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Newly developed method for determination Indomethacin using Phosphotungstic acid by continue flow injection analysis via homemade ISNAG-fluorimeter.

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Abstract: *A new, simple, sensitive and fastdeveloped methodfor the determination of Indomethacinin pure form and drugs (tablets)by continuous flow injection diverged light. The method based on the reaction of the Indomethacin with Phosphotungstic acid to formoff-white precipitate, using homemade ISNAG- fluorimeter. Optimum parameter have been studied to increase the sensitivity for developed method. The linear dynamic range for the instrument response versus Indomethacinconcentration was 0.01- 5mmol/L while the L.O.D was 320.222ng/sample from the step wise dilution for the minimum concentration of lowest concentration in the linear dynamic range of the calibration graph. The correlation coefficient (r) was 0.9952while percentage linearity (R^2%) was 99.05%. RSD% for the repeatability (n=7) was lower than 0.5% for the determination of Indomethacin, with concentration of 1, 5mmol/L respectively. The methods: developed method was applied successfully for the determination of Indomethacinin pharmaceutical tablets. A comparison was made between the newly developed method with the classical method (uv-spectrophotometer method) of analysis using the standard addition method via the use of paired t-test. It shows that there was no significant difference between the quoted values of each individual company with calculated t-value at 95% confidence interval from developed method.*

Keyword: *Indomethacin, flow injection diverged light, homemade instrument.*

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I. Introduction

The chemical name of Indomethacin 1-(p-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid and a molecular formula(fig. 1) of $C_{18}H_{16}ClN0_4$**[1-4].**Indomethacin is a nonsteroidal, anti-inflammatory agent with antipyretic [**3-5**]. Indomethacin is an odorless, pale yellow to yellow tan crystalline substance. It is lipid-soluble, practically insoluble in water and sparingly soluble in alcohol. Indomethacin has a pKa of 4.5 and is stable in neutral or slightly acidic media and decomposes in strong alkali. The suspension has a pH of 4.0–5.0 and it has a melting point between 155°C and 161°C and has molecular weight of 357.79.[**2-9**].

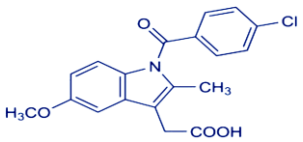


Fig. 1 Chemical structure of indomethacin].

It has been identified as a nonselective inhibitor drug of cyclooxygenase enzymes COX1 and COX2, whichinhibits the synthesis of prostaglandins [**2–7**]. Its solubility in water is very low but acceptable enough to carry out UV-Vis- Spectroscopy determination of some of its physicochemical properties in solution [**4, 10- 11**]. For instance, so far, the p*K*a value of the Indo H has been found by UV-Vis and density functional theory (DFT) based methods, among other approaches [**8, 10–12**]. On the other hand, copper is present in around 100 enzymes in living organisms [**13**], the reason why it is a very important biochemical agent; there are abundant observationsabout altered levels of copper in various acute diseases; particularly, lack of copper is identified in illness resultingin inflammatory processes. It has been documented that the administration of compounds of NSAIDs with metal ions,especially with Cu(II), produces an enhanced therapeuticaction as well as less negative side effects than the parentNSAID [**14-15**].Complexes ofCu(II) and carboxylic acids exist in solutionand in their crystalline forms either as monomeric or dimericstructures, although a marked preference for the formationof dimeric structures of the type of Cu(II)-Acetate has beenidentified [**7, 16–21**]. The Cu(II)–Carboxylate monomersexist in a trans-configuration with incidental bis(carboxylate)bond and the metal

centerCu(II) with a coordination numberranging from four to six [**7**]. On the other hand, the widelystudied, dimeric compounds of Cu(II) have structures type"paddle wheel," where the Cu–Cu bond distance is 2.64 ˚ A,Whichmarginally exceeds the distance Cu–Cu in the metalliccopper (2.56 ˚ A) [**7**]. Those Cu(II)–NSAID complexes, wherethe NSAID contains carboxylate groups, tend to have similarstructures, with solvent molecules in the position trans tothe Cu–Cu bond. In such complexes, each d^9 ion donates anelectron to give rise to an important metal-metal interactionshortening the Cu–Cu distance. Interestingly, it has alsobeen found that Cu(II)–drugs dimeric complexes seem to betherapeutically more effective and less toxic thanmonomericcomplexes, which in turn are less lipophilic andmore reactive[**21**]. Thus, the monomeric-dimeric distribution was revealas an important factor in the toxicity and effectiveness of thecomplexes. That distribution was determineby the values ofequilibrium constants of different complexes, that is, by thethermodynamics of the process of formation of the stablespecies in solution.Regarding the geometries adopted by the Cu(II)–Indomethacincomplexes, the X-ray study of the compoundCu(II)2[Indo]4L2 (L = H2O, DMSO, and DMF) has beenreported [**19**-**20**], where the characteristic known paddlewheel structure of binuclear Cu(II)–Carboxylate is present[**7, 22-23**]. Important information about the formed complexesis found from the X-ray characterization, but to discussthe pharmacological action of the Cu(II)–Indomethacincomplexes, it is necessary to identify those species predominatingunder physiological conditions as they play a centralrole in displaying the biological effect.As far as we know, Cu(II)–Indomethacin species formedin solution either mononuclear or binuclear have not yet beenidentified and/or characterized. A study of the speciation ofCu(II)–Indomethacin system in solution represents a firstapproach to know the distribution and formation (stability)constants of the complexes. Given the low solubilityof Indomethacin in water, the UV-Visible spectrophotometrybecomes a suitable technique for the speciation ofthe Cu(II)–Indomethacin systems; in this respect, molarratios and continuous variations methods supported on UV-VisibleSpectrophotometry have been successfully applied forthe speciation of Fe(III)–Tenoxicam and Fe(III)–MeloxicamSolutions [**24- 25**]. The determination of the structure of thecrystallized complexes can be performed either by X-raymethods or by applying quantum-chemistry based methods;the applicability of theoretical methods allows the structuralcharacterization of the species in solution.Molecular modelingbased on the density functional theory helps us to knowthe structure of those species, in the different stoichiometryexperimentally recognized in solution[**22-25**].The official (**BP**) method**[26]** for the Indomethacin pure form is assayed by titration in acetone using NaOH, while the capsules and suppositories were assayed by a UV-VIS spectrophotometric method, many of these methods already described for the assay of Indomethacin require prior hydrolysis, extensive extraction, heating, and all of these compromised accuracy gave a higher Beer's law range.. In this work using flow injection scattering method, the diverged light is measured at 0-90^o angle will be detected by homemade ASNAG fluorimeter via low pressure mercury lamp as a source and using 2[4 x 2.5cm] solar cell.

II. Experimental

Reagent and chemical

All chemicals were use of analytical-reagent and distilled water was use to prepare all the solutions. A standard solution 50mmol/L of Indomethacin molecular formula $C_{18}H_{16}ClN0_4$, molecular weight 357.79g/mole and SDI-Iraq was prepared by dissolving 1.7890g in 100 ml of NaOH. A stock solution 12mmol/L of Phosphotungstic acid molecular formula(**$H_3PW_{12}O_{40}$**) molar mass 2880.2 g/moleand Merck-USA was prepared by dissolving 8.6406g in 250 ml of distilled water.

Sample preparation

Twenty capsules were weight then crushed and mixed. capsules containing 25mg of Indomethacin were weighted 7.3597g, 21.3815g, 12.5785g, 12.8733g (equivalent to 1.7890g of active ingredient, 50mmol/L) forIndomethacinaTROGE-MEDICALGMBH- Germany andIndoflam-Ajanta pharma Limited-India,Indylon Medochemie LTD–Cyprus and Indomal Uni-pharma- UEA, respectively. Each one from The four kinds of sample dissolved in NaOH. The solution was filtered to get rid of undissolved materials, the residue was washed with NaOH and completed the volume to 100ml with the same solvent(NaOH).

Apparatus

Tow line design system (Fig.2) was used for Indomethacin determination. Firstline is the carrier line stream (1.7ml/min) that will take and introduce the sample loop segment(179µL, open valve mode, 5mmol/L) into the reaction stream by combining with the secondline (1.7ml/min) that carry the reagent (Phosphotungstic acid) that will form the precipitate. This formedprecipitate will be irradiated by Low presser mercury lamp have two main wavelengths namely 184.9nm and 253.7nm. Theseboth two line are easily diverged due to its high frequency. The divergence (all kind ofscattered light) of this beam of incident light will be detected at0- 90o through a line of 2mmoptical operture extended for 100mm distance using 2[4 x 2.5cm] solar cell.. Peristaltic pump two channels variable speed (Ismatec, Switzerland). Valve 6 – port medium pressure injection valve (I

D E X corporation , USA) with sample loop (1 mm i.d. Teflon ,variable length).2[4 x 2.5cm] solar cells are used as detector for collecting signal via sample travel through a line of 2mm optical openture extended for 100mm distance.The output signals were recorded by potentiometric recorder (Siemens, Germany)(1- 5 Volt,1000-5000 mV). Peak height was measured for each signal. absorbance readings by uv-spectrometer, UV-spectrophotometer (UV-1800 Shimadzu) (Japan).

