Continuous Online Flow Analysis of the Chromium (III) Ion in Drugs using Five Snow White LEDs as Radiation Sources Arranged in a Single Row Matrix Cell Photometer

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Summary: A new spectrophotometric flow injection analysis method has been successfully developed to measure chromium (III) ion levels. This approach relies on the oxidation of the chromium (III) ion by hydrogen peroxide in a basic environment, leading to the formation of the chromate ion. This ion subsequently reacts with 1,5-diphenylcarbazide in an acidic solution, resulting in a striking blue-violet complex. Under optimal conditions, the method demonstrates a linear response within the range of 0.05 to 15 mmol.L-1, achieving a high correlation coefficient (r) of 0.9912. The detection limit is impressively low at 51.996ng/100µL, and the precision, quantified by relative standard deviation (RSD%) across six replicate measurements, remains below 1% for Cr(III).

This analytical technique has been effectively utilized to quantify chromium (III) ion content in three different pharmaceutical preparations: Vitaking kft-200µg from Hungary, GTF-200µg from the USA, and AdvaCare Pharma-200µg from the USA, all produced by different manufacturers using a homemade NAG-SSP analyzer. In comparing this novel method with a traditional spectrophotometric approach, statistical analysis revealed no significant differences between the two at a 95% confidence level, as determined by the paired t-test and one-way ANOVA. These results indicate that the new method can be confidently adopted as a reliable routine alternative for analyzing Cr(III) in various pharmaceutical products.

Keywords: Flow injection analysis, Chromium (III) ion, 1,5-diphenylcarbazide, Spectrophotometric method, Pharmaceutical analysis.

Introduction

Chromium is a transition element that displays a wide range of oxidation states, from −4 to +6, of which the +3 state is the most stable and the trivalent and hexavalent states being the most commonly observed in chromium species[1]. The roles of chromium and other elements in the human body are primarily seen in the metabolism of proteins, carbohydrates, lipids and, in particular, in cholesterol levels. Chromium's biological function is closely correlated with that of insulin, and indeed the majority of biological chromium reactions are also insulin-dependent. Appropriate chromium nutrition leads to a decreased requirement for insulin and, also, an enhanced blood lipid profile. Good sources of dietary chromium include fresh and minimally processed foods[1,2]. Chromium species in the +3 oxidation state are typically found in enzymes, which accelerate blood coagulation, and increase the activity of B-glucuronidase and ribonucleic acids. The properties of chromium play key roles in the synthesis of certain vitamins and hormones[3]. Due to its biological importance, chromium content is estimated via various analytical methods, such as spectrophotometry[4-6], atomic absorption spectrometry[7,8], and voltametry[9]. Continuous flow injection analysis (CFIA) techniques are considered the most significant of the analytical methods used, however, and are coupled with other techniques, in particular UV-VIS spectrometry, and with the extensive development in reactions that form colored solutions[7-9]. In the latter instance, the analysis is based on injection of a sample through a continuous movable stream of a solution with high reproducibility, which is then mixed with continuous flow solutions of chemical reagents in the measuring cell; this occurs without any loss of signal because the mixing and reaction processes occur in front of a detector that is fixed in front of the cell[10]. The importance of CFA is that it is a fast, sensitive and inexpensive method[10,11]. FIA, coupled with other techniques[12-14], in particular UV-Vis spectrophotometry, has led to one of the major problems with colorimetric analysis using traditional

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instruments being solved, namely the speed at which one can conduct analysis, which avoids issues due to the instability of the colored complex to be measured, in addition to the ability to obtain results with high accuracy, precision and sensitivity with low detection limits, and across a wide range of concentrations as compared to traditional spectroscopic methods[6,15-18]. In the present work, a flow injection spectrophotometric method was developed for the determination of chromium (III) ion concentrations using a patented homemade NAG-SSP analyzer[19], as based on the formation of the blue-violet-colored chromium complex necessary for spectrophotometric measurements.

Reagents

- Chromium chloride hexahydrate solution, 0.05 $molL^{-1}$ (266.48 $g.mol^{-1}$, BDH). Dissolve 3.331 g/250 mL distilled water
- 1,5-diphenylcarbazide solution, 0.05 mol. L^{-1} (242.28 g/mol, Fluka). Dissolve 3.0285 g $C_{13}H_{14}N_4O$ in 5 mL glacial acetic acid and make up the solution to 250 mL with 245 mL distilled water.
- H_2O_2 solution, 100 mmol. L^{-1} (BDH). Dilute 19.44 mL of 35% H_2O_2 in 2 L distilled water (standardized with standard $KMnO_4$ solution).
- CH_3COOH, 1 mol. L^{-1} (BDH- Sigma-Aldrich). Dilute 28.7 mL of 99.5% CH_3COOH (specific gravity 1.05) with distilled water in a 500 mL flask. Standardized with NaOH solution.
- Sulfuric acid solution, 1 mol. L^{-1} (BDH- Sigma-Aldrich). Dilute 27.8 mL of 96% H_2SO_4 (specific gravity 1.84) in distilled water in a 500 mL flask. Standardized with Na_2CO_3 solution.
- NaOH solution, 0.05 mol. L^{-1} (40 g/mol, Fluke). Dissolve 0.5 g/250 mL distilled water. Standardized with HCl solution.
- KOH solution, 0.05 mol. L^{-1} (56.106 g/mol). Dissolve 0.7013 g/250 mL distilled water.
- Sodium carbonate solution, 0.05 $mol.L^{-1}$ (105.99 g/mol, BDH). Dissolve 1.3249 g/250 ml. Dry overnight in an oven before weighing.

