

**Application of Flow injection spectrophotometric determination in some pharmaceutical analysis**

Research Project

Submitted to the Department of (Chemistry) in partial fulfillment of the requirements for the degree of **BSc.** in **chemistry**

***By***

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# CERTIFICATE

This research project has been written under my supervision and has been submitted for the award of the **BSc.** degree in **chemistry** with my approval as a supervisor.

Signature

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# DEDICATION

This effort I dedicate to **Allah** Almighty, my lord, my powerful foundation, my source of inspiration, wisdom, knowledge, and understanding. Throughout this project, he was the source of my energy.

***Arkan***

# ACKNOWLEDGMENTS

To begin with, I thank (**Allah**) for His blessing, which made me able to complete and perform this study with success, the lord of the universe, blessing, and peace be on **Muhammad** (Allah’s peace and prayers be upon him).

Finally, I want to say thanks to my Supervisor Dr. Sirwan and all those I forgot them here to mention his/her name, who assisted me even by one useful scientific word directly or indirectly.

**Abstract**

Flow injection analysis (FIA) is a sensitive and quick method for determining drug analysis in pharmaceuticals. Sample injection, consistent timing, and controlled dispersion of sample are three criteria for flow-injection analysis, which is based on a different technique than continuous-flow analysis (rather than a dispersion retarded with gas bubbles), In addition, the method has been optimized and thoroughly validated in terms of selectivity and accuracy, detection and quantitation limits, precision, linearity, and range. Drug analyses referred to the chemical testing of drug compounds. Analyzing pharmaceutical products is a branch of chemistry that includes purification, determination, quantification, and identification of a material, structural determination of compounds, and separation of the parts of a solution or solution.

**Keyword:** flow injection analysis, spectrophotometry, pharmaceutical drug.

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# **1. Introduction**

## 1.1 Flow-injection analysis and pharmaceutical

For a non-equilibrium dynamic response, flow injection analysis (FIA) is a very successful methodology that also served as an important bridge between human methods and automation instrument analysis(G.-Q. Zhang et al., 2020). For a non-equilibrium dynamic response, flow injection analysis (FIA) is a very successful methodology that also serves as a good bridge between human methods and automation instrument analysis. Flow injection analyzers (FIA) have become an increasing essential tool for pharmaceutical investigations due to its simplicity, low cost, and rapid approach. FIA is a continuous flow method that includes injecting a smaller batch plug into a stream of flowing reagents (Hadad et al., 2011) and (Sanchez-Pedreno et al., 1995). The FIA method is rigorous and based on three major factors: sample injection, reproducible timing, and controllable dispersion of the sample(Ranger, 1981). Drugs are important for human growth because they can be used to treat, repair, reduce, or prevent diseases in humans and animals(Felix & Angnes, 2010). One of the most important areas of analytical chemistry is pharmaceutical analysis. The discovery of new medications and the ongoing updating of international safety and effectiveness regulations for pharmaceutical formulations involves the continuous development of new analytical methods (Tzanavaras & Themelis, 2007). Detectors based on electrochemical and optical detection, as well as flow injection systems, have been widely used in a variety of applications. UV-Vis spectrophotometry is used in the analytical laboratory (Martinović et al., 2008). A sample is injected into a flowing carrier solution that mixes with reagents before reaching a detector in FIA, an automated method of chemical analysis. FIA techniques have evolved into a wide range of applications during the last 30 years, utilizing mass spectrometry, atomic absorption spectroscopy, spectrophotometry, fluorescence spectroscopy, and other instrumental analysis methods for detection (Ruzicka & Hansen, 2000). There have been a lot of random methods for determining these medications created (Solich et al., 2000). Apart from flow injection analysis. There are several methods for analyzing pharmaceutical drugs as High-Performance Liquid Chromatography (Kazakevich & Lobrutto, 2007), chemiluminescence (C. Zhang et al., 1999) ,NM Resonance (Malet-Martino & Holzgrabe, 2011), Thin-Layer Chromatography(Wagner & Bladt, 1996), liquid chromatography/mass spectrometry (Kumazawa et al., 2003), Gas chromatography-mass spectrometry(Belal et al., 2009) , fluorometry (Zeeb et al., 2010). Today, Drug Research publishes up-to-date information on all aspects of pharmaceutical discovery, including lead identification, molecular targets, gene therapy, drug delivery, lead optimization and related technologies, vaccine production, and clinical trials, as well as overviews of the current status of component classes and approaches in specific therapeutic areas or disease states(Koh et al., 2003). The study's goal was to develop a rapid and easy method for determining medications on a regular basis.

