Development and Application of Fully Automated Microfluidic Devices for the Determination of Phosphate in Natural Waters of South IRAQ: A Comparative Study with Classical Analytical Methods

Rana T. Shibli1, K. H. Al-Sowdani2
1-Chemistry Department, College of Education for Pure Sciences, University of Basrah, Basrah, Iraq
2-Department of Pathological Analysis Science, Shatt Al-Arab University College, Basrah, Iraq
Corresponding Author Email: **rana.tariq@uobasrah.edu.iq**

Abstract:

Microfluidic devices are widely used for various applications in environmental monitoring, including the assessment of water quality. These tools have become indispensable for detecting ecological pollutants due to portability, ease of use, and rapid response capability. Although previous studies have explored the utility of microfluidic technology, this study developed a custom automated microfluidic spectrophotometer system to determine phosphate ions in environmental water samples collected from the Shatt Al-Arab River in southern Iraq. The system, designed with three Arduino-controlled pumps, incorporates a fabricated microfluidic chip consisting of three channels with a total volume of 25µL and a length of 10 Cm. Two of these channels have a volume of 10 µL each, while the third channel has a volume of 5µL. Data were recorded as peak heights using Microsoft Excel 2016, with the peak heights corresponding to the sample concentrations. The range obtained for the linearity standard curve was (0.05-3) µgmL-1, and eight points were chosen to find the regression coefficient (0.9997). A detection limit of 0.005 µgmL-1 was achieved, indicating high sensitivity in phosphate determination. The relative standard deviation (R.S.D. %) from ten replicate measurements was 0.052 % demonstrating the precision and reliability of the analytical method. One sample required 67µL of chemical reagents; hence, the samples consumed 16 mL of chemical reagents each hour, meaning the throughput in one hour was 240 samples. This indicates the amount of waste and chemicals. The reagents consumed were very low, making the proposed method for monitoring phosphate ions eco-friendly. The analytical performance of the system was evaluated by comparing the results with those obtained using conventional approaches, the Murphy method, and the standard additions method. The findings confirmed that the proposed system provides a cost-effective, rapid, and reliable alternative with high analytical efficiency for phosphate ion determination

Keywords: monitoring phosphate ions, microfluidic, ecological pollutants, fully automated, standard additions method

Introduction

Phosphorus (P) is essential for growth and development in aquatic life. There are two forms of Phosphorus: Dissolved and particulate. The dissolved form has a variety of organic and inorganic materials. The most common type of phosphorus in water and wastewater is phosphate $(PO4)^{3-,}$ which is divided into three main groups: organic phosphorus compounds, condensed phosphates (pyro, meta, and poly), and the inorganic orthophosphate form, which is considered the stable state [1-3]. The mixing of untreated wastewater, raw wastewater, and agricultural runoff fosters the growth of microorganisms in large quantities. All these processes are related to eutrophication, photosynthesis, and decomposition [4], human activities are the primary source of eutrophication, resulting in an overabundance of nutrients in lakes, rivers, and other bodies of water, the key nutrient components include nitrogen (N) and (P) Phosphorus which is also essential for Photosynthesis the main production process in aquatic environments and the marine carbon cycle so phosphorus levels in aquatic bodies should be measured [5]. The basic step for determining orthophosphate involves colorimetric detection using the Spectrophotometric technique, which is widely regarded as the most favorable approach due to its excellent detection limits, dynamic range, and cost efficiency. When analyzing water samples to assess phosphorus there are two fundamental procedural steps to follow [6], the major method used is the molybdenum blue method known for the deep blue colored complex it formed, which is perfect for visual titrations, it is also suitable for a region of the visible spectrum because the molybdenum blue complex absorbs in the 650 -800 nm [7]. Another colorimetric method that has emerged as an attractive alternative is the vanadomolybdo phosphoric acid method, also referred to as the yellow method. This process involves a combination of reagents and the sample containing orthophosphate, resulting in the formation of ammonium phosphor-vanadomolybdate, a heteropoly acid represented as (NH_4) $3PO_4.VO_3$ $16MoO_3$ produced a distinct yellow color. This complex exhibits strong absorbance below 400 nm under acidic conditions. Ammonium molybdate $(NH_4)_6MO7O_{24}$-$7H_2O$, and ammonium metavanadate NH_4VO_3 are reacted [8]. The assessment of environmental waters continues to rely largely on manual sampling, which is subsequently analyzed in laboratories utilizing conventional methods such as colorimetry, atomic absorption spectroscopy, mass spectrometry, and ion chromatography [9]. In recent years, advancements in microfluidic systems

have led to a growing trend in developing lab-on-a-chip automated devices for processing and analyzing various environmental samples [10]. Microfluidic emerging technologies offer significant customization options and present commercially viable alternatives to traditional detection methods used in ecological monitoring. They enhance toxin detection through the production of low-cost, miniaturized, and highly stable solutions, thereby increasing sensitivity [11,12]. This study aimed to develop a portable, efficient, and affordable analytical system for the comprehensive detection of phosphate in environmental samples. The automated system employs colorimetric detection of phosphate using the molybdenum blue method.