Sample preparation

A batch of 20 tablets from Vitaking kft (VITAKING -Hungary), now-GTF (NOW Foods), and Adva Care Pharma (Adva Care Pharma-200 USA, LLC), containing 200 µg of Cr (III) were weighed, i.e., 0.6453, 0.3159, and 0.3336 g, respectively, equivalent to 0.51996 mg active ingredient (0.1 mmol. L^{-1}). The tablets of drug were then ground, followed by sieving through a standard 200 mesh and dissolved in distilled water[20]. The solution was then filtered to remove any undissolved residue. The volume was made up to 100 mL with the same solvent.

Apparatus

The flow system used for the determination of Cr (III) is shown in Fig. 2, which consists of a three-channel manifold. A peristaltic pump (Ismatec, Switzerland) was used to transport the carrier solutions. For the provision of adjustable sample volumes, a rotary six-port medium-pressure injection valve made of Teflon (1 mm inner diameter), INDEX Corporation-USA, was utilized. Various lengths of Teflon tubes with an inner diameter of 1 mm were employed to serve as an oxidation coil delay, to facilitate the completion of reactions. Absorbance measurements were carried out on a NAG-SSP analyzer[19] which is composed of four parts: the metal incubator, flow tube, five WLED sources, and single solar cell as a detector (Fig. 1). A flow cell's full length is 105 mm and is composed of two parts that form a single piece, each of which is 55 mm in length and of which the middle region it exposed to irradiation and detection (fig.1.A).

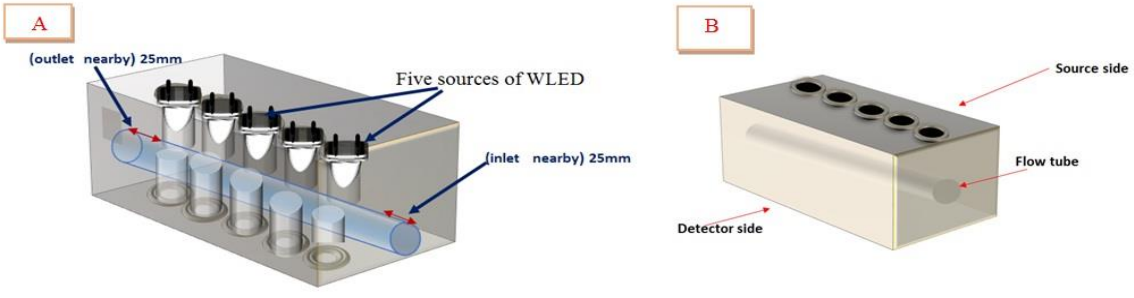


Fig. 1: A- Complete alignment of five radiation sources in front of the solar cell at 0–180° and B-The location of the solar cell, flow cell, and flow tube in the incubator

Experimental

The NAG-SSP instrument is a multipurpose photometric device that allows for the possibility of multiple measurements[21] at 0-180°. In this research, the response was recorded to measure the absorbance of a blue-violet colored chromium species in order to determine the amount of chromium (III) ions, as shown in the chemical equations below[22] (scheme no.1). The colored complex formed due to the oxidation of an injected sample of Cr (III) (8 mmol. L^{-1}, 100 µL) via hydrogen peroxide solution (line no.1) in a basic medium (NaOH, 0.7 mmol. L^{-1} line no. 2), which were used in oxidation coil no. 1 (125 µL volume) to complete the oxidation process, which leads to the formation of the chromate (VI) ion, CrO_4^{2-}. This, in turn, reacts with the highly sensitive chemical reagent 1,5-diphenylcarbazide (DPC) in acidic media (1 mmol. L^{-1} in 0.8 mmol. L^{-1} H_2SO_4, line no. 3) to form the blue-violet complex (Cr (III)-diphenyl carbazone). Reaction coil no. 2 (50 µL volume) was used to take the reaction between chromate (VI) ion and DPC to completion and lead to the formation of the colored complex. The incident light is passed through a flow cell with an absorption path of 5 mm over a distance of 105 mm, and the transmitted light is received by a single solar cell that acts as a detector[19]. The flow feeds consist of three lines that allow for a flow rate of 1.5 mL.min^{-1}, as shown in Fig. 2.

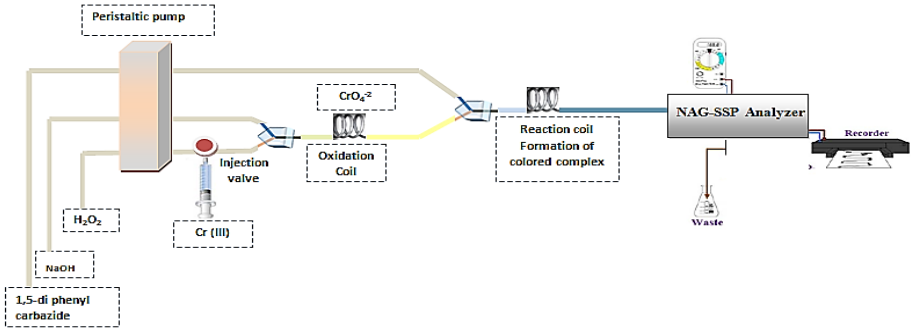


Fig. 2: Three-line manifold system design for the determination of the chromium (III) ion.