## 1.2 Spectrophotometric Technique

Spectrophotometry is a technique for determining the amount of light that interacts with a substance. Light can be dispersed, reflected, transmitted, or absorbed by a material, and light can be emitted by it either because it has absorbed and re-emitted some light because it has received energy in some other way (e.g., electroluminescence), or because it produces light owing to its temperature (incandescence). Spectrophotometric approaches such as spectral reflection, propagation, uptake, emittance, dispersion, and fluorescence can be classed as phenomenological optical features of the material (Germer et al., 2014). Spectrophotometry is the most practical analysis technique because of its inherent simplicity, low cost, and wide availability in most quality control laboratories (Ebraheem et al., 2011). Every chemical substance absorbs, transmits, or reflects light across a given wavelength range (electromagnetic radiation). Spectrophotometry is a measurement of how much a chemical material absorbs or transmits light. It is extensively used for quantitative analysis in a variety of fields (e.g., chemical engineering, biochemistry, clinical applications, biology, material and chemistry, industrial applications, physics, etc). Any app that interacts with chemical elements or chemicals can benefit from this technique. In biochemistry, for example, it is used to detect enzyme-catalyzed reactions. In clinical applications, it is used to evaluate blood or tissues for clinical diagnosis. Spectrophotometry of atomic emissions spectrophotometry and atomic absorption spectrophotometry are two examples of spectrophotometry variations(Filip et al., 2012). The visible spectrophotometer is a device that measures absorption and conducts quantitative visible light analysis (400 to 760 nm)(Wilson & Wilson, 2017). The UV-VIS spectrophotometer is used for the computation and quantitative examination of the absorption material at (200 760nm) and is one of the most extensively used pharmaceutical analysis procedures. Two light beams in the ultraviolet-visible area are used in ultraviolet-visible spectrophotometers(Behera et al., 2012). More over 760 nm is considered infrared. Infrared spectrometers come in a range of shapes and sizes, but they always have a few characteristics. All of the fascinating infrared radiation has a source. Spectrophotometer for Fluorescence The Fluorescence Principle is explained as follows: When light energy is incident on such fluorescence-capable aromatic-conjugated molecules, the incoming light is initially absorbed by the electron systems of these molecules. As a result, the electrons in this system transition from a ground to an excited state of energy. Furthermore, the electrons jump to different vibrational levels of the excited state. The electrons subsequently pass to the lowest vibrational energy level in the excited state due to thermal loss of energy (indicated by red vibrations of the molecule ). The electrons now move from the lowest energy level of the excited state to distinct vibrational energy levels of the ground state via the emission of a specific quantum of energy to produce of light because these molecules may fluoresce(Mehta, 2013). Atomic absorption spectrometry (AAS) is a very sensitive Atom analysis technology that allows metals to be determined at the picogram level in a variety of substances. Thousands of applications for a wide range of samples have been completed with it. The atomic absorption phenomenon is defined as the reduction in the intensity of optical radiation after passing through a cell containing gaseous atoms. Modern AAS instruments typically include a hollow cathode light source, which emits specific wavelengths of light that the analyte can ideally only absorb; a 'atomic cell,' which converts samples into gaseous atoms that can reflect radiation from the HCL; a 'Detection system,' which isolates and quantifies wavelengths of interest; and a computer system for controlling and collecting and processing data on instruments(Butcher, 2005).

