Individual and Simultaneous Determination of Cr^{6+} and Mo^{6+} in Binary Mixtures by Spectrophotometry and First-Derivative Spectrophotometry

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In this study, individual and simultaneous determination conditions of Cr^{6+} in the presence of Mo^{6+}, the latter being a known interferent at ≥5 mole-to-mole ratios, by the common spectrophotometric method with 1,5-diphenylcarbazide(DPC) reagent were investigated. An optimal period of sixty minutes at room temperature or ten minutes at 40°C in a water bath was required after DPC reagent addition, and ligand/metal mole ratio had to be at least forty for maximum color development in the presence of Mo^{6+}. The ordinary and first-derivative spectra of complexes were recorded for both single ions and their binary mixtures. The Cr^{6+} complex had a maximum absorbance at 540 nm while the Mo^{6+} complex had three peaks at 540, 665 and 755 nm in ordinary spectra. So, 510 or 590 nm for the Cr^{6+} complex, and 690 or 820 nm for the Mo^{6+} complex (for individual determination), might be selected as working wavelengths in the first-derivative spectra. At wavelengths mentioned above, linear equations for calibration, linear ranges and relative standard deviations were determined for both methods. Cumulative linear equation of first-derivative absorbance (^1D) values at 590 nm was used to calculate Cr^{6+} concentration in binary mixtures after calculation of Mo amount from linear equation at 820 nm.
Under experimental conditions proposed, Cr^{6+} can be determined individually and simultaneously in the presence of Mo^{6+} at higher than forty molar excess. On the other hand, in the range of 5-40 Mo^{6+}/Cr^{6+} mole ratios, Cr^{6+} can not be determined in the presence of Mo^{6+} by these procedures. But at high Mo^{6+} concentrations, the interference effect of molybdenum might be converted to an advantage of simultaneous analysis due to newly emerging peaks at 665 and 755 nm arising from possibly molydenum blue. Both spectrophotometric and first-derivative spectrophotometric methods were suitable for determination of Cr^{6+} and Mo^{6+}, individually and simultaneously.

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Chromium is both an essential trace element (as Cr^{3+} ion) and an environmental toxicant (as Cr^{6+} ion). Trivalent chromium (50-200 μg per day) is necessary for normal development of humans and animals because of its role in glucose, cholesterol and fat metabolisms. On the other hand, ecological problems with hexavalent chromium are related to the development of chromium-based industry. Presently, Cr^{6+} has been recognized as a suspected agent of lung cancer, and it also produces gastrointestinal disorders, dermatitis and ulceration of skin in man. Although the exact mechanism of Cr^{6+} toxicity is not known, intracellular chemical intermediates (Cr^{5+}, carbon-based radicals) formed during the reduction of Cr^{6+} to Cr^{3+} probably cause the genotoxicity of hexavalent chromium compounds. Chromium compounds are extensively used in several commercial applications including leather tanning, corrosion inhibition, chrome alloys, plating, glassware-cleaning solutions, wood preservatives, manufacture of safety matches, metal finishing, production of pigments, etc. During the process of production a relatively large quantity of chromium is released to the atmosphere, soil and water supplies.[1-3] Thus, for reliable evaluation of environmental contamination with chromium, selective and sensitive analytical tools are needed for discrimination and quantitation of different chromium species. A great majority of the procedures reported for chromium speciation in environmental and biological samples includes preconcentration and/or separation step prior to detection. Among of the preconcentration and/or separation techniques, extraction,[4-9] HPLC (and IC),[10-13] sorption in microcolumns[14-17] are generally used. Spectrophotometry[9,16-19] and atomic spectrometry[4,10-12,14,15] are common techniques to detect chromium species after preconcentration and/or separation step in both traditional and flow systems. In many of chromium determination methods, especially in spectrophotometric ones, molybdate ion was reported as interferent.