$Cr^{3+}+OH^- \longrightarrow Cr(OH)_3$

$Cr(OH)_3+OH^- \rightleftharpoons Cr(OH)_4^-$

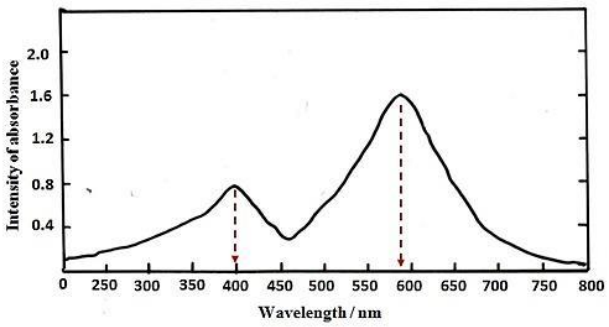
Yellow solution is obtained, owing to the oxidation of Cr(III) to chromate

$2Cr(OH)_4^-+3H_2O_2+2OH^- \longrightarrow 2CrO_4^{2-}+ 8H_2O$

1,5-diphenyl carbazide

diphenyl carbazone

Blue-violet colour



Scheme-1: Proposed mechanism for the formation of the colored complex and the absorbance spectrum for the determination of Cr(III).

- The first absorption peak: appears at a wavelength of about 400 nm, where the absorption intensity is about 0.8.
- The second absorption peak: It appears at a wavelength of about 600 nm, where the absorption intensity is about 1.6, which is higher than the first peak.

Chemical Variables

Effect of H_2O_2 concentration on the formation of colored complex in three different basic mediums

Various concentrations of hydrogen peroxide, ranging from 0.1-4.0 mmol. L^{-1}, were utilized as a carrier stream and as a strong oxidizing agent in the oxidation of Cr (III) (8 mmol. L^{-1},75 µL) to chromate species using three different basic mediums namely NaOH, KOH, and Na_2CO_3 (0.5 mmol. L^{-1}, 1.2 mL.min^{-1}) respectively. It appears that the highest peak response of blue-violet species increases with increasing H_2O_2 concentration up to 0.9 mmol. L^{-1}, while for concentrations > 0.9 mmol. L^{-1} a decrease in response height was observed (Table 1). This decrease can be attributed to the dissociation of some of the colored complex. The results indicate a significant decrease in absorbance when using Na_2CO_3 and KOH solutions as the basic mediums compared to NaOH solution because sodium hydroxide provides an optimally basic medium that facilitates the oxidation process (Fig. 3).

Effect of NaOH concentration

Eight solutions of sodium hydroxide were prepared, ranging in concentration from 0.1 to 1.0 mmol·L^{-1}, and were used as a basic medium to study the effects of increasing NaOH concentration on the Cr (III) 8 mmol·L^{-1}–H_2O_2 0.9 mmol·L^{-1}/OH^-–DPC 0.6 mmol·L^{-1}/1 mmol CH_3COOH system. From the results obtained (Fig. 4), it can be seen that increasing NaOH concentration leads to an increase in the NAG-SSP analyzer response up to a concentration of 0.7 mmol. L^{-1}. Higher concentrations (> 0.7 mmol.L^{-1}) lead to a decrease in intensity of the recorded peaks due to the increasing NaOH concentration precipitating Cr(III) as chromium hydroxide, $Cr(OH)_3$, which in turn reacts with DPC to yield another complex (a red-colored species), in the process consuming some of the reagent (reducing the amount of DPC) that reacts with the chromate ion to form the blue-violet-colored complex.

In acidic media (pH < 4), the reaction between Cr (III) and H_2O_2 shows minimal or no formation of hydroxyl radicals (. OH), making the system largely inactive other oxidation processes. The Cr (III)/H_2O_2 system becomes more reactive and efficient at neutral to alkaline pH levels, where a redox cycle between Cr (III) and Cr (VI) can be established to generate reactive oxygen species like hydroxyl radicals. The DPC (1,5-Diphenylcarbazide) spectrophotometric method can be used to measure H_2O_2 in this system in an acidic medium by monitoring the oxidation of Cr (III) to Cr (VI)[23].

Table-1: Effect of variation in H_2O_2 concentration on response using three different basic mediums.

$[H_2O_2]$ mmol. L^{-1}	[Base] = 0. 5 mmol. L^{-1}		
	Type of basal medium		
	NaOH	KOH	Na_2CO_3
	$\bar{y}i$ (n = 3) (mV) ± C.I*		
0.1	200 ± 2.83	185 ± 1.98	143 ± 1.68
0.3	368 ± 2.65	288 ± 2.23	195 ± 1.32
0.5	487 ± 1.98	372 ± 2.46	255 ± 1.76
0.7	510 ± 2.03	398 ± 1.97	290 ± 2.24
0.9	575 ± 1.90	467 ± 2.08	298 ± 2.67
1.0	568 ± 1.97	475 ± 2.97	310 ± 2.91
3.0	560 ± 2.33	490 ± 2.90	378 ± 1.92
4.0	525 ± 2.49	510 ± 1.88	394 ± 2.80

$$C.I^* = t_{0.05/2,n-1,SD/\sqrt{n}}$$

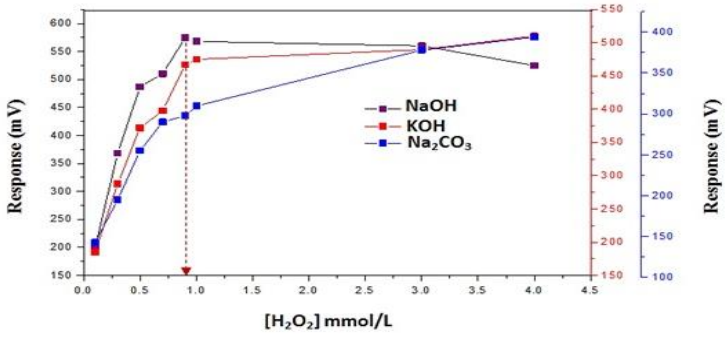


Fig. 3: Effect of hydrogen peroxide concentration on the output response of the NAG-SSP analyzer.