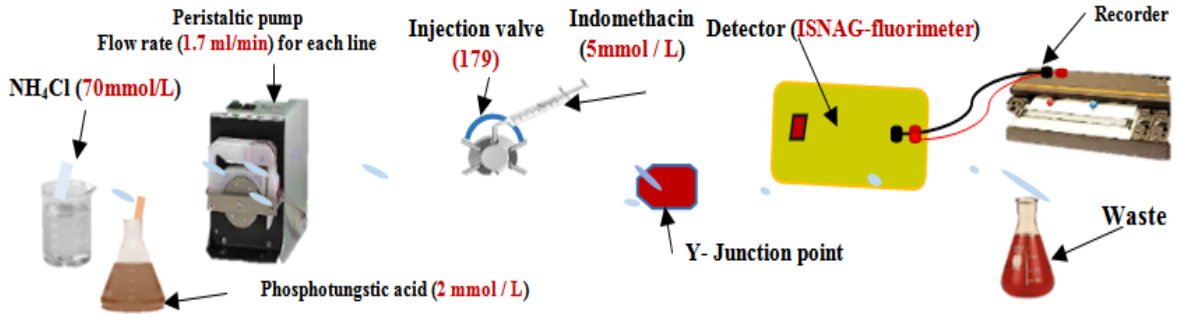
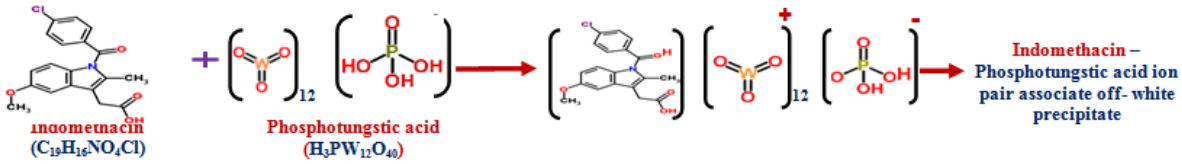


Figure 2: Schematic diagram of flow injection instrument analysis system for determination of Indomethacin.

III. Methodology

Tow lines designsystem (Fig. no. 2) was used for Indomethacin determination. First line is the carrier stream(1.7ml/min) from aqueous medium (H_2O) that will take and introduce the sample loop segment (329µL, 30mmol/L) from Indomethacin into the reaction stream by combining with the second line (1.7ml/min) that will form the precipitate in Y-junction zone (mod of PMMA plexiglass). This formed precipitate will be successive measurements were used ASNAG- fluorimeter via low pressure mercury lamp, it's give two main wavelengths namely 184.9nm and 253.7nm. These both two line are easily diverged due to its high frequency. The divergence of this beam of incident light will be detected at 90^o through a flow cell of 2mm path length that extend for 100mm distance by using 2[4 x 2.5cm] solar cell. While the proposed probable reaction pattern is expressed inscheme 1. [**27-28**]



Scheme 1:Proposed mechanism of the reaction between Indomethacin and Phosphotungstic acid.

IV. Result and Discussion

Study of the optimum parameters

The flow injection manifold system as shown in fig.2 was investigated in the relation of chemical and physical variables, in order to obtain optimum conditions for the react Phosphotungstic acid with Indomethacin and formed off- white precipitate. They were optimized by making all variables constant and varying one at a time i.e. fixed variable optimization.

Variation of chemical parameters

Phosphotungstic acid concentration

Using variable concentration of precipitating agent 0.05-3mmol/L for Phosphotungstic acid1.3ml/min for each line and sample volume of 179µL from Indomethacin (5mmol/L), it was noticed that an increase of diverged light in terms of peak height up to 2mmol/L for Phosphotungstic acid(fig. 3.-A). An excess increase will lead to decrease the amount of diverged light thus might be probably blocking any diverged light due to lump formation prevently effective light to reach the detector. Therefore, 2mmol/L for Phosphotungstic acid(fig. 3-B)is the most appropriate concentration for the work at hand. Table 1 summarises all the obtained data.

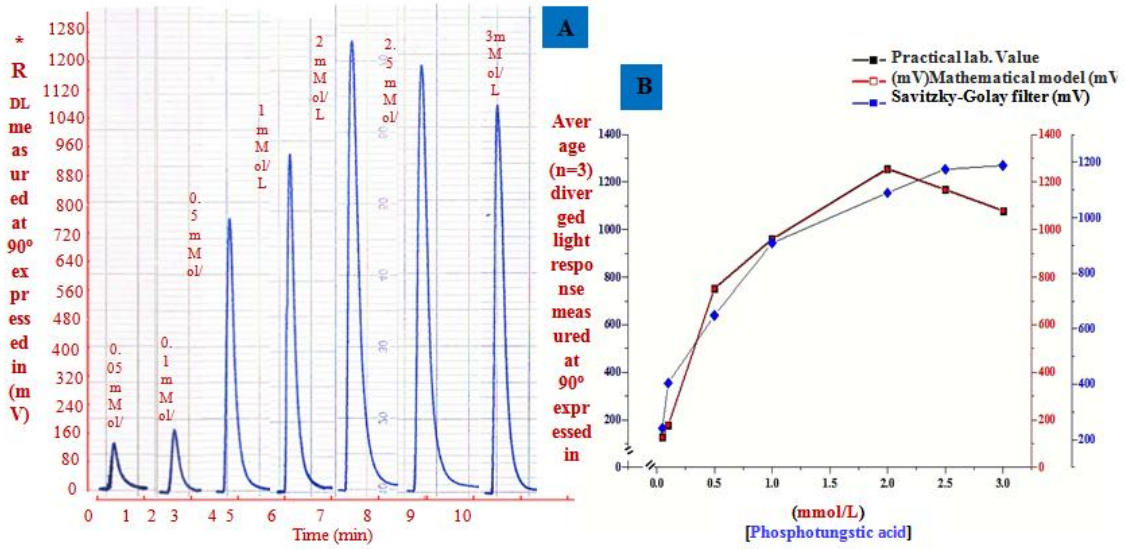


Figure 3: A- Response –time profile of Indomethacin with variable concentration of Phosphotungstic acid.
B-Plot of averaged peak height responses ʋs. Phosphotungstic acid solution concentration.

Table 1: Effect of Phosphotungstic acid concentration on response function expressed as an average peak heights ȳi (n=3) and tabulation of all available data obtained practically, calculated as obtained by best fit mathematical model, and smoothed digital filtering using Savitzky-Golay data treatment

Independent variable [Phosphotungstic acid] mmol/L	Dependent variable Average (n=3) diverged light response measured at 0-90°expressed in mV				
	Practical lab. value			Mathematical model $\hat{y}_i$	Savitzky-Golay filter $\hat{y}_{i\,(S\text{-}G)}$
	Average peak height($\bar{y}_i$)	RSD%	Reliability(two tailed) $\bar{y}_i$(mV)± $t_{0.025,2}$ $\sigma_{n-1}/\sqrt{n}$		
0.05	128	1.52	128 ± 4.82	125.256	242.349
0.1	176	1.05	176 ± 4.60	178.572	403.686
0.5	752	0.27	752 ± 4.70	751.789	647.344
1	960	0.21	960 ± 4.92	960.087	908.521
2	1256	0.08	1256 ± 1.24	1251.969	1089.691
2.5	1168	0.12	1168 ± 3. 43	1168.021	1174.330
3	1080	0.14	1080 ± 3.95	1079.995	1188.225

$t_{0.025,2}$ =4.303, $\bar{Y}j = \sum_{i=-\frac{m-1}{2}}^{\frac{m-1}{2}} (Ci\ \bar{y}j + i)$ **, m= convolution coefficient,** $\frac{m+1}{2} \leq j \leq n - \frac{m-1}{2}$

Carrier stream effect
Acidity Effect

At a sample volume 179µL from Indomethacin (5mmol/L) reacted with Pho (30mmol/L), study different types of acids medium used a carrier stream (H_2SO_4, HNO_3, HCl, and CH_3COOH) at 50mmol/L concentration in addition to aqueous medium as a carrier stream at flow rat 1.3ml/min for each line (using open valve mode). Study the effect of every one of these acids on reaction and compared with (H_2O) response recorded. Table 2 Tabulate the average of responses recorded for every one of carrier using in this system. Figure4 show the pest response recorded to aqueous medium (H_2O) because it was gave high and Sharpe response compared with the other acids carrier stream used.

