity Calculations for chromium (III) complexes[141]

Electrical conductivity is directly proportional to the number of charged ions in a solution that can transport an electric current. It can be taken on low values that are very close to zero when the complex solution lacks any ionic characteristics. However, the charge of the soluble solid complex in ethanol was determined by measuring the conductivity of the formed complex, the metal ion complex molar conductance should be determined at room temperature. Table (3-44) provides the data for the molar conductivity of the reagent in complex with chromium (III).

Table (3-44) The molar conductivity measured for chromium (III) complexes

No.	Complex	Λ_m (μS/cm)
1	$[Cr(NC)_3]^{3+}$	370

As a result, shown above that the chromium (III) complex has an ionic property that is mean the complex [Cr (III)-Neocuproine] was charged.

3.35 The Envisioned Composition of Chromium (III) Complexes

The stoichiometry of our result complex (M: L) for chromium (III) is (1:3). However, depending on the findings of this investigation. The Figure (3-25) show the suggested structures for Complex.

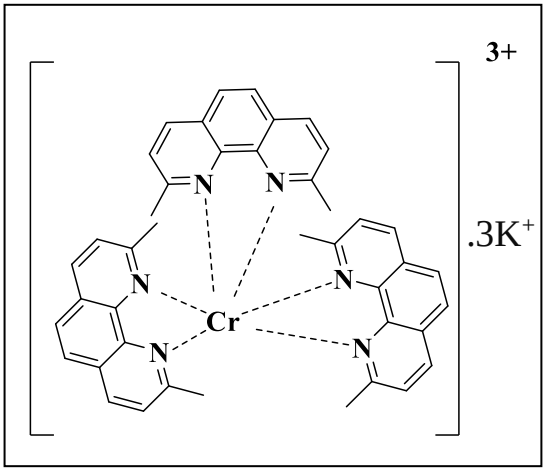


Figure (3-25) The proposed structure of the chromium (III) complex

3.36 Application of the suggested method to MFA analyses in pharmaceutical formulation

The method could be used with pharmaceutical formulations including MFA, such as Ponstidin Capsule (250 mg) GMBH, Germany , Ponstidin Capsule (250 mg) N.D.I-IRAQ, and mefril (250 mg) India, MFA determination in pharmaceutical samples was accomplished successfully and with good recovery rates using the proposed method [141]as we describe below in Table (3-45).

Table (3-45) Application of the novel framework for analysis of commercial MFA formulations in tablet dosage form

Drug type	Mefenamic acid µg/2mL		Mean	SD	RSD%	Eerror%	%Recovery
	Present	Measured					
Ponstidin Capsule N.D.I-IRAQ 250mg	10	9.88	0.130	0.0020	1.5152	-1.20	98.80
	20	19.15	0.180	0.0017	0.9569	-4.25	95.75
	30	28.96	0.233	0.0012	0.4942	-3.46	96.54
	40	41.00	0.298	0.0006	0.1935	2.50	102.50
	50	48.96	0.341	0.0006	0.1695	-2.08	97.92
Ponstidin Capsule GMBH, Germany 250 mg	10	9.88	0.130	0.0025	1.9017	-1.20	98.80
	20	19.70	0.183	0.0015	0.8287	-1.50	98.50
	30	30.40	0.241	0.0006	0.2392	1.30	101.30
	40	39.51	0.290	0.0006	0.1993	-1.20	98.80
	50	50.40	0.349	0.0000	0.0000	0.80	100.80
Mefril Bangalore-India 250 mg	10	10.07	0.133	0.0021	1.5612	0.70	100.70
	20	19.70	0.183	0.0015	0.8287	-1.50	98.50
	30	30.25	0.240	0.0012	0.4818	0.83	100.83
	40	39.88	0.295	0.0021	0.7049	-0.30	99.70
	50	49.88	0.345	0.0015	0.4432	-0.24	99.76

Above clearly shows that the developed UV technique provided good recovery beliefs in accordance with the marked quantities for every one of the analyzed samples collected from different pharmaceutical industry. Furthermore, quantities analyzed within range of USP-specified permissible 90–110% of the MFA designated quantity[120].

Part Four : Flow Injection Analysis For Determination of Mefenamic Acid in by using chromium (VI)

At the first we studed the optimum design of the flow injection system from eight flow injection system model designed in our laboratory for determination of mefenamic acid in aqueous solution and Pharmaceutical Formulations by using chromium VI.

At the first we should choose the optimum flow injection system model designed from(8 designed FIAs) system model as shown below

3.37 The Design of the System Applied for Reaction and the Fundamental Concept in its Work

The type of chemical system employed and the designed system for it are the key determinants of the flow injection system's design. In order to identify mefenamic acid by using chromium VI we will use the following reaction which describe below in scheme 3-2 ,in this part, we aimed to design and build an integrated flow injection system.However, it is an indirect method with a two-step reaction designed to determine mefenamic acid, The resultant yellow-orange coloured complex shows a maximum of absorbance at 430 nm.

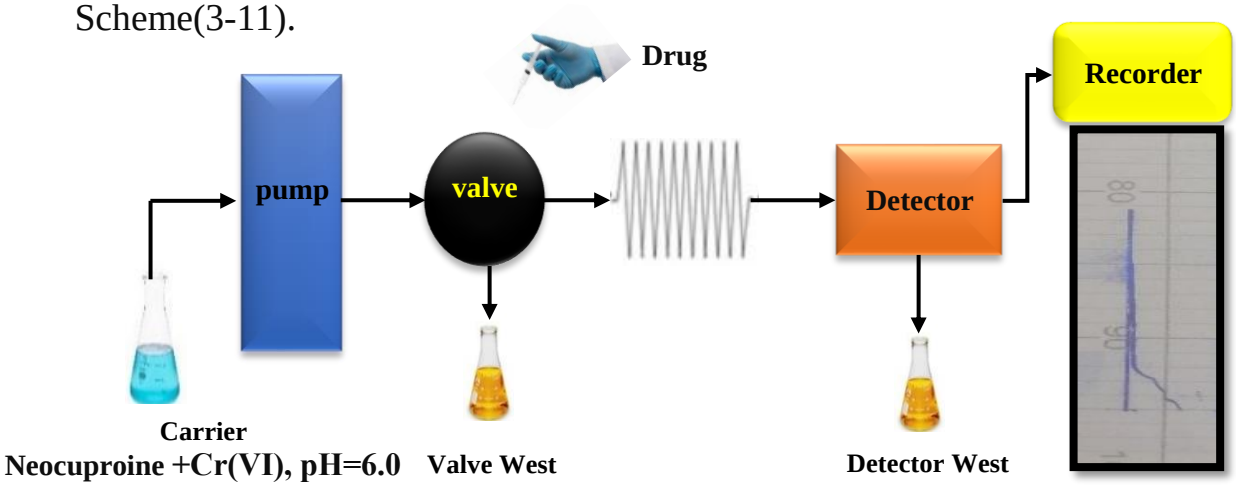
3.38 Flow Injection System Model Designed

3.38.1 Model Designed (1)

In the first model designed, In step (1), the metal ions Cr(VI) and neocuproine react in an acidic solution to generate a Cr(VI)-Neocuproine Colorless complex . The produced Cr(VI)-Neocuproine Colorless complex then reacted with mefenamic acid in step (2) to yield Cr(III)-Neocuproine yellow-green Complex, whose absorbance is measured at the maximum wavelength. As a result, a flow injection system with a single stream manifold was designed, which operates on both concepts:

1.introducing a carrier into the system.

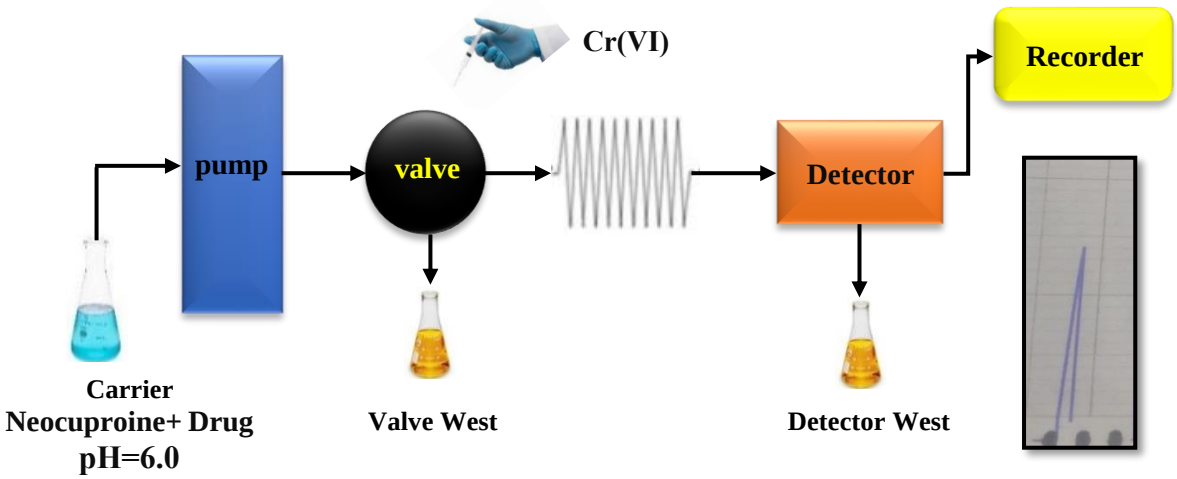
2.Sample injection into the sample loop, followed by carrier delivery to the unit. The flow injection system design for this work is depicted in Scheme(3-11).