Experimental

Reagents and chemicals:

-Chemicals

Several chemicals were used in this research, including: Potassium dihydrogen phosphate (Hiimedia), ammonium molybdate (BDH), Nitric acid (Riedel-deHaen), Ascorbic acid (BDH)

-The Solutions:

All stock and standard solutions were prepared from primary materials using deionized water (DI) and high-purity, analytic-grade chemical reagents

- 100 μgmL^{-1} KH_2PO_4 Potassium dihydrogen phosphate was prepared by dissolving 0.1432 g in 1000 mL of the stock solution, and the standard solutions in the range were prepared by serial diluting between 0.01- 3.5 μgmL^{-1}.
- 0.01 M $(NH_4)_6Mo7\ O_{24}.4H_2O$, was prepared by dissolving 6.175g in 0.4M of HNO_3 and completing to 500 ml with 0.4M of HNO3.
- 0.35% w/v ($C_6H_8O_6$) ascorbic acid solution was prepared by dissolving 0.875g in 250 ml of deionized water, and the concentration solution ranging between (0.1-0.3) % w/v was prepared by serial dilution.

The principle of the Analytical Methods

This study investigated spectrophotometric methods for monitoring phosphate levels in water samples from environmental sources. The approach utilizes a fully automated microfluidic system to identify the optimal parameters for flow rate, sample volume, and suitable reagent concentrations, thereby determining phosphate concentration. The results obtained with this

method was compared with those from the standard additions, as well as Murphy and Riley methods. They are based on the reaction of orthophosphate with ammonium molybdate in acidic medium to form phosphomolybdic acid, Then, it was reduced by ascorbic acid to form a blue-colored complex measured at 690 nm. The absorbance is proportional to the phosphate concentration. [13-15]

Methodology

Three Arduino pumps, were operated and controlled via custom-developed software which used to regulate the flow of reagents and samples through the manifold of the microfluidic system (Fig. 1). The flow rate of these pumps specifically ranged from 1 to 5 $mLmin^{-1}$. The chip used to determine phosphate ions consists of three channels for acidified ammonium molybdate, the sample solution, and for ascorbic acid. These solutions mix and react inside the chip, and the resulting complex is directed through a detector supplied with a flow cell. The detector is a photoresist sensor type which convert the signal to peak height through the microcontroller, The peak height corresponding with phosphate concentration in the water samples. Finally, the result was recorded on a laptop screen equipped with custom software and Microsoft Excel 2016, for spectral data analysis.

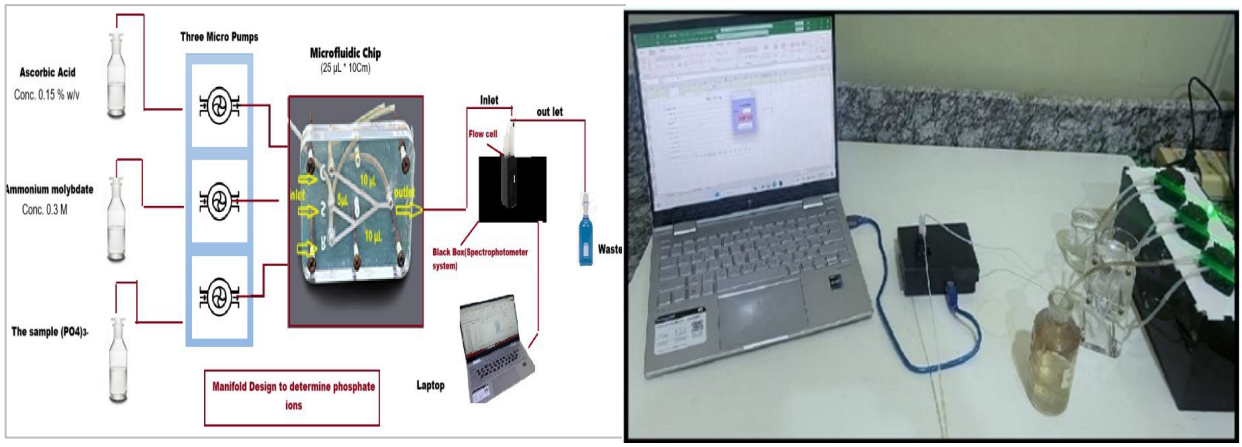


Figure 1. The automated microfluidic system. b - The live screen of the automated microfluidic system

The Microfluidic Chip Design:

The microfluidic chip which dimension was 6 cm, 10 cm and 0.8 cm for width, length, and thickness respectively., was made from two layers of Polymethylmethacrylate (PMMA), The upper piece acted as a cover without any drilling, while the other was drilled using a specialized tool to create three channels. Samples and reagents are introduced into the chip through the three-input channel

which emerge into a single output channel, through a Y-shaped configuration. Suitable for the working method, the two pieces were bonded together using an adhesive material. This was applied only to the outer parts of the chip to avoid affecting the internal flow and reactions. The layers were also tightly fixed with mechanical screws to ensure the structure is more stable for the working method. The volume of these channels was determined by injection of highly concentrated dye, where found two channels having a volume of 10 µL each, while the third had a volume of 5 µL with a total volume of 25 µL(Figure 2).