Table2: Effect of carrier stream type on precipitate response Expressed as an average peak heights $\bar{y}_i$ (n=3)

type of [acid] mmol/L	Dependent variable ($\bar{y}_i$) Average (n=3) diverged light response measured at 0-90°expressed in mV	RSD%	Reliability of average response(two tailed) at 95% confidence level $\bar{y}_i$ (mV)± $t_{0.025,2}$ $\sigma_{n-1}/\sqrt{n}$
H_2O	1248	0.06	1248 ± 1.74
H_2SO_4	576	0.34	576 ± 4.84
HNO_3	888	0.17	888 ± 3.68
HCl	760	0.21	760 ± 3.93
CH_3COOH	208	0.63	208 ± 3.25

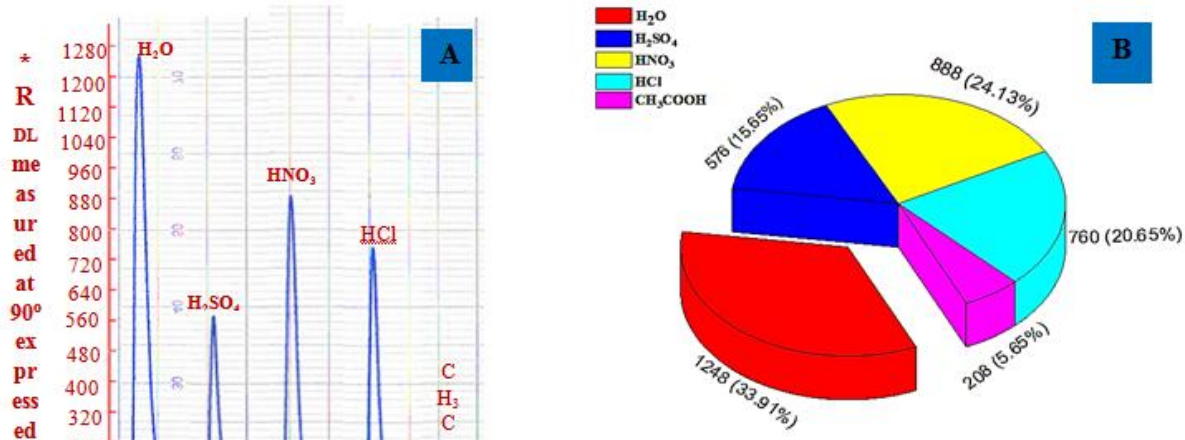


Figure 4: Effect of acid solution used as a carrier stream on profile (A), and pie percentage Representation of the contribution of each acid solution (B).

Salts effect

Series of salts ((50mmol/L) NaCl, KCl, KNO_3, NH_4Cl, CH_3COONH_4, CH_3COONa) solution in addition to distilled water were used as a carrier stream. It was found that while using Phosphotungstic acid as a precipitating agent an increase of peak height from 1256mV to 2400mV in the prescence of **NH_4Cl** (fig. 5). It might be probably due to aggregation precipitating particulate and increase their sizes thus increase amount of diverged lightOn the above inferred experiments that the ammonium chloride was used as a carrier stream when using Phosphotungstic acid as precipitating agentThe obtained results were summarized in table 3.

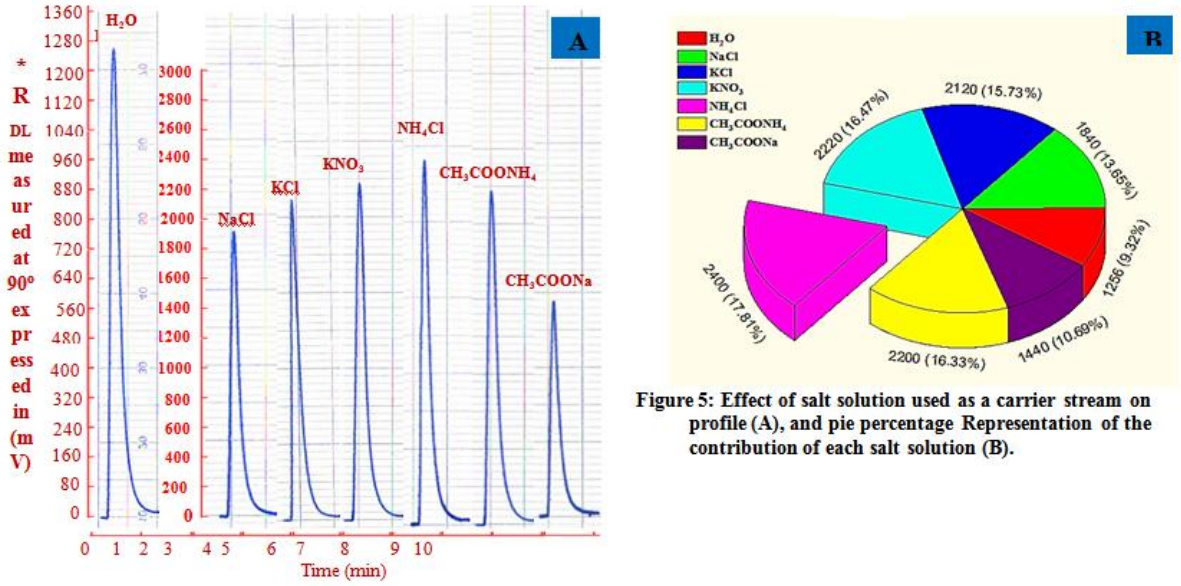


Figure 5: Effect of salt solution used as a carrier stream on profile (A), and pie percentage Representation of the contribution of each salt solution (B).

Table 3: Effect of different salt as a carrier stream on diverged light response at 0- 90°.

Independent type of salt as a carrier stream	Dependent variable ($\bar{y}_i$) Average (n=3) diverged light response measured at 90°expressed in mV	RSD%	Reliability of average response(two tailed) at 95% confidence level $\bar{y}_i$ (mV)± $t_{0.025,2}$ $\sigma_{n-1}/\sqrt{n}$
H_2O	1256	0.15	1256 ± 4.82
NaCl	1840	0.11	1840 ± 4.75
KCl	2120	0.08	2120 ± 4.20
KNO_3	2220	0.062	2220 ± 3.40
NH_4Cl	2400	0.04	2400 ± 2.36
CH_3COONH_4	2200	0.07	2200 ± 3.55
CH_3COONa	1440	0.13	1440 ± 4.62
H_2O	1256	0.15	1256 ± 4.82

Effect of ammonium chloride concentration on diverged light for Indomethacin- Phosphotungstic acid system.

Sets of series solutions of ammonium chloride(10-100)mmol/L as a carrier stream, it was carefully following that an increase in ammonium chloride solution causes an increase in peak response profile up to 70mmol/L; above that causes a negatively decrease in height of response profile. This above affect might be due to an increases with lots of spaces that light divergence is happened while an excess increase (above 70mmol/L) agglomerate combination of formed grainules which in turn to causes a decrease in diverged light in a munner similar to inner filter effect. Thus diverged light can not reach to the detector. Therefor 70mmol/L was regard as the concentration of choice for this work (fig. 6). The result were tabulate in table 4.

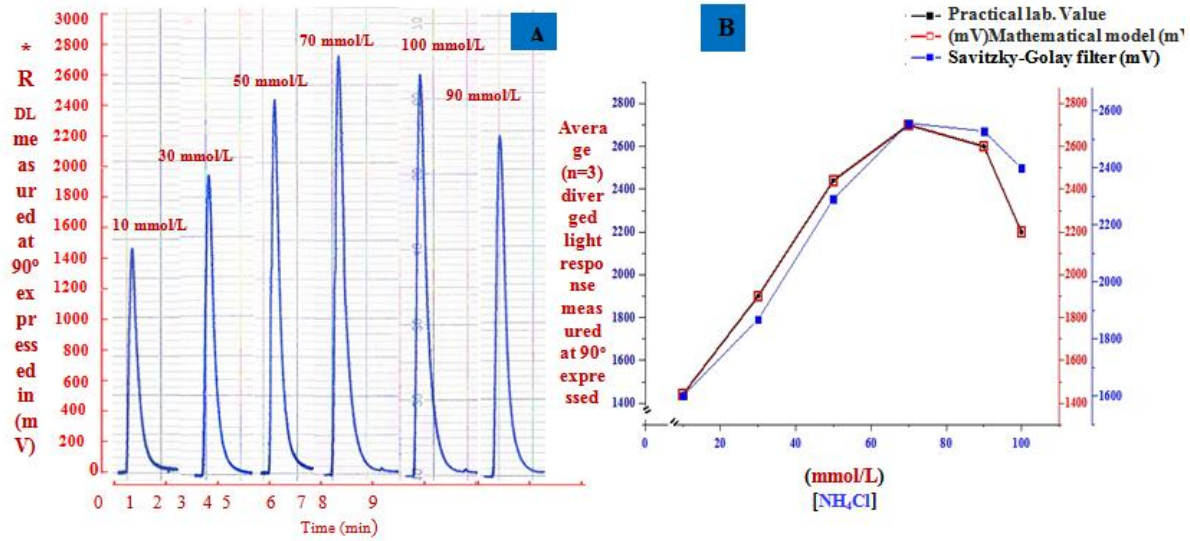


Fig. 6: (A) Response– time profile of Indomethacin with variable concentration of ammonium chloride solution
(B) Plot of averaged peak height responses ʋs. concentration of ammonium chloride solution using 179µL of Indomethacin (5mmol/L)- Phosphotungstic acid (2mmol/L).