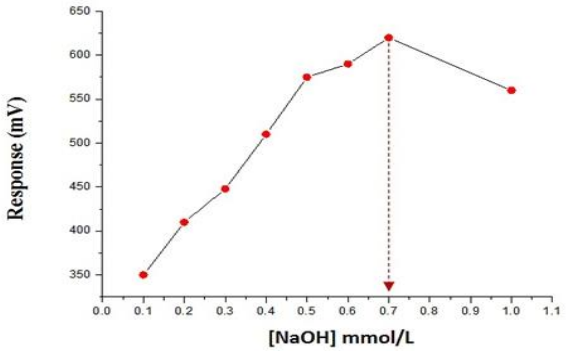


Fig. 4: Variation of the effect of sodium hydroxide concentration on recorded analyzer response.

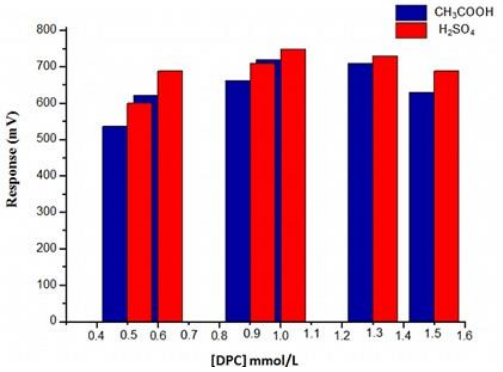


Fig. 5: Effect of two different acids with variation in DPC concentration on output.

Effect of DPC concentration in different acidic mediums

The reaction between Cr (III)-H_2O_2/OH^- with the DPC regent to form the blue-violet-colored complex was studied in two different acidic media, H_2SO_4 and CH_3COOH; the use of HCl and HNO_3 was avoided since the chloride ion is a reducing agent and the nitrate ion is an oxidizing agent, both of which would themselves affect the formation of the colored complex. The stock solution of the reagent was diluted by adding glacial acetic acid to a portion of a concentrated stock, and the volume was then brought to the mark with distilled water. A set of solutions of DPC ranging from 0.5-1.5 mmol. L^{-1} in concentration were prepared by transferring different volumes from the stock solution and making up the volume using 1 mmol. L^{-1} H_2SO_4. The same dilution process was repeated using 1 mmol. L^{-1} acetic acid. The results tabulated in Table 2 indicate a significant increase in peak height with increasing DPC concentration, up to 1.0 mmol. L^{-1}; thereafter, the decrease in the responses obtained can be attributed to an increase in the density of the colored segment, which acts as an internal filter. It was noticed that H_2SO_4 was the most favorable acidic medium used (Fig. 5).

Table-2: Effects of DPC concentration with two different acids on the formation of the colored complex.

[DPC] mmol. L^{-1}	$[H_3O^+]$ = 1 mmol. L^{-1}	
	CH_3COOH	**H_2SO_4**
	$\bar{y}i$ (n = 3) (mV) ± C.I*	
0.5	**536 ± 2.00**	**600 ± 1.83**
0.6	**620 ± 2.12**	**688 ± 1.68**
0.9	**662 ± 1.94**	**710 ± 1.95**
1.0	**720 ± 2.14**	**748 ± 1.78**
1.3	**710 ± 2.17**	**730 ± 1.83**
1.5	**629 ± 1.99**	**688 ± 1.48**

$$C.I^{*}=t_{0.05/2,n-1,SD/\sqrt{n}}$$

Response

Under the same experimental conditions, a range of concentrations from 0.2-1.5 mmol. L^{-1} of H_2SO_4 were prepared in order to determine the most favorable concentration of acid for the formation of the colored complex. It is clearly shown in Fig 6-A, B that a concentration of 0.8 mmol. L^{-1} is the optimal concentration, as an excess of sulfuric acid would lead to a weakening in the recorded peaks., which can be attributed to the dissociation of the complex formed.

Effect of flow rate

The optimum chemical parameters determined above were used to study the effect of flow rate on the response intensity. Table 3 reports the variation in the average of three successive measurements with flow rate (mL.min^{-1}) and the time required for a single measurement (time required for the Cr (III) segment (75 µL) to run from the injection valve to the measuring cell and subsequently leave the NAG-SSP instrument). As a comparison between the clarity of response, dispersion effect, sensitivity, and the time required for the analysis, 1.5 mL.min^{-1} was selected as the optimal flow rate for the three lines.

Effect of sample segment

The injected sample volume was varied between 25 and 150 µL by changing the length of the injection valve (sample loop), while fixing all other physical and chemical parameters. Fig. 7-A shows the increase in the response of the NAG-SSP analyzer with increasing injection volume. In samples with large amounts of Cr (III), a distortion of the peak and irregular response profile was noticed (Fig. 7-B). This may be due to an increased amount of the blue-violet colored species acting as an internal filter, preventing the remaining light from reaching the solar cell detector post-absorption, and elongation of the colored segment in front of the detector point.