Scheme (3-11) Optimum system design for the estimation of mefenamic acid Model (1) Cr(VI) =3×10^{-3}M, NC=1.0×10^{-3} M, MEF Conc= 50µg.2mL^{-1}

3.38.2 Model Designed (2)

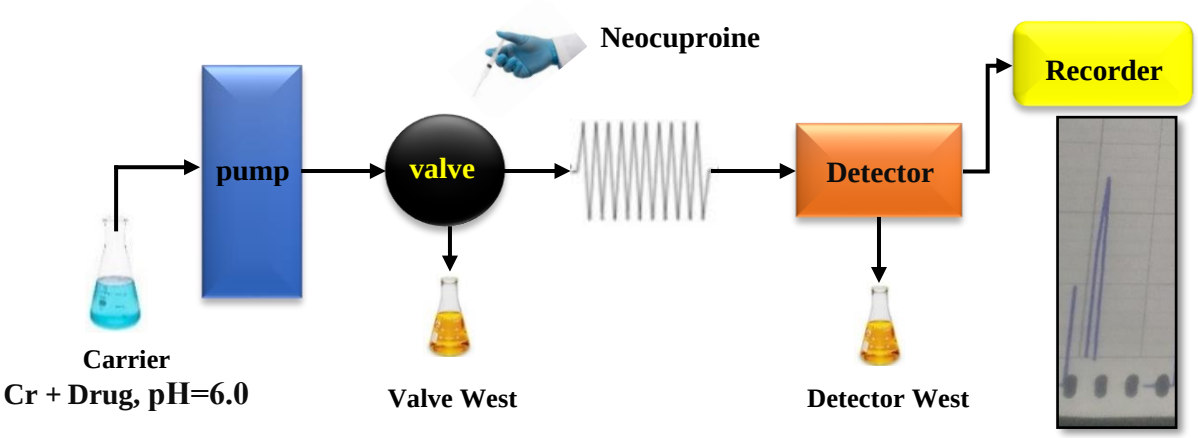
In the second model designed, In the step (1), the neocuproine react with mefenamic acid in an acidic solution. Then Cr(VI) injected in loop of valve in step (2), whose absorbance is measured at the maximum wavelength at 430 nm. As shown in Scheme(3-12).



Scheme (3-12) Optimum system design for the estimation of mefenamic acid Model (2) Cr(VI) =3×10^{-3}M, NC=1.0×10^{-3} M, MEF Conc=50 µg.2mL^{-1}

3.38.3 Model Designed (3)

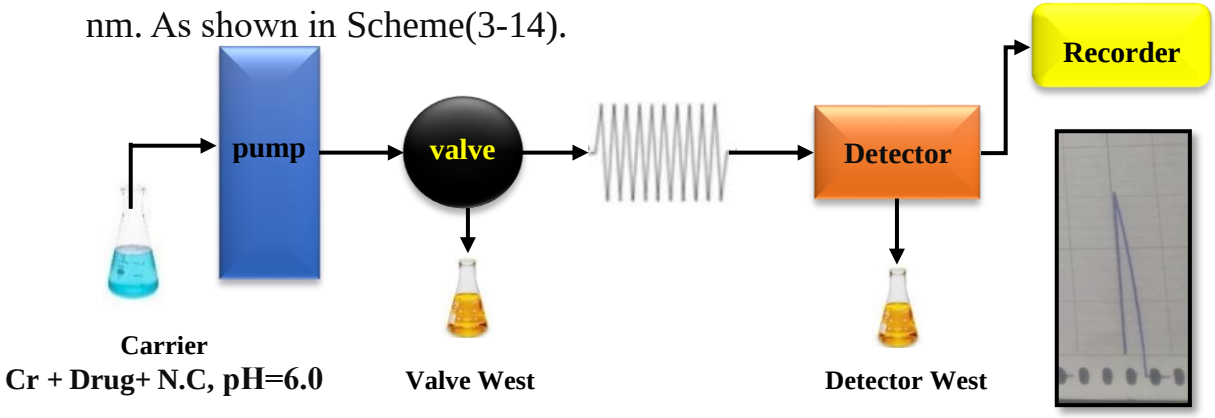
In the third model designed, In the step (1), the Cr(VI) react with mefenamic acid in an acidic solution. Then neocuproine injected in loop of valve in step (2), whose absorbance is measured at the maximum wavelength at 430 nm. As shown in Scheme(3-13).



Scheme (3-13) Optimum system design for the estimation of mefenamic acid Model (3)
Cr(VI) =3×10^{-3}M, NC=1.0×10^{-3} M, MEF Conc=50 µg/2mL

3.38.4 Model Designed (4)

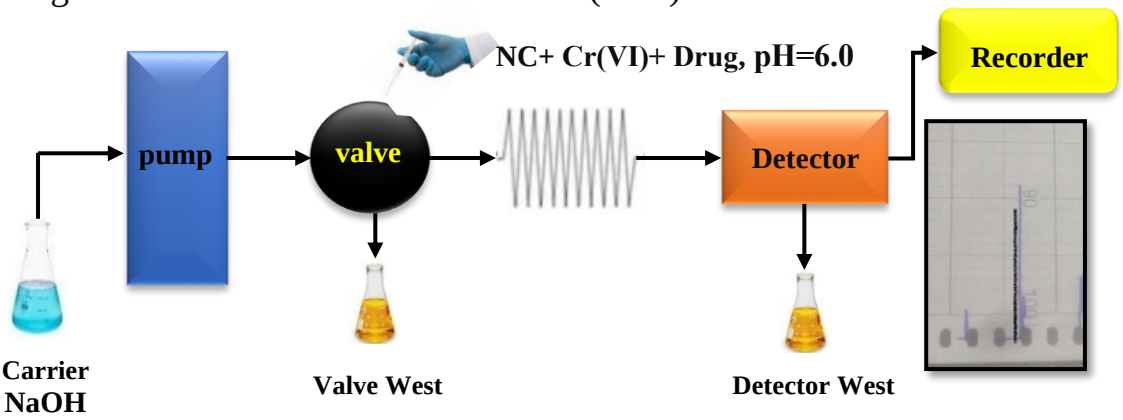
In forth flow injection system model designed, it is a one-step reaction (all three solutions are mixed with each other) in which the oxidation and reduction reaction is occurred between mefenamic acid and Cr(VI) to form Cr (III) then the resultant reacted with Neocuproine reagent to form a yellow-green color complex of Cr(III)-Neocuproine which absorb the light at 430 nm. As shown in Scheme(3-14).