Table 4: Effect of potassium chloride concentration on Indomethacin expressed as an average peak heights $\bar{y}_i$ (n=3) in mV

Independent variable [NH_4Cl]mmol/L	Dependent variable. Average (n=3) diverged light response measured at 0 -90°expressed in mV				
	Practical lab. value			Mathematical model $\hat{y}_i$	Savitzky-Golay filter $\hat{y}_{i\,(S\text{-}G)}$
	Average peak height($\bar{y}_i$)	RSD%	Reliability(two tailed) $\bar{y}_i$(mV)± $t_{0.025,2}\ \sigma_{n-1}/\sqrt{n}$		
10	1440	0.13	1440 ± 4.62	1440.000	1600.096
30	1900	0.09	1900 ± 4.35	1899.999	1868.782
50	2440	0.06	2440 ± 3.68	2440.002	2290.282
70	2700	0.029	2700 ± 1.92	2699.996	2555.882
90	2600	0.04	2600 ± 2.43	2600.004	2528.710
100	2200	0.07	2200 ± 3.58	2199.998	2398.422

Physical parameters

Electronic filter effect.

This study was carried out for the determination of preferred low band pass electronic filter using 179µL sample volume of Indomethacin (5mmol/L) at 1.3ml/min flow rate at each line and NH_4Cl(70mmol/L) as a carrier stream. Variable RC- filters were used to establish optimum response sensitivity and response profile with the sake for increased S/N ratio.. While data smoothing cannot really gave an improved data profile to choose form. As there were no large fluctuation in the measurements. Therefore, no digital filtering was used on RC- response filter. On the above based on measurement and profile response of S/N signals. Direct measurements was the choice of this part of research work. Table 5 tabulate all the results obtained

Table 5: Effect of electronic filters on precipitate response expressed as an average peak heights $\bar{y}_i$ (n=3)

Independent variable of electronic filter response (Sec.)	Dependent variable ($\bar{y}_i$) Average (n=3) diverged light response measured at 0-90°expressed in mV	RSD%	Reliability(two tailed) at 95% confidence level $\bar{y}_i$(mV)± $t_{0.025,2}$ $\sigma_{n-1}/\sqrt{n}$
Without filter	2703	0.03	2703 ± 2.14
0.1632	2660	0.05	2660 ± 3.40
0.3196	2620	0.06	2620 ± 3.88
0.68	2600	0.07	2600 ± 4.50
0.8364	2540	0.08	2540 ± 4.92
1.6728	2420	0.09	2420 ± 5.22
3.974	2400	0.10	2400 ± 5.71

Flow rate effect

Variable flow rate study were carried out using 0.4- 3.5 ml/min for carrier stream line while 0.4- 3.6 ml/min was used for precipitating reagent solution line were used at Indomethacin (5mmol/L)- Phosphotungstic acid (2mmol/L)- and 179µL sample volume (open valve mode) (i.e. allowed permissible time for sample segment to be injected from injection valve). It can be noted from fig. 7-A, B that at slow flow rate (0.4-1ml/min) caused an irregularity of flow, which in turn causes the deformed or broad of response- time profile due to irregular passage of precipitated plug of sample to be dealt with the detector for 100mm distance of 2mm path length. Therefore, a 1.7 ml/min for each line was the optimum choice to compromize between sensitivity, response profile and consuption of chemicals since a response is a function of physical and chemical variable. All results tabulated in table 6.

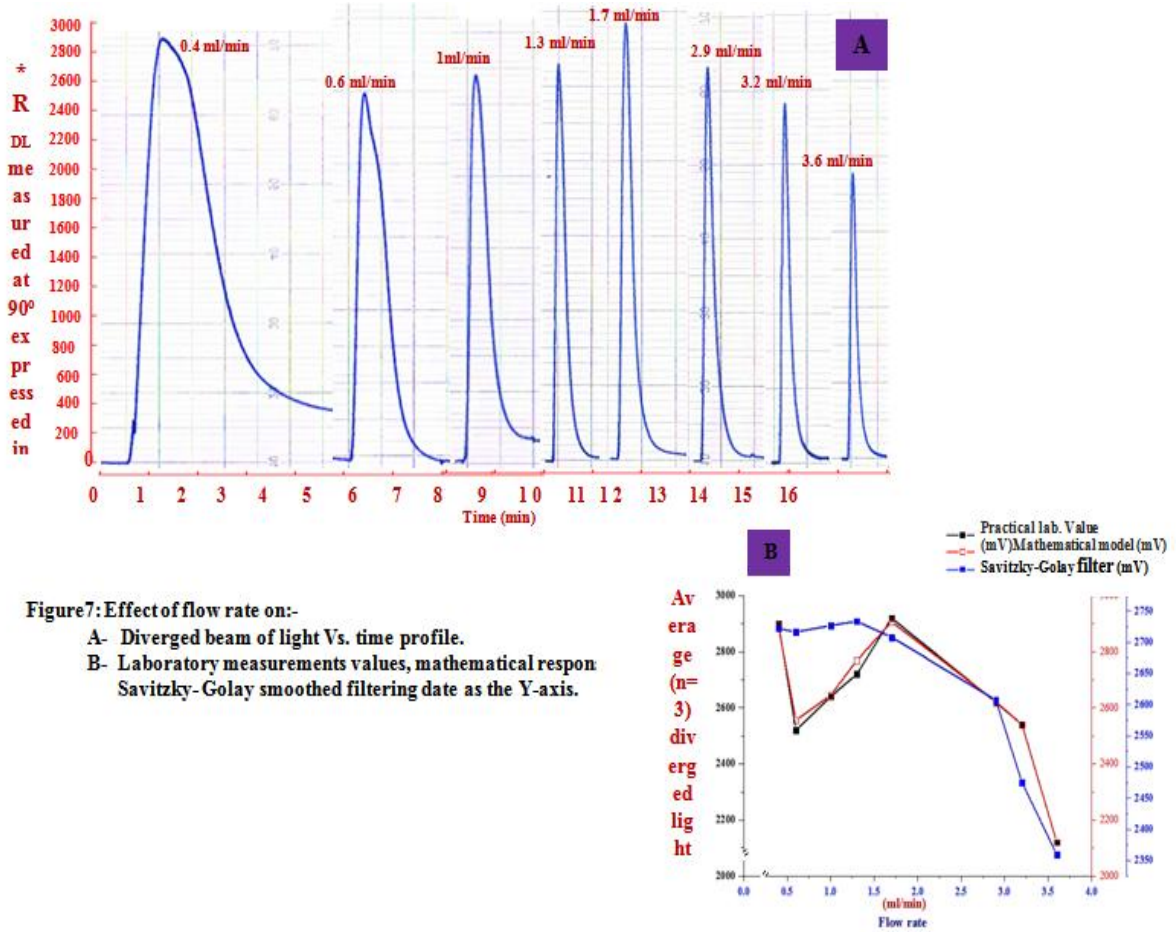


Figure7: Effect of flow rate on:-
A- Diverged beam of light Vs. time profile.
B- Laboratory measurements values, mathematical respon Savitzky- Golay smoothed filtering date as the Y-axis.

