Lab-Build Very Simplefluorimeterflow injectionsystem with Assistanceof 3D printer

Ali S. Namea and K.H.Al-Sowdani

Chemistry Department - Education and pure science college - Basrah University/Iraq
alisna343@gmail.com

Abstract:

This study focusing on build-up and designing a fluorimeter flow injection system with assistance of a 3D printer.. The fluorimeter as new detector in our laboratory is a very simple fluorimeter was designed and built-up by the aid of a 3D printer to housing in which all the components are supplied from the local market. It consists of three major parts. Firstly, a simple, low-voltages, and long-lived light-emitting diodes (LED) with 0.25 watt. The second parts is 450 µL flow cell QS Hellman with four faces in order to select precisely the 90 ° angle between the source and third components, the LED arrays detector . All these components were put in small plastic box which the internal and external covers were made from black plastic which thought to prevents the unwanted light from reaching the detector. The device is very light weight (165.5 g) and the fluorimeter box dimensions were 11cm in length , 8cm in width and 3cm in height.Two Microcontrollers Mega and UNO types were used as data a data-logger and controlled the home-made mini peristaltic pump, respectively. A direct fluorimetric determination of fluorescein dye as an example which was carried out in this work. This thought will be made the construction system a very simple fluorimeter since the dye excited and fluorescent in the visible range.Linearity was found in the range ($0.2\text{-}1.6 \times 10^{-4}$mol/L) with a regression coefficient of eight points was 0.9989, r.s.d% for ten replicates of was 0.84% and detection limit was 0.1×10^{-4}mol/L. The sample throughput was 200 samples per hour. The dispersion coefficient of the manifold was 1.04. The lab-build semi-automated fluorometeric FI system was reliable, fast and low cost which is matching our undergraduate laboratories with low budget.

Keywords: Lab-made fluorimeter, 3Dprinter, Flow injection,Fluorescein, Arduino.

1. Introduction

Photoluminescence is generally defined as the emission of photons from electronicallyexcited molecule that initially energized by photon absorption [1-2]. It is divided into two categories fluorescence and phosphorescencewere discussed in details somewhere else [3-4]. Fluorescencesensing isa major and important chemistry topics incorporating in undergraduate curriculum whichoften omitted from the laboratory coursesthis can be due to expensive and sophisticated commerciallyavailable fluorimeter, which cannotbe afforded by developed countries with a lack of resources. So, therewas huge effort, which can be cleary notice in the literature, for designing and build-up a low-cost homemade fluorimeter [5-10].One of the most popular continuous flow techniques is the flow injection analysis (FIA) is regarded as an attractive and most popular continuous flow techniques. It is characterized by versatility, simplicity, high reproducibility, and accuracy [10-13]. Itcan be easily build-up and tocouplewith several instrument techniques which can be equipped with a flow cell [8,9].In recent times, a Lab-built semi-automated FI system operational with microcontrollers which were constructing and build-up in our laboratory [14 – 17]. So, thought using a single manifold FI systems, in this case as sample processing will offer a semi-automated FI Fluorimeter system which can be easy to construct and building-up to be adapted in the teaching laboratory[18,19].

At the beginning of this work, there was no fluorimeter FI system existed in the laboratory, so the main aim of this project focused on constructing and build-up a very simple, inexpensive, reliable, and easy to use by an undergraduate student. This mini-fluorimeter FI system was build-up from available components in the local markets with the aid of 3D printers.A direct fluorimetric determination of fluoresecein dye as an example which was carried out in this work. This thought will be made the construction system a very simple fluorimeter since the dye excited and fluorescent in the visible range[20].

2. Experimental

2.1 Chemicals

During analytical application with lab-build fluorimeter flow injection system deionized distilled water was used throughout and all reagents employed were analytical grade unless otherwise stated .Value of measurements as peak height was the average of three successive measurements.A stock fluorescein sodium salt dye solution of 0.001 mol/L was prepared by dissolving 0.0752 g in an appropriate amount ofdeionized distilled water, and then the volume was completed to 200 ml. A series of fluorescein dye concentrationswere prepare from dilution the stock solution with deionized distilled water.

2.2 Instrumentation

The figures (1-4) show the lab-buildfluorimeter flow injectionsystem, the components, ofthe electronic components andthe components of the Miniperistaltic pump of fluorimeter devicethe system respectively.