Scheme(3-14) Optimum system design for the estimation of mefenamic acid Model (4)
Cr(VI) =3×10^{-3}M, NC=1.0×10^{-3} M, MEF Conc=50 µg.2mL^{-1}

3.38.5 Model Designed (5)

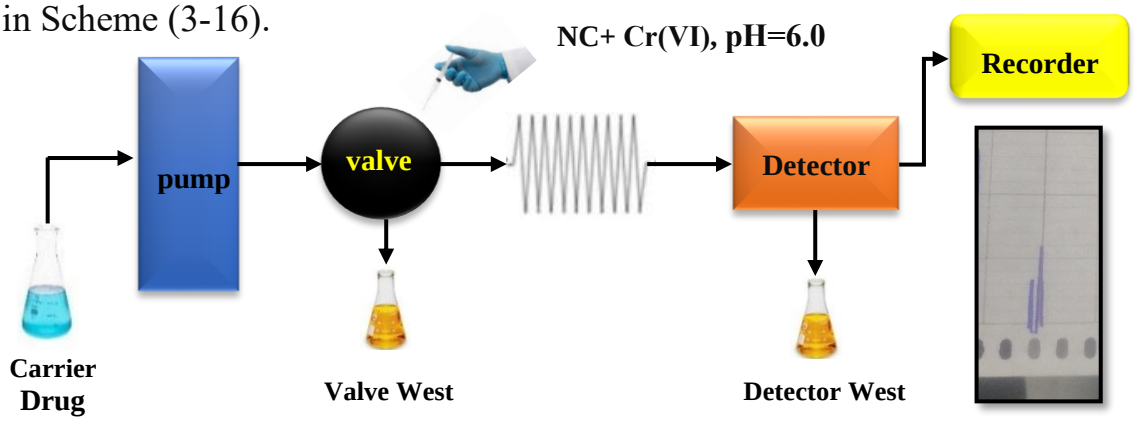
In fifth flow injection system model designed, the reaction is occurred between mefenamic acid and Cr(VI) and Neocuproine reagent, these three-reactant mixed together and injected in loop of valve, and NaOH consider as Carrier solution, the resultant is a light green color complex which absorb the light at 430 nm. As shown in Scheme(3-15).



Scheme(3-15) Optimum system design for the estimation of mefenamic acid Model (5) Cr(VI) =3×10-3M, NC=1.0×10-3 M, MEF Conc=50 µg.2mL^{-1}

3.38.6 Model Designed (6)

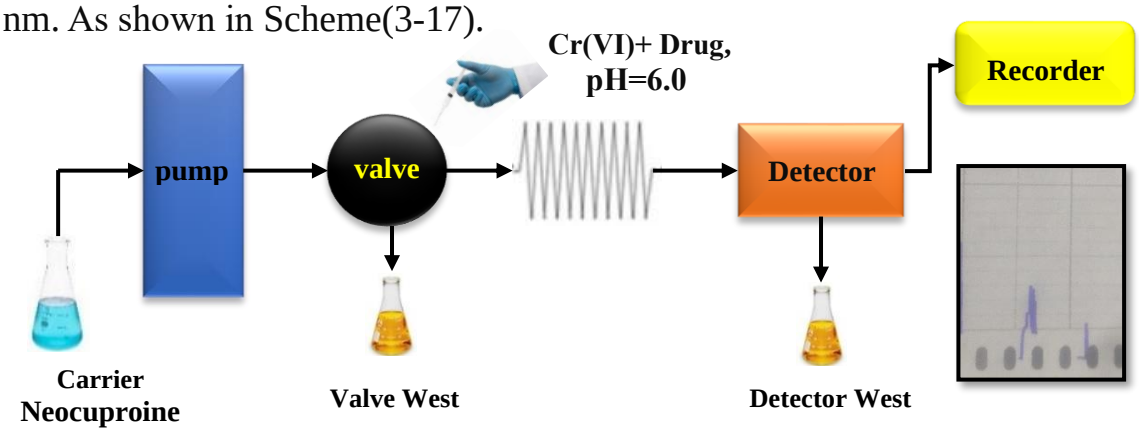
the sixth flow injection system model designed are two step reaction in which mefenamic acid consider as carrier solution, in first step the mefenamic acid is pumped through peristaltic pump as carrier solution, in the second step the Neocuproine reagent and Cr (VI) is mixed in an acidic solution and injected in loop of valve, the resultant is a light green colour of Cr (III)-Neocuproine complex which absorb the light at 430 nm. As shown in Scheme (3-16).



Scheme(3-16) Optimum system design for the estimation of mefenamic acid Model (6) Cr(VI) =3×10^{-3}M, NC=1.0×10^{-3} M, MEF Conc=50 µg/2mL^{-1}

3.38.7 Model Designed (7)

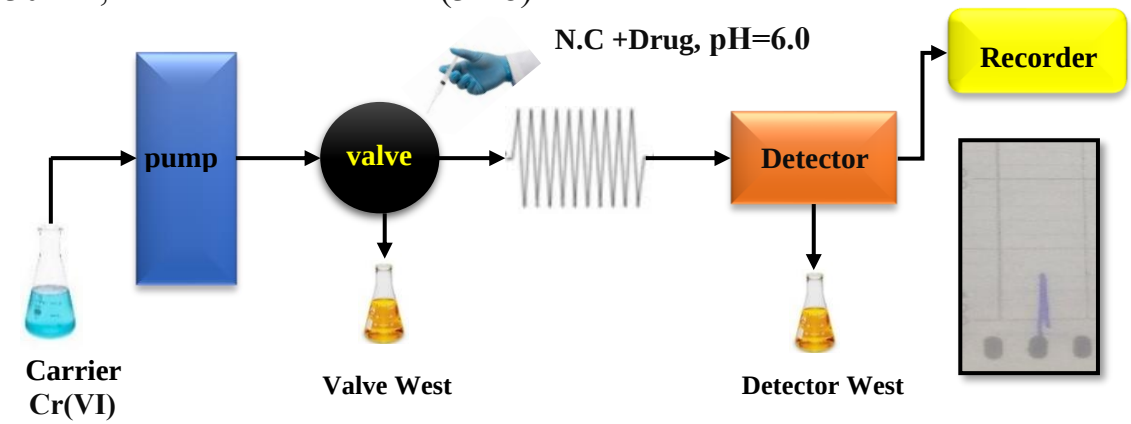
In step (1), the neocuproine reagent consider as carrier solution which is pumped through peristaltic pump, then in second step the metal ions Cr(VI) is reduced by the action of mefenamic acid to Cr (III) which is act as reducing agent and then injected into loop of valve. However, the resultant complex Cr (III)-Neocuproine Color Complex, which absorb the light at 430 nm. As shown in Scheme(3-17).



Scheme(3-17) Optimum system design for the estimation of mefenamic acid Model (7) Cr(VI) =3×10-3M, NC=1.0×10-3 M, MEF Conc=50 µg.2mL^{-1}

3.38.8 Model Designed (8)

In the third model designed, In the step (1), the Cr(VI) consider as carrier solution which is pumped through peristaltic pump, then in the second step the reaction is accrue between neocuproine and mefenamic acid in an acidic solution then injected in loop of valve then the absorbance read at 430 nm, As shown in Scheme (3-18).



Scheme(3-18) Optimum system design for the estimation of mefenamic acid Model (8) Cr(VI) =3×10-3M, NC=1.0×10-3 M, MEF Conc=50 µg.2mL^{-1}

As shown above in scheme (3-11) to (3-18) and according to resultant peaks height, the optimum system's design for the estimation of mefenamic acid in model designed (1) scheme (3-11) which give best peak height (3.4cm).

3.39 Stages of The System's Operation for the Determination of Mefenamic Acid by Cr(VI)

3.39.1 The first phase

Start the first phase of the work of multiple peristaltic pump channels operating on the pumping carrier solution containing Cr(VI) and Neocuproine in which they're pumping them across their own channel to the system through the loop of the sample at the injection valve for Cr(III)-Neocuproine complex yellow-green , subsequently to a cell output and finally to the waste stream (Waste).

3.39.2 The Second Phase,

The second phase typically consists of two phases of loading and injection, as seen below:

3.39.2.1 loading phase:

As the sample solution and other typical substances in the chemical reaction are loaded into the known loops of the valve (with known lengths and diameters), they are then emptied through the tube last linked to the valve gap. At the same time, another channel of the peristaltic pump attached to the inlet valve operates the recycling carrier stream continuously through the proper pipelines for paying the sample and other substances in the reaction to the exit port.

3.39.2.2 Injection phase

With the assistance of a peristaltic pump, the carrier solution (Cr(VI) and Neocuproine) moves through the sample (mefenamic acid) in a loop of the valve loaded as being pushed for the sample to the detector. As shown in Figure 3-34.

3.40 Optimization of Experimental Conditions:

To determine the best potential experimental conditions for the experiment, we monitored a single parameter and its influence on the absorbance of the colored species.