Molybdenum is necessary in trace amounts in plant nutrition and is also valuable in human metabolism. Molybdenum is also widely used in a variety of industrial processes, being an important constituent of metal alloys, e.g., ultra-high strength steels, pigments, lubricants and chemical catalysts, etc.[20] Due to its chemical importance, numerous analytical procedures for molydenum determination can be found in the literature.[20-27] Spectrophotometric thiocyanate method is one of the most common techniques in various modified manners.[20,21]

Also, simultaneous determination methods of chromium and molybdenum by suppressed ion chromatography,[28] electrothermal atomic absorption spectrometry[29] and capillary ion electrophoresis[30] exist in the literature.

Derivative spectrophotometry in the UV-Vis region is a useful technique in extracting qualitative and quantitative information from overlapping bands of the analyte and interferents due to incompletely resolved peaks.[31-33] Extractive spectrophotometric determination of Cr^{6+} using internal standard and first-derivative spectral data was reported.[19]

Aside from the more sensitive and relatively interference-free techniques requiring highly sophisticated instruments and well-trained operators such as atomic spectrometric or hyphenated techniques like HPLC or FIA coupled to spectrophotometric or atomic spectrometric detection techniques mentioned above, the simpler and cheaper spectrophotometric method with 1,5-diphenylcarbazide(DPC) reagent is still commonly used for Cr^{6+} determination. This method has been found more suitable for determination of total chromium as Cr^{6+} in tannery waste water when compared with ICP-AES and FAAS using an air-acetylene

flame[3]. The basis of this spectrophotometric method is a redox reaction between chromate ions and diphenylcarbazide. In an acidic solution, chromate ions oxidize diphenylcarbazide to diphenylcarbazone and the Cr^{3+} ions thus occured form a red-violet complex with diphenylcarbazone.[18] The aim of this study is to investigate individual and simultaneous determination conditions of Cr^{6+} and Mo^{6+}, the latter being a known interferent[18,19] at ≥5 mole-to-mole concentrations, using its most specific colorimetric reagent, 1,5-diphenylcarbazide, in binary mixtures. Meanwhile, spectrophotometric and first-derivative spectrophotometric measurements were compared in established conditions.

Experimental

Instruments

Ordinary and derivative spectra were recorded with a Varian Cary 1E spectrophotometer utilizing quartz cells. pH measurements were made with a Metrohm E-512 pH-meter equipped with a glass electrode.

Reagents and solutions

All chemicals (E. Merck) were of analytical reagent grade and were used without further purification. A 0.1 M solution of DPC ($C_{13}H_{14}N_4O$) was prepared daily in acetone. Cr^6 ($2.0x10^{-4}$ M) and Mo^{6+} ($2.0x10^{-2}$M) solutions were prepared by dissolving calculated amounts of $K_2Cr_2O_7$ and $Na_2MoO_4.2H_2O$ in distilled water, respectively. Concentrated H_2SO_4 was diluted with distilled water for preparing a 0.5 M solution of H_2SO_4.

Procedures

For single Cr^{6+} determination, 1 ml of sample solution containing preferably $2.0x10^{-5}$-$2.0x10^{-4}$ mmol (1.0-10.4 μg) hexavalent chromium was taken into a stoppered test tube; 1 ml of H_2SO_4 solution followed by 5 ml 0.1 M DPC solution were added.

For single Mo^{6+} determination, 1 ml of sample solution containing preferably $2.0x10^{-3}$-$9.0x10^{-3}$ mmol (0.2-0.9 mg) molybdate ion was taken into a stoppered test tube; 1 ml of H_2SO_4 solution followed by 5 ml 0.1 M DPC solution were added.

For simultaneous determination of Cr^{6+} and Mo^{6+}, 1 ml of sample solution containing Mo^{6+} which is more than 40-fold of Cr^{6+} (at a total concentration within linear ranges of the two ions) was taken into a stoppered test tube (for example, solutions containing $2.8x10^{-5}$-$1.4x10^{-4}$ mmol Cr^{6+} in the presence of $6.0x10^{-3}$ mmol Mo^{6+}, and $2.0x10^{-3}$-$9.0x10^{-3}$ mmol Mo^{6+} in the presence of $5x10^{-5}$ mmol Cr^{6+} are suitable for simultaneous analysis of Cr^{6+} and Mo^{6+}); 1 ml of H_2SO_4 solution followed by 5 ml 0.1 M DPC solution were added.