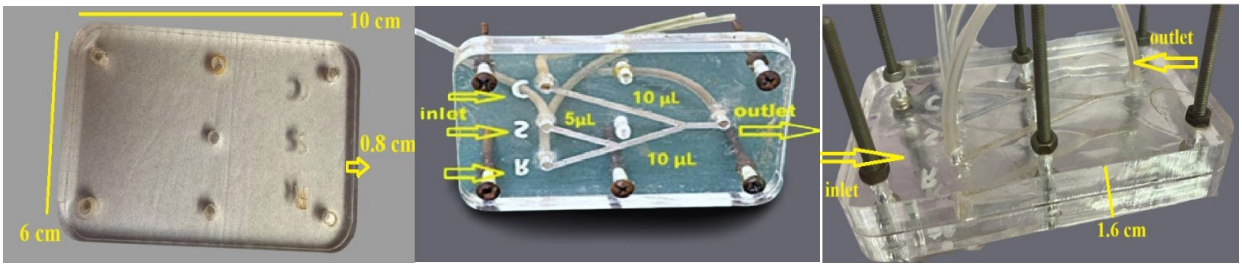


Figure 2. Microfluidic chip design

Design and Main Components of the Developed Microfluidic Spectrophotometric System: Table 1 and Figure 3 show the fundamental components of the laboratory-fabricated microfluidic system include three 12V DC micro pumps type DIY, controlled via an L298N motor driver to regulate solution flow rate, a 32 µ L flow cell high precision high quality customize flow through cuvette quartz cell (Centre height: 8.5mm, Light Path: 10mm). Outer dimensions: H35mm x W12.5mm x D12.5mm), a 28 V LED lamp operating in visible region, and photoresist sensor functioning as a detector to convert light intensity into an electrical signal. An Arduino microcontroller linked to a laptop running a custom Microsoft Excel 2016 served as a data logger, used for real-time acquisition and display of peak heights related to the analyte concentration.

Table 1. Main components of the fabricated microfluidic system

Components	Work	type	Origin
ARDUINO	Micro controller	UNO	Italy
Driver Motor	Micro controller	L298N	China
Three micro pumps	With drawing carrier and reagent	INTLLAB12VDCDIY	China
Power supply	Device power controlling	-	China
On/off button	on/off	-	China
Flow cell 32 µL	Sample container	SOS	China
LED-Lamp	Light source	-	China
Photoresistor sensor	Detector	-	China