3.40.1 The Effect of Flow Rate

The effect of flow rate on the production of coloured products was examined by varying the flow rate (1.3 - 4.2) mL/min and determining the absorption of the coloured product produced. The results of this study showed that the highest peak was found at a peak height of 4.1 cm, and the ideal flow rate of 2.5 mL/min produced the best-looking beak shape and maximal absorption. Two were chosen over the other possibilities because they required more time to complete as shown in Table (3-46) and Figure (3-26)

Table (3-46) Effect of Flow Rate on Peack Hight

Flow Rate (mL/min)	1.3	2.1	2.5	2.7	3.3	3.7	4.2
Peack Hight(cm)	3.2	3.5	4.1	3.7	3.4	3.0	2.0

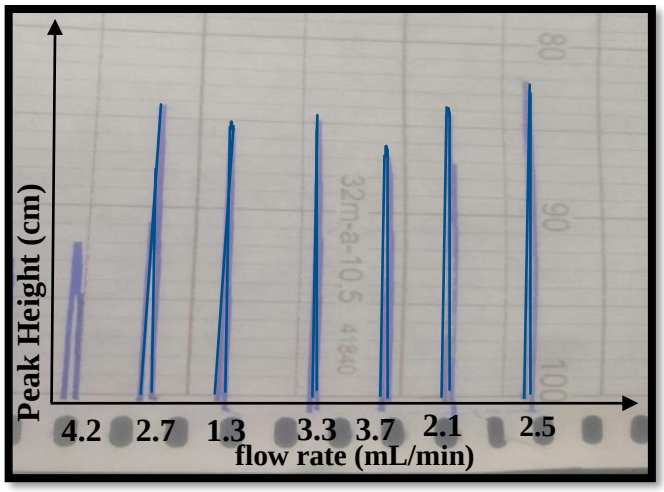


Figure (3-26) The Effect of Flow Rate, λmax= 430nm, NC. Conc. = 300 µg.mL^{-1}, Cr(VI)Conc.=100 µg.mL^{-1}, Flow rate =2.5 mL/min, MEF Conc.=50 µg.2mL^{-1}

3.40.2 The Influence of pH Changes

To investigate the effect of pH (1.0-9.0) on peak Height (cm) and coloured complexes, various amounts of hydrochloric acid and sodium concentration solutions were added to an aliquot of solution containing 50 µg.2mL^{-1} of MFA as shown in Table (3-47) and Figure (3-27).

Table (3-47) Effect of pH changes on Peack Hight

pH	Peack Hight(cm)
1.0	1.6
2.0	1.4
3.0	2.2
4.0	2.9
5.0	3.2
6.0	3.9
7.0	3.6
8.0	2.3
9.0	1.5

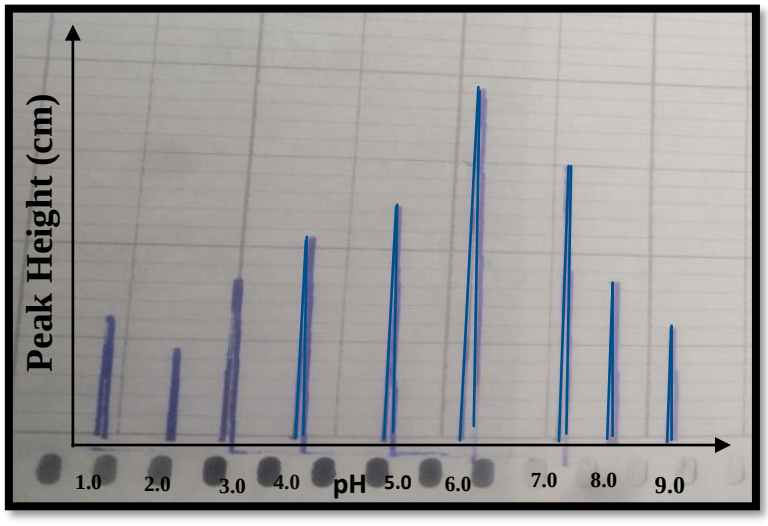


Figure (3-27) The Effect of pH changes, λmax= 430nm, NC. Conc. = 300 µg.mL^{-1}, Cr(VI)Conc. =100 µg.mL^{-1}, Flow rate=2.5 mL/min, MEF Conc.=50 µg.2mL^{-1}

As can be seen above, at pH 6.0, there is a higher absorption intensity at a peak height of 3.9 cm because of good intensity.

3.40.3 Effect of Cr (VI) Concentration

The Effect of Cr (VI) concentration (50-400) µg.mL^{-1} on complex's peak height was studied.

It was observed that 100 µg.mL-1 of Cr (VI) gave the highest absorption with peak height 4.0 cm which is strongly suggested for experimental procedures [75] as shown in Table (3-48) and Figure (3-28).

Table (3-48) Effect of chromium(VI) concentration on Peack High

Cr Conc.(ppm)	50.0	100.0	150.0	200.0	250.0	300.0	350.0	400.0
Peack Hight(cm)	3.4	4.0	3.3	2.5	2.0	1.4	1.2	1.0

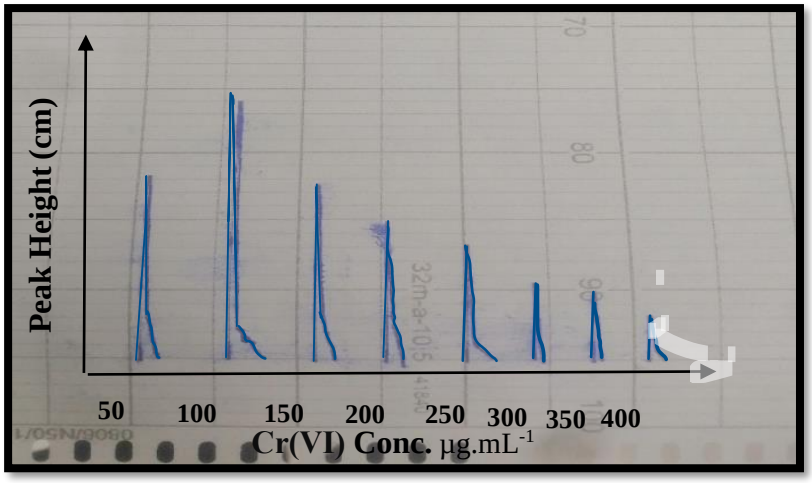


Figure (3-28) The Effect Cr(VI) Conc.(50-400) µg.mL^{-1}, λmax= 430 nm , NC Conc. = 300 µg.mL^{-1}, pH=6.0, Flow rate =2.5 mL.min^{-1}, MEF Conc.=50 µg.2mL^{-1}

as shown above the optimum Cr(VI) Conc µg.mL^{-1} with higher absorption intensity at peak height 4.0 cm due to good intensity.

3.40.4 The Influence of Neocuproine Reagent Concentration

The impact of adjusting the reagent concentrations on the reaction was investigated in this study. Data was collected using a variety of Neocuproine reagent concentration (50-500) µg.mL^{-1}. However, It was also noted that high concentrations cause a decrease in response. However, this

was possibly caused by an increase in particle density, which could have resulted in the buildup of precipitate particles in front of the detector as seen in Table (3-49) and Figure (3-29)

Table (3-49) Effect of neocuproine concentration on Peack Hight

NC. CONC. µg.mL^{-1}	Peack Hight(cm)
50.0	1.3
100.0	1.8
150.0	2.1
200.0	2.7
250.0	3.5
300.0	3.8
350.0	3.3
400.0	2.5
450.0	1.9
500.0	1.2

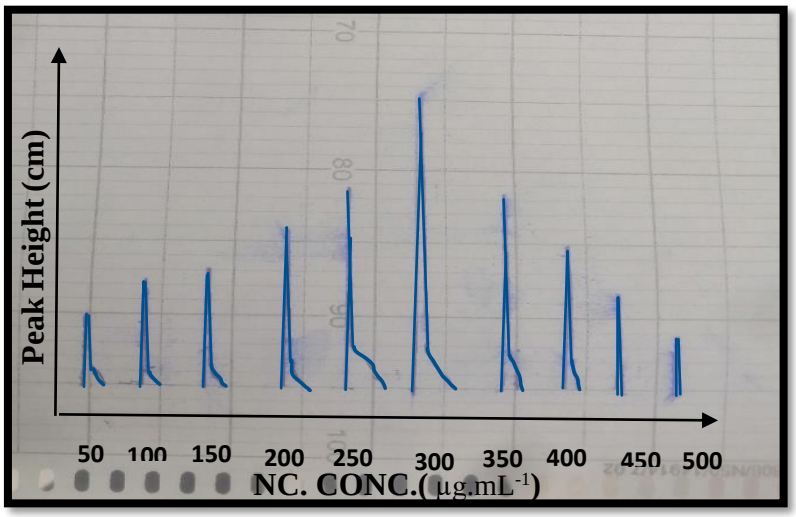


Figure (3-29) The Effect of Neocuproine reagent concentration (µg.mL^{-1}), λmax= 430 nm Cr(VI). Conc. = 100 µg.mL^{-1}, pH=6.0, Flow rate =2.5 mL/min, MEF Conc.=50 µg.2mL^{-1}

The findings indicated that the optimal absorption occurs when the reagent concentration is 300 µg.mL^{-1}.