# **2. Flow Injection Methods**

The main source flow was stopped, and the sample was transferred through the flow path either by secondary source flow. The main flow was then restarted to transfer the reagent to the detecting system, and this was then stopped. In order to improve this technique, a multi-position switching valve was used in sequential injection analyses. (b). The multi-position switch is used to select the sample to be inhaled into the column 1 reaction, the sample is then moved to the reaction column 2, and the inserted plug(s) is transported to the detector via the carrier liquid transport mechanism. The transport process to the detector is paused in this implementation while the individual samples are inserted into the detector. The transport mechanism to the detector is paused while the individual samples are inserted into the reaction column, as shown in this diagram. These reaction columns are typically utilized as reservoirs to hold large amounts of samples for transport or as dilution columns (figure1) (Idinyang et al., 2017).

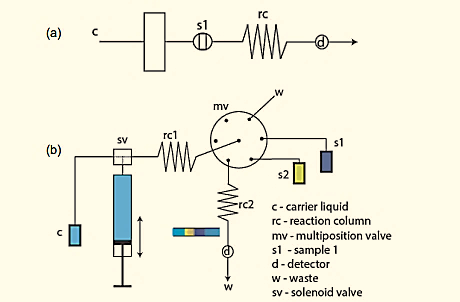


Figure 1: Flow Injection Methods (Idinyang et al., 2017).

# **3. Flow Injection System for Analysis**

A sample is injected into a solution of a flowing carrier, which mixes with reagents before reaching a detector in an automatic chemical analysis process known as FIA. In the last 30 years, FIA techniques have also been employed in a wide range of applications, including mass spectrometry, atomic absorption spectroscopy, spectroscopy fluorescence, and spectrophotometry, among others. High repeatability, automated sample processing, waste reduction, adaptation to micro-miniaturization, chemical isolation, and reagent economy in a microliter-scale system are all valuable qualities that contribute to the use of flow injections in real-world tests. The well-defined concentration gradient that occurs as the analyte is injected into the reagent stream (which allows an infinite number of well-reproduced analyte/reagent ratios) and the precise timing of fluidic manipulations are the flow injection's major resources (which provide exquisite control over the reaction conditions) (Ruzicka & Hansen, 2000).

## 3.1. Pumps

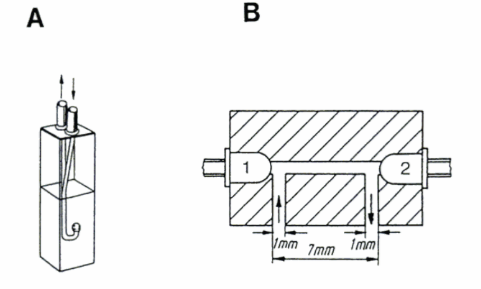
The consistent, steady flow of fluids required for a repeatable FIA can be produced in a variety of ways. Constant-head systems and various pumps, including peristaltic, syringe, and reciprocating types, have been employed. Because there are no gas bubbles in the system, pulsation is never a big issue; however, some workers consider it as a small issue, and deposing devices have been developed to address it. However, more recently, it has been suggested that a modest amount of flow pulsation may improve the peak forms produced during an analysis. Most clinical laboratories' chemistries feature a spare peristaltic pump ("back up") that would work perfectly in an experimental FIA system. Pumps for liquid chromatography are similarly acceptable, but each stream would require its own pump. Typical applications need 0.5 mL/min for 5 mL/min flow rates (Rocks & Riley, 1982).

## 3.2 Injection valve

The injection valve must also be constructed to place the wide distribution wave bolus of the sample in the carrier stream. The flow of the stream is not affected in this way with an injection event. The sample size ranges from less than 1μl to 200 μl for flow injection analysis, with 10 to 30μl being used for most applications(Kulkarni & Vaidya, 2015).

