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Spectrophotometric determination of Salicylamide one of the novel COVID -19 protocols via new design Flow Injection with Merging-Zone Technique

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Abstract:

Recently, attention has turned to drugs like salicylamide for treating COVID-19. Several studies have reported simple, fast, and sensitive methods for measuring salicylamide levels. However, potential limitations remain regarding interference from other drugs and accuracy of results. To tackle this issue, researchers combined the standard addition technique with flow injection analysis. As part of this investigation, the reaction involving Fe (III), salicylamide, and 1,10-phenanthroline at a temperature of 55°C was examined using a spectrophotometer set at a wavelength of 528 nm. Thus, the new design was able to show an excellent linearity over the concentration range of (2-30 µg/mL) and (25-300 µg/mL), with correlation coefficients (r^2) of 0.9669 and 0.9781, respectively. In addition, physical and chemical variables were optimized to obtain the best results. There was a significant positive correlation between the new design and $SCÏO$ NIR micro-spectrometers device (handheld) outcomes.

Keywords: Salicylamide, COVID-19 protocol, Merging zone flow injection.

التقدير الطيفي للساليسيلاميد أحد بروتوكولات COVID −19 الجديدة باستخدام التحليل بالحقن الجرياني واندماج المناطق

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الخلاصة:

في الآونة الأخيرة ، تحول الاهتمام إلى عقاقير مثل الساليسيلاميد لعلاج فيروس كرونا 19. أفادت العديد من الدراسات عن طرق بسيطة وسريعة وحساسة لقياس مستويات الساليسيلاميد ومع ذلك لا تزال هناك قيود محتملة فيما يتعلق بالتداخل من الأدوية الأخرى ودقة النتائج لمعالجة هذه المشكلة ، قام الباحثون بدمج تقنية الإضافة القياسية مع تحليل حقن التدفق. كجزء من هذا التحقيق ، تم فحص التفاعل الذي يشتمل. تفاعل للحديد (III) مع الساليسيلاميد بوجود1−10فينانثرولين عند درجة حرارة 55 درجة مئوية باستخدام مقياس طيف ضوئي مضبوط على طول موجي يبلغ 528 نانومتر. وهكذا ، كان التصميم الجديد قادرًا على إظهار خطية ممتازة على مدى التركيز (2−30 ميكروغرام / مل) و (25−300ميكروغرام / مل) ، مع معاملات الارتباط 0.9669 و0.9781، على التوالي. بالإضافة إلى ذلك ، تم تحسين المتغيرات الفيزيائية والكيميائية للحصول

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على أفضل النتائج فكان هناك ارتباط إيجابي كبير بين التصميم الجديد ونتائج جهاز المطياف الدقيق استشعار مطيف (محمول).

1. Introduction:

The novel COVID-19 treatment protocol assumes a pivotal role in disease management, encompassing a variety of pharmaceutical agents, among which salicylamide features prominently. This compound is harnessed to impede the propagation of the COVID-19 virus, serving as an imperative countermeasure [1]. In this capacity, salicylamide assumes the role of an aspirin alternative and is further integrated into an over-the-counter analgesic regimen in synergy with aspirin and caffeine [2]. Salicylamide's utility extends broadly, particularly in conjunction with paracetamol or vitamin C, as a remedy for cold-related symptoms, correlating with myalgia and pyrexia [2]. Consequently, the quantification of salicylamide assumes significance, given precedent studies elucidating its conjunction with vitamin C [3-4] or paracetamol and employing spectrophotometric techniques [5-7]. In addition, salicylamide was determination by spectrofluorometric as salicylic acid[8] or chemiluminescence[9] and estimated using HPLC, as previously reported [10].

While the determination of salicylamide has been accomplished proficiently through diverse analytical approaches, certain methodologies, notably the flow injection analysis (FIA), have displayed a heightened level of sensitivity. It is worth noting that FIA has emerged as a prevalent technique for the quantification of substances within the temporal span from 1975 to 2022 [11]. However, most of these methods [12-17] depend on early analyses to determine the optimal based on pure materials. It is then applied to natural or manufactured materials without considering their interactions with other materials, which plays a major role in the calculation [14-18] . Despite the successful use of more than one injection to explain merging zone, they still neglect material interference and flexibility in the calculation of the results and accuracy. As a result, the standard addition [19] method and more than one injection were used. On the other hand, the reaction used in this study was based on Fe(III) with salicylamide by relying on the new design of the work system [6,20]. This based on a the idea of merging zone in our previous designs system [13].

2. Materials and Methods

2.1. Material

Two types of samples (drug and blood - human serum – (H.S)) were used in this study. Pharmaceutical model [analgesics, salicylamide] according to [1]. The thin powder was obtained by pulverizing the compressed medication (with prior measurement of each tablet's weight) [21], Similar to the procedure outlined in [6] the preparation of the H.S samples followed a corresponding methodology involving three discrete stages. Initially, a centrifugation step (at 300 rpm) was undertaken to segregate the H.S from the blood. Subsequently, 0.5 mL of the resultant supernatant was carefully transferred into a new glass test tube (with a diameter of 75 mm), into which a small quantity of NaCl crystals and 1.0 mL of ethyl acetate were introduced, followed by vigorous mixing for a duration of 30 seconds. Lastly, a volume of 0.5 mL derived from the organic layer was meticulously drawn into a pipette and subsequently transferred into a 10 mL volumetric flask. The reagents were prepared, according to [6 ,13] using ultra-pure deionized water (DOW) (18.2 MΩcm at 25°C) , as shown in Table 1.