Fig (1) Lab-made fluorimetricflow injection system

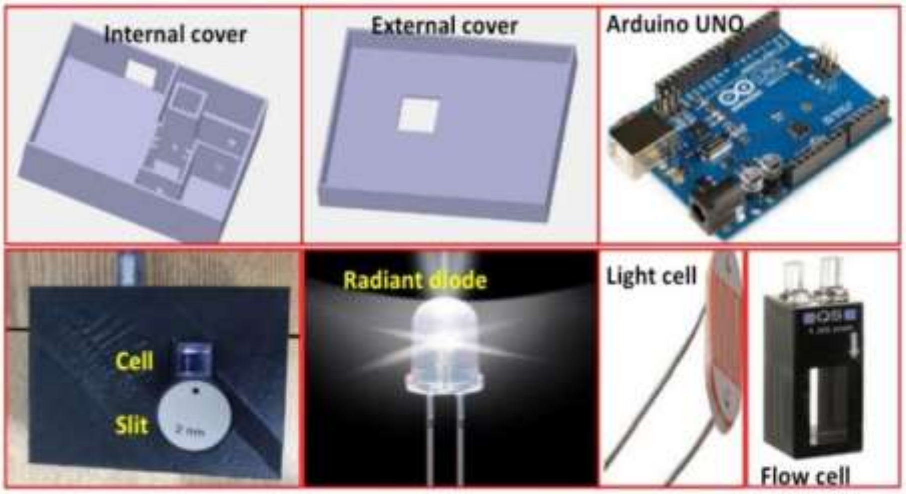


Figure (2) the components of the system

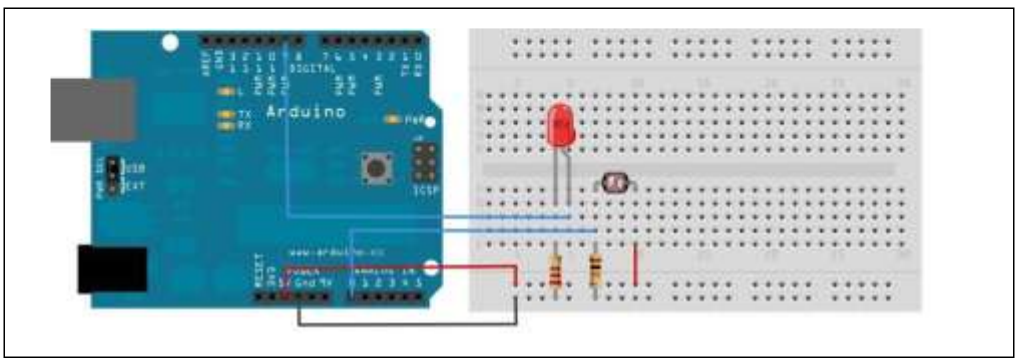


Fig (3) the electronic components of fluorimeter device

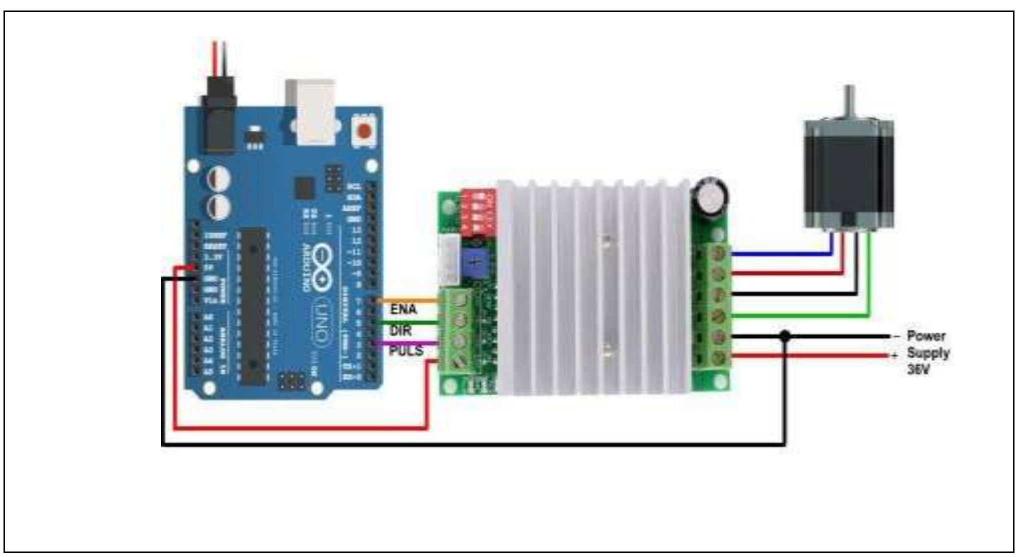


Fig (4) the components of the Mini peristaltic pump

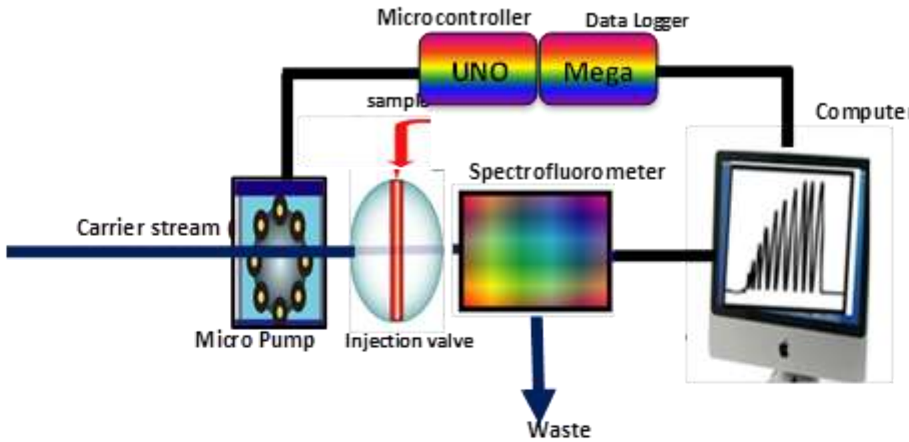


Fig (5) A singlemanifold for fluorescein determination by the lab-buildfluorometeric-FIA

A very simple fluorimeter was designed and built-up by the aid of a 3D printer to housing in which all the components are supplied from the local market. It consists of three major parts. Firstly, a simple, low-voltages, and long-lived light-emitting diodes (LED) with 0.25 watt were used as a light source. It gives ultra-bright light which is suitable for excited the fluorescein dye which was used in this study. A 10 nm slit can be but between the source and the flow cell (the second components) which thought be able to minimize the noise in the proposed system [21,22]

The second parts is 450 µL flow cell QS Hellma with four faces in order to select precisely the 90 ° angle between the source and third components, the LED arrays detector . This configuration can be functionally design bythe aid of 3D printer which constructed the main body of the fluorimeter by housing each component in right position [23]. The detector was a LED diode arraywhich response to visible light to invert the photon power to electric current [24, 25].

All these components were put in small plastic box as shown in fig.1 which the internal and external covers were made from black plastic which thought to prevent the unwanted light from reaching the detector.The device is very light weight (165.5 g) and the fluorimeter box dimensions were 11cm in length, 8cm in width and 3cm in height.