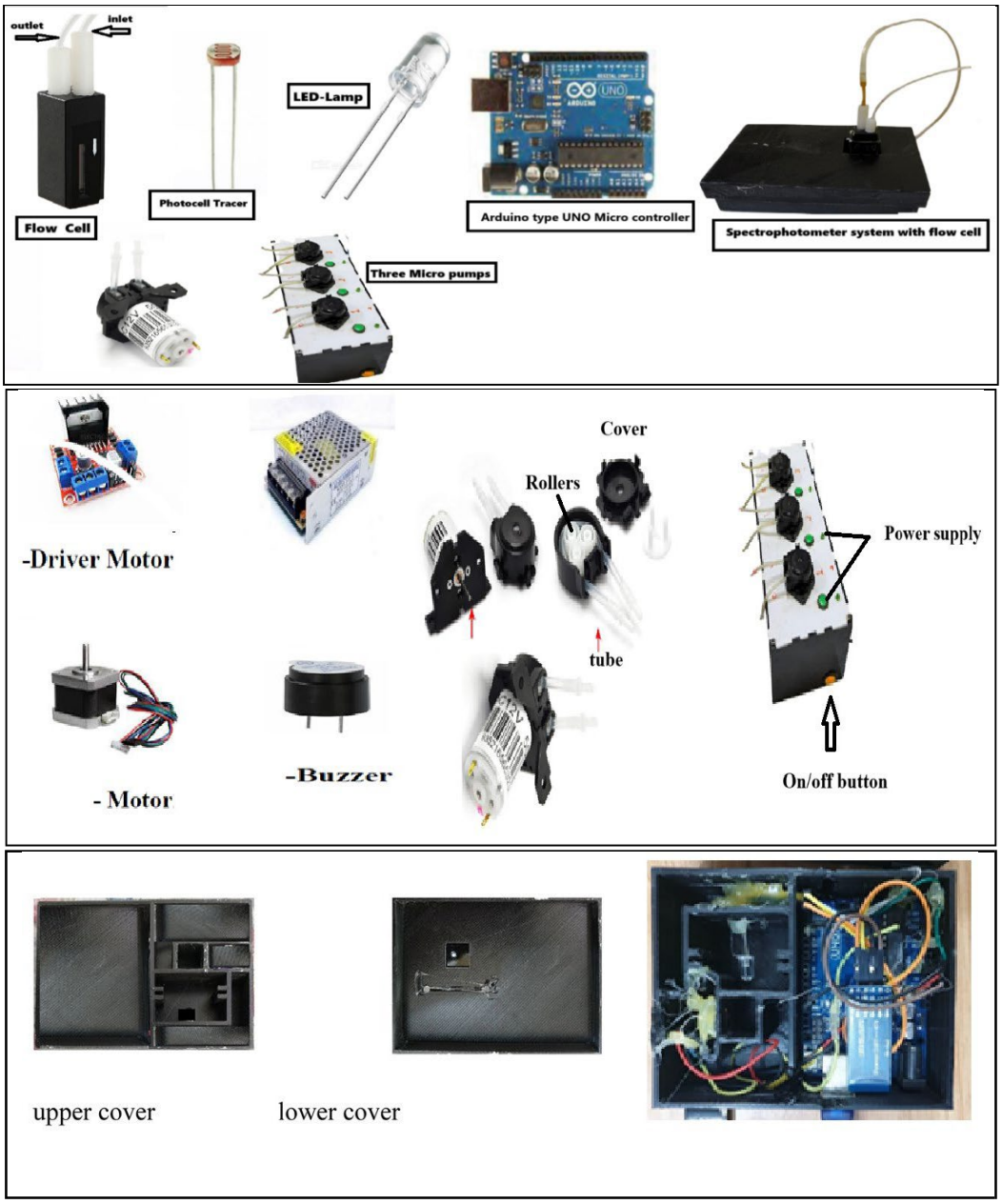


Figure 3. All Components of Microfluidic system

Result and Discussion

Optimum conditions:

The optimal operating conditions were established through a comprehensive series of experiments designed to evaluate the parameters influencing the analytical signal. Focusing on peak height, which corresponds to phosphate concentration detection as we described perversely [16]. These experiments enabled to identification the key variables, including sample volume, total flow rate, and reagent concentrations. Under the established optimized conditions,

The reaction proceeded with high efficiency, leading to the selection of a of. The system exhibited its best analytical performance at sample volume, total flow rate, ammonium molybdate and ascorbic acid were 12 µL, 2.5 mL/min, 0.003 M and 0.15 % w/v. respectively. These findings are supported by the experimental data, presented in Figures 4 and Table 2.

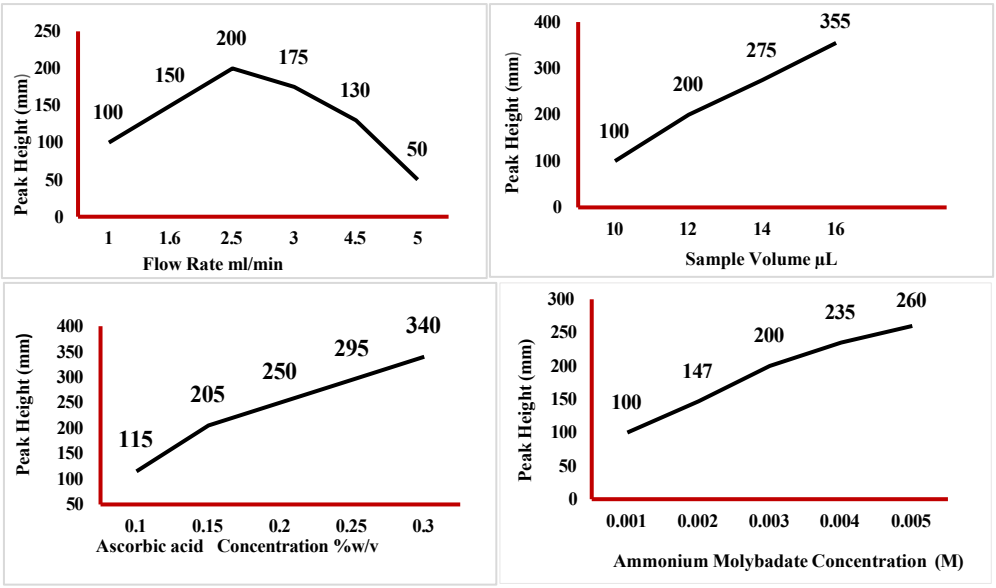


Figure 4. Effect of (total flow rate, sample volume, ascorbic acid concentration, ammonium molybdate concentration)

Table 2. Optimum conditions for phosphate ion concentration determination

Determination	Parameters Values
Chip Volume	25µL
Total Flow Rate	2.5 mL/min
Sample Volume	12 µL
Ascorbic Acid Concentration	0.15 %w/v
Ammonium Molybdate concentration	0.003 M
Throughput	240 sample/h
Tube diameter	0.18mm

Linearity Studied

The proposed microfluidic system was used to establish a calibration curve for phosphate determination under the optimized conditions, as presented in Table 2. The method exhibited excellent linearity over the concentration range of (0.05-3) µgmL^{-1}, with a regression coefficient (R^2) of 0.9997. The relative standard deviation (R.S.D.%) and detection limit are 0.052 % and 0.005 µg mL^{-1} respectively (shown in Table 3 and Figure 5.). The R.S.D.% values are

below 1% confirming the high precision, repeatability, and sensitivity of the method, which fall within acceptable limits. The linear calibration curve for the Murphy method was established over the concentration range of 0.005-3 µg /mL, as illustrated in Figure 6. Additionally, standard additions method was employed to accurately measure the concentration of phosphate ions, eliminating all interferences, by using a standard phosphate concentration within the range of 0.05 to 2.5 µg mL^{-1}, as shown in Figure 7. All measurements were conducted at a wavelength of 690 nm [17,18].