Table 1: The concentration of the prepared standard solution

Reagents	Weight dissolved in Volume liquid	Completed dissolved	Concentration required
Salicylamide	0.25g in 5% ethanol	Completed by250 ml ultrapure DOW up to volume	3.645×10^{-2}M
Fe $(NO_3)_3.9H_2O$	0.125 g in 21mL HCl (12N)	Completed by 250 ml ultrapure DOW up to volume	1.23×10^{-3}M
10-Phenanthroline	0.24g	Completed by 100 ml methyl alcohol	0.01212M
Acetic acid	30mL of con 17.4125	Completed by 100 ml ultrapure DOW up to volume	5.22M

2.2. Instrument

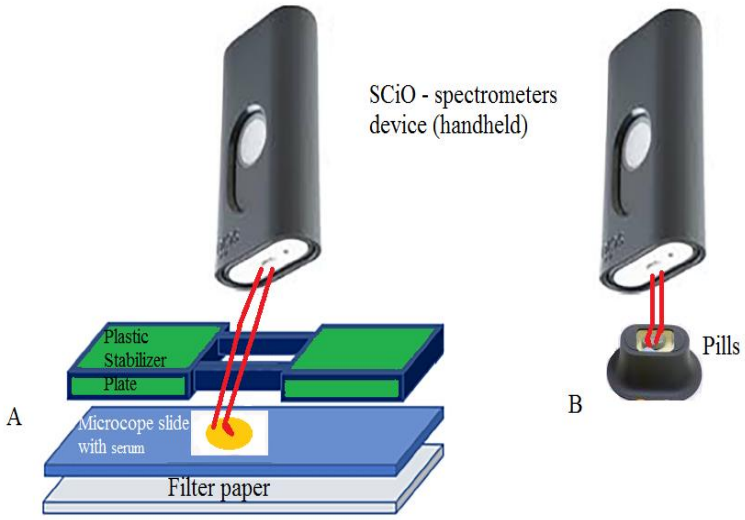
In this section explain types of apparatus that use in both Flow injection method or in compare method

2.2.1. The manifold system used for flow injection method

A peristaltic (B07WNFV584), four valve (homemade) with volume (12, 10, 5, and 7) µL, four mixing coils (35, 40, 50, and, 70) cm, and colorimeter (homemade)

2.2.2.*SCÏO - Spectrometers device (handheld)*

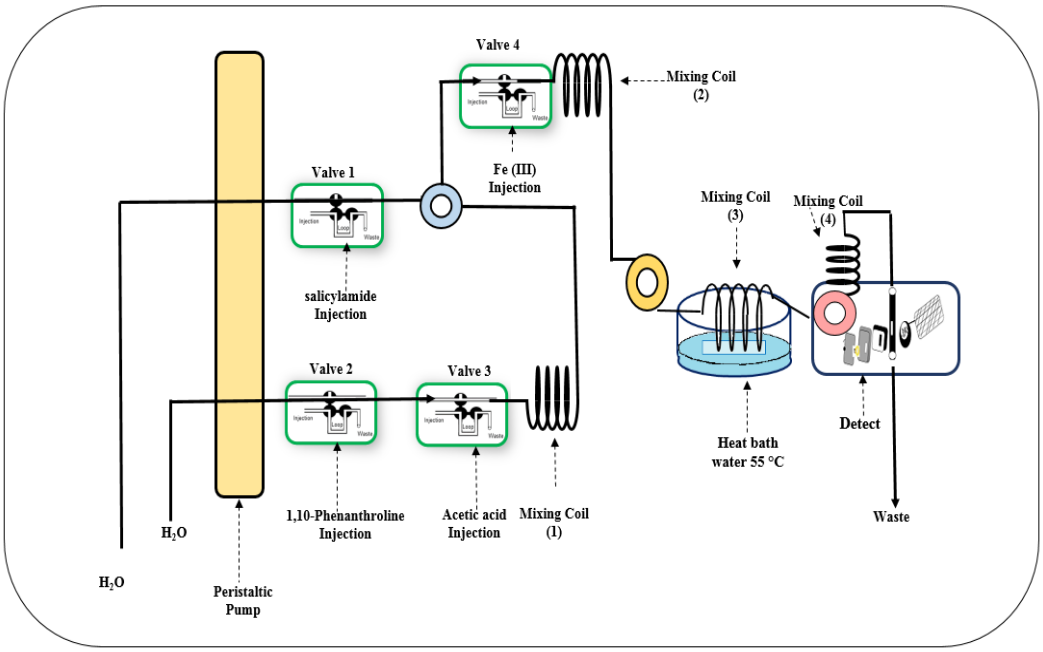
The $SCÏO$ is an apparatus that use to determination salicylamide in serum [22-23] as shown in A Schematic 1 ,and in pill as shown in B Schematic 1.



Schematic diagram (1): The SCI˙O pocket infrared scanner (A) A drop of serum, and (B)pills

2.3. Experimental arrangement

Illustrated in Schematic Diagram 2 is the experimental configuration comprising dual flows of deionized water, a peristaltic pump, a quartet of valves, four mixing coils, a heat bath, and a custom-crafted colorimeter operating at a wavelength of 528 nm.



Schematic diagram 2. Experimental system of merging zone via flow injection analysis system.

2.4. Standard addition- merging zone via flow injection analysis experiments

The standard addition sample method is associated with merging zone flow injection analysis, to avoid interference from other materials [24].Therefore, the results were determined using the following equation.

$$\frac{C_0}{A_1 - A_2} = \frac{C}{A_1} \quad \ldots \quad \ldots\ldots\ldots\ldots (1)$$

where, $C_0, and\ C$ denotes the concentrations of the extra material and the sample respectively, whileA_1 , $and A_2$, denotes the signal values of the extra material and the sample respectively.

2.5. The statistical analysis

This section to display the statistics that were used in this study based on [25]. In a comprehensive approach, the mean, standard deviation, relative standard deviation (RSD%), and a 95% confidence interval were computed based on the acquired data. Additionally, the correlation coefficient (r^2) and the calculated t-value, utilizing a significance threshold of 95% and degrees of freedom (n-2) pertinent to calibration, were determined. Further enriching the analytical framework, two distinct statistical methodologies were employed, namely the t-test and the F-test. These procedures were systematically undertaken to evaluate the veracity and accuracy of the method under scrutiny.