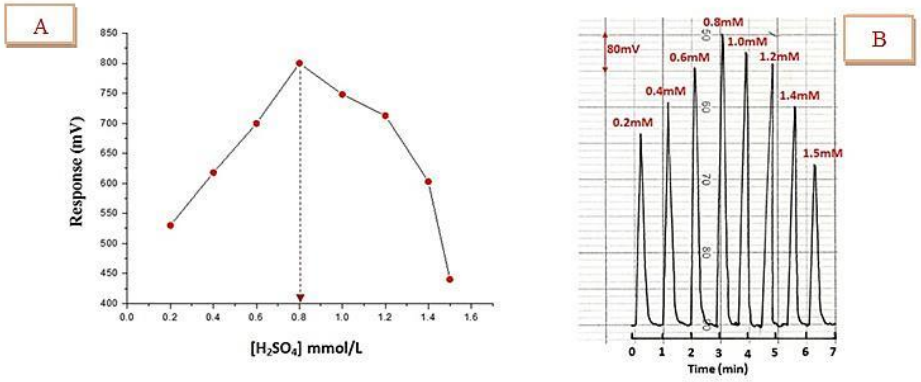


Fig. 6: Effect of sulfuric acid concentration on: A-Output response of NAG-SSP Analyzer; B-Response profile obtained by the NAG-SSP instrument.

Table-3: Effect of flow rate on the three average output responses and time of analysis used for the determination of Cr (III).

	Output of response ȳi (n=3) (mV) ± C.I*	**Analysis time in NAG-SSP instrument (sec)**
0.5	**700 ± 2.32**	**60**
0.9	**775 ± 1.79**	**52**
1.2	**800 ± 1.69**	**45**
1.4	**850 ± 1.90**	**40**
1.5	**888 ± 1.58**	**32**
1.6	**789 ± 1.77**	**28**
1.7	**710 ± 1.93**	**25**
1.8	**680 ± 1.85**	**21**
2.0	**530 ± 1.82**	**20**

$$C.I^{*}=t_{0.05/2,\ n-1,\ SD/\sqrt{n}}$$

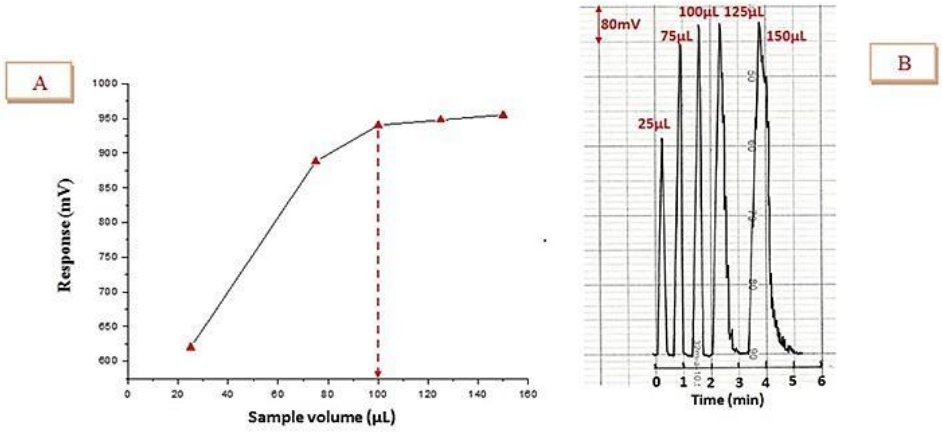


Fig 7: Effect of volume of injected sample on: A-Output response of NAG-SSP Analyzer.

B-Response profile recorded

Effect of reaction coil volume on the completion of the oxidation reaction and formation of the colored complex

A range of volumes between 0-200 µL for the oxidation coil (coil no.1) and between 0-100 µL for the reaction coil (coil no. 2) were considered. All the results obtained are tabulated in Table 4, whilst Fig. 8 shows the effects of reaction coil on the completion of the reaction, i.e., conversion of 8 mmol.L^{-1} Cr(III) to Cr(VI) as the chromate ion, CrO_4^{2-}, in oxidation coil no. 1, which in turn mixes with DPC at the second junction point, and then on to reaction coil no. 2 to form the blue-violet-colored complex and complete the reaction. An oxidation coil volume of 125 µL and a reaction coil volume of 50 µL were much more expressive in terms of peak profile, height, and clarity, and were thus considered the optimal choices. Increasing the coil length results in an increase in dilution and dispersion of the colored species, which in turn leads to the attenuation of the incident light.

Table 4: Effect of reaction coil volume and oxidation coil volume on output response

Type of coil					
Volume of coil (µL)					
To complete oxidation	To complete the formation of the coloured complex				
	Without coil	50	70	90	100
	Output of response ȳi (n=3)(mV)±C.I				
Without coil	940±1.92	980±2.33	889±2.32	733±2.52	700±2.24
75	968±1.93	990±2.00	895±2.23	800±2.05	778±1.95
100	995±1.53	1000±1.59	980±1.97	878±1.94	805±1.94
125	1050±1.72	1150±1.92	1020±1.82	998±2.07	830±2.32
150	1000±2.30	1080±2.22	998±2.33	900±1.93	810±2.02
175	886±2.52	920±2.48	880±2.48	798±1.97	688±2.32
200	730±2.25	888±2.34	730±1.97	678±2.34	598±2.34

