Article

A Rapid Method for Measurement of Nickel and Chromium at Trace Level in Aqueous Samples

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Abstract. A rapid, simple and portable technique to measure Ni(II) and Cr(VI) at trace level in aqueous samples has been proposed. Colored spots were developed by interacting micro-liter drops of metal solutions with suitable reagents on TLC plates. Soon after, TLC having spots was scanned to import the image into the computer. Color densities of spots were calculated with the help of homemade software. Simple regression was practiced on calibration between standards' concentrations and their color densities. For both metals, correlation coefficients were approaching to one, which pointed out a good correlation between color densities and corresponding analyte concentrations. Real samples from electroplating unit were analyzed with the present and conventional spectrophotometric method (employed as reference), and the results were compared to evaluate the pertinence of the proposed technique in the real analysis.
Key words: Chromium, densitometry, image scanning, nickel, spot test quantification.

Resumen. Se ha propuesto una técnica rápida, sencilla y portátil para medir Ni(II) y Cr(VI) a nivel de trazas en muestras acuosas. Puntos de color de los metales se han desarrollado mediante la interacción de microlitros con reactivos adecuados en las placas de cromatografía de capa fina, TLC. Posteriormente se escanea el cromatograma para importar la imagen al ordenador. Se calcularon densidades de color de los puntos con la ayuda de un software realizado en nuestro laboratorio. Una sencilla regresión se utilizó para correlacionar las concentraciones de los estándares y las densidades de color medidas. Para ambos metales, los coeficientes de correlación se acercaban a uno, lo que indicaba una buena correlación entre la densidad del color y las correspondientes concentraciones de analito. Muestras reales provenientes de procesos electroquímicos se analizaron con este método y con el método espectrofotométrico convencional, y se compararon los resultados para evaluar su aplicación en el análisis de muestras reales.
Palabras clave: Cromo, densitometría, escaner, níquel; prueba de cuantificación de puntos.

Introduction

Techniques based on analysis of colored spots produced by reacting suitable reagents with analyte, were developed mostly up to 1960s but essentially from a qualitative point of view [1]. Quantification of spot-tests has never been a common practice because of difficulty involved in handling minuscule samples accurately and precisely. One more factor that discouraged spot-test analysis for quantification purposes was the existence of several instrumental techniques, such as all modern forms of chromatography that provided good qualitative and quantitative results. However, when simplicity of technique, portability, and low cost are necessary conditions, techniques like the present one become a preferred choice.

For micro-determinations of metals, spectrophotometry is preferred since it is simple, sensitive and relatively less sophisticated in comparison to atomic absorption spectroscopy and inductively coupled plasma based optical emission spectroscopy. However, spectrophotometry has its own limitations [2]. For instance, precise and accurate results cannot be achieved in the case of micro-samples. In addition, when precipitates are produced, the technique cannot be directly employed. Efforts have been made all over the world to develop new methods to overcome the limitations of spectrophotometry [3-5]. A reasonable alternative to negative aspects of spectrophotometry is to use quantitative spot test technique. For that reason, it could

be interesting to develop new quantifiable spot-test procedures that could be applied in the areas of clinical, geological, and environmental analysis.

Abraham developed a method for the micro-determination of allantoin in pathological samples of serum and urine by producing yellow spots with dimethylaminobenzaldehyde on TLC [6]. Ueno employed scanning densitometry for the estimation of dyes in foods and coal tar [7]. Teasdale used conventional flatbed scanner and image software for micro-determination of sulfides [8]. Kompany-Zareh developed a simple method for determination of iron by producing colored spots with NaSCN [9]. Mohammad and Zehra developed a new TLC system for the separation of two amino acids, i.e. *l*-histidine and *dl*-tryptophan. The system comprises an anionic surfactant supported on silica layer as a stationary phase and borate buffer (pH 2.3) as mobile phase [10]. Paramasivam analyzed pharmacologically active curcuminoids by densitometry [11].

Recently, our research group working at University of the Punjab, Pakistan developed a simple and sensitive technique to determine arsenic, lead, and mercury in micro-samples in the concentration range 1-5 µg L^{-1} [12]. Only 1 µL sample was used that was applied on TLC plate with micro-pipette, subsequently coated with suitable reagent(s) to produce colored spots. Afterwards, the density of spot was measured using a flatbed colored scanner connected to a computer having specially developed software. Working on the same principle, a

method was developed to measure Ni(II) and Cr(VI) at trace level. The selectivity of the method depends on the reagents employed. Therefore, dimethylglyoxime and diphenylcarbazide were used as color developing reagents for nickel and chromium respectively. In 1997, Tubino and coworkers [13] developed a quantitative spot test method for the measurement of Fe(III), Ni(II) and Cr(VI). The method was similar to the present one in terms of approach (reagents used to develop spots) but different in quantification methodology. The present method makes use of software installed on a scanner-connected computer to quantify spots (on TLC), whereas previous method utilized HP diode array spectrophotometer and a Labsphere reflectance accessory (for spots on filter paper). The present technique can be made portable by using laptops, digital cameras, and USB scanners.