3. Results and Discussion

3.1. Physical variables

3.1.1. The effect of time reaction

In this investigation, two distinct time intervals, ranging from 2 to 16 minutes, were employed in conjunction with specific concentrations: salicylamide (40 mg/mL, 5 mL), Fe $(NO_3)_3.9H_2O$ (5×10^{-4} M, 5 mL), 1,10-Phenanthroline (5×10^{-2} M, 3 mL), pH set at 4, and a temperature of 50°C. This experimental design was undertaken to explore the temporal influence on the absorption spectrum of the resulting complex. Illustrated in Figure 1 is the absorption spectrum of the generated complex, emanating from the reaction between ferroin

and salicylamide, as assessed by spectrophotometric means. Within this spectrum, distinct peaks are discernible, marked as peak (1) exhibiting comparatively lower absorption and peak (2) displaying elevated absorption levels, manifesting at time points of 2 and 16 minutes, respectively. The result indicated the use of 16 min enhance the absorption, therefore the design of system requires mixing coil.

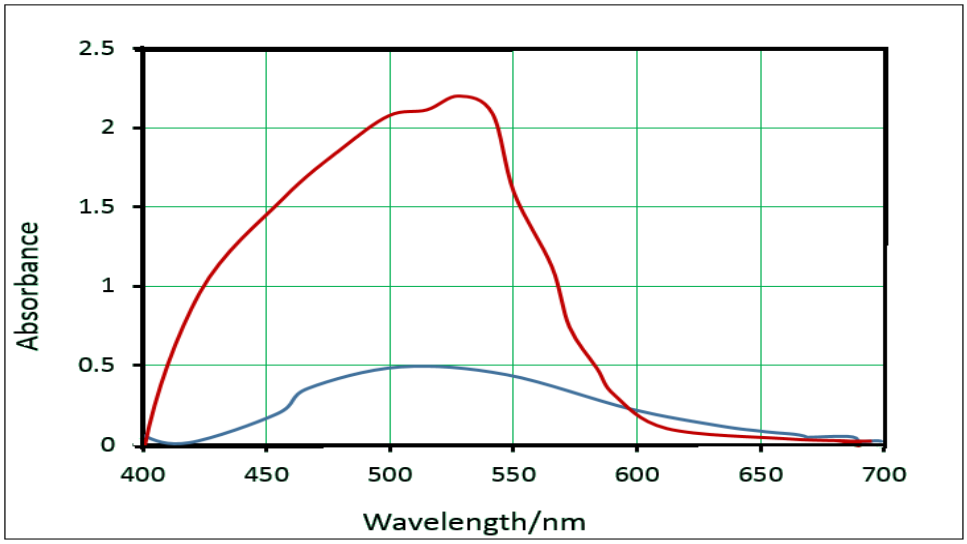


Figure 1: Absorbance opposite wavelength (nm) of complex produced from reaction ferroin, and salicylamide; (1) 2min reaction time, while (2) 16min reaction time.

3.1.2.The effect of Flow rate

Initially, the flow rate was manipulated to determine the optimal pump rate for both lines (no.1 and no.2) as well as the duration for injecting the loop volume. This preliminary step was essential since the sample reacts concurrently with the injected loop volume of the reagent. This synchrony ensures the attainment of the most favorable absorbance for the resultant coloration. An ambit flow rates have been applied for line no1 (range between 0.65to 4.3 mL/min), and no2 (range between 0.55to3.9 mL/min), with concertation salicylamide (40 mg/mL), Fe $(NO_3)_3.9H_2O$ $(5 \times 10^{-4}M)$, 1, 10-Phenanthroline (5×10^{-2}), and PH (4), Table 2, and Figure2.

Table 2: The effect of use different speed of pump on absorbance for reaction:

Speed of pump	Flow rate of H_2O line .no 1 (mL/min)	Flow Rate of H_2O line .no.2 (mL/min)	Absorbance (n=3)	RSD %	Confidence interval at95% mean± $^*t_{0.05}\delta n\text{-}1/\sqrt{n}$	
5	0.65	0.55	0.85	1.18	0.87	0.83
10	1.45	1.35	0.98	2.04	1.03	0.93
15	2.2	1.95	1.25	1.60	1.30	1.20
20	3.2	2.6	1.34	1.49	1.39	1.29
25	3.8	3.2	1.56	1.28	1.61	1.51
30	4.3	3.9	1.42	1.86	1.49	1.35

$^*t_{0.05}$=4.303 at n=3

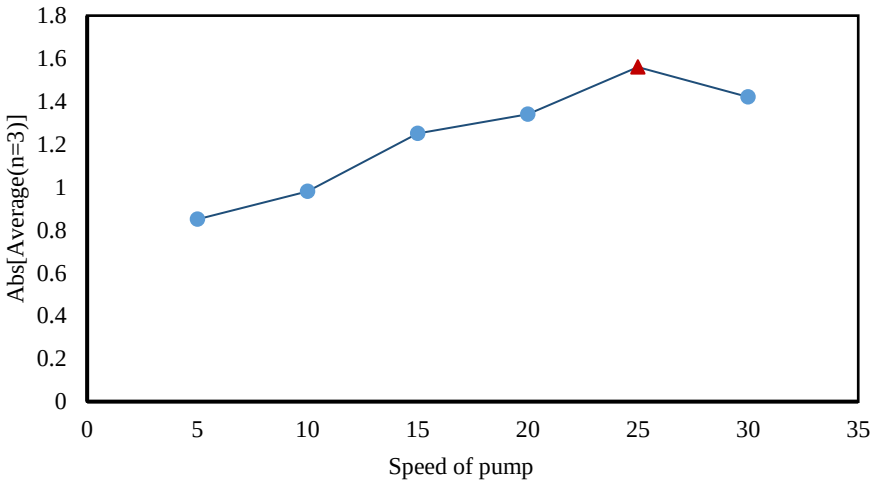


Figure 2: The effect of use different speed of pump on absorbance of reaction

3.1.3. The effect of sample loop

The primary focus of this study was to examine the impact of the sample volume. To achieve this, five different loop sample volumes (5, 7, 10, 12, and 15 µL) were employed. These volumes were utilized in conjunction with salicylamide (40 mg/mL), Fe $(NO_3)_3.9H_2O$ (at a concentration of 5×10^{-4} M), 1,10-Phenanthroline (at a concentration of 5 × 10^(-2)), and a pH of 4. The experiment was conducted with a flow rate of 3.8 mL/min for the first carrier and 3.2 mL/min for the second carrier. The result shown the enhance on the as depicted in the Table3 and Figure3 The volume of loop sample 12 was corresponding to the flow rate of the reaction complete.