A microcontroller MEGA type was used as a data-logger to manipulate and recording the fluorescence response aspeaks height corresponding to the fluorescein concentration by using a PC computer supplied with a home-made single to peak program [10, 11] and Microsoft Excel 2010 program. On other hand a second microcontroller type UNO used to control the speed of the home-made single channels mini-peristaltic pump which pumping the deionized distilled water as carrier solution to the detector at flow rate in the range of 0.4-5 ml/min.The Sample was injected through an injection valve supplied with variable loops 15-35 µL. In order to minimize the size of the manifold, the rest of themanifold and the reaction coil were made from a 0.2 mm i.d tube[26-28].

3. Results and Discussion

3.1Optimum conditions for fluorescein determination by the FI fluorometer system.

Several experiments were carried out to find the optimum conditions for fluorescein determination by the proposed FI fluorimeter system. Figure 6 shows the effect of flow rate on the peak height of $1.6x10^{-4}$ of fluorescein over the range 4-8 ml/ min which illustrated that increasing of the flow rate leads to decrease the peak height. This was thought to be due to increase of the dispersion with increasing flow therefore, a

6.1 ml/min flow rate was chosen for subsequent work[11].This flow rate is very high but was suitable to be used for this single manifold(Fig. 5) to move the sample through the flow cell with avery large volume (450 µL) and also to clean-up the cell for next sample. It is also the results cleary indicated that the home-made mini peristaltic pump is suitable to be used for this FI system.