3.41 Standard Calibration Curve for Mefenamic Acid

This is accomplished by creating a range of solutions with concentrations between 0.1 µg.mL^{-1} (4.144×10^{-7}) and 60.0 µg.mL^{-1} (2.486×10^{-4}) and by employing the ideal circumstances that are employed in this project. The final results we are seeing are displayed in Table (3-50) and Figures (3-30) and (3-31).

Table (3-50) Calibration Curve for Mefenamic Acid

MEF Conc. µg/2mL	MEF Conc (M)	peak height(cm)
0.1	$4.144X10^{-7}$	0.6
0.5	$2.07X10^{-6}$	0.7
1.0	$4.144X10^{-6}$	0.8
5.0	$2.072X10^{-5}$	1.2
10.0	$4.144X10^{-5}$	1.7
20.0	$8.288X10^{-5}$	2.8
30.0	$1.243X10^{-4}$	4.0
40.0	$1.866X10^{-4}$	5.0
50.0	$2.072X10^{-4}$	6.1
60.0	$2.486X10^{-4}$	7.2

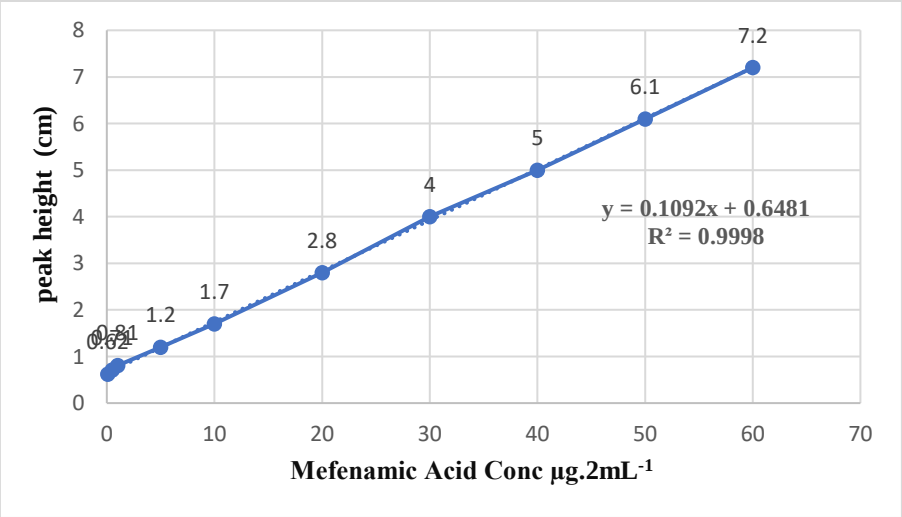


Figure (3-30) Calibration Curve for Mefenamic Acid, λmax= 430 nm, Cr(VI) Conc.=100 µg.mL^{-1}, NC Conc. = 300 µg.mL^{-1}, pH=6.0, Flow rate =2.5 mL/min, MEF Conc.=0.1-60 µg.2mL^{-1}

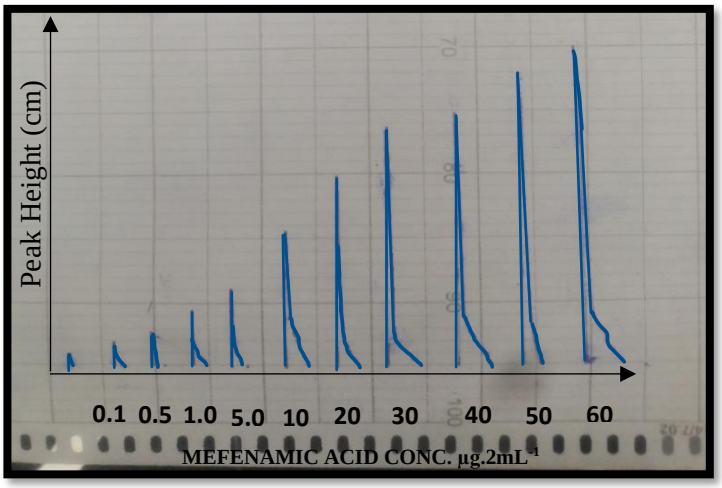


Figure (3-31) Calibration Curve for Mefenamic Acid

The statistical characteristics of the ideal conditions and our suggested approach are shown in Table (3-51).

Table (3-51) The ideal circumstances and statistical characteristics for the suggested approach

Analytical Data	Value
λ_{max}	430 nm
Flow Rate	2.5 mL/min
pH of carrier solution	6.00
Neocuproine Conc.	300 µg.mL^{-1}
Cr(VI) Conc.	100 µg.mL^{-1}
Linearity range	0.1-60.00 µg.2mL^{-1}
Regression equation	y = 0.1092x + 0.6481
Linearity coefficient (R^2)	R^2 = 0.9998

3.42 Rate of Sample Analysis

The time from the detection point of the maximum absorbance value was measured under the optimum physical and chemical conditions and used to calculate the Sampling speed. It was discovered that this absorbance only appears after (30) second, so the sampling speed rate was (120) sample.h^{-1}

3.43 Repeatability[130]

The Repeatability of the proposed approach tested by using six sample solutions containing 30 µg.2mL^{-1} of mefenamic acid under optimum conditions. The results were very good and highly repeatable and degree of repeatability are extremely excellent, as Table (3-52) and Figure (3-32) illustrates.

Table (3-52) Repeatability of Mefenamic acid

Drug Conc. µg.2mL^{-1}	**Peak Height(cm)**						**Mean $\bar{X}$**	**SD**	**RSD%**
30	4.1	4.1	4.0	4.1	4.2	4.1	4.1	0.0577	1.4082

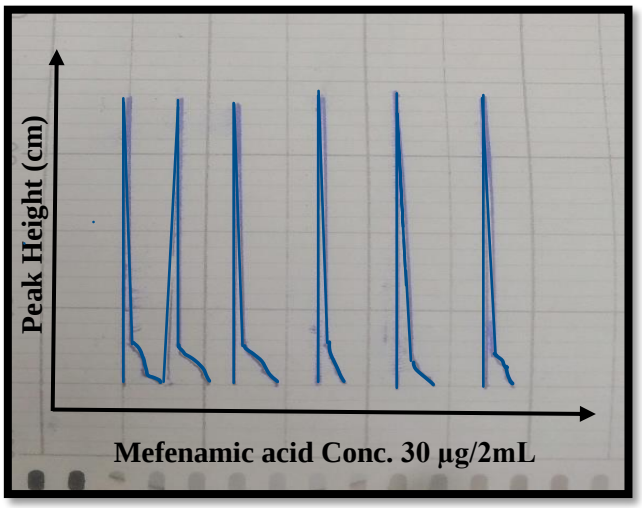


Figure (3-32) Repeatability of Mefenamic acid, λmax= 430 nm, Cr(VI) Conc. =100 µg.mL^{-1}, NC Conc. = 300 µg.mL^{-1}, pH=6.0, Flow rate =2.5 mL.min^{-1}, MEF Conc. =30 µg.2mL^{-1}.

3.44 Dispersion of Mefenamic Acid

The dispersion of mefenamic acid tested by doing two experiments to estimate the dispersion value of MEF present in (30 µg.2mL^{-1} and 50 µg.2mL^{-1}). First, Mefenamic acid with concentration (30 µg.2mL^{-1}),The current experiment represents the sample's intensity response that passes into the investigation (H_{max})[123]. the two reactants (mefenamic acid and Neocuproine) have been mixed and then passed into a manifold unit, and the result shows no dispersion effect due to convection or diffusions.[124] This illustration depicts (H^0). It is possible to compute dispersion (D) by applying dispersions equation as shown: ((D ° = H^0/H_{max})) ,D= 0.875 and by doing the same procedure for the second mefenamic acid concentration (50 µg.mL^{-1}) we get dispersion value equal to D= 0.909 under optimum conditions[125], as shown below in and Table (3-53) and Figure (3-33).