Results and discussion

Several methods are available to estimate metals, but these methods are either expensive or not precise at micro levels, particularly when the sample size is small. On the other hand, spot test is capable of detecting metal concentrations in just a drop of solution by contacting it with a suitable reagent. Classic qualitative inorganic tests acquire an unexpectedly large sensitivity when they are used as spot reactions on filter paper or TLC, reaching micro-analytical goals [1].

In the present work, an effort has been made to develop an economical, convenient, and accurate method to determine concentrations of nickel and chromium in aqueous solutions at micro levels. The main advantages are elimination of expensive gadgets, achievement of high sensitivity and accuracy and easy handling. Extremely small sample volume can be incorporated that is the most important feature of the technique [12]. A fixed volume of each standard (1 µL) was applied to TLC plate (chromatography paper can also be used) moistened with suitable reagent to produce colored spot. Afterwards, image of the plate/paper was imported into the computer using a flatbed scanner at 300 dpi, 24 bits RGB mode. The image was then analyzed with specially developed software by selecting the spots inside a circular marquee. The software measures the red, green, and blue components of each pixel inside the boundary and totals the values to give a final color depth.

For quantification of nickel and chromium, dimethylglyoxime and diphenylcarbazide have been used as developing reagents, respectively. Compounds like dimethylglyoxime having general formula R–C(NOH)–C(NOH)–R react with Ni(II) to give bright red water insoluble products. Complex formed between Ni(II) and dimethylglyoxime is shown in Figure 1. Cr(VI) reacts with diphenylcarbazide to give violet red coloration. In distinction to nearly all other reactions of chromate ion, there is no production of chromic ions. Instead chromous ions are formed, which react with enol form of carbazone to yield violet-red inner complex salt (Fig. 1). Formation of complex not only stabilizes an anomalous valence state but also brings out an anomalous oxidation-reduction process in this unique reaction [1]. Since, Cr(III) can be converted to Cr(VI)
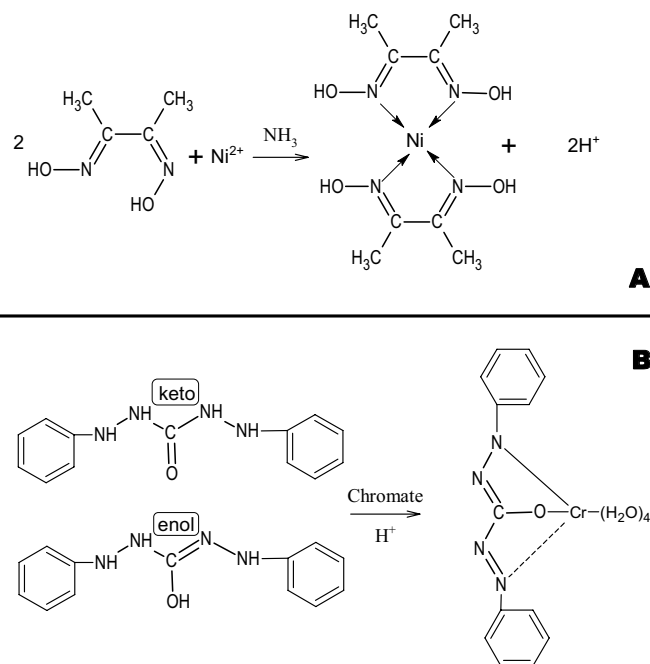

Figure 1. Complex of nickel with dimethylglyoxime (A) and of chromium with diphenylcarbazide (B).

by oxidation, diphenylcarbazide can be utilized as a sensitive developing reagent for both forms of chromium.