Table 3: The effect of use different volume of sample loop

Sample loopµL	Absorbance(n=3)	RSD%	Confidence interval at95% mean±*$t_{0.05}$δn-1/√n	
5	0.75	0.44	2.27	2.23
7	0.83	0.61	2.54	2.46
10	0.87	0.38	2.63	2.59
12	1.43	0.36	4.34	4.26
15	0.93	0.55	2.82	2.74

*t0.05=4.303 at n=3

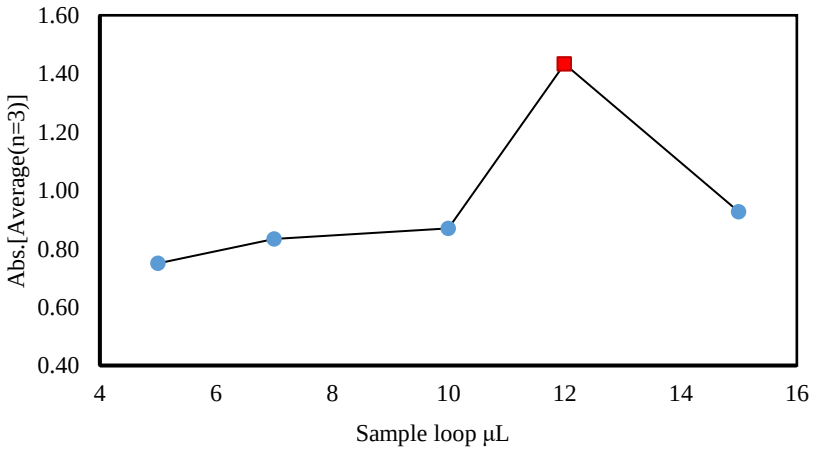


Figure3: The effect of use different volume of sample loop

3.1.4. The effect of Temperature

As referenced in the existing literature [6], the reaction necessitates an elevated temperature surpassing ambient conditions. To explore this, a heat bath was introduced, spanning a temperature range of 25 to 60 °C. In conjunction with this, salicylamide (40 mg/mL), Fe $(NO_3)_3.9H_2O$ (at a concentration of 5 × 10^{-4}M), 1,10-Phenanthroline (at a concentration of 5 × 10 -2)), and a pH level of 4 were employed. The experiment was carried out with flow rates of 3.8 mL/min and 3.2 mL/min for the first and second carriers, respectively, and a loop sample volume of 12 µL. The findings from this study underscore that a temperature of 55°C engenders a stable and comprehensive complex formation, as corroborated by the outcomes presented in Table 4 and Figure 4.

Table 4: The effect of using variable Temperature on the absorbance of complex

Temperature,° C	Absorbance(n=3)	RSD%	Confidence interval at95% mean±*$t_{0.05}$δn-1/√n	
25	0.50	3.03	0.54	0.47
30	0.56	1.79	0.58	0.54
35	0.76	0.76	0.77	0.74
40	0.81	1.23	0.83	0.79
45	1.02	2.46	1.09	0.96
50	1.24	0.81	1.26	1.22
55	1.45	0.69	1.47	1.43
60	1.46	0.39	1.48	1.45

*t0.05=4.303 at n=3

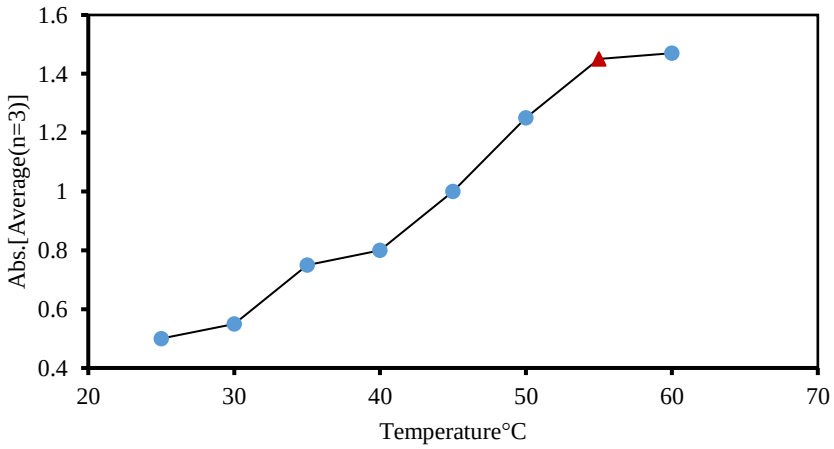


Figure 4: The effect of using variable Temperature °C on the absorbance of complex

As a result from previous experiments of physical variables, the operating conditions that use in further experiments listed in Table 5

Table 5: The operating conditions of Physical variables

Variables	Conditions
Speed of pump	25
Flow rate($line_1, and Vline_2$)	(3.8 ,and 3.2 respectively)(mL/min)
Volume of valve loop($V_1, V_2, V_3, and V_4$)	(12, 10, 5, and 7 respectively) µL
Length of mixing coil($C_1, C_2, C_3, and C_4$)	(20,20,35,and35 respectively) cm
Temperature a heat bath	55°C

3.2. Chemicals variables

A series of solutions (Fe $(NO_3)_3.9H_2O$), reagent (1, 10-Phenanthroline), and buffer solution (Acetic acid) were prepared.