Ordinary and first derivative absorbance values of the above mixtures at 540, 665, 755 nm of absorbance maxima and 510, 590, 690, 820 nm of ^1D maxima were measured, respectively, against a reagent blank after heating for 10 min in 40°C water bath. The concentrations of Cr^{6+} and Mo^{6+} were estimated from the linear calibration curves of absorbance (A) and first-derivative absorbance (^1D) versus concentration (C) drawn with standard solutions of a suitable concentration range.

Analysis of a real sample (dental alloy)

A 0.5 g amount of Ni-Cr-based dental alloy Wiron 99 (Bego, Germany) containing Ni, 65%; Cr, 22.5%; and Mo, 9.5%; and traces of Ce, Fe and Mn (amounts were declared by the producer) was dissolved using aliquots of 10 ml aqua regia for 5 repetitions, evaporated to dryness, and then dissolved with 50 ml of 0.5 M H_2SO_4.

For simultaneous determination of Cr and Mo, a 5 ml aliquot of this solution was boiled by adding 5 ml concentrated NH_3 solution and 2 ml concentrated H_2O_2 (35%) solution so as to guarantee that all chromium is converted to Cr^{6+}, evaporated to dryness and dissolved in 20 ml of 0.5 M H_2SO_4 and diluted to 100 ml with distilled water. Then this solution was diluted 25 times, again. Since the final solution did not sufficient Mo for simultaneous determination by the proposed method, the solution was spiked with $2.0x10^{-2}$ M Mo^{6+} solution. For this purpose, 8 ml of the last diluted sample solution and 2 ml $2.0x10^{-2}$ M Mo^{6+} solution were mixed and analyzed.

Results and Discussion

Absorption spectra

The ordinary and first-derivative spectra of colored solutions obtained in the presence of Cr^{6+} and Mo^{6+} were recorded for individual ions and mixtures covering Cr^{6+}:Mo^{6+} molar ratio range between 1:1 and 1:240. The ordinary and first-derivative spectra for Cr^{6+} and Mo^{6+} are presented in Figs. 1 and 2, respectively. The Cr^{6+} complex had a maximum absorbance at 540 nm while Mo^{6+} complex had three peaks where the last two might be due to molybdenum blue formed from the redox reaction between Mo^{6+} and diphenylcarbazide at 540, 665 and 755 nm in ordinary spectra. So, 510 or 590 nm for the Cr^{6+} and Mo^{6+}, and 690 or 820 nm for only the Mo^{6+}, were selected as possible working wavelengths in the first-derivative spectra. Among possible quantitative evaluations, the zero-to-peak method was selected where the vertical distance of ^1D to the zero line was measured.

Selection of optimal parameters for analysis

The ^1D values were obtained at wavelength intervals ($\Delta\lambda$) varying 1-25 nm. Optimal $\Delta\lambda$ was chosen as 20 nm for further evaluations in the search of increased sensitivity at the cost of spectral distortion.[34]

It was established that it had been necessary to incubate the mixture of Mo^{6+} and DPC solution for 60 min at room temperature or for 10 min at 40°C in a water bath to complete color development, i.e., redox reaction (molybdenum blue formation) and complexation between Mo^{6+} and DPC. In fact, the absorbance of Mo^{6+}-DPC solution slightly increased at the end of 40 min in a 40°C water bath. But, at that time interval, the absorbance of Cr^{6+}-DPC solution decreased relatively faster. So, 10 min was selected as optimum incubation period in a 40°C water bath. However, Cr^{6+}-DPC complex was stable for 120 min at room tempature. Nevertheless, it was prefered to use 10 min incubation at 40°C (water bath) for further evaluations.