## 3.3 Detector

FIA-based systems Each detector that employed the measuring system's operations should be marked so that they can be tracked as closely as possible. The lit cell volume is aided by the lowest available dead volume and the A photometric detector. However a large volume of light reduces detection sensitivity and peak growth, it causes weak reproducibility of the flow injection peak's height and form. The most extensively used commercial detectors are geometry flow cuvettes that fit traditional spectrophotometers.(figure 1) , Generally with a volume of 50-60μI for a few and a path length of 10 mm. In comparative research, it was shown that the unfavorable effect of an increase of cuvette volume above 25µI, It is particularly critical at low flow rates. The impulse response function of the FIA system, which indicates the detector's contribution expanding to the peak, may be used to optimize detector geometry numerically. The utilization of a capillary flow cell with optical fibers to transmit light with a small illuminated volume(<1μI) allows the dynamic spectrum of sensitivity to be expanded and used in extraction systems without phase separation. The system with a working volume of 115nI and a light path of 0.5 cm was a result to be none colorimeter flow. Measurement of FIA 0.1 cm path through a thermal cross-beam photometer lens depends on the use of a single laser (Trojanowicz, 2000).



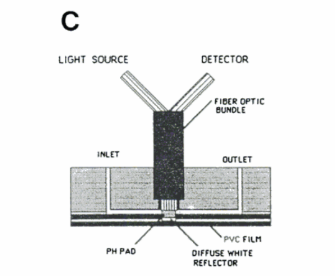


Figure 2: Spectrophotometric detection of flow-through cells used in FIA:- (commercial flow-through cell for conventional spectrophotometers); (B) With light-emitting diode(1) and phototransistor, flow-through cell;(c) Bifurcated optical cell flow-through light source, detector and fibrous indication pad (Trojanowicz, 2000) . The flow reverse injection analysis is one of the modes of FIA to minimize the usage of reagents, reduce the dispersion of the samples, and enhance mixing efficiency(Mansour & Danielson, 2012).

## 3.4 Flow injection description and performance or related system analysis

The parameters necessary for the application of the FIA or SIA system must be stated clearly, including sample and reagent flow rates of transport and reagent streams, multicommutator sequences or injection quantities, tubing diameter, and mixing coil length. Although traditional FIA uses a continuous flow of carrier and reagents, other modes of operation, including SIA, sometimes use stopped-flow, flow reversal, transient, pulsed, and gradient flows, and the time and flow sequences for each should be described, ideally in table form (Zagatto et al., 2002). Similarly, the reagent and carrier composition should be described with the flow cell volume specification, the detector used should be reported, and the entrance window opening, super for photometric measurements must be reported, or LEX and LEM for fluorescence measurements must be proposed like a technique flow-based analysis process. The following analytical merit graphs should be defined in terms of linear dynamic range: precision, precision, sensitivity, detection limit, and selective (McKelvie, 2008).

# **4.** **Flow-injection spectrophotometric techniques for pharmaceutical samples**

Spectrophotometric techniques are the most extensively used techniques in chemical analysis due to their availability of methodologies, procedural simplicity, sensitivity, and extensive applicability. The rules controlling absorption and emission phenomena can be used to compute the concentrations of Mixtures in solutions, particularly those of pharmacological, chemical, or biological importance(Skoog et al., 2017). Analysis of medicines, including pharmaceutical preparations or the raw materials used in their manufacture, and drug determination in combination with metabolites samples from biological (urine, serum, saliva, plasma, and certain secretions) samples make up a large part of clinical and pharmaceutical laboratories' work. Spectrophotometry, which is based on light absorption or chromogenic reactions by the analyte, is the most extensively used method for drug determination. Redox processes and the creation of charge-transfer complexes Drug chromogenic reactions are made up of metal-ion complexes(Costa et al., 2017). Some of the processes for the quantification of pharmaceutical samples for flow-injection spectrophotometry are detailed in Table 1.