3.2.1. The effect of PH value (Buffer solution Acetic acid)

In this section of study variation pH value (Buffer solution Acetic acid), concentrations affected absorbance values within the range of PH $3.9-5$as shown Table 6 and Figure5. The result shown the pH 4.7 enhance the reaction and this result, significantly close with the result (pH 4.5) has been mentioned in previous studies[6].

Table 6: Effect of variation PH value (Buffer solution Acetic acid) on absorbance value.

pH	Absorbance(n=3)	RSD%	Confidence interval at95% mean± $^{*}t_{0.05}\delta n\text{-}1/\sqrt{n}$	
3.9	0.28	3.57	0.30	0.26
4	0.35	2.86	0.37	0.33
4.3	0.43	2.33	0.45	0.41
4.5	0.55	1.82	0.57	0.53
4.7	0.57	1.01	0.59	0.56
4	0.52	1.92	0.54	0.50
4.9	0.44	2.27	0.46	0.42
5	0.42	2.77	0.45	0.39

*t0.05=4.303 at n=3

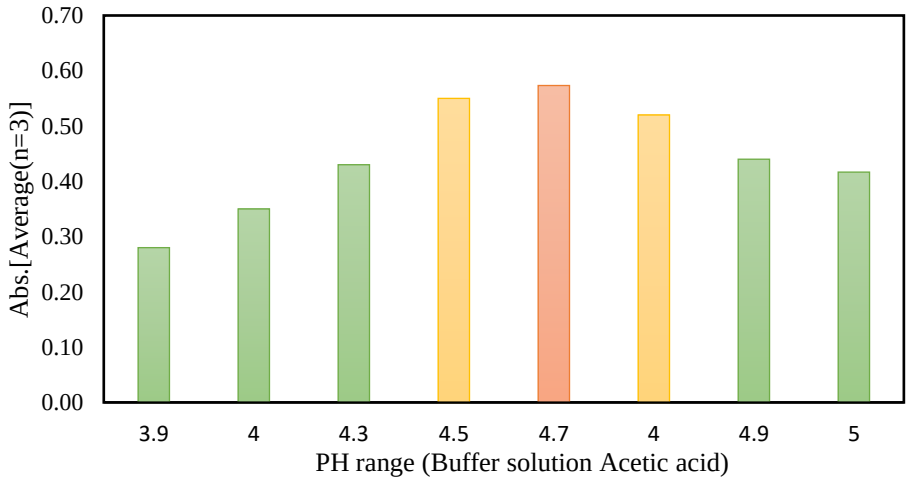


Figure5: Effect of variation PH value (Buffer solution Acetic acid) on absorbance value.

3.2.2. The effect of Ferric nitrate Nona hydrate Fe (III)

In this investigation there are different concentrations of Fe(III) reagent affected absorbance values within the range of $2\times10^{-3}\_7\times10^{-5}M$. The result indicated that concentration$7\times10^{-4}M$ given significantly absorbance as depicted in Table7, and Figure6.

Table 7: The effect of using variable of Fe(III)reagent concentration

Con.Fe(III) *M*	Absorbance(n=3)	RSD%	Confidence interval at95% mean± $^{*}t_{0.05}\delta n\text{-}1/\sqrt{n}$	
0.00001	0.55	1.82	0.57	0.53
0.00007	0.56	0.41	0.56	0.55
0.0001	0.74	1.35	0.76	0.72
0.0007	0.86	1.55	0.89	0.82
0.0015	0.85	0.31	0.86	0.84
0.002	0.85	0.18	0.85	0.84
0.005	0.84	0.42	0.85	0.83
0.007	0.83	0.37	0.84	0.83

*t0.05=4.303 at n=3

3.2.3. The effect of reagent (1, 10-Phenanthroline)

Various concentrations of (1,10-Phenanthroline) spanning from 0.5×10^{-2} M to 12×10^{-3} M were subjected to analysis, leading to the observation of altered absorbance values. Notably, the outcomes demonstrate an augmentation in response at a concentration of 1.1×10^{-2} M, as depicted in Table 8 and Figure 7.

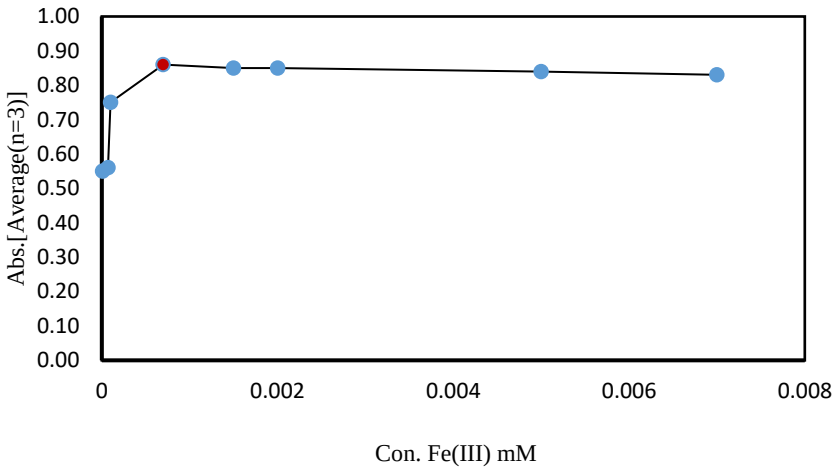


Figure 6: The effect of using variable of Fe(III)reagent concentration

Table 8: The effect of using variable of reagent 1, 10-Phenanthroline

1, 10-Phenanthroline Con. M	Absorbance(n=3)	RSD %	Confidence interval at95% mean± $^{*}t_{0.05}\delta n\text{-}1/\sqrt{n}$	
0.005	0.10	5.65	0.12	0.09
0.006	0.15	6.67	0.17	0.13
0.008	0.21	4.76	0.23	0.19
0.009	0.56	1.79	0.58	0.54
0.01	0.65	1.54	0.67	0.63
0.011	0.85	1.18	0.87	0.83
0.012	0.85	1.18	0.87	0.83