Concentration of H_2SO_4 was important for both Cr^{6+} and Mo^{6+} color formation. While the absorbance of Cr^{6+}-DPC solution decreased, the absorbance of Mo^{6+}-DPC solution increased with increasing H_2SO_4 concentration first, but decreased later. So, 0.07 M was chosen as a suitable H_2SO_4 concentration which is slightly lower than the 0.1 M H_2SO_4 stated in the literature.[18]

The DPC-to-Mo^{6+} mole ratio dependence of absorbances at working wavelengths (540 and 665 nm) are shown in Fig. 3. When DPC/Mo^{6+} ratio was varied between 40-100, absorbances at the indicated wavelengths did not change appreciably enabling the selection of this molar ratio interval as suitable for Mo^{6+} and Cr^{6+} determination. Also, at that DPC concentration, DPC/Cr^{6+} mole ratio was very excessive and the absorbances of Cr^{6+}-DPC solutions did not change, practically.

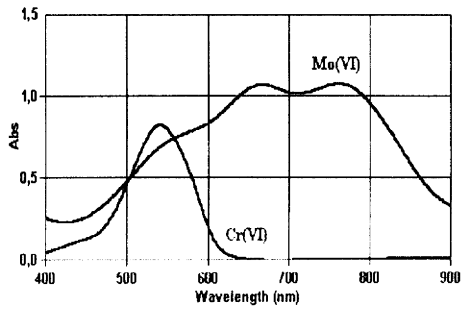

Fig. 1 Ordinary spectra of Cr^{6+} and Mo^{6+}complexes with DPC against reagent blank. $[Cr^{6+}]_{final}$ = 2.0x10^{-5} M, $[Mo^{6+}]_{final}$ = 1.14x10^{-3} M, $[DPC]_{final}$ = 7.14x10^{-2} M

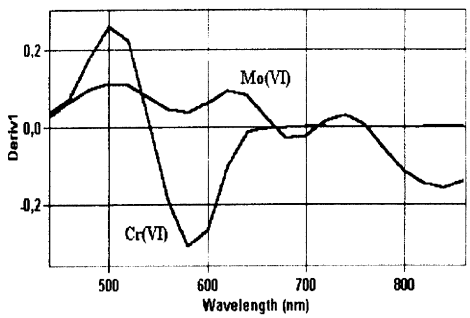

Fig. 2 First-derivative spectra of Cr^{6+} and Mo^{6+} complexes with DPC against reagent blank. $[Cr^{6+}]_{final}$ = 2.0x10^{-5} M, $[Mo^{6+}]_{final}$ = 1.14x10^{-3} M, $[DPC]_{final}$ = 7.14x10^{-2} M, $\Delta\lambda$ = 20 nm

The linear plots (calibration curves)

The calibration curves for both Cr^{6+} and Mo^{6+} were drawn with both ordinary and first-derivative absorbance values at the indicated wavelengths. Linear equations with the corresponding correlation coefficients (r) were obtained at 540 nm for Cr^{6+} and Mo^{6+}, also at 665 and 755 nm for only Mo^{6+} by using ordinary absorbance values.

For chromium,

$A_{540} = 4.0 \times 10^4 C_{Cr} - 0.009$ (r = 0.9993) Eq. (1)

For molybdenum,

$A_{540} = 6.7 \times 10^2 C_{Mo} - 0.085$ (r = 0.9995) Eq. (2)
$A_{665} = 1.2 \times 10^3 C_{Mo} - 0.270$ (r = 0.9991) Eq. (3)
$A_{755} = 1.2 \times 10^3 C_{Mo} - 0.272$ (r = 0.9993) Eq. (4)

Because Eq.3 and Eq.4 was practically equal, 665 nm was preferred as working wavelength.

Linear equations with the corresponding correlation coefficients (r) were obtained at 510 and 590 nm for both Cr^{6+} and Mo^{6+}, and also at 690 and 820 nm for only Mo^{6+} by using first-derivative absorbance values.

For chromium,

$^1D_{510} = 1.1 \times 10^4 C_{Cr} + 0.006$ (r = 0.9993) Eq. (5)
$^1D_{590} = -1.3 \times 10^4 C_{Cr} - 0.003$ (r = 0.9994) Eq. (6)

For molybdenum,

$^1D_{510} = 1.0 \times 10^2 C_{Mo} - 0.010