Table 1:Some techniques for the pharmaceutical samples are represented by flow-injection spectrophotometry.

|  |  |  |
| --- | --- | --- |
| Samples | linear range | Detection limit |
| Gemfibrozil (Tzanavaras & Themelis, 2005) | 20–100  µg/ml | 1.4 mg /L |
| Lansoprazole (Yeniceli et al., 2004) | 5.4×10−6  - 5.4×10−5 M | 5.8×10−7 mol /L |
| Ketoprofen (Özlü et al., 2005) | 7.5–75 μg/ml. | 0.303 µg/ml ampoules. |

# **5.** **Determination of some pharmaceutical by** **flow injection spectrophotometry**

Table 2; Flow injection-spectrophotometry for determination of pharmaceutical analysis

|  |  |  |
| --- | --- | --- |
| Sample | Linearity range | Limits of detection |
| Amoxicillin Trihydrate (Al-Abachi & Subhi, 2013) | 50-1200μg/ml | 14.21 μg/ml PHP. 34.00μg/ml AMOX. |
| Clonazepam (Al-Abachi & Hammoudi, 2015) | 50-400 µg/ml | 0.193, 22. µg/ml |
| Azithromycin (Rufino et al., 2008) | 50 – 1600 µg/ml | 6.6 μg µg/ml |
| Ciprofloxacin (Palamy & Ruengsitagoon, 2018) | 0.5- 50 µg/ml | 0.20μg /mL |
| Nitrofurantoin (Hadi & Mouayed, 2017) | 5–300 µg/ml | 1.9-4.8 mg/mL |
| Imipramine (Pérez-Ruiz et al., 1994) | 0.79 - 25.3 µg/ml | --------- |
| Piroxicam (Abed & Hadi, 2020) | 1 - 35 and 10 – 250 μg/mL | 0.2 and 3.4μg/mL |
| Novalgina (Los Weinert et al., 2007) | 1.45×10–6 - 2.90×10–5 mol/ L | 1.31 × 10–7 mol/ L |
| Adrenaline (Ferreira et al., 2008) | 0.1 - 0.8 mmol /l | 8×10−3 Mmol/ l |
| Phenylephrine hydrochloride (Knochen & Giglio, 2004) | 5.8–160 mg /L | ------ |
| Tenoxicam (Garcı́a et al., 1999) | 7.0–320 ,0.5–8.5 mg/L | -------- |
| Methyldopa (Ribeiro et al., 2005) | 50–200 mg /L | ------- |
| Diclofenac sodium (Garcı́a et al., 1998) | 0.20–8.0 mg /L | ------- |
| N-Acetyl-L-Cysteine (de Toledo Fornazari et al., 2005) | 3.5×10–6 - 4.3×10–4 M | 6.3×10–7 M |
| Diazepam (Liawruangrath et al., 2006) | 2-110 mg l−1 | 0.6 mg L−1 |
| Paracetamol (Bouhsain et al., 1996) | 1 x10-5 - 3 x 10-5 M | 0.2 µg ml |
| Dipyrone (Marcolino Júnior et al., 2005) | 5 × 10-4 - 8 × 10-3 mg /L | ------- |
| Methyldopa and dopamine hydrochloride (Al-Abachi et al., 2009) | 1 to 100 µg/ml | 0.769- 0.560 µg/ml |

# **6. Conclusion**

The methodology suggested is particularly suitable for analyzing pharmaceutical drug analysis. As a result, Flow-injection spectrophotometric is one of the most essential approaches. suggested FI spectrophotometric system for pharmaceutical drug analysis. This technique is sensitive, stable, inexpensive, accurate, quick, and easy to use, with only a few reactions and sequences required; it could be of great interest to quality control laboratories or the pharmaceutical industry for frequent quantitative drug analysis. There has been an emphasis on enhancing throughput for expedited drug discovery by improving technique or instrument.

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