*t0.05=4.303 at n=3

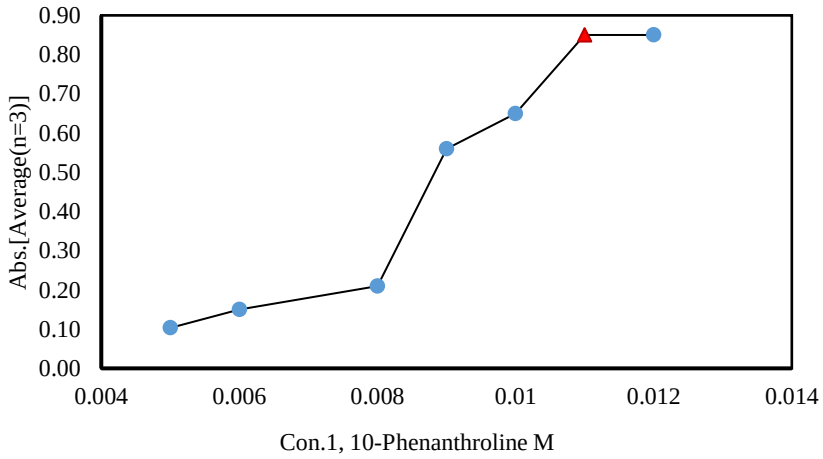


Figure 7: The effect of using variable of reagent 1, 10-Phenanthroline

Result, from previous experiments of chemicals variables, the best conditions listed in Table 9.

Table 9: The best obtained results of chemicals variables

Chemicals variables	Range	The best	Absorbance
pH	3.9-5	4.7	0.56
Fe $(NO_3)_3.9H_2O$,	2×10^{-3}_$7 \times 10^{-5} M$	$7 \times 10^{-4} M$	1.22
1, 10-Phenanthroline,	$0.5 \times 10^{-2} - 12 \times 10^{-3} M$	$1.1 \times 10^{-2}\, M$	1.33

3.3. Calibration graph of the proposed system

Two different sets of concentrations of salicylamide were prepared using deionized water (D.W). The first set ranged from 2 to 30 µg/mL, and the second set ranged from 25 to 300 µg/mL. Each concentration of salicylamide was tested ten times, for a total of 150 analyses. The average values of these analyses are presented, along with correlation coefficients of 0.9669 and 0.9781 for the two concentration ranges. These coefficients are labeled as A and B in Figure 8. Additionally, Table 10 includes calculated t-values and tabulated values at a 95% confidence level with degrees of freedom (n-2) for both calibration ranges. The results indicate that the calculated t-values are significantly higher than the tabulated t-values, suggesting a linear relationship between the variables.

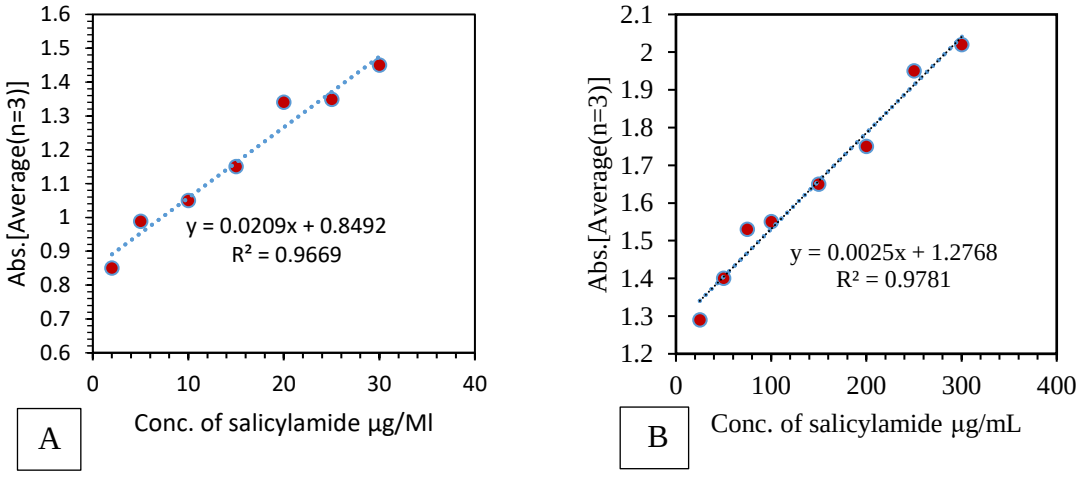


Figure 8: The calibration curves obtained from new system for determination of salicylamide ,with range (2-30µg/mL) for A,and with range(25-300 µg/mL) for B.

Table 10: The statistical analysis of the proposed system under optimum conditions, with range (2-30µg/mL) for A, and with range(25-300 µg/mL) for B.

Type ,concertation range with number of Test	$[Y = bx + a]$	r, r^2 , $R^2\%$	t_{tab} at 95%,n-2	$t_{cal} = \frac{\lvert r \rvert \sqrt{n-2}}{\sqrt{1-r^2}}$
A,(2-30µg/mL), n=10	$[Y = 0.0209x + 0.8492]$	0.9833, 0.9669, 96.69	2.3060 <	15.287
B,(25-300 µg/mL), n=10	$[Y = 0.0025x + 1.2768]$	0.9889 0.9781 97.81	2.3060 <	18.695

4. Method validation

After exploring the quantification of concentrations within two distinct sample types-human serum and pharmaceutical preparations – the specimens were formulated in accordance with references [26] and [27], respectively. Furthermore, this procedural methodology is elucidated within the "Materials" section. The results of human serum by using new system flow injection with merging-zone technique under optimum condition that listed in both Tables 5 and 9 as shown in Table 11, and for estimated determination of Salicylamide in pharmaceutical preparations Table 12.

Table 11: Estimated determination of Salicylamide in human serum by use flow injection with merging-zone technique under optimum condition

Human serum number test	Spiked, mg/mL	Found, mg/mL	Recovery,%	Relative error, %
1	6	6.5	108.33	-8.33
2	12	12.3	102.5	-2.50
3	18	18.6	103.33	-3.33
4	24	23.9	99.58	0.42
5	30	29.2	97.33	2.67

It is clear from result of human serum, that there was no significant interference in this method of determination salicylamide in this samples, because the recoveries were nearly 100%, and also the result of pharmaceutical preparations very close to claimed label.