Calibration lines, shown in figure 2, were plotted for both metals between concentrations of metals (abscissa) and respective color densities (ordinate). Corresponding statistical parameters are given in Table 1. Higher slope for Ni(II) in comparison to Cr(VI) points out relatively more sensitivity of Ni-DMG system. In the concentration range 5-80 ng/µL, the relationship between concentrations of standards of Cr(VI) and associated color densities was described by linear regression in comparison to that of Ni(II) where non-linear regression (second order polynomial quadratic) fitted better the experimental data. Cor-
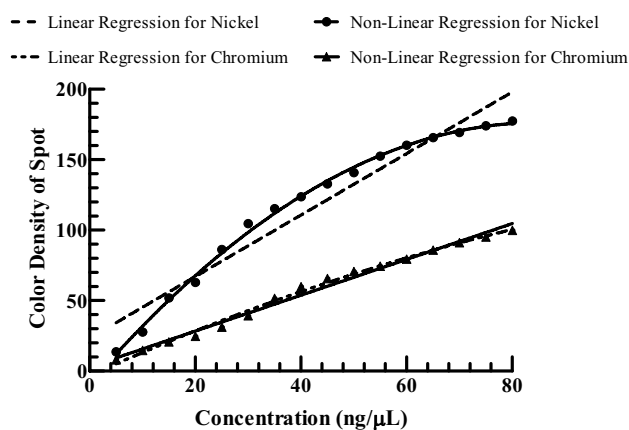

Figure 2. Calibration lines for Ni(II) and Cr(VI) obtained by new quantitative spot test method (Respective statistical parameters are given in Table 1).

Table 1. Linear Regression, Non-linear Regression, and Correlation Parameters for Ni(II) and Cr(VI).

Linear Regression									
Metal	Slope	Y-intercept	X-intercept	1/slope	Slope (95% CI)	Y-intercept (95% CI)	X-intercept (95% CI)	r²	Sy.x
Ni(II)	2.262 ± 0.1346	18.97 ± 6.315	−8.387	0.4421	1.975 to 2.549	5.515 to 32.43	−16.16 to −2.199	0.95	13.6
Cr(VI)	1.278 ± 0.03374	2.580 ± 1.583	−2.02	0.7827	1.206 to 1.349	−0.7921 to 5.953	−4.898 to 0.5917	0.99	3.41

Non-Linear Regression (Second Order Polynomial)								
Metal	B0	B1	B2	B0 (σ)	B1 (σ)	B2 (σ)	Sum of Squares	R²	Sy.x
Ni(II)	−4.022	4.102	−0.02299	1.081	0.06267	0.0007558	1135	1	3.72
Cr(VI)	−1.27	1.586	−0.00385	0.7501	0.04349	0.0005245	546.6	0.99	2.58

Correlation and Calibration Parameters							
Metal	Pearson r	95% CI	P value	R²	LOD	LOQ	Dynamic Range
Ni(II)	0.974	0.929 to 0.991	*p* < 0.0001	0.95	8.74 ng/μL	26.47 ng/μL	26-60 ng/μL
Cr(VI)	0.995	0.985 to 0.998	*p* < 0.0001	0.99	5.97 ng/μL	18.09 ng/μL	18-80 ng/μL

relation coefficients (r^2) were 0.95 and 0.99 for Ni(II) and Cr(VI), respectively, meanwhile, standard deviations of regression or standard errors of estimate (Sy.x) were 13.6 and 3.41 for Ni(II) and Cr(VI), respectively. Limits of detection (LOD) were 8.74 and 5.97 ng/μL for Ni(II) and Cr(VI), respectively. Limits of quantitation (LOQ) or lower limits of quantitation (LLOQ) were 26.47 and 18.09 ng/μL for Ni(II) and Cr(VI), respectively (LOD and LOQ were calculated by the standard method provided in [14]). Other correlation and calibration parameters are also provided in Table 1.

To check the applicability to real samples and to evaluate the performance of the new method, five real samples (from two electroplating units) of both metals were analyzed. The concentration of metal was calculated by interpolating the software calculated color density in the respective linear calibration line. Furthermore, the conventional spectrophotometric methods to estimate nickel and chromium were practiced, and the results were compared with those of newly described method. The reason to select spectrophotometric method for comparison was its resemblance with the new method (colorimetry). In Table 2, average values of each sample by both methods are provided along with standard deviations for seven replications. New method is more precise than conventional spectrophotometric method as indicated by standard deviations. It is interesting to note that present method is also valid for systems involving precipitations, where the spectrophotometric method fails. Using laptops and portable scanners (those can operate simply through USB ports), the technique can be easily applied for on-field estimations of nickel and chromium.

Conclusion

Statistical analysis and comparison of the results of the currently reported method with the conventional spectrophotometric method revealed that the computational quantification of spot tests by image scanning is a better technique in terms of handling, precision, accuracy, and cost. It has many advantages like a requirement of extremely small sample volume, portability, capability to analyze precipitated samples, elimination of expensive instruments without bringing down precision and accuracy. Limits of detection (LOD) were 8.74 and 5.97 ng/μL for Ni(II) and Cr(VI) in that order whereas limits of quantitation (LOQ) were 26.47 and 18.09 ng/μL, respectively.