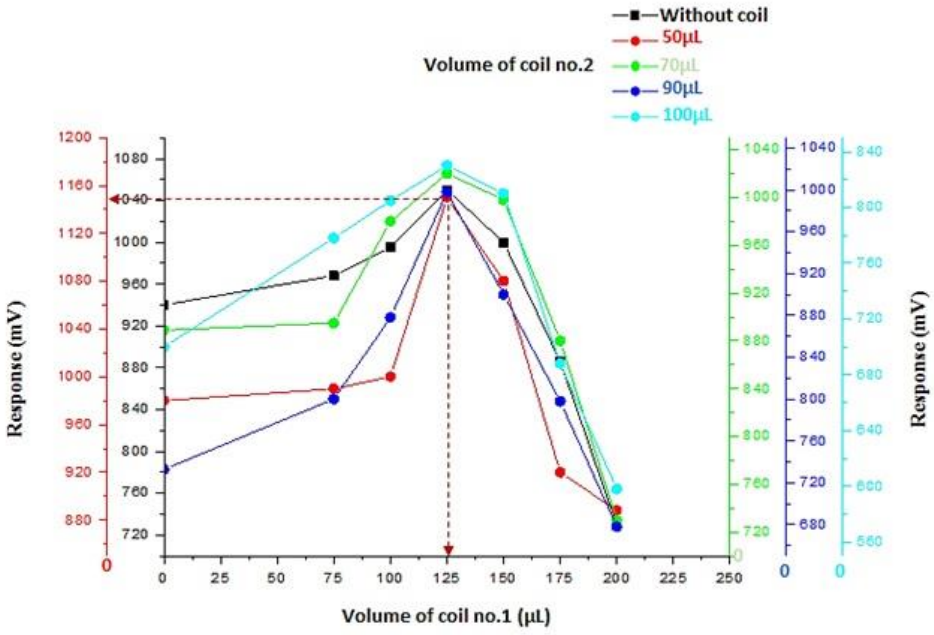


Fig. 8: Effect of the variation of reaction coil volume and oxidation coil volume on the response of the NAG-SSP analyzer.

Study of calibration curve, detection limit, and repeatability

A series of concentrations (ranging from 0.05-15 mmol. L^{-1} in concentration) of chromium (III) ion solutions were prepared under the established optimum conditions. Each response was obtained three times. All prepared concentrations were injected and measured using the NAG-SSP instrument (Fig. 9-A). It was noticed that at greater than 15 mmol. L^{-1}, a broad distortion in the peak maxima could be seen, as well as an increase in the base width of the response, resulting in deviation from linearity. This was most probably due to the increasing intensity of the colored segment in front of single solar cell and the internal filtering effect of the blue-violet species present in the solution as a result of the Cr (III)-H_2O_2/OH^--DPC/H_2SO_4 reaction system, decreasing the light transmitted to the detector. Plotting a linear plot diagram for the chromium (III) ion concentration from 0.05-15 mmol. L^{-1} gave a correlation coefficient of r = 0.9912, which correlates to the response (dependent variable) expressed in mV versus concentration (independent variable). The linear plot equation explains 98.25% of the relation as the value of R^2 (coefficient of determination) was greater than 90%. It can be clearly seen that the new method satisfies both the use of low as well as high concentrations (Fig. 9-B). This indicates that the chosen concentration range with the linear equation used can be considered the optimum range for the application of this newly developed method. The assessment of the newly developed instrument for the determination of Cr (III) was compared with a classical instrument (UV-Vis spectrophotometer)[7, 9]. Spectrophotometric measurement is based on the same Cr (III)-H_2O_2/OH^--DPC/H_2SO_4 chemical reaction under optimal conditions for the classical instrument (absorbance measurement at 580 nm). Fig. 9-C shows a calibration graph with an R^2 of 99.85% and with an r of 0.9993 for a linear regression equation of the form of Response (Absorbance) = a + b (Cr (III) mmol. L^{-1}) over a concentration range of 0.02-2 mmol. L^{-1}. The study was conducted to determine the extent to which the results can be trusted and are repeatable for the developed (Fig. 10) and classical devices through six successive measurements for two particular analyte concentrations (7 and 10 mmol. L^{-1}). This gave a variation in RSD% of less than 0.5%, indicating the two methods are both characterized by high precision with good repeatability, in addition to allowing a study of the detection limits using each. It was noted that the NAG-SSP analyzer allowed for a sensitivity towards low concentration of 84%, meaning that the detection limits were improved compared to the traditional instrument, with a gain equivalent to 52 times that of the UV-Vis spectrophotometer. All the results obtained are tabulated in Table 5.

% = 51.996 -327.575 /327.575 x 100 = 84.127%
84.127/100 x 327.575 = 275.579
327.575-275.579 = 51.99 ~ 52