Table 12: Estimated determination of Salicylamide in pharmaceutical preparations by use flow injection with merging-zone technique under optimum condition

	Pharmaceutical preparations	Nominal value, Salicylamide (Claimed Label)mg/mL	Found[(a)], mg/mL
1	EXAPRIN(Seattle WA98124)	152	152±1.67
2	PAIN RELIEVER(USA)	152	152±1.75
3	Pain Stoppers (Honeywell Safety Products USA)	152	152±1.45
4	Pain Zapper(USA)	152	152±0.98

[(a)] Mean±S.D(n=3)

In comparison to the reference method, the $SCÏO$ - spectrometers device (handheld) was use as reference as mentation in section method see schematic diagram (1) A, which explain how use to determination salicylamid concentration in drop of serum, while part B explain how use to determination of salicylamide in pharmaceutical preparations pills. Table 13 and Table 14 listed the result of estimated determination of salicylamide in human serum and pills respectively.

Table 13: The statistical analysis summary of assess the method trueness with SCI-O - spectrometers device, , via, calculated T-test for Pharmaceutical preparations

Type of sample	Type of system	Mean	t_{Cal}
Pharmaceutical preparations	Claimed Label	M=152	1.999
Pharmaceutical preparations	FIA	X=153.46	
Pharmaceutical preparations	SCI-O	M=152	1.999

$t_{cal} = (X - M) * \frac{\sqrt{n}}{\delta}$; t_{tab}at 95%,n-1= 2.571

Table 14: Estimated determination of salicylamide in pharmaceutical preparations by SCI-O - spectrometers device

Human serum number test	Spiked, mg/mL	Found, mg/mL	Recovery,%	Relative error, %
1	6	6	100	0.00
2	12	12.01	100.08	-0.008
3	18	18.0	100	0.00
4	24	24	100	0.00
5	30	29.99	99.96	-0.04

As mentioned in the statistical section two types of statistical procedure was used include, t-test, and F-test in order to assess the method trueness, via the calculated of comparison form both system new flow injection with merging-zone technique and $SC\ddot{I}O$ - spectrometers device, as depicted in Table 15 for serum sample and Table 16 for pharmaceutical preparations. The results showed no significant differences between the developed and reference methods because the value calculation for T and F less that table vale.

Table 15: The statistical analysis summary of assess the method trueness with SCI-O - spectrometers device, , via, calculated T-test and F-test for Human serum

Type of sample	Type of system	Mean	t_{Cal}	F_{Cal}
Human serum	FIA	X=18.1	0.02716	1.1056
Human serum	SCI-O	M=18.0		

$t_{cal} = (X - M) * \frac{\sqrt{n}}{\delta}$; t_{tab}at 95%,n-1= 2.571

F_{tab}at 95%,n-1=5.05;$F_{cal} = \frac{\delta_1^2}{\delta_2^2}$;(n=6); $\delta_{1=}^2$89.91 ; $\delta_2^2 = 81.3249$

Table 16: The statistical analysis summary of assess the method trueness with SCI-O - spectrometers device, via, calculated T-test for Pharmaceutical preparations

Type of sample	Type of system	Mean	t_{Cal}
Pharmaceutical preparations	Claimed Label	M=152	1.999
Pharmaceutical preparations	FIA	X=153.46	
Pharmaceutical preparations	SCI-O	M=152	1.999

$t_{cal} = (X - M) * \frac{\sqrt{n}}{\delta}$; t_{tab}at 95%,n-1= 2.571

Conclusion

The incorporation of Salicylamide into emerging COVID-19 protocols presents a promising avenue for mitigating the substantial viral impact on human health. This paper delves into the exploration of operational parameters encompassing both physical and chemical variables, complemented by the application of statistical analysis. The present study entails the assessment of Salicylamide quantification using an innovative flow injection approach, integrating the merging-zone technique and the SCI˙O-spectrometry device. This investigation was conducted across two distinct sample types: pharmaceutical preparations and human serum. This presents opportunities to utilize the new design ensures the detection of salicylamide in different samples without separation in contrast to other methods as well as present new device SCI˙O. The new model of determination Salicylamide provides further evidence to support flow injection Merging-Zone Technique, therefore it will be the key support modern analytical chemistry.

References:

[1] K. H. Chowdhury, M. R. Chowdhury, and S. Mahmud, "Drug repurposing approach against novel coronavirus disease (COVID-19) through virtual screening targeting SARS-CoV-2 main protease", *Biology (Basel).*, vol. 10, no. 1, pp. 1–14, 2021.

[2] R. Kumar, N. Gupta, P. Kodan, A. Mittal, M. Soneja, and N. Wig, "Battling COVID-19: using old weapons for a new enemy", *Trop. Dis. Travel Med. Vaccines*, vol. 6, no. 1, pp. 1–10, 2020.

[3] E. A.-O. Diego José Zanini1, Matheus Henrique Silva1, "Spectrophotometric analysis of vitamin c in different matrices utilizing potassium permanganate", *Eur. Int. J. Sci. Technol.*, vol. 7, no. 2304–9693, pp. 70–84, 2018.

[4] N. W. A. E. Z. M. Abdelrahman, "Novel spectrophotometric methods for determination of Salicylamide and Ascorbic acid in their binary mixture", *J. Chem. Soc. Pakistan*, vol. 36, no. 6, pp. 988–995, 2014.

[5] C. Sullivan and J. Sherma, "Determination of salicylamide in pharmaceutical tablets by high-performance thin-layer chromatography with ultraviolet absorption densitometry", *Acta Chromatogr.*, no. 16, pp. 153–163, 2006.