The peak height increases gradually with increasing of the sample volume in the of (50-250µL), this due to decreasing in the dispersion(Fig. 7)[12] . Therefore, 50µL of sample volume was chosen as the best volume for subsequent studies to increase the sample throughput, obtain a suitable sensitivity and smart peak shape. The influence of mixing coil length on peak height was studied in the range (10-110cm) as shown in Fig 8. The results indicated the increasing of the mixing coil length leads to decrease peak height due to increasing dispersion [12] therefore, a 10 cm was chosen for subsequent work.

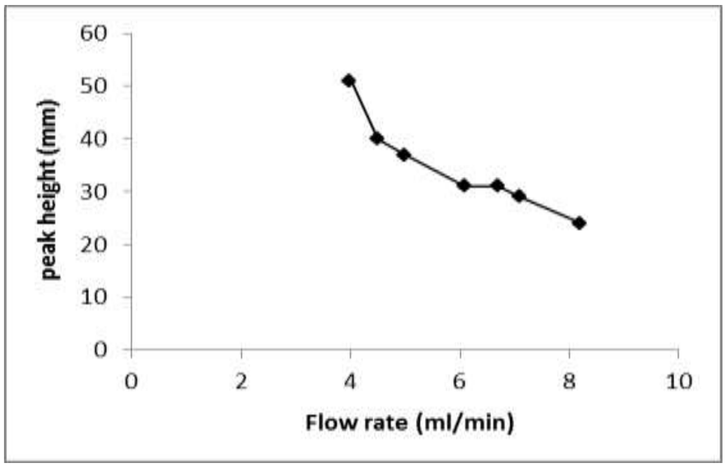


Fig (6) Effect of flow rate on peak height

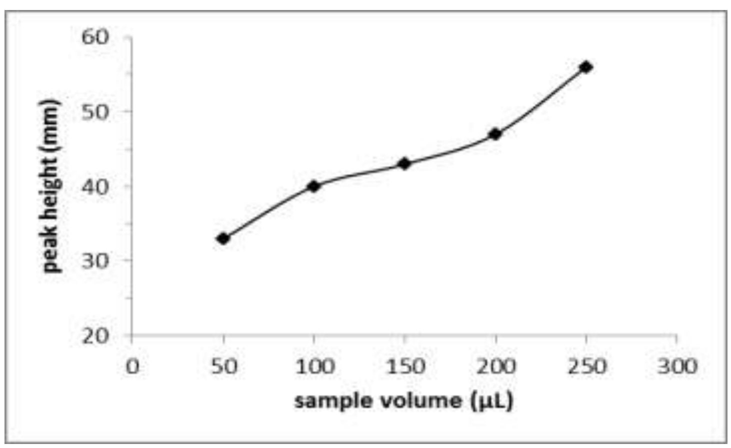
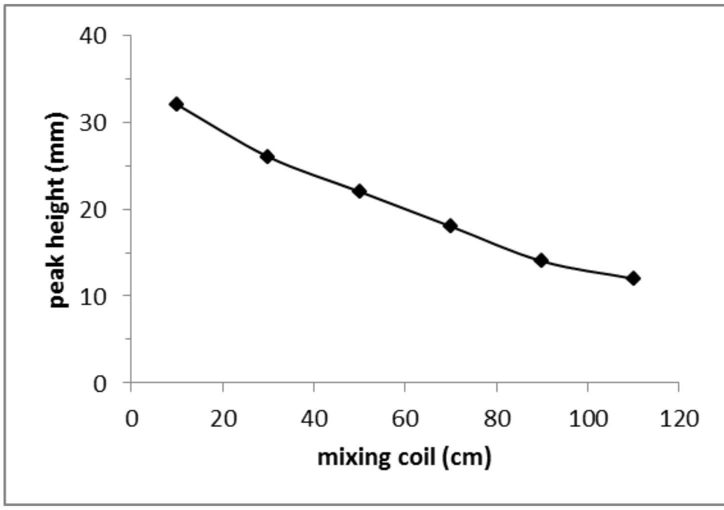