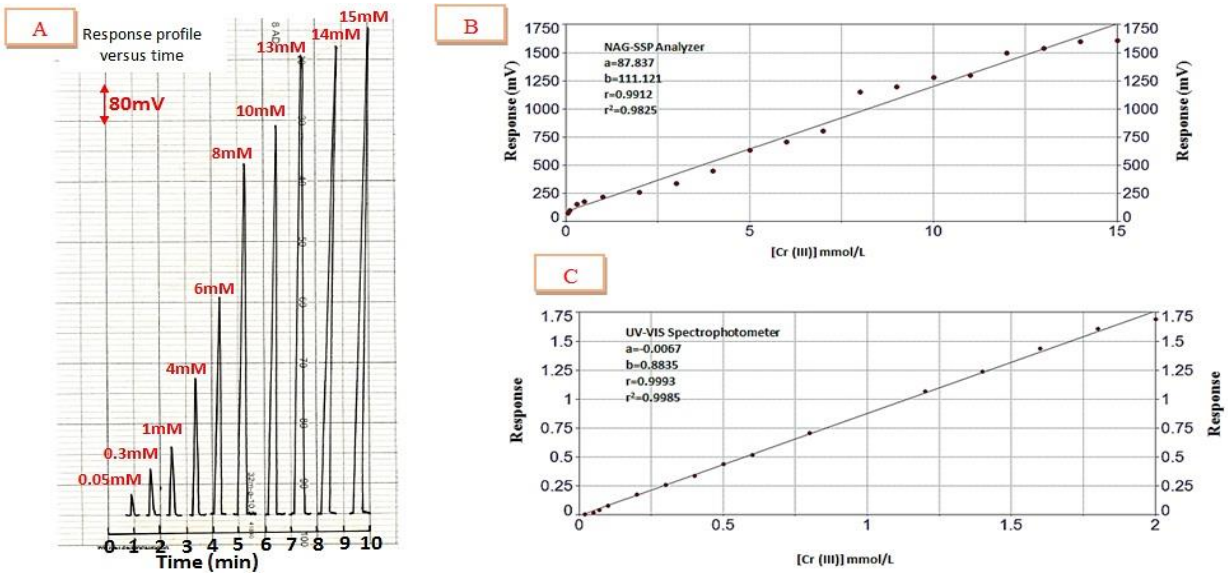


Fig. 9: A-Response profile of the calibration curve recorded by the NAG-SSP analyzer.
B-Linear dynamic range of the calibration curve obtained from the NAG-SSP analyzer.
C-Linear dynamic range of the calibration curve obtained from the UV-Vis spectrophotometer

.Table-5: Comparison between the NAG-SSP and UV-VIS spectrophotometer methods.

Result parameter	NAG-SSP Analyzer	UV-VIS spectrophotometer	Repeatability of six successive injected sample measurement (n = 6) RSD%	
Linear range	0.05-15 mmol. L^{-1}	0.02-2.0 mmol. L^{-1}		
			NAG-SSP Analyzer	UV-VIS spectrophotometer
Slope	111.121	0.8835	7 mmol. L^{-1}	
Intercept	87.837	-0.0067	0.24%	0.13%
Correlation coefficient (r)	0.9912	0.9993		
			10 mmol. L^{-1}	
coefficient of determination (R^2)	0.9825	0.9985	0.48%	0.24%
percentage linearity (R^2%)	98.25%	99.85		
Limit of detection	0.01 mmol. L^{-1} (51.996 ng/100 µL)	0.018 mmol. L^{-1} (327.575 ng/350 µL)		

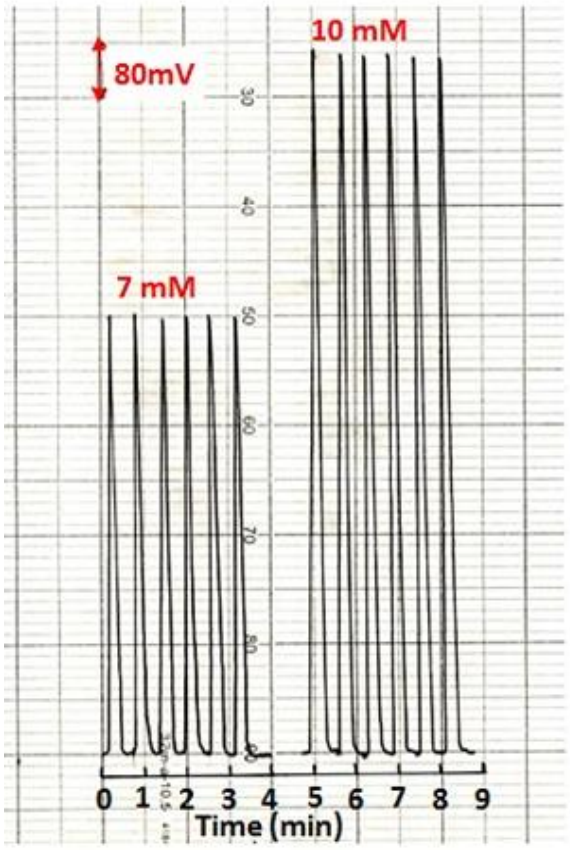


Fig 10: NAG-SSP response to repeatability test for Cr (III) (7 and 10 mmol. L^{-1})

Application of the adopted methodology for the assay of the chromium (III) ion and statistical data treatment

The two methods (developed and classical) were applied for the determination of certain drugs on the basis of the formation of the blue-violet-colored complex. The colored solution formed represents the way in which the three drugs (Vitaking kft-200µg-Hungary, now GTF-200µg-USA, and AdvaCare Pharma-200µg-USA**)** will be assayed in various formulations. A standard addition procedure was adopted by preparing a series of solutions from drug samples and measuring them via both the developed and classical instruments. Table 6 reports the Cr(III) content of each drug sample using the two different methods and the recovery (efficiency of determination). The analysis was conducted based on the extent to which the methods are correlated and whether there are any significant differences that detract from the developed method. On this basis, two statistical tests were made: the paired t-test at $\alpha = 0.05$ (two-tailed) for the comparison of the NAG-SSP analyzer and UV-VIS spectrophotometer, as shown in Table 6 (column 7). Null hypothesis:

Ho = $\mu_{\text{NAG-SSP}} = \mu_{\text{UV-VIS Spectrophotometer}}$

Alternative hypothesis

H1 = $\mu_{\text{NAG-SSP}} \neq \mu_{\text{UV-VIS Spectrophotometer}}$

The results obtained clearly indicated that there were no significant differences between the newly developed and the UV-VIS spectrophotometer (classical) methods at the 95% confidence level, therefore the null hypothesis was accepted and the alternative rejected.