Table (3-53) Dispersions of mefenamic acid at optimum conditions

Drug Conc. µg.2mL^{-1}	**Peak Height (cm)**		**dispersion(D)**
	H^0	**Hmax**	
30	4.2	4.8	0.875
50	6	6.6	0.909

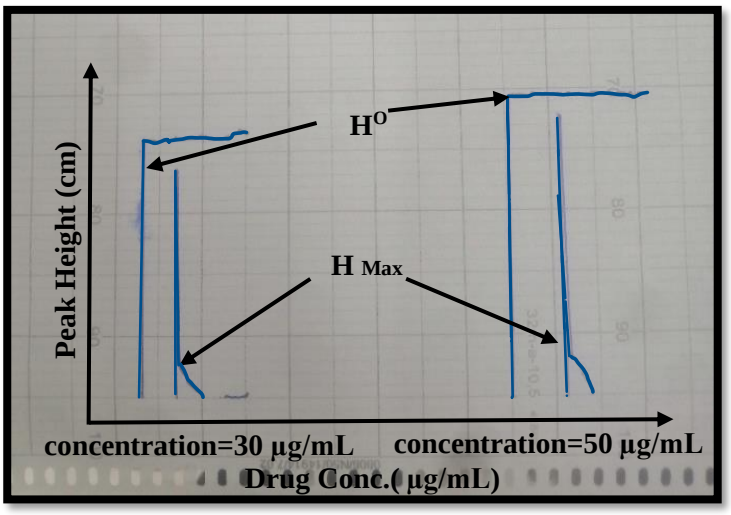


Figure (3-33) Disperssions of mefenamic acid, λmax= 430 nm, Cr(VI) Conc. =100 µg.mL^{-1}, NC Conc. = 300 µg/mL, pH=6.0, Flow rate =2.5 mL/min, MEF Conc. =30-50 µg.2mL^{-1}

3.45 Sensitivity of Spectrometric Approach in Mefenamic Acid Estimation

The presented limit of detection (L.O.D.) and limit of quantification (L.O.Q.) demonstrated the sensitivity of the approach used in this procedure to determine mefenamic acid. The lowest mefenamic acid concentration that could be estimated using this FIA approach was 0.1 µg.mL^{-1}[129-130]

Table (3-54) Limit of Detection and Limit of Quantification

Peak height(cm)	SD	LOD µg.mL^{-1}	LOQ µg.mL^{-1}
6.1033 cm	0.0047	0.1	0.4274

n*=3

3.46 Studying The Dead Volume

Three experiments have been done. When water was utilized in the loop as the carrier solution in the first experiment rather than neocuproine, there was no reaction. As demonstrated in Figure (3-34), the system's efficacy was tested in the second experiment when water was used as the carrier instead of Cr(VI), and no reaction happened. In the third experiment, water was used in the loop instead of mefenamic acid. This demonstrates the system's effectiveness[127].

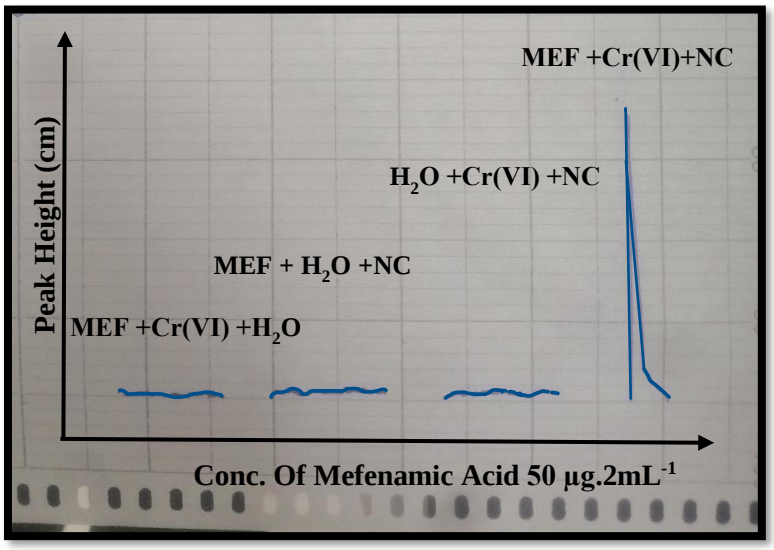


Figure (3-34) Dead Volume's Influence on response times, λmax= 430 nm, Cr(VI) Conc. =100 µg/mL, NC Conc.= 300 µg.mL^{-1}, pH=6.0, Flow rate =2.5 mL.min^{-1}, MEF Conc. =50 µg.2mL^{-1}

3.47 Accuracy and Precision

By applying five different concentrations of standard solutions of mefenamic acid, the accuracy and precision of the suggested procedure for the determination of mefenamic acid by flow injection technique have been investigated under ideal conditions. Table (3-55) provides the results of calculating the E%, Rec.%, and RSD% of three readings of each of five distinct concentrations (10, 20,30, 40, 50 µg.2mL^{-1}).

Table (3-55) Accuracy and Precision for Proposed Method by Flow Injection Technique

Concentration of MFA µg/2mL		RSD%	Rec%	Error %
Present	Found			
10	10.24	3.2680	102.40	2.4
20	19.55	1.0371	97.75	-2.25
30	30.08	1.4678	100.26	0.26
40	40.4	1.1395	101.23	1.23
50	48.73	0.4838	97.50	-2.5

n*=3

The findings demonstrate good accuracy and precision.[128]

3.48 Application of Mefenamic Acid in Pharmaceutical Preparation

Commercial dosage forms from various brands were used to test the suitability of the suggested flow injection approach. Moreover, the outcome demonstrates that the location was constant as indicated on the medication dosage form label, as indicated in Table (3-56) and Figures (3-35,36,37).

Table (3-56) Application of FIA Method in Capsule Formulations

Drug Type	Conc. µg/2mL		peak height(cm)	Eerror%	Rec%	SD	RSD%
	Present	Found					
Ponstane Capsule N.D.I-IRAQ 250mg	10	10.09	1.75	0.90	100.90	0.050	2.857
	20	19.55	2.78	-2.25	97.75	0.076	2.744
	30	29.93	3.92	-0.23	99.77	0.104	2.657

n*=3

Drug Type	Conc. µg/2mL		peak height(cm)	Eerror%	Rec%	SD	RSD%
	Present	Found					
Ponstidin Capsule GMBH, Germany 250mg	10	9.54	1.69	-4.60	95.40	0.010	0.591
	20	20.31	2.87	1.55	101.55	0.029	1.007
	30	30.08	3.93	0.26	100.26	0.058	1.467

n*=3

Drug Type	Conc. µg/2mL		peak height(cm)	Eerror%	Rec%	SD	RSD%
	Present	Found					
Mefril Bangalore-India250mg	10	10.24	1.77	2.40	102.40	0.058	3.268
	20	19.55	2.78	-2.25	97.75	0.029	1.037
	30	30.08	3.93	0.26	100.26	0.058	1.467

n*=3

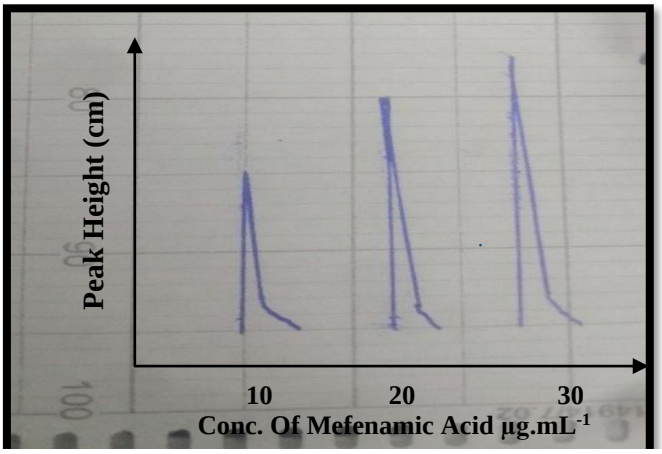


Figure (3-35) Analysis of mefenamic acid in capsule formulations Ponstane Capsule (250 mg), λmax= 430nm, NC.Conc. = 300 µg.mL^{-1}, pH=6.0, Cr(VI)Conc. =100 µg.mL^{-1} Flow rate =2.5 mL.min^{-1}, MEF Conc. =10,20,30 µg.2mL^{-1}