Table 3. Standard Calibration graph of phosphate standards

Phosphate Concentration µgmL^{-1}	Peak Height (mm)	Relative Standard Deviation R.S.D% (N=5)
0.05	15.157	0.518
0.5	100.632	0.314
1	200.085	0.021
1.5	312.645	0.103
2	415.886	0.107
2.5	511.198	0.215
3	612.883	0.154

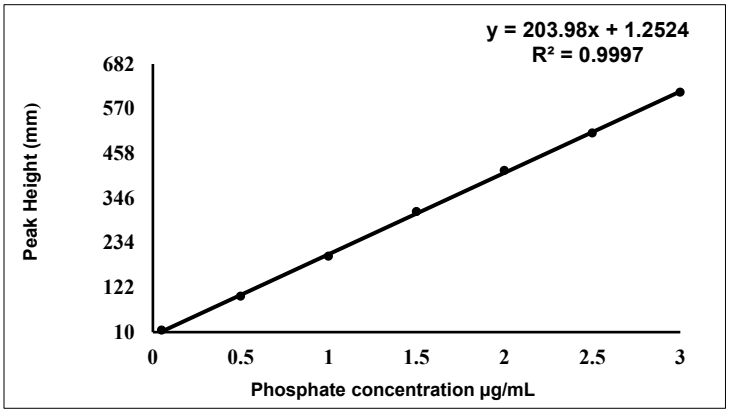


Figure 5. Standard calibration curve for phosphate determination by a microfluidic device

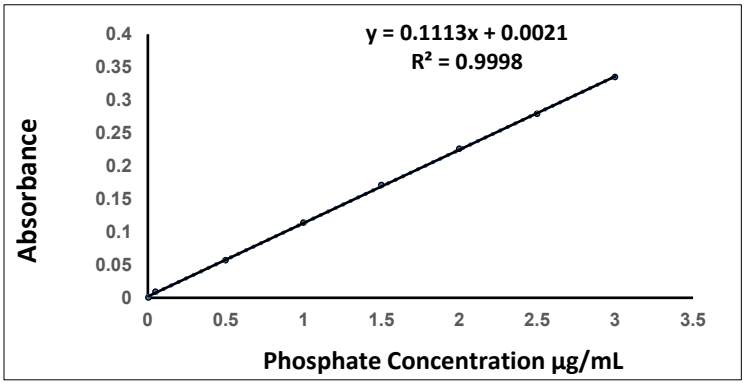


Figure 6. Standard calibration curve for phosphate determination by the Murphy Method

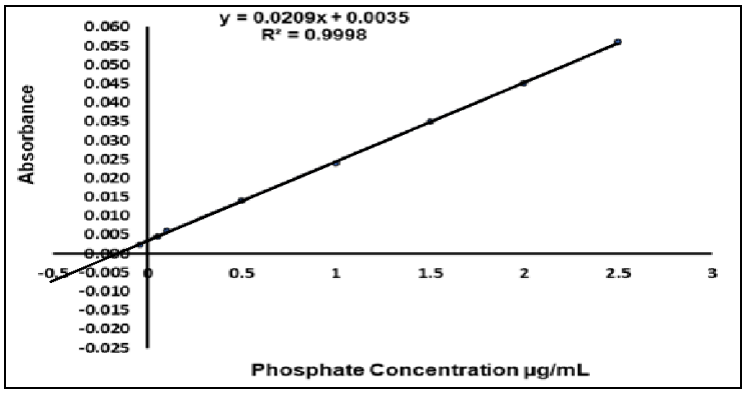


Figure 7. Standard calibration curve for phosphate determination by the Standard additions Method

Repeatability & Dispersion Coefficient (D):

The repeatability was assessed by performing at least ten measurements of the sample injections under optimal conditions [19-21]. With five consecutive injections of seven different concentrations, as shown in Figure 8. A throughput of 240 samples per hour was achieved, with a total reagent consumption of 16 mL per hour, corresponding to 67 µL of chemical reagent per sample. This indicates that the proposed method is characterized by reduced consumption of chemical reagents in addition to its economic and operational efficiency. The accuracy and stability of the results were confirmed by evaluating the coefficient of dispersion of the manifold unit, which was found to be 1.25, as shown in Figure 9. The dispersion coefficient values were calculated by dividing H^0 by H max, where H^0 is the highest peak measurement obtained by mixing all components, and H max is the typical peak produced during the loading and injection process, representing the highest peak. The coefficient of dispersion was measured under optimal conditions

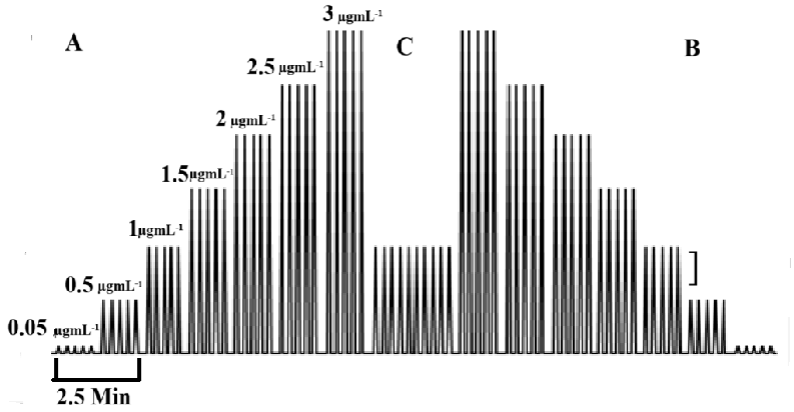


Figure 8. (A). Standard calibration graph for phosphate determination (B). The Peaks obtained by injecting phosphate Standards (C). Ten replicates of 1 μgmL^{-1} standard phosphate