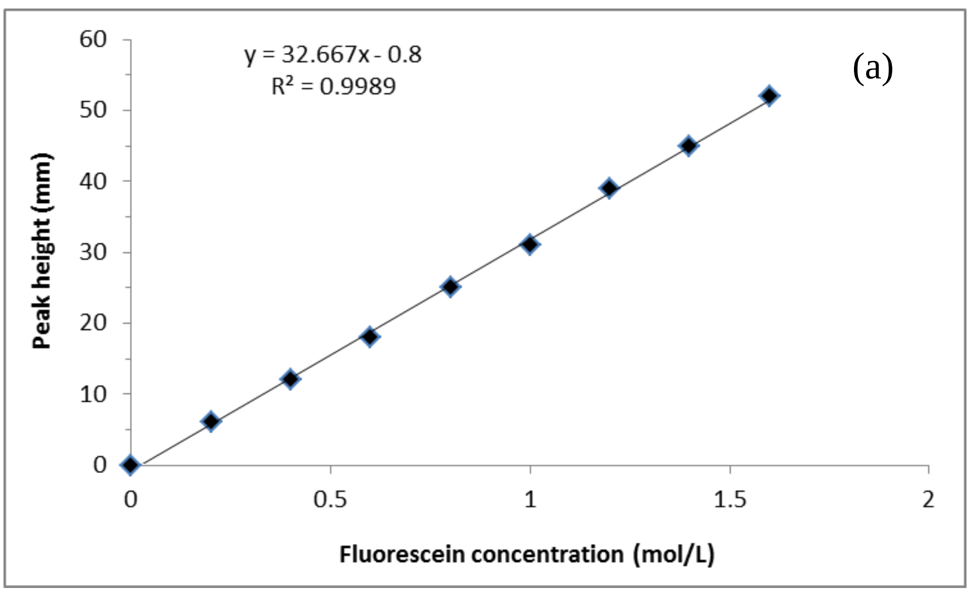
Fig (7) Effect of sample volume on peak height



Fig(8)Effect of mixing coil on peak height

3.2 Calibration curve

Under the established conditions a corresponding calibration graph for fluorescein determination was obtained. Linearity was found in the range (0.2-1.6× 10^{-4} mol/L) as shown in Fig (9a and b).The regression coefficient of eight points was 0.9989, r.s.d% for ten replicates of was 0.84% as shown in Fig (9-c) and detection limit was 0.1×10^{-4} mol/L.The equation y=32.667x-0.8 gives the relationship between the peak height and the concentration where y and x are the peak height and the concentration of fluoresceinrespectively. The sample throughput was 200 samples per hour. The dispersion coefficient of the manifold was 1.04 as shown in Fig (10).



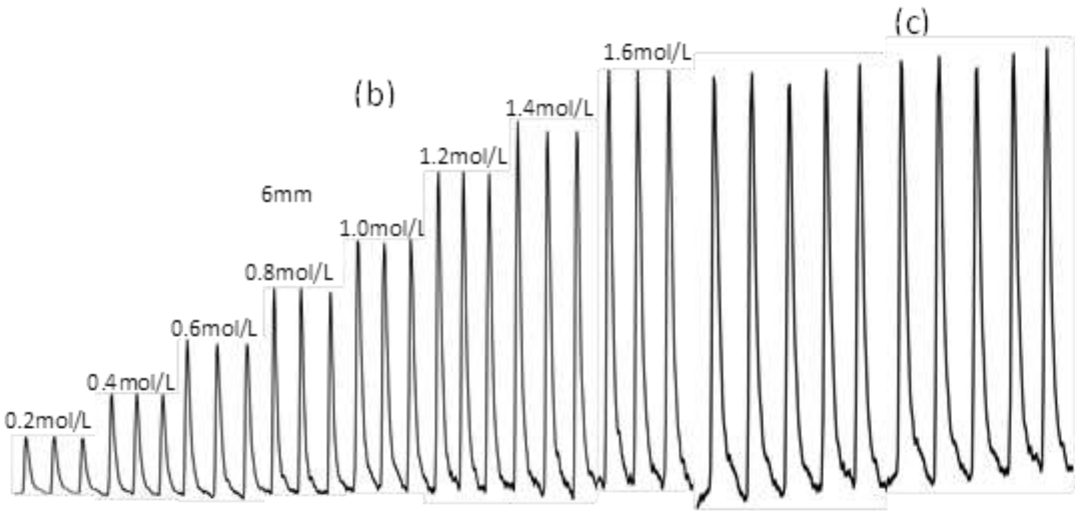


Fig (9) (a)Corresponding calibration graph of fluorescein (b) Peaks obtained by injected fluorescein standard(c) Ten injection of 1.6×10^{-4} mol/L fluorescein