Table 2. Analysis of real samples (electroplating effluents) by new and conventional spectrophotometric method

Sample ID	Spectrophotometric method		New method	
	Ni(II)	Cr(VI)	Ni(II)	Cr(VI)
A*	32.33 ± 0.12**	41.12 ± 0.14	33.12 ± 0.02	42.09 ± 0.01
B	37.39 ± 0.14	44.22 ± 0.11	38.19 ± 0.06	46.12 ± 0.04
C	39.67 ± 0.15	45.71 ± 0.18	40.12 ± 0.05	46.99 ± 0.04
D	32.39 ± 0.09	52.41 ± 0.11	33.41 ± 0.07	54.47 ± 0.08
E	31.67 ± 0.13	51.12 ± 0.14	32.12 ± 0.05	51.33 ± 0.06

* Real samples were collected by two different electroplating units around the Lahore city.
** Value = Average concentration (ng/μL) ± Standard deviation (seven replications).

Experimental

Equipment and apparatus

Micropipette (Pipettman, 0.2-2 μL) was used for developing spots on TLC plates (Merck, Aluminum sheets, 20 × 20 cm, silica gel 60 F$_{254}$). Reflective flatbed scanner (HP-3670) was used to scan and carry the image of the spotted TLC to the computer (windows XP, Service Pack 2) while Visual Basic 6 based computer graphic software program with circular picture box, selection marquee and flex grid control was used to measure color density of the spot. UV/Vis spectrophotometer (Labomed UVD-3500) was used in reference method for comparison.

Analysis of nickel

A stock solution of nickel (1000 μg/mL) was prepared by dissolving 4.48 g of nickel sulfate in 1000 ml of double distilled water. Standards (5-80 μg/mL) were prepared by successive dilution of the stock solution. A TLC (3×5 cm) was impregnated with 1% ethanolic solution of dimethylglyoxime. Next, it was placed in an ammonia desiccator for 5 min. After that, TLC was taken out of desiccator and 1 μL of each standard was applied on the reagent plate with the help of micropipette. Rose red spots were obtained. The plate was scanned on a flatbed scanner to carry the image of the spotty TLC to a computer in which indigenously developed software was installed. Software was capable of quantifying the color density of the spot. Regression line was plotted between concentration of metals in spots (abscissa) and their corresponding colored densities (ordinate). Standard statistical methods were applied to measure different parameters like standard error of estimate and correlation coefficient.

Analysis of chromium

A stock solution of chromium (1000 μg/mL) was prepared by dissolving 2.82 g of potassium dichromate in 1000 ml of double distilled water. Standards in the range 5-80 μg/mL were prepared by successive dilution of the stock solution. A TLC (3×5 cm) was impregnated with 0.5% acetone/ aqueous solution of diphenylcarbazide and sulfuric acid (2 N) one after another. One micro-liter of each standard was applied on the plate. Violet spots were obtained. The plate was scanned on a flatbed scanner to carry the image of the spotted TLC to a computer. Color density of each spot was calculated with software. Regression line was plotted between concentration of chromium in spots (abscissa) and their corresponding colored densities (ordinate). Standard statistical methods were applied to measure different parameters such as standard error of estimate, and correlation coefficient.

Comparison of proposed method with conventional spectrophotometric method

For comparison, conventional spectrophotometric method was employed to evaluate the accuracy and efficiency of the presently described method.

Nickel was determined colorimetrically by reaction with dimethylglyoxime. In a 50 mL volumetric flask, standard or sample solution of nickel (10 mL) was added followed by addition of concentrated ammonia solution (3 mL) drop by drop. Two milliliters of dimethylglyoxime solution were added to the above solution, mixed, and diluted to the volume to 50 mL with distilled water. The solution was allowed to stand for 5 min to permit full development of color. A portion of the solution was transferred into the cuvette of spectrophotometer to measure the absorption of light having wavelength 445 nm.

Hexavalent chromium was determined colorimetrically by reaction with diphenylcarbazide in acid solution. In a standard or sample solution of chromium (50 mL), 6N sulfuric acid was added to adjust the pH to 1.0 + 0.3. Afterwards, ethanolic solution of diphenylcarbazide (4.0 mL) was added to the aforementioned solution followed by proper dilution (to 100 mL) with double distilled water. After few minutes, the red-violet colored solution was transferred to the quartz cell of 1 cm path length that was placed in cell compartment of the spectrophotometer where absorption of light was measured at 540 nm.

Five real samples of electroplating effluents (from local industry) were tested by both methods, and the outcomes were compared. To check the reproducibility of the results, each sample was analyzed seven times. The concentration was calculated by interpolating the software calculated color density and absorption from the respective calibration lines of present and spectrophotometric method, in that order.

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