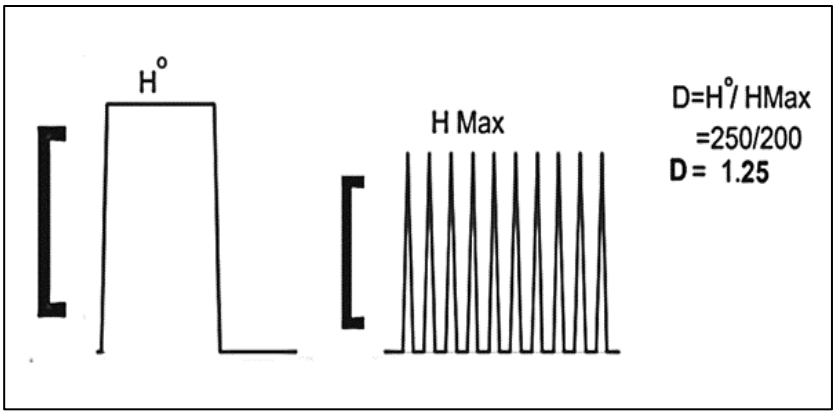


Figure 9. The dispersion coefficient in the microfluidic system

Application:
Sampling

Before starting the sample collection, it is essential to confirm the suitability of the sampling strategy by selecting appropriate sampling sites that consider safety and accessibility. Therefore, we collected samples 30 cm below the water surface using a water sampler and placed them in 1L plastic containers for storage. To preserve the samples, 10 mL of chloroform was added, and they were stored in a freezer at 4 ˚C. Four locations along the Shatt Al-Arab River in the Basra governorate of southern Iraq were selected for sampling in October. [22] The Sampling sites were strategically chosen to represent the diversity of pollution sources, including boating and fishing activities, residential area, agricultural lands, and drainage discharges. Although nutrient levels are generally low during this month[23], the sampling sites were distributed as follows: The first site (R_5) was located at the at the Al-Saad bridge**(30°44′51.03″N,47°41′ 59.64″ E**), , the second site (R_6) was situated in the Al- Ashar area, **(30°30'44.0"N,47°51'05.5"E**) the third site (R_7) was selected from Abu Flous (**30°27'54.2"N 48°00'36.5" E**). while the fourth site (R_8) was located at Al-Seeba (**30°20'14.9"N, 48°15'38.1"E)**, representing the downstream end of the studied section. (Figure 10). Several processes occurred after collection at the laboratory, such as filtration and analysis, before storing in the freezer. 0.45 mm filter paper (GF/C Whatman) was used for filtration [24].

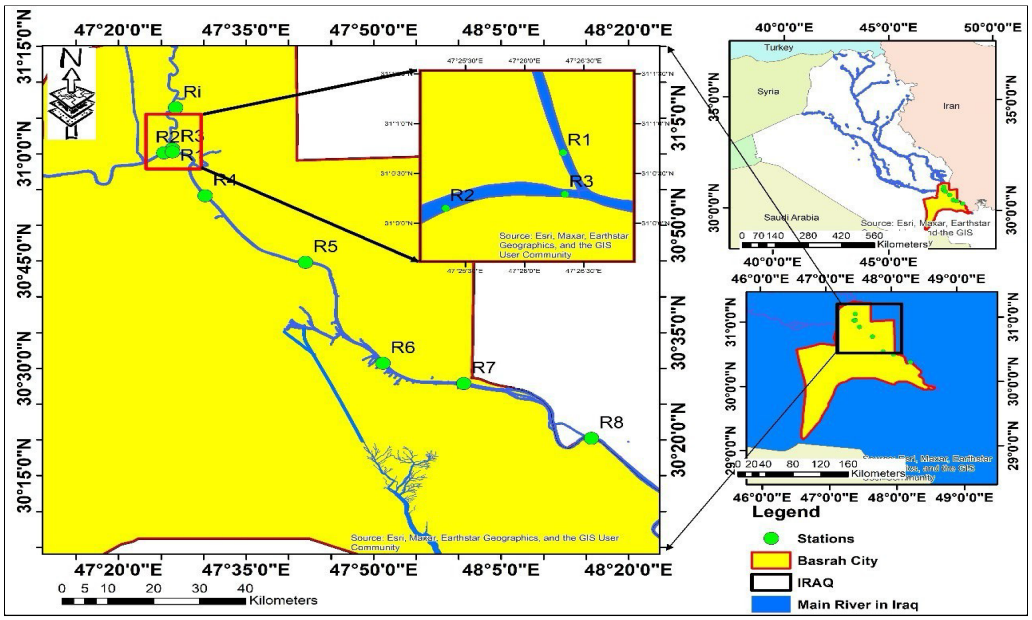


Figure 10. The Location of the samples selected in southern Iraq

Results

The proposed microfluidic system was successfully used to detect phosphate ions in various water samples. This approach helped reduce interferences from other compounds in complex matrices, enabling a comparison of the results with those obtained using the standard additions method and the Murphy method on the same samples. The results showed consistency among the three methods, as indicated by the relative standard deviation and recovery according to the tables (4).