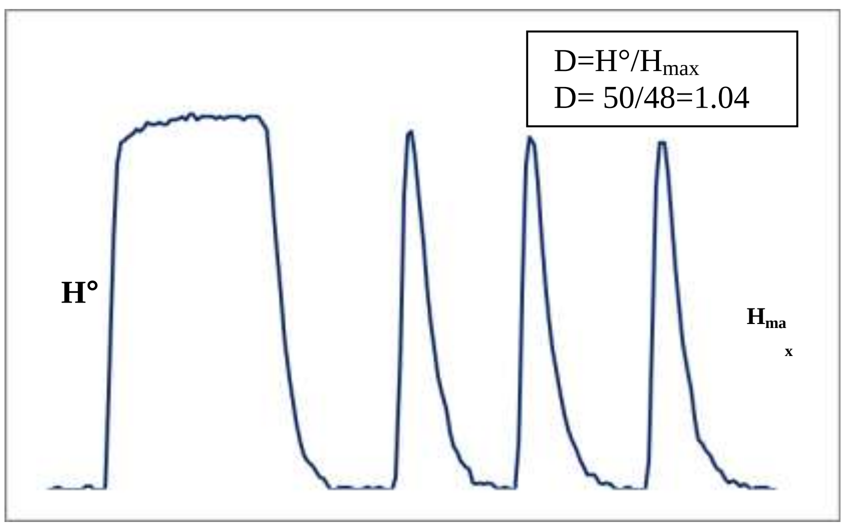


Fig (10) Dispersion cofficient in fluorometer FIA system

3.3 Accuracy

The accuracy of lab-built FI fluorimeter system for determination of fluorescein in three representative samples was carry outrecoveries experiments using the standard editions method .This method was used to eliminated all expected interferences [29]. The range of recoveriesresults whichwere obtained by the proposed system as shown in Table (1) and Fig (11) are acceptable [30].

Table (1) Recoveries study of three representative fluorescein by standard addition standard method

Sample	Added (mol/L) $\times 10^{-4}$	Found (mol/L) $\times 10^{-4}$	Recovery ± r.s.d%
1	0.6	0.6	100±1.93
2	0.8	0.88	100.2±1.52
3	1.0	0.99	98.66±0

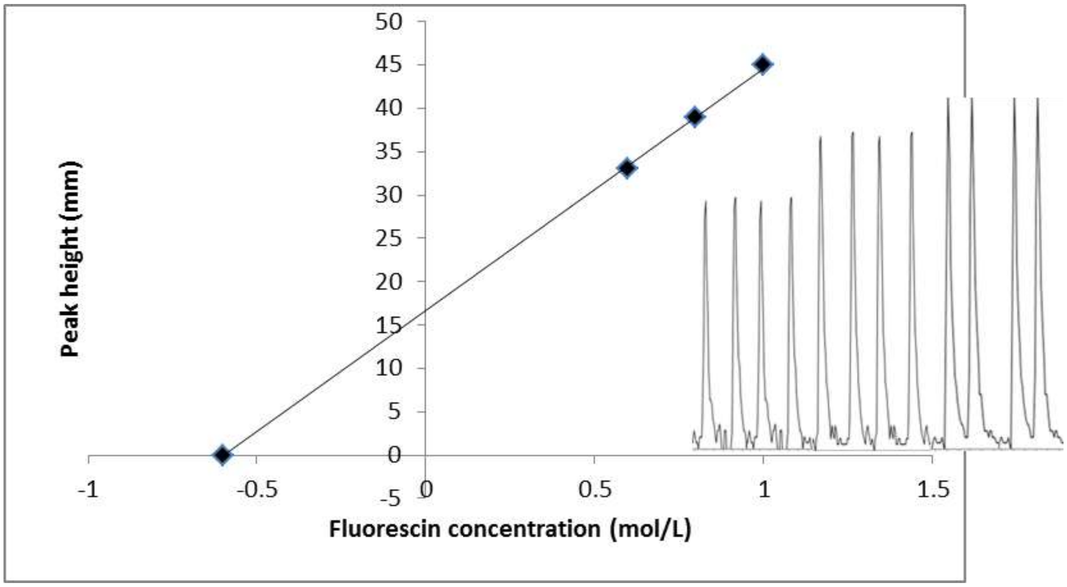


Fig. (11)0.6 mol/L fluorescein by standard additions method

Conclusion

The Lab-Build flow injection fluorimeter system with aid of 3D printer was designed for the first time in our Advance analytical laboratory, college of education and pure science, Basrah University. This simple, low cost, accurate and reproducible system can be easily assembled and joined with FI technique .This proposed system will offered an excellent educational tool to be used inundergraduate laboratory.It can be applied successfully for fluorescein determination for the undergraduate students.