Table6: Effect of flow rate on the variation of diverged light response and tabulate all available data obtained practically, calculated as obtained by best fit mathematical model, and smoothed digital filtering using Savitzky-Golay data treatment

Independent variable of pump Speed	Dependent variable. Average (n=3) diverged light response measured at 90°expressed in mV							
	Practical lab. value						Mathematical model $\hat{y}_i$	Savitzky-Golay filter $\hat{y}_{i(S-G)}$
	Flow rate (mL/min)		Average peak height($\bar{y}_i$)	RSD %	Reliability(two tailed) $\bar{y}_i$(mV)$\pm t_{0.025,2}\ \sigma_{n-1}/\sqrt{n}$	Δt_b (Sec)		
	Line no. 1	Line no. 2						
5	**0.4**	**0.4**	2900	0.10	2900 ± 6.83	161	2890.049	2722.501
10	**0.6**	**0.6**	2520	0.09	2520 ± 5.79	123	2555.723	2716.588
15	**1**	**1**	2640	0.07	2640 ± 4.52	87	2645.262	2727.200
20	**1.3**	**1.3**	2720	0.05	2720 ± 3.38	56	2768.949	2733.860
25	**1.7**	**1.7**	2920	0.03	2920 ± 1.42	48	2906.849	2708.260
30	**2.9**	**3**	2620	0.05	2620 ± 3.03	39	2621.749	2607.301
35	**3.2**	**3.3**	2540	0.07	2540 ± 4.40	35	2539.223	2474.979
40	**3.5**	**3.6**	2120	0.09	2120 ± 4.50	31	2119.954	2359.557

Δt_b (sec) : Time lapse for the preciptate response within measuring cell or peak base width
Line no.1 = carrier stream (NH_4Cl), Line no. 2= Phosphotungstic acid (2mmol/L)

Effect of sample volume

Using variable sample volume starting from 79µL up to 329µL, which represent 10cm up to 42cm, Teflon tube length of ϕ (diameter) 1mm. It was clearly noticed that up to 179µL; there were a constant increase in light divergency followed by a little decrease with an increased width of maximum peak height and increased base width; that was in Indomethacin(5mmol/L)- Phosphotungstic acid(2mmol/L)- NH_4Cl(70mmol/L) system.Therefore, 179µL was the choice of the successive system at this stage (fig. 8).Table 7 summarises all the obtained data for the system.

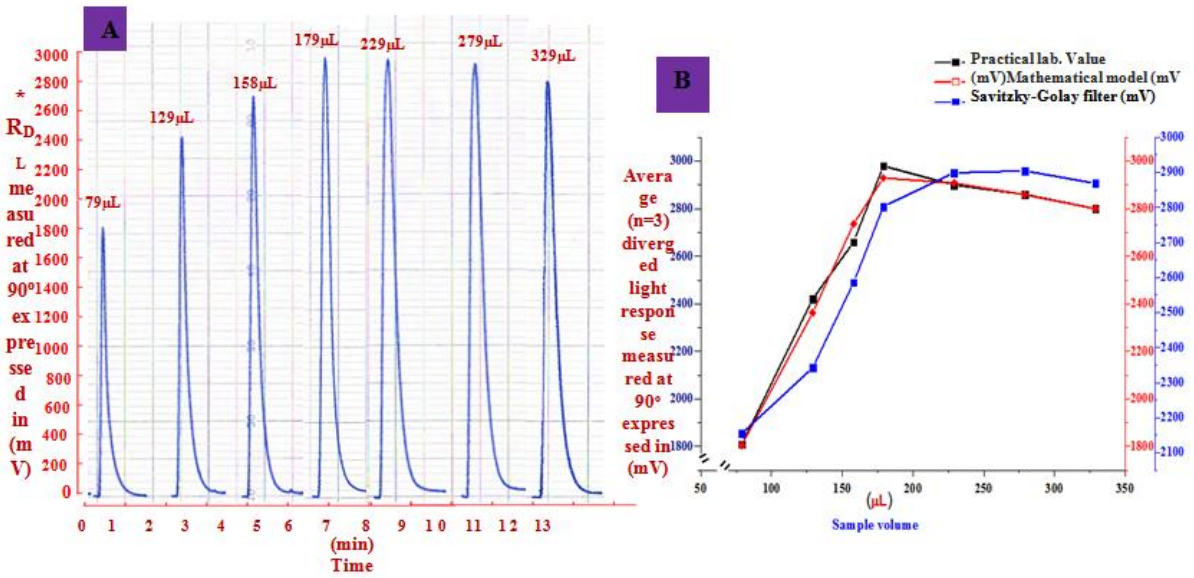


Figure7: Effect of sample loop volume on:- A- Diverged beam of light Vs. time profile. B- Laboratory measurements values, mathematical response values, and Savitzky- Golay smoothed filtering date as the Y-axis.

Table 7: Variation of injected sample volume on diverged light response tabulation all available data obtained practically, calculated as obtained by best fit mathematical model, and smoothed digital filtering using Savitzky-Golay data treatment.

length of sample loop(cm) r=0.5mm	Independent variable sample loop volume µL	Dependent variable. Average (n=3) diverged light response measured at 0- 90o expressed in mV					
		Practical lab. Value				Mathematical model $\hat{y}_i$	Savitzky-Golay filter $\hat{y}_{i\,(S\text{-}G)}$
		Average peak height($\bar{y}i$)	RSD %	Reliability(two tailed) $\bar{y}i(mV) \pm t_{0.025,2}\ \sigma_{n-1}/\sqrt{n}$	Δtb (Sec)		
10	79	1810	0.13	1810 ± 5.74	41	1805.986	2155.078
16.43	129	2420	0.08	2420 ± 4.89	43	2362.075	2342.345
20.13	158	2660	0.05	2660 ± 3.577	45	2736.037	2585.518
22.80	179	2980	0.03	2980 ± 2.26	48	2929.318	2802.760
29.17	229	2900	0.045	2900 ± 3.25	56	2907.721	2898.961
35.54	279	2860	0.06	2860 ± 4.07	59	2858.762	2904.473
41.91	329	2800	0.07	2800 ± 4.65	61	2800.101	2868.712

Purge time effect

Using optimum parameters that were achieved in the previous sections, purge time of the sample volume to be injected via the carrier stream (NH_4Cl 70mmol/L) was studied. Using different purge time (5-45 sec) for the sample segment to pass through injection valve at pre- selected time interval as shown in table 8, it can be noticed that an evacuation of sample segment from injection valve of less than 40 sec. gave weak response. This is caused by not achieving complete purge of sample. In complete precipitation of reactant was accomplished by incomplete introduction of sample segment. Therefore, a disturbed response- time profile can be noticed or a weakening response might happen (fig. 9-A, B). A vice versa will insure a complete discharge and a full purge of the sample plug from injection valve. So, 45 second was found a time that compromize a suitable purge time and through output with a good response profile avoiding any irregularty.

Table 8: variation of purge time on diverged light response and tabulate all available data obtained practically, calculated as obtained by best fit mathematical model, and smoothed digital filtering using Savitzky-Golay data treatment

Independent variable of Purge time (Sec.)	Dependent variable. Average (n=3) diverged light response measured at 90°expressed in mV				
	Practical lab. value			Mathematical model $\hat{y}_i$	Savitzky-Golay filter $\hat{y}_{i(S\text{-}G)}$
	Average peak height($\bar{y}_i$)	RSD%	Reliability(two tailed) $\bar{y}_i(mV) \pm t_{0.025,2}\ \sigma_{n-1}/\sqrt{n}$		
5	800	0.30	800 ± 5.96	800.000	960.970
10	1240	0.18	1240 ± 5.44	1240.004	1202.241
15	1540	0.13	1540 ± 5.07	1539.930	1591.543
20	2120	0.09	2120 ± 4.87	2120.491	1991.388
25	2240	0.09	2240 ± 4.77	2238.346	2264.843
30	2420	0.08	2420 ± 4.50	2423.028	2429.760
35	2540	0.06	2540 ± 3.80	2536.913	2565.982
40	2620	0.06	2620 ± 3.65	2621.651	2699.075
Open valve (45)	2988	0.03	2988 ± 2.26	2939.639	2795.041

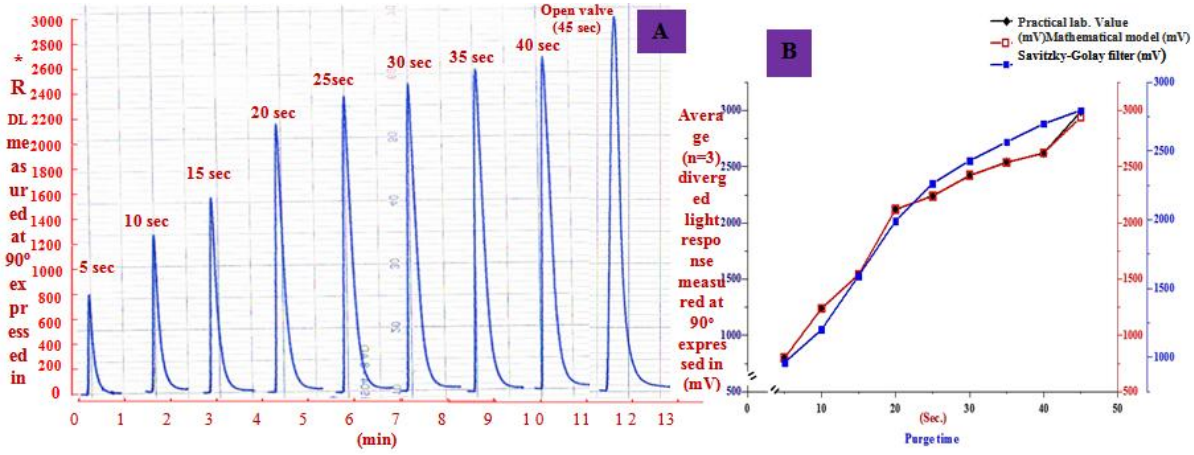


Figure 8: Effect of purge time on:- Diverged beam of light Vs. time profile (A), Laboratory measurements values, mathematical response values, and Savitzky- Golay smoothed filtering date as the Y-axis (B).