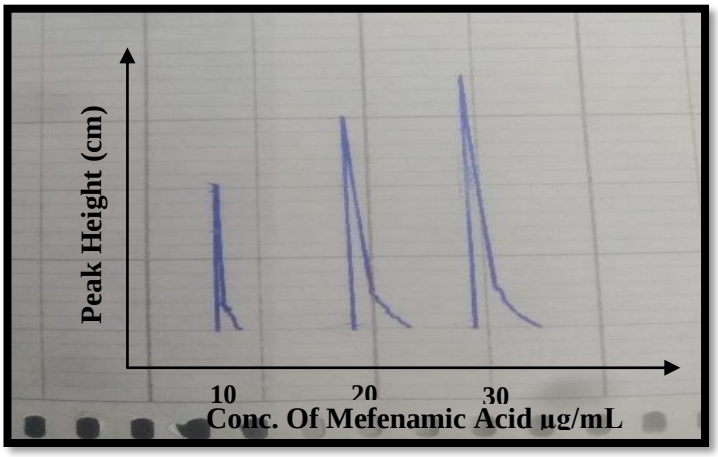


Figure (3-36) Analysis of mefenamic acid in capsule formulations Ponstidin Capsule (250 mg) , λmax= 430nm, NC.Conc. = 300 µg.mL^{-1}, pH=6.0, Cr(VI)Conc. =100 µg.mL^{-1} Flow rate =2.5 mL.min^{-1}, MEF Conc. =10,20,30 µg/2mL^{-1}

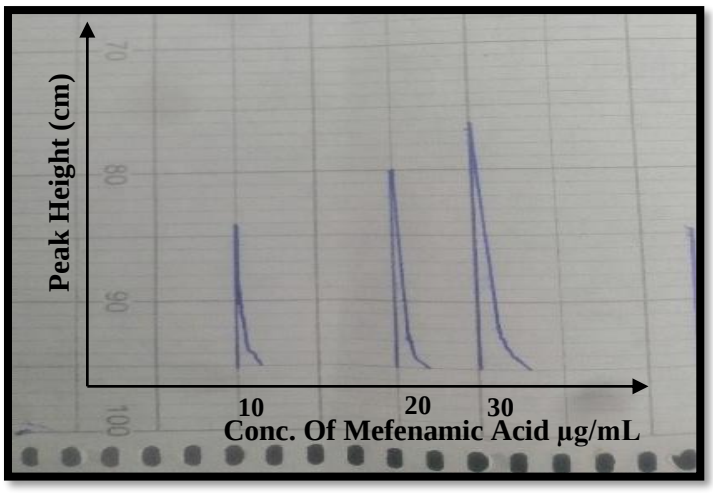


Figure (3-37) Analysis of mefenamic acid in capsule formulations Mefril Capsule (250 mg) , λmax= 430nm, NC.Conc. = 300 µg.mL^{-1}, pH=6.0, Cr(VI)Conc. =100 µg.mL^{-1} Flow rate =2.5 mL/min^{-1}, MEF Conc. =10,20,30 µg.2mL^{-1}

3.49 Results Comparison of Two Systems

The physical and chemical properties measured by using the new systems are summarized in Table (3-57)

Table (3-57) Results comparison of two systems

Analytical Data	Spectrophotometric Method for Cr(VI)	Flow Injection Analysis Method for Cr(VI)
λmax	430 nm	430 nm
flow rate	.....	2.5 mL/min
Regression equation	y=0.0054x+0.0765	y = 0.1092x + 0.6481
Cr(VI) Concentration	3.0×10^{-3} M	1.9×10^{-3} M
Neocuproine Concentration	1.0×10^{-2} M	1.4×10^{-3} M
Mefenamic acid concentration	50 µg.2mL^{-1}	50 µg.2mL^{-1}
pH	6.0	6.0
Temperature	25 C^{O}	25 C^{O}
Rate of Sample Analysis	12 sample.h^{-1}	120 sample.h^{-1}
Slope	0.0054	0.1092
Intercept	0.0765	0.6481
Linearity Range	4.0-70.0 µg.2mL^{-1}	0.1-60.0 µg.2mL^{-1}
Linearity coefficient (R^2)	0.9998	0.9998
RSD % at (10.0-50.0 µg. mL^{-1})	0.433-0.909	0.4838-3.2680
%Error at (10.0-50.0 µg. mL^{-1})	-0.1.00 -1.00	-2.5-2.4
%Recovery at (10.0-50.0µg. mL^{-1})	99.90-101.10	97.50-102.40
LOD	0.9166 µg. mL^{-1}	0.1424µg. mL^{-1}
LOQ	2.7498 µg. mL^{-1}	0.4274 µg. mL^{-1}

The Spectrophotometric system for determination of mefenamic acid by using Cr(VI) in aqueous solutions and pharmaceutical dosage form gave best value of SD, RSD% rather than flow injection analysis innovate system.

The flow injection analysis system for determination of mefenamic acid by using Cr(VI) in aqueous solutions and pharmaceutical dosage form gave best value of Cr(VI) Concentration(ppm), Neocuproine Concentration(ppm), sampling speed, Slope, Intercept, Linearity Range, Recovery%, Error%, LOD, LOQ rather than Spectrophotometric system.

There is no any previous study for (Spectrophotometric and flow injection analysis) determination of mefenamic acid by using Cr(VI)-Neocuproine complex in aqueous solutions and pharmaceutical dosage form.

When compared the outcomes of both suggested methods to those of the global spectrophotometric and flow injection systems demonstrate the effectiveness and superiority of the aforementioned new design systems for determining mefenamic acid traces in various pharmaceutical, environmental, and aqueous solutions. In addition to the materials' and chemicals' accessibility, simplicity, speed, and ease of maintenance.

3.50 Conclusions

1. The FIA systems and spectrophotometric were distinguished by their sensitivities, excellent efficiency, simplicity, and quickness. Mefenamic acid and other amino drug concentrations can be determined with great precision using a wide range of liner concentrations, which is easy to handle, cheap operating cost, high sampling speed, and minimal sample and reagent consumption.
2. The Linearity coefficient (R^2) of the calibration graph is the same regardless of the flow injection or spectrophotometric system design for the same reaction. But the LOD, LOQ, Error % and Recovery% become better in flow injection then spectrophotometric method.
3. The suggested techniques are employed to analyze mefenamic acid in pharmaceutical preparations and aqueous solutions indicating that they can be used as reliable and useful alternative to the other techniques that were previously exported for routinely analyzing amino drugs in these samples.
4. FIA system has successfully detected mefenamic acid and produced precise, accurate results with a dead volume of zero, a dispersion factor inside the first quarter of the first class, and a very low detection limit.
5. Mefenamic acid can be estimated by formation a complexes of Cu(II)-Neocuproine and Cr(VI)-Neocuproine by both flow injection analysis system and spectrophotometric method with very good limit of detection and highly precision.
6. The new FIA system and the new spectrophotometric system were both utilized in the same reaction to determine the presence of trace mefenamic acid. The FIA system met the majority of the physical and chemical requirements, confirming the precision and effectiveness of the new FIA system.

7. The suggested techniques' main advantages are their simplicity, strong selectivity, outstanding recovery, SD and RSD%, and acceptable repeatability.
8. The suggested FIA methods have dead volume values of zero for both complex Cu(II)-Neocuproine and Cr(VI)-Neocuproine , which indicates improved outcomes and demonstrates the methods' effectiveness for flow injection analysis.
9. The interferents effect on the determination of mefenamic acid was minimized by using the new flow injection system and spectrophotometric method comparatively with other methods.
10. The flow injection system and the new spectrophotometric system were both give correlation coefficient, Linear Range, better than the results of literatures [38-48] [106-114]
11. The new spectrophotometric system gives the L.O.D and L.O.Q better than the results of literatures [40-48]
12. The new flow injection system gives the L.O.D and L.O.Q better than the results of literatures [37][106-114,]

3.51 Future Prospects

Based on the knowledge that was obtained from this project, the following recommendations can be recommended for future study:

1. Application of suggested techniques to identify biological samples containing mefenamic acid or its derivatives or other amino compounds.
2. Using the same Spectrophotometric reagent (Neocuproine) for determination of other organic amino drug compounds.
3. Use of the suggested techniques to identify amino drug compounds, such as mefenamic acid, by using other metal like Co and Mn.
4. other detection tools can be employed to enhance measurement sensitivity and other features like fluorescence and chemiluminescence, etc.

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[Cr(III)-Neocuproine] يكون مشحون، حيث تم قياس دقة وتوافقية الطريقة الطيفية المستخدمة باستخدام خمس محاليل بتراكيز مختلفة فكانت قيمة النسبة المئوية للانحراف القياسي النسبي بين (0.418% - 0.909%) ونسبة التوافقية بين (98.85% -101.10%)، و تم إيجاد حد الكشف والحد الكمي وكانت ($0.8433\ \mu g.mL^{-1}$) ($2.5299\ \mu g.mL^{-1}$)على التوالي مما يدل على ان الطريقة الطيفية ذات حساسية عالية، وتم تطبيق الطريقة المقترحة على محاليل مائية ومستحضرات صيدلانية ووجد ان الطريقة المتبعة في التقدير ذات حساسية ودقة عالية.