References:

[1]Harvey D. (2000) Modern Analytical Chemistry,McGraw-Hill Companies.

[2]Bernard V. (2012) Molecular Fluorescence: Principles and Applications Hardcover, 2nd ed.Willy-Vch.

[3] Jameson, D. M. (2014) Introduction to Fluorescence; Taylor & Francis: Boca Raton, FL.

[4] Lakowicz, J. R. (2011) Principles of Fluorescence Spectroscopy, 3rd ed.; Springer: New York.

[5]Farooq M. Wahab (2007)Journal of Chemical Education84(8), 1312A.

[6] Mark A. Milanick(2011)Journal of Chemical Education *88* (3), 260-260.

[7]Stokes G.GOn the change of refrangibility of Light*Philosophical Transactions of the Royal Society of London* 1852 *142,* 479.

[8] Lon A., Porter, Jr., Cole A. Chapman, and Jacob A. Alaniz (2017) Journal of Chemical Education 94 (1), 105-111

[9] Farooq M. Wahab (2007). Journal of Chemical Education,*84* (8) , 1308.

[10] NagamS.Turkie and H.Q.Munshed (2018),Iraqi Journal of Science ,59, 240-250

[11] Trojanowicz M.(2008) Advances in Flow AnalysisWiley-VCH Verlag GmbH &Co.KGaA Weinheim Germany.

[12] Ruzicka J and HansenE.H. (2005)Flow Injection Analysis3rded (J Wiley NewYork).

[13] Mark Trojanowicz, (2000) Flow Injection AnalysisInstrumentationAND application,World Scientific Singapore.

[14] K.H. Al Sowdani and Mohammad TH.K.Al-Balaawi (2019)Journal of physics:(2nd International Science Conference IOP Conf.Series 1294(2019)072010.

[15] Y. Shafi Al-Jorani and K. H. Al-Sowdani (2020) Material science and engineering,871, 012032.

[16] K.H. Al Sowdani and NajahZ.H.Al-Hisnawe (2020) Test Engineering and ManagementNo16732 -16737.

[17] K.H.Al-Sowdani and Mohammad Th.k.Al-Balaaai (2019),Research Journal of Pharmacy and Technology, 12, 1-3.

[18] K. H. Al-Sowdaniand Alan Townsend(1987)Analytica Chimica Acta,201, 339-343.

[19] K H. Al-Sowdani and Alan Townsend(1987, ,Analytica Chimica Acta, 201(1987)339-343..

[20] Turner Biosystems, A Modulus™ Micro plate Fluorimeter Method for Fluorescein Measurement, Application Note, Turner Bios stems, Inc.645 N. Mary Avenue Sunnyvale, CA 94085 USA

[21]Mirek M., Tomasz P. and Purnendu K. Dasgupta (2014) Annu. Rev. Anal. Chem. 7:183–207

[22] Prince Technologies, (2020) On-board DAD for PrinCE-C 700 series, P Prince Technologies inCE Next|870 and Next|875.

[23] Hellma GmbH & Co. KGKlosterrunsstraße 579379 MüllheimGermany, Product Catalog BestCellersCELLSTRAYCELL® MICRO VOLUME ANALYSIS(2018).

[24]JD Lee, SD Dabell - US Patent 6,852,986, 2005(US6852986B1) - Fluorometer with low heat-generating light source - Google Patents.;

[25]V. V. Povazhny, (2014)Oceanology, 54, 387–391.

[26]Mohammad Th.k.Al-Balaaai , M. Sc. University of Basarah , 2018.

[27]Ali Yaaqoop Jaafer Sagar ,M. Sc. Thesis, University of Basarah , 2019.

[28]Mohammad Th.k.Al-Balaaai (2019) Iraq patientNo 6099, Design Low Cost the field spectrophotometer for nutrient determination.

[29] Harvy D. (2000) Modern Analytical chemistry, Bosten, McGraw-Hill Higher Education.

[30] Peter C. Meier and Richard E. Zünd (2000) Statistical Methods in Analytical Chemistry Chemical Analysis: A Series of Monographs on Analytical,450.