V. Calibration graph

Selling all achieved experimental parameters that at the end will lead to establish a new methodology regarding the assessment and determination of this crucial drug. In previous section physical as well as chemical variable were set at their optimum values (2mmol/L concentration Phosphotungstic acid, 179µL sample volume, and 1.7 ml/min flow rate for each line). Set of series (0.01-10mmol/L) solutions were prepared an output came was depicted in fig. 9-A. All the prepared concentration was used. An increase in Indomethacin concentration causes an increase number of nuclei formed up to 5mmol/L. In which it will lineup and densification with entraped water molecule, which might cause a diverged beam of light. All what is received by the ISNAG- fluorimeter detector is 0 - 90^{o}. An increase in Indomethacinconcentration more than 5mmol/L cause a much more intensification caused by the effect of agglomerate formation which form in this short period of time a relatively more intensified massive precipitate. Which in turn prevent the penetration of light only affecting the reflection of light at a certain extend. Therefore, a shift from linearity is un avoidable affecting the correlation coefficient. Choosing allpoints (fig. 9-A) that were measured trying to fit a linear equation of the form **y= a+ b x**in which a correlation coefficient of **r = 0.9664**while capital squared- **R**gave **93.40%** for the whole chosen range (0.01-10mmol/L). Searching for better representation, a shorter range should be used to improve the assessment mathematical formulation. The best fit linear equation representing the diverged response light as dependent variable against concentration of Indomethacin (0.01-5mmol/L) (fig. 9- B) has a correlation coefficient of **r =0.9952**with a capital squared- **R** of **99.05%**. This indicate that the linear equation chosen: **R_{DL}(mV) = a+slope[Indomethacin] mmol/L.** Was able to explain this much of the obtained results, this chosen eleven points were the outcome of scatter plot. All results summed up in table 9.

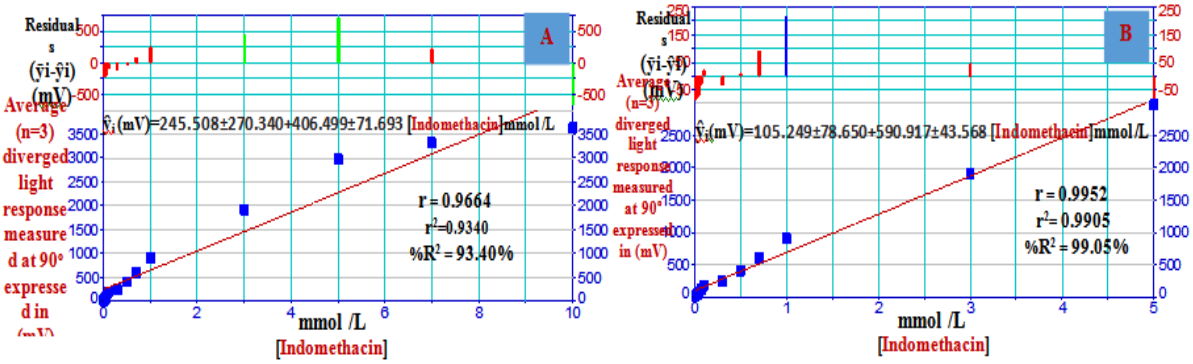


Figure9:
A- variation of scattered diverged light at range (0.01-10mmol/L), n=13 against Indomethacin concentration
B- Calibration graph deduced from scatter point plot at range (0.01-5mmol/L), n= 11.
Residual=($\bar{y}_i$-$\hat{y}_i$)in mV,$\bar{y}_i$= practical value, $\hat{y}_i$=estimated value

The assessment evaluation of the new developedmethodology for the determination of Indomethacin was compared with the available literature cited method namely uv-spectrophotometry method. Spectrophotometric method based on the measurements of absorbance for the range of concentration (0.05-1mmol/L) at max wavelength (λmax =226nm)[23],(fig. 10-A) using quartz cell.From fig. 11-B, the best linear range extend from 0.01- 1mmol/L with correlation coefficient of **0.9832** and capital square-R = 96.76%, n= 7 (no. of measurement). Table 9 shows the variable data treatments. Table 10 tabulated the detection of limit and repeatability with minimum of the RSD% . It can be clearly noticed that the new adopted methodology satisfies both the use of low as well as high concentration with high precision and repeatability with minimum of the relative standard deviation (fig..11).

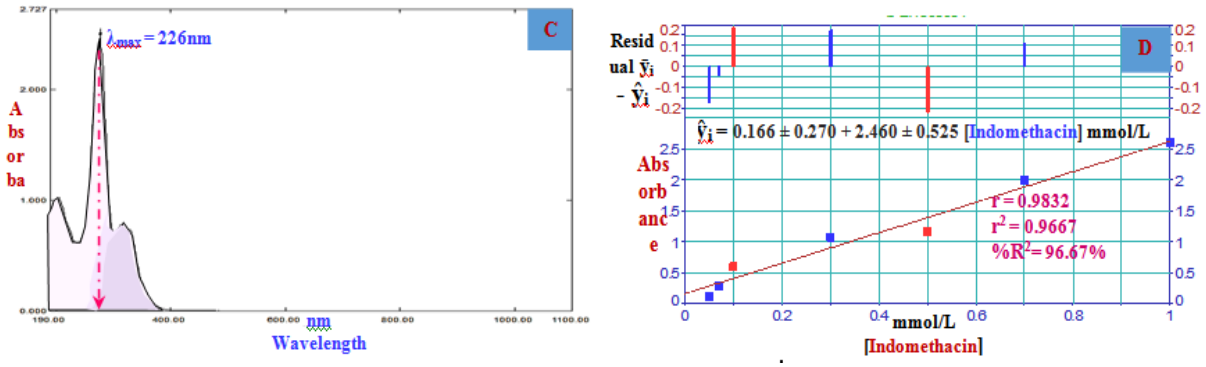


Fig. 10: A- Absorbance UV-spectra of Indomethacin standard solutions (0.05mmol/L) dissolved in NaOH (0.1M) against NaOH (0.1M) as a reference.
B- Calibration graph deduced from scatter point plot of spectrophotometry method (classical method) at range (0.05-1mmol/L), n=7 against Indomethacin concentration.

Table 9: Summary of calibration curve results for the determination of Indomethacin precipitate response with Phosphotungstic acid .

Method		No. of measurements	Measured mmol/L	Linear dynamic range mmol/L	ŷ(mV)=(a± S_at)+ (b± S_bt) [Indomethacin]mmol/L at confidence level 95%, n-2	r r^2 R^2 %	t_{tab} at 95% confidence level, n-2	$t_{cal} = \frac{\lvert r\rvert\sqrt{n-}}{\sqrt{1-r^2}}$
		Phosphotungstic acid						
Newly developed methodology	ISNAG- fluorimeter	13	0.01 - 10	0.01 – 10	245.508±270.340+406.499 ±71.693 [Indomethacin] mmol/L	0.9664 0.9340 93.40%	2.201<< 12.480	
		11		0.01 – 5	105.249±78.650+590.917 ±43.568 [Indomethacin] mmol/L	0.9952 0.9905 99.05%	2.262 << 30.680	
Used method based on	uv-spectroscopy λ_{max} = 226 nm	7	0.05 - 1	0.05 - 1	0.166±0.270+2.460±0.525 [Indomethacin]mmol/L	0.9832 0.9667 96.67%	12.0402.571 <<	

$\hat{y}_i$ = Estimated value in mV for newly developed method, and without unite for spectrophotometry method, [Indomethacin]: Concentration (mmol/L), r:Correlation coefficient, r^2:Coefficient of determination, R^2%: percentage Capital R-square. $t_{tab} = t_{0.025}$, n-2

Table 10: Limit of detection and repeatability for Indomethacin at optimum parameter in of Indomethacin using Indomethacin – [Phosphotungstic acid] (2mmol/L) system.