أما الجزء الرابع فقد تضمن تصميم منظومة حقن جرياني متطورة لتقدير حامض الميفيناميك في المستحضرات الصيدلانية (Mefril، Ponstan،Ponstidin) والمحاليل المائية. حيث اعتمدت الطريقة المقترحة لتقدير حامض الميفيناميك باستخدام الحقن الجرياني المستمر على تفاعل الكاشف الطيفي النيوكوبروين مع الكروم (VI) بوجود (حامض الميفيناميك) بوسط حامضي لتكوين معقد كروم(III)–نيوكوبروين الملون (أصفر–أخضر) وقياس امتصاصية المعقد الملون المتكون عند الطول الموجي الاعظم لهnm 430 وتم دراسة الظروف الكيميائية و الفيزيائية الفضلى للمنظومة المقترحة لغرض الوصول الى اعلى حساسية وناتج اكثر استقرار وللحصول على احسن تصميم يعطي افضل استجابة ، حيث أظهر منحني المعايرة مطاوعة لقانون لامبرت– بير في مدى التركيز ($0.1–60.0\ \mu g.2mL^{-1}$) وكان معامل الخطية ($R^2= 0.9998$) ،كما تم قياس دقة وتوافقية طريقة الحقن الجرياني المستمر المستخدمة باستخدام خمس محاليل بتراكيز مختلفة فكانت قيمة النسبة المئوية للانحراف القياسي النسبي بين (0.4838%–3.2680%) ونسبة التوافقية بين (97.50%–102.40%) وكان حد الكشف والحد الكمي ($0.1424\ \mu g.mL^{-1}$) ($0.4274\ \mu g.mL^{-1}$) على التوالي، وأظهرت الطريقة المقترحة خصائص جيدة مثل السرعة والحساسية والموثوقية، وتم تطبيق الطريقة المقترحة على محاليل مائية وصيدلانية ووجد ان الطريقة المتبعة في التقدير ذات حساسية ودقة عالية.

على تفاعل الكاشف الطيفي النيوكوبروين مع النحاس (II) بوجود (حامض الميفيناميك) بوسط حامضي لتكوين معقد نحاس(I)–نيوكوبروين وقياس امتصاصية المعقد الملون المتكون عند الطول الموجي الاعظم له454 nm ، وتم دراسة الظروف الكيميائية و الفيزيائية الفضلى للمنظومة المقترحة لغرض الوصول الى اعلى حساسية وناتج اكثر استقرار وللحصول على افضل تصميم يعطي افضل استجابة، حيث أظهر منحني المعايرة مطاوعة لقانون لامبرت– بير في مـــدى التركيز (1.0–80.0µg/mL) وكان معامل الخطية (R^2= 0.9998) ، كما تم قياس دقة وتوافقية الطريقة (الحقن الجرياني المستمر) المستخدمة باستخدام خمس محاليل بتراكيز مختلفة فكانت قيمة النسبة المئوية للانحراف القياسي النسبي بين (0.0235%–0.0115%) ونسبة التوافقية بين (99.94%-99.30%)، وكان حد الكشــف والحد الكمي ($0.1983\ \mu g.\ mL^{-1}$) . ($0.6543\ \mu g.\ mL^{-1}$)على التوالي، وأظهرت الطريقة المقترحة خصائص جيدة مثل السرعة، الحساسية ،الموثوقية والتطابقية، مما يجعلها مناسبة للتقدير الكمي لحامض الميفيناميك في المستحضرات الصيدلانية والمحاليل المائية وتم تطبيق الطريقة المقترحة على محاليل مائية وصيدلانية ووجد ان الطريقة المتبعة في التقدير ذات حساسية ودقة عالية.

تضمن الجزء الثالث تطوير طريقة طيفية ، سريعة، مباشرة، حساسة، دقيقة وفعالة لتقدير حامض الميفيناميك في المحاليل المائية والأدوية الصيدلانية، حيث اعتمدت الطريقة على تكوين معقـدات باستـــخدام تـــفاعل الأكســدة والاختزال وتـــحويل مـــعقد Cr(VI)-2,9DMP إلى معـــقد Cr(III)- 2,9DMP الملون ،وتم اختيار الظروف التجريبية الفضلى للتفاعل مثل الدالة الحامضية، زمن استقرار المعقد المتكون، المحاليل المنظمة، حجم وتركيز الكروم(VI) ، حجم وتركيز الكاشف (النيوكوبروين)، درجة الحرارة، ترتيب الإضافة، حيث أظهر منحني المعايرة مطاوعة لقانون لامبرت–بير في مدى التركيز($4.0–70.0\ \mu g.2mL^{-1}$) كذلك تم قياس معامل الخطية وكانت قيمته R^2= 0.9998 ،وتمت دراسة تكافؤية المعقدين من خلال ايجاد نسبة الايون الفلزي الى الكاشف (M:L) باستعمال طريقة التغيرات المستمرة والنسب المولية وأشارت النتائج الى ان هذه النسبة كانت تساوي (1:3) كما تم حساب ثابت الاستقرارية Ksta للمعقد المتكون وكانت قيمته تساوي (1.369×10^{10})، وتم تحديد شحنة المعقد الصلب الذائب في الايثانول عن طريق قياس توصيلية المعقد المتكون حيث أظهرت النتائج الى ان معقد

الخلاصـــــــــــــة

الجزء الاول تضمن تطوير طريقة طيفية مباشرة ،بسيطة ، دقيقة ،سريعة ،انتقائية وعالية الحساسية باستخدام معقد النحاس(I)–النيوكوبروين لتقدير الكميات الضئيلة من حمض الميفيناميك في المحاليل المائية والادوية وتعتمد الطريقة على إنشاء معقدات باستخدام تفاعل الأكسدة والاختزال حيث اعتمدت الطريقة المقترحة على اختزال معقد Cu(II)- 2,9DMP إلى معقد Cu(I)-2,9DMP الملون، وتم اختيار الظروف التجريبية الفضلى للتفاعل مثل الدالة الحامضية، زمن استقرار المعقد المتكون، المحاليل المنظمة، حجم وتركيز النحاس(II) ، حجم وتركيز الكاشف (النيوكوبروين)، درجة الحرارة، تعاقب الإضافة وتأثير المتداخلات على تقدير المعقد، حيث أظهر منحني المعايرة مطاوعة لقانون لامبرت – بير في مـــدى التركيز ($\mu g.\ mL^{-1}$ 5.0-60.0) وقيـــمة الامتصاصية المولارية (ε) تســـاوي ($L.moL^{-1}.cm$ 0.238) عند 454 نانومتر ، كذلك تم قياس معامل الخطية وكانت قيمته(R^2=0.9999) ، وتمت دراسة تكافؤية المعقدين من خلال ايجاد نسبة الايون الفلزي الى الكاشف (M:L) باستعمال طريقة التغيرات المستمرة والنسب المولية وأشارت النتائج الى ان هذه النسبة كانت تساوي (1:2).كما تم حساب ثابت الاستقرارية Ksta للمعقد المتكون وكانت قيمته تساوي (3.5×10^8)، وتم تحديد شحنة المعقد الصلب الذائب في الايثانول عن طريق قياس توصيلية المعقد المتكون حيث أظهرت النتائج الى ان معقد[Cu(I)-Neocuproine] يكون مشحون ، كما تم قياس دقة وتوافقية الطريقة الطيفية المستخدمة باستخدام خمس محاليل بتراكيز مختلفة فكانت قيمة النسبة المئوية للانحراف القياسي النسبي بين (0.000%–0.552%) ونسبة التوافقية بين (100.32%– 99.50%) ، وكان حد الكشف والحد الكمي(0.7142µg/mL) (2.3568µg/mL) على التوالي مما يدل على ان الطريقة الطيفية ذات حساسية عالية وتم تطبيق الطريقة المقترحة على محاليل مائية وصيدلانية ووجد ان الطريقة المتبعة في التقدير ذات حساسية ودقة عالية.

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جامـــــــعة كـــــــــربلاء
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قســـــــــم الكيميـــــــــاء

نظام حقن جرياني وطيفي لتقدير حامض الميفانيميك باستخدام معقدي انتقال الشحنة بين النحاس والكروم مع كاشف النيوكابريون

اطروحة مقدمة الى مجلس كلية العلوم – جامعة كربلاء وهي جزء من متطلبات نيل درجة دكتوراه فلسفة في الكيمياء

من قبل

علـــي ناصـــــر نايـــــف

بكالوريوس علوم كيمياء/ جامعة كربلاء/ كلية العلوم 2009
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