Table 6: Practical content, standard addition results, recovery, and paired t-test summary for the two different methods of sample analysis for Cr(III) in drugs

Sample No.	Commercial name content and company country	Chromium (III) ion sample weight equivalent to 0.51996 mg (1 mmol. L^{-1}) of the active ingredient (g)	Standard addition equation at 95% for n-2 $\hat{Y}_{(mV)}=a \pm s_a t+b \pm s_b t$[Cr (III)] mmol. L^{-1} r,r^2,R^2%	Practical content $Wi \pm 4.303\sigma_{n-1}/\sqrt{n}$ (µg) for (n=3) ,at 95%	Recovery, %	Paired t-test comparison between two methods Sig. 2-tailed
		NAG-SSP Instrument				
		UV-VIS spectrophotometer				0.176>0.05 No sig.
1	Vitaking kft 200 µg Hungary	0.6453	22.8 ± 0.282 + 444 ± 3.283 0.9975, 0.9951, 99.51%	205.319 ± 3.528	102.69	
			0.0544 ± 0.0023 + 1.064 ± 0.0213 0.9947, 0.9894, 98.94%	204.502 ± 3.251	102.25	
2	now GTF 200 µg USA	0.3159	27.820 ± 1.224 + 564.300 ± 3.458 0.9989, 0.9978, 99.78%	197.185 ± 2.583	98.59	
			0.0492 ± 0.0023 + 1.0061 ± 0.018 0.9928, 0.9857, 98.57%	195.592 ± 4.219	97.79	
3	AdvaCare Pharma 200 µg USA	0.3336	25.382 ± 1.583 + 515.894 ± 8.232 0.9979, 0.9958, 99.58%	196.773 ± 3.162	98.39	
			0.0492 ± 0.0021 + 0.9959 ± 0.0232 0.9909, 0.9819, 98.19%	197.583 ± 3.982	98.79	

Secondly the analysis of variance test (ANOVA test) was carried out at the 95% confidence level ($\alpha = 0.05$). The results of testing the hypothesis, i.e., ANOVA results, are given in Table 7.

Table-7: Results of ANOVA test for comparison between different methods of determination of the chromium (III) ion

Source	Sum of Squares	df	Mean Square	F_{cal}	F_{tab}	Sig.
Between group	0.942	2	0.471	0.031 < 5.14		0.969
Within groups	90.197	6	15.033			
Total	91.139	8	-			

Since all values obtained for F_{calc} (0.031) < F_{tab} (5.14) at the 95% confidence level and the degree of freedom is n-1, the alternative hypothesis was rejected and the null accepted. Thus, there is a no significant difference between the NAG-SSP and UV-Vis spectrophotometer methods.

Table-8 shows another ANOVA test for comparison between the differences of the means of the samples (i.e., Vitaking kft-200µg-Hungary, now GTF-200µg-USA, and AdvaCare Pharm-200µg-USA**)**.

Table-8: ANOVA test for the comparison between three different samples for the analysis of Cr(III) n drugs

Source	Sum of Squares	df	Mean Square	F_{cal}	F_{tab}	Sig.
Between group	59.129	2	29.564	5.542 > 5.14		0.043
Within groups	32.010	6	5.335			
Total	91.139	8	-			

Since the values of sig.(0.043) < 0.05 and F_{cal} (5.542) > F_{tab} (5.14), there is a significant difference between the three different samples.

Table-9: Tukey and Scheffe test results for the three different Cr(III) samples.

Type of test	Type of comparison		Std. Error	Sig.
	(I) measurements	(J) measurements		
Tukey HSD	1 Vitaking kft	2	1.885924	0.054
		3	1.885924	0.076
	2 now GTF	1	1.885924	0.054
		3	1.885924	0.958
	3 AdvaCare Pharma	1	1.885924	0.076
		2	1.885924	0.985
Scheffe	1 Vitaking kft	2	1.885924	0.063
		3	1.885924	0.088
	2 now GTF	1	1.885924	0.063
		3	1.885924	0.962
	3 AdvaCare Pharma	1	1.885924	0.088
		2	1.885924	0.962

For the three drugs, the F-test can only indicate whether a difference exists among the three samples; it cannot, however, reveal where this difference actually lies. So, if the F-test indicates that there is a difference between the samples, other tests must be used to determine where this actually is. The most commonly used tests, to this end, are the Scheffe and the Tukey tests (Table 9).

The Scheffe and Tukey tests show that there is a very slight but significant difference between sample no.1 and the other samples.

Conclusion

The method described in this research is based on two reactions. The first involves the generation of chromate ions from Cr(III)-H_2O_2-NaOH, in which the reaction is completed in the oxidation coil. The second involves the reaction between chromate ions with 1,5-diphenylcarbazide in an acidic medium to form a blue-violet-colored complex, with the reaction occurring prior to the solution reaching the detector.

Three drugs of particular importance were analyzed using two different instruments, one of them characterized by high technology (NAG-SSP analyzer) while the second uses the same chemical reaction using classical instrumentation. The drugs were analyzed and compared in detail using a simple homemade instrument that is capable of producing results similar to the high-quality instrumentation manufactured by a well-known manufacturer. Statistical analysis confirmed that there was no significant difference between the traditional UV-Vis spectrophotometer and the NAG-SSP instrument. The simplicity of the latter, however, allows it to be used as a successful, reliable alternative to traditional instrumentation, that itself allows for the rapid determination of chromium (III) in pharmaceutical samples.

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