Method		No. of measurements	Linear dynamic range mmol/L	Detection limit Weight / sample		[Indomethacin] mmol/L	Repeatability at 95% confidence level $\bar{y}i \pm t0.025, n-1 \sigma n-1/\sqrt{n}$ at 95% , n = 7	RSD %
				Practically based on the gradual dilution of the minimum concentration	Theoretical based on slope X =3SB /slope			
Newly developed methodology	ISNAG-fluorimeter	Phosphotungstic acid						
		11	0.01 – 5	(5µmol/L) 320.222ng/sample	(0.5076 µmol/L) 32.514ng/sample	1	910 ± 1.2948	0.153
						5	2984 ± 1.6278	0.059
Usedmethod based on	UV-spectroscopy λmax = 226 nm	7	0.05 - 1	(10µmol/L) 14.312µg/sample	(36.588µmol/L) 52.364µg/sample	0.7	1.98 ± 0.0278	1.515

X= value of L.O.D. based on slope, S_B= standard deviation of blank repeated for 11 times, $\bar{y}_i$ = practical value for (n=3) in mV for newly developed method, and without unite for spectrophotometry method, $t_{tab} = t_{0.025}$, n-1 = $t_{0.025}$, 6 = 2.447.

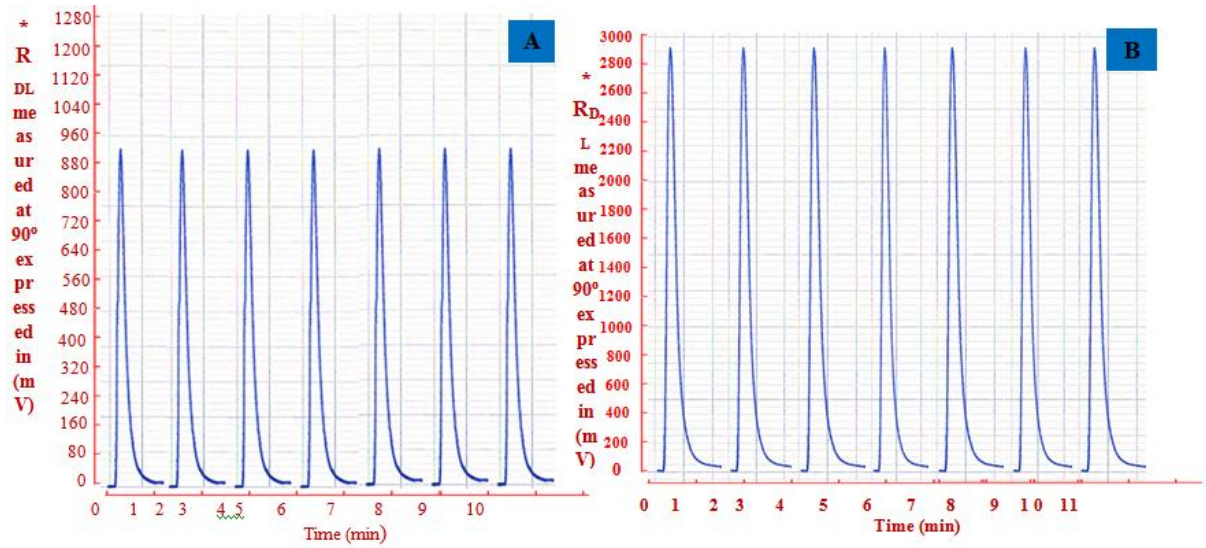


Fig. 11: Response profile of repeatability of Indomethacin (1mmol/L and 5mmol/L- with for Phosphotungstic acid (2mmol/L)- NH_4Cl (70mmol/L) system.

VI. Application

The continuous flow injection analysis via diverged light response using low pressure mercury lamp that used in ISNAG fluorimeter achieved in this work was used for the analysis of Indomethacinin the four different drug manufactures (Indometacina- 25mgTROGE-MEDICAL GMBH- Germany,Indoflam- 25mg Ajanta pharma Limited-India,Indylon- 25mgMEDOCHEMIE LTD–CYPRUS, and Indomal- 25mgUni pharma-UEA). In addition, was compared with UV-spectrophotometric via the measurement of absorbance at λ_{max} = 226nm [**5**] by UV-1800, UV-spectrophotometer- Shimadzu,[**4**]. A series of solutions were prepared of each drug (5mmol/L) (0.178895gof active ingredient in 100ml) by transferring 5ml to each five volumetric flask (10ml), followed by the addition of gradual volumes of standard solution of Indomethacin (0, 0.1, 0.2, 0.3, and 0.4ml) of 50mmol/L to obtain (0, 0.5, 1, 1.5, and 2mmol/L) when use ISNAG fluorimeter.While transferring 0.5ml to each five volumetric flask, followed by the addition of gradual volume of standard solution of Indomethacin (50mmol/L) (0, 0.01, 0.03, 0.05, and 0.07ml) to obtain (0, 0.005, 0.015, 0.025, and 0.035mmol/L) concentration. Figure 12-A, B, C and D shows standard addition calibration graphs using ISNAG-fluorimeter(newly developed methodology). The results were summed in table11at confidence level 95% (2-tailed), showing practically content of Indomethacin in each sample of drug using two different methods and efficiency of determination.

VII. Conclusion

The developed newly adopted methodology in this research work was put into a paired t-test (the tool comparison) for the sake of accepting it as an alternative method for analysis and assessment of Indomethacin with standard used method. Mainly British Pharmacopoeia (B.P) [**26**], and UV-spectrophotometric (scheme 2), or rejecting it as an alternative method.The assessment is made on how much they are correlated as a methods and if there is any significant difference that will work against the developed method. On this basis three assumption statisticallyis made [**29**-**30**]. There is no significant difference between the means of all used four methods (i.e.; undistinguishable differences between the method) and if**μ** indicates the mean then it will annotated with specified term representing the method used as such

H_o= Nullhypothesis= No significant difference between

$\mu_{ASNAG\ fluorimeter} = \mu_{B.P} = \mu_{UV\text{-}spectrophotometry}$ OR$\mu_{ASNAG\ fluorimeter} - \mu_{B.P}$ = zero, $\mu_{ASNAG\ fluorimeter} - \mu_{UV\text{-}spectrophotometry}$ = zero

$\mu_{UV\text{-}spectrophotometry} - \mu_{B.P}$= zero. The alternative hypothesis **H_1:-**

$\mu_{ASNAG\ fluorimeter} \neq \mu_{B.P}$, $\mu_{ASNAG\ fluorimeter} \neq \mu_{UV\text{-}spectrophotometry}$

Conducting paired t- test will all possible pairs (i.e.; 3- pairs)three that are as follows: **ISNAGVs**. **BritishPharmacopoeia** (**BP**), **ISNAGVs**. **UV- spectrophotometry**, and**UV- spectrophotometry Vs BP**. As ISNAG being the suggested alternative or equivalent method of assessment of the drug which challenges the available official method as ISNAG as an instrument is new in its whole properties of working and presenting results for determination.So therefore, it is the one who is its capability is under question and its approval as a method with the existing method and the used ones. Following table no.12, it can be found that there is threecomparison. As it compare, ISNAG method with the other there standard method as shown above. Which

significance test indicate that at 95% confidence (α = 0.05/2 two tailed) there is no significant difference between the newly developed method and standard method. Therefore, the analyst should be able to choose any method for analysis i.e.; **ISNAG** or **UV-spectrophotometry**. Thus accepting null hypothesis. This indicate that the high efficiency of ISNAG as a reliable instrument for analysis of Indomethacin.