Table 4. The determination of phosphate ions in real samples by Microfluidic methods

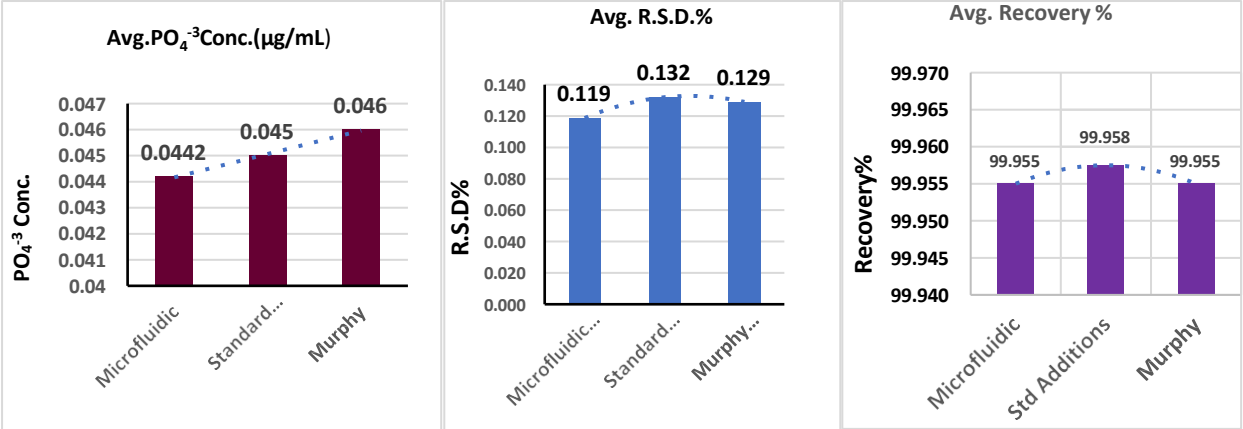
No.	sample	Microfluidic Method			Standard additions method			Murphy method		
		Conc. Po4 μgmL^{-1}	R.S.D. % N=5	Recovery %	Conc. Po4 $\mu g\ mL^{-1}$	R.S.D % N=3	Recovery %	Conc. Po4 $\mu g\ mL^{-1}$	R.S.D % N=3	Recovery %
R5	Saad Bridge/ Shatt al-Arab River	0. 079	0.126	99.92	0.075	0.134	99.93	0.08	0.130	99.92
R6	Al-Ashar	0. 019	0.132	99.98	0.02	0.145	99.98	0.022	0.142	99.98
R7	Al-Seeba	0. 009	0.075	99.99	0.012	0.102	99.99	0.01	0.089	99.99
R8	Abu Flous	0. 07	0.142	99.93	0.073	0.147	99.93	0.071	0.155	99.93

Statistical Analysis

The phosphate (PO_4^{3-}) concentrations were determined for three analytical Method (Microfluidic, Standard Additions, and Murphy) show very close agreement in concentration

values with average results ranging between 0.044–0.046 µg/mL, and the results were statistically analyzed in terms of mean, standard deviation (SD), relative standard deviation (R.S.D%), and recovery efficiency. The recovery percentages are nearly identical (≈99.95%), indicating excellent accuracy and negligible systematic error across all methods.

Regarding precision, the Microfluidic method demonstrated slightly lower RSD values (0.119%) compared to Standard Additions (0.132%) and Murphy (0.129%), suggesting that it provides more consistent measurements. The standard deviation analysis also supports this trend, as the Microfluidic approach exhibits smaller data spread. Although the differences are small, this highlights the potential of the Microfluidic method for high- precision analysis in environmental water samples. In conclusion, all three methods are reliable for phosphate determination However, the Microfluidic approach offers a slight advantage in precision while maintaining equivalent accuracy compared to the conventional Standard Additions and Murphy methods.



Conclusion:

Water is an essential resource for human survival, and its conservation is crucial to ensure the sustainability of human life. Therefore, monitoring certain environmental factors is necessary for protection. However, the high operation cost, the requirement for large sample volumes, the need for highly skilled personnel, and the extensive space required limit the applicability of many classical methods. Therefore, the LOC microfluidic devices provide promising features for detecting water contaminants, highlighting their importance in environmental diagnostics and water quality management. Their features include cost-effectiveness, compact size, and minimal sample volume requirements. The results of this study demonstrate that the proposed method provides accurate and reliable results when compared to standard conventional techniques.

References:

1. Schipanski M.E., Eennett M. B., Fundamentals of ecosystem science, 2021, The phosphorus cycle Fundamentals of Ecosystem Science (Second Edition), Pages 189-213, (2021). https://doi.org/10.1016/B978-0-12-812762-9.00009-5.

2.Rout P.R. et al, Nutrient removal from domestic wastewater: A comprehensive review on conventional and advanced technologies, Journal of Environmental Management 296 113246. (2021) https://doi.org/10.1016/j.jenvman.2021.113246

3. Worsfold P. et al. Determination of phosphorus in natural waters: A historical review, Analytica Chimica Acta 918 8e20, (2016). Doi: 10.1016/j.aca.2016.02.047

4.Abonyi, M.N., Obi, C.C., N wabanne, J.T. et al. Emerging and eco-friendly biological methods for agricultural wastewater treatment. Environ Syst Res 13, 45 (2024). https://doi.org/10.1186/s40068-024-00373-45.