[6] A. R. Zarei, A. Afkhami, and N. Sarlak, "Simultaneous spectrophotometric determination of paracetamol and salicylamide in human serum and pharmaceutical formulations by a differential kinetic method", *J. AOAC Int.*, vol. 88, no. 6, pp. 1695–1701, 2005.

[7] A. Ruiz Medina, M. L. Fernández De Córdova, and A. Molina Díaz, "A very simple resolution of the mixture paracetamol and salicylamide by flow injection-solid phase spectrophotometry", *Anal. Chim. Acta*, vol. 394, no. 2–3, pp. 149–158, 1999.

[8] K. W. Street and G. H. Schenk, "Spectrofluorometric determination of acetylsalicylic acid, salicylamide, and salicylic acid as an impurity in pharmaceutical preparations", *J. Pharm. Sci.*, vol. 70, no. 6, pp. 641–646, 1981.

[9] Y. Fuster Mestre, L. Lahuerta Zamora, and J. Martínez Calatayud, "Direct flow injection chemiluminescence determination of salicylamide", *Anal. Chim. Acta*, vol. 394, no. 2–3, pp. 159–163, 1999.

[10] M. S. El-din, M. Eid, and A. M. Zeid, "Chromatography Development and Validation of RP-HPLC Method for Simultaneous Determination of Ascorbic Acid and Salicylamide in their Binary Mixtures : Application to Combined Tablets", *Chromatogr. Sep. Tech.*, vol. 3, no. 5, pp. 1–7, 2012.

[11] M. Trojanowicz and M. Pyszynska, "Flow - Injection Methods in Water Analysis — Recent Developments", *Molecules*, vol. 27, no. 1410, pp. 1–31, 2022.

[12] S. B. Al-badri, "Newly Developed system for the Analysis of Micro Quantites of Molybdenum (VI) using Flow Injection Analysis", *Baghdad Sci. J.*, vol. 4, no. 2, pp. 180–190, 2007.

[13] S. B. Al-badri and Y. K. Al-bayati, "A new system of technology integration in the areas of analysis and continuous injection Jeriana use spectrometer plant locally to estimate the drug paracetamol", *Al-Nahrain J. Sci.*, vol. 1, no. 17, pp. 1–9, 2014.

[14] N. S. Turkey and J. N. Jeber, "Flow injection analysis with turbidity detection for the quantitative determination of mebeverine hydrochloride in pharmaceutical formulations",

Baghdad Sci. J., vol. 19, no. 1, pp. 141–154, 2022.

[15] V. N. Mehta, S. K. Kailasa, and H. F. Wu, "Sensitive and selective colorimetric sensing of Fe3+ ion by using p-amino salicylic acid dithiocarbamate functionalized gold nanoparticles", *New J. Chem.*, vol. 38, no. 4, pp. 1503–1511, 2014.

[16] I. M. A. Shakir and R. F. Hassan, "New Approach for Determination of Ciprofloxacin by Quenched Fluorescence of Analytically Interested Species Using Continuous Flow Injection Laser Diode Fluorimeter Analyser", *Iraqi J. Sci.*, vol. 57, no. 1, pp. 522–533, 2016.

[17] M. Q. Al-Abachi and Z. A. Al-Nedawi, "Batch and Flow Injection Spectrophotometric Determination of Doxycycline Hyclate in Pharmaceutical Preparations", *Iraqi J. Sci.* , vol. 56, no. 2A, pp. 909–920, 2015.

[18] I. M. Shakir and M. K. Hammood, "Novel turbid metric-continuous flow injection analysis method for the determination of tetracycline HCl in pharmaceutical formulation using homemade linear array ayah 5SX1-T-1D-CFI analyses", *Int. J. Res. Pharm. Chem.*, vol. 4, no. 4, pp. 946–957, 2014.

[19] P. Steliopoulos, "Extension of the standard addition method by blank addition", *MethodsX*, vol. 2, no. 2, pp. 353–359, 2015.

[20] M. M. W. Wani and D. G. K. Chandok, "Interaction of Salicylamide and Salicylic Acid with Iron and Cobalt and their Comparative Study by IR and NMR", *Int. J. Adv. Res. Sci. Commun. Technol.*, vol. 2, no. 2, pp. 53–62, 2021.

[21] S. Sud and A. Kamath, "Methods of Size Reduction and Factors Affecting Size Reduction in Pharmaceutics", *Int. Res. J. Pharm.*, vol. 4, no. 8, pp. 57–64, 2013.

[22] M. P. Bickler and L. J. Rhodes, "Accuracy of detection of carboxyhemoglobin and methemoglobin in human and bovine blood with an inexpensive, pocket-size infrared scanner", *PLoS One*, vol. 13, no. 3, pp. 1–12, 2018.

[23] C. McVey, U. Gordon, S. A. Haughey, and C. T. Elliott, "Assessment of the analytical performance of three near-infrared spectroscopy instruments (Benchtop, handheld and portable) through the investigation of coriander seed authenticity", *Foods*, vol. 10, no. 5, pp. 1–16, 2021.

[24] N. Cimetiere, I. Soutrel, M. Lemasle, A. Laplanche, and A. Crocq, "Standard addition method for the determination of pharmaceutical residues in drinking water by SPE-LC-MS/MS", *Environ. Technol. .UK*, vol. 34, no. 22, pp. 3031–3041, 2013.

[25] J. N. Miller and J. C. Miller, "*Statistics and Chemometrics for Analytical Chemistry Sixth*. "2010.

[26] A. Afkhami, N. Sarlak, and A. R. Zarei, "Spectrophotometric determination of salicylamide and paracetamol in biological samples and pharmaceutical formulations by a differential kinetic method", *Acta Chim. Slov.*, vol. 53, no. 3, pp. 357–362, 2006.

[27] M. A. R. D. Fauzi, E. Hendradi, P. Pudjiastuti, and R. T. Widodo, "Analysis of dissolution of salicylamide from carrageenan based hard-shell capsules: A study of the drug-matrix interaction", *Indones. J. Chem.*, vol. 21, no. 1, pp. 148–156, 2021.