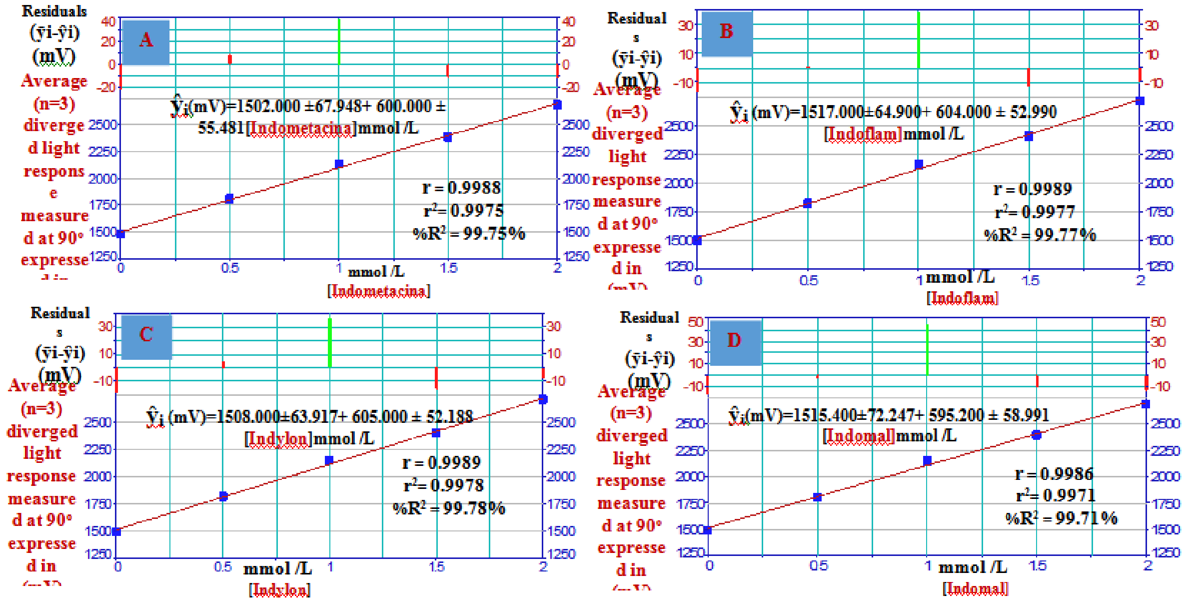
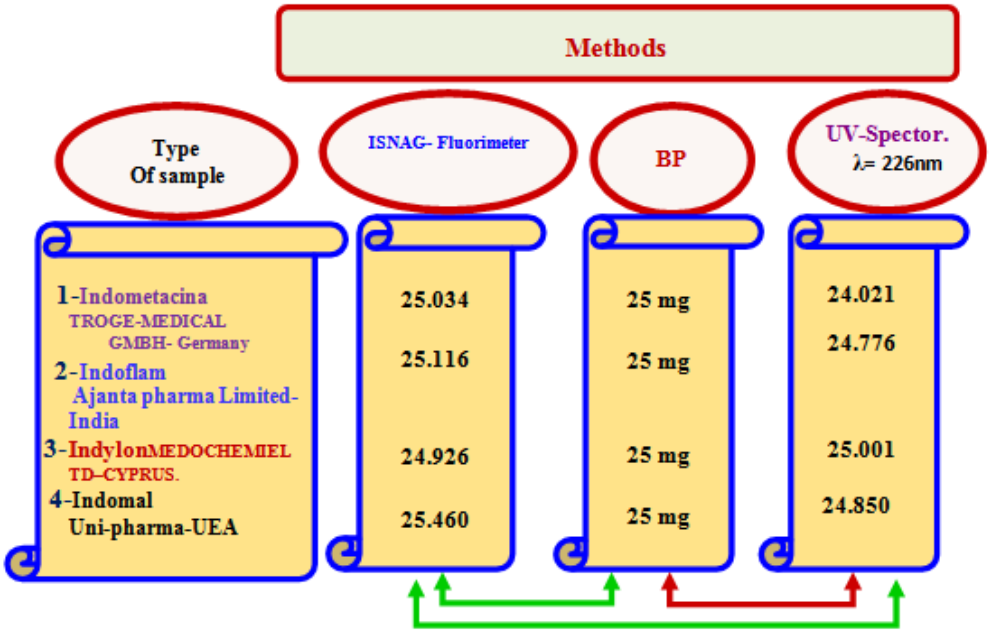


Fig.12: Standard addition calibration graph using ISNAG- fluorimeter for: A- Indometacina,B- Indylon, C- Indoflam, D- Indomal reacted using Indomethacin– [Phosphotungstic acid] (2mmol/L)-NH4Cl(70mmol/L) system.

Table 11: Summary of results by standard additions method for the determination of Indomethacin by different methods:- ISNAG fluorimeter method, and UV-spectrophotometermethod

Number of sample	Name, coted value, company And country	Confidence interval for the average weight of tablets $\overline{Wi}$ (g) ±1.96 $\sigma_{n-1}/\sqrt{n}$ at 95%	Theoretical content for the active ingredient $\overline{Wi}$ (mg)±1.96 $\sigma_{n-1}/\sqrt{n}$ at 95%	Sample weight equivalent to 0.178895 g (5mmol/L of the active ingredient. w; (g)	ISNAG- fluorimeter / UV-spectrometer											
					Volume of Indomethacin (ml) / [Indomethacin] mmol/L					$\hat{y}_i$= a ± S_at+ b ± S_bt [Sample]mmol/L at confidence level 95%, n-2	r r^2 R^2%	Practical content of active ingredient				Efficiency of determination REC%
					0	0.1	0.2	0.3	0.4			Concentration (mmol/L) prepared sample in 10ml	100ml	Weight in 100ml $\overline{Wi}$(g)±$t_{0.025}$ $\sigma_{n-1}/\sqrt{n}$ at 95%, n-1	In tablets $\overline{Wi}$ (mg) ±$t_{0.025}\sigma_{n-1}/\sqrt{n}$ n = 3	
					0	0.5	1	1.5	2							
					0	0.01	0.03	0.05	0.07							
					0	0.005	0.015	0.025	0.035							
					ӯi (mV) for n= 3											
					ӯi (mV) for n= 3											
1	Indometacina 25 mg TROGE-MEDICAL GmbH- Germany	0.1029±0.0202	25 ± 0.0049	0.73597	1480	1810	2140	2390	2690	1502.000 ±67.948+ 600.000 ± 55.481 [Indometacina]mmol /L	0.9988 0.9975 99.75%	2.5033	5.0067	0.17913 ± 0.0149	25.034 ± 2.082	100.13%
					0.80	0.90	1.34	1.70	1.89	7.959 ±1.667+ 33.134 ± 8.133 [Indometacina]mmol /L	0.9912 0.9825 98.25%	0.2402	4.8041	0.17189 ± 0.0191	24.021 ± 2.669	96.08%
2	Indoflam 25 mg Ajanta pharma Limited-India	0.2988± 0.0171	25 ±0.0014	2.13815	1500	1820	2160	2410	2715	1517.000±64.900+ 604.000 ± 52.990 [Indoflam] mmol /L	0.9989 0.9977 99.77%	2.5116	5.0232	0.17972 ± 0.0186	25.116 ± 2.599	100.46%
					0.83	0.98	1.35	1.70	1.98	8.312±0.611+ 33.549 ± 2.978 [Indoflam] mmol /L	0.9989 0.9977 99.77%	0.2478	4.9551	0.17729 ± 0.0196	24.776 ± 2.739	99.10%
3	Indylon 25 mg MEDOCHEMIE LTD–CYPRUS	0.1758± 0.0184	25 ± 0.0026	1.25785	1490	1815	2150	2400	2710	1508.000±63.917+ 605.000 ± 52.188 [Indylon] mmol /L	0.9989 0.9978 99.78%	2.4926	4.9851	0.17836 ± 0.0199	24.926 ± 2.781	99.70%
					0.82	0.98	1.38	1.69	1.97	8.342±0.853+ 33.366 ± 4.153 [Indylon] mmol /L	0.9977 0.9954 99.54%	0.2500	5.0003	0.17891 ± 0.0204	25.001 ± 2.851	100.01%
4	Indomal 25 mg Uni-pharma-UDA	0.1799± 0.0206	25 ± 0.0029	1.28733	1498	1810	2155	2398	2692	1515.400±72.247+ 595.200 ± 58.991 [Indomal] mmol /L	0.9986 0.9971 99.71%	2.5460	5.0921	0.18219 ± 0.0241	25.460 ± 3.368	101.84%
					0.82	1.00	1.36	1.69	1.99	8.346±0.481+ 33.585 ± 2.348 [Indomal] mmol /L	0.9993 0.9986 99.86%	0.2485	4.9701	0.17782 ± 0.0162	24.850 ± 2.264	99.40%

$\hat{y}_i$: Estimated response value (mV) for ISNAG fluorimeter,and without unit for uv-spectrophotometer for (n=3), [sample]: drug concentration (mmol/L), r: correlation coefficient, r^2:coefficient of determination& R^2%: percentage capital R square, $t_{0.025,\ \infty}$= 1.96 at 95%$t_{0.025,\ 2}$ = 3.182. For n-2.



Scheme 2: Summed up the path for comparison between three different methods using paired t-test

Table 12: Paired t-test for the comparison between four different methods of four samples for the analysis of Indomethacin in drugs for n= 4 at 95% confidence level (α = 0.05) and DF = 3

Paired	Correlation coefficient r	Paired differences				Significant (2 tailed)
		$\bar{X}d$	Standard Deviation (σ_{n-1})	t_{cal}	t_{tab}	
Pair- 1 BP- ISNAG fluorimeter	**0.9889**	**- 0.134**	**0.231**	**- 1.161< 3.182**		**0.330> 0.05 Not significant**
Pair- 2 ISNAG fluorimeter- UV-Spector.	**0.9892**	**0.472**	**0.458**	**2.063 < 3.182**		**0.131 > 0.05 Not significant**
Pair- 3 UV-Spector- BP	**0.9804**	**0.338**	**0.438**	**1.545< 3.182**		**0.220> 0.05 Not significant**

DF: Degree of freedom (n-1) = 3, $\bar{X}d$: average of difference between two methods, t_{tab} = $t_{0.025,\ 3}$ = 3.182, tcal= **$\bar{X}d\ \sqrt{n}\ /\ \sigma$ n-1 at 95 %**

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