5.Suresh K.et al. Recent advancement in water quality indicators for eutrophication in global freshwater lakes, Environ. Res. Lett. 18, 063004 (2023), https://doi.org/10.1088/1748-.

6.Habibah N., Dhyanaputri G. A., et.al, A Simple Spectrophotometric Method for the Quantitative Analysis of Phosphate in the Water Samples, Journal Sains dan Teknologi, Vol. 7, No. 2, (2018). DOI: 10.23887/jst-undiksha. v7i2.13940

7.Fones G. R. & Bakir A et.al, Using high-frequency phosphorus monitoring for water quality management: a case study of the upper River Itchen, UK, Environ Monit Assess (2020) 192: 184.https://doi.org/10.1007/s10661-020-8138-0.

8.M.M. Taha and M.A. Zayed, Efficient comparative evaluation of spectrophotometrically determined phosphate in synthetic and in actual human embryos' culture medium, Egypt. J. Chem. Vol. 65, No. 2 pp. 113 - 121 (2022). DOI: 10.21608/ejchem.2021.80863.4006

9. Cleary, J., Maher, D., Diamond, D. Development and Deployment of a Microfluidic Platform for Water Quality Monitoring. In: Mukhopadhyay, S., Mason, A. (eds) Smart Sensors for Real- Time Water Quality Monitoring. Smart Sensors, Measurement and Instrumentation, vol 4. Springer, Berlin, Heidelberg. (2013). https://doi.org/10.1007/978-3-642-37006-9_6.

10.Maguire I., Fitzgerald J., et. al, Novel Microfluidic Analytical Sensing Platform for the Simultaneous Detection of Three Algal Toxins in Water, ACS Omega, 3, 6624−6634, (2018). doi:10.1021/Acs omega .8b00240.

11. Solanki Sh., Mouli Ch., et. Al, Emerging Trends in Microfluidics, Based Devices, Biotechnology Journal,15(5):1900279, (2020). doi: 10.1002/biot.201900279.

12.Aboud, M. N., and Al-Sowdani K. H. A smartphone serves as a data logger for a fully automated lab-constructed microfluidic system." Methods X 12 (2024): http://dx.doi.org/10.1016/j.aca.2015.07.030

13. Mogashane T. M., et.al, A Review of Recent Developments in Analytical Methods for Determination of Phosphorus from Environmental Samples, Molecules, 30, 1001, (2025),https://doi.org/10.3390/molecules30051001

14.Dadamos T.R.L., Damaceno A.J., et .al, Standard addition method with cumulative additions: Monte Carlo uncertainty evaluation, Analytica Chimica Acta. 1059, Pages 28-35, ISSN 0003- 2670, (2019). DOI: 10.1016/j.aca.2019.02.002

15.Murphy, J., & Rilly, J. A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta, 27, 31–36. (1962) https://doi.org/10.1016/S0003-2670(00)88444-

16.Shibli R.T., Al-Sowdani K.H. Analytical Design of an Automated Microfluidic System and Its Application for the Determination of Nitrite Ions in Environmental Samples, The International Journal of Environmental Impacts 8(4):807-815, (2025). DOI:10.18280/ijei. 08041

17. Al-Sowdani K. H.; et al. Flow-injection determination of europium after on-line reduction. 201(none), 339–343(1987). doi:10.1016/s0003-2670(00)85356-8

18. Al-sowdani K. et al., Simultaneous spectrofluorimetric determination of cerium (III) and cerium (IV) by flow injection analysis, Analytica Chimica Acta 179:469-473, December (1986). DOI: 10.1016/S0003-2670(00)84494-3

19. Yousif Sh. J. Al-Jorani 1 and K. H. Al-Sowdani , Lab-Made Semi-Automated Microfluidic Flow Injection Spectrophotometric System for Determination of Nitrite in Natural Water , Journal of Physics: Conference Series 1818 (2021) 012050 IOP Publishing,doi:10.1088/1742-6596/1818/1/012050

20.Ahmed F., et al.; Design and validation of microfluidic parameters of a microfluidic chip using fluid dynamics. AIP Advances; 11 (7): 0752241 July (2021). https://doi.org/10.1063/5.0056597

21. B.O. Isiuku and C.E. Enyoh, Pollution and health risks assessment of nitrate and phosphate concentrations in water bodies in South-Eastern, Nigeria, Environmental Advances 2 100018, (2020). https://doi.org/10.1016/j.envadv.2020.100018

22.Wilde, F.D., Sandstrom, M.W., Skrobialowski, S.C. Chapter A2. selection of equipment for water sampling. In Techniques of Water-Resources Investigations. US Geological Survey (2014). https://doi.org/10.3133/twri09A2

23.Lateef Z. Q., Al-Madhhachi A.-S. T., et.al, Evaluation of Water Quality Parameters in Shatt AL-Arab, Southern Iraq, Using Spatial Analysis, Hydrology, 7, 79, (2020). http://dx.doi.org/10.3390/hydrology7040079.

24.DES. Monitoring and Sampling Manual: Environmental Protection (Water) Policy. Brisbane: Department of Environment and Science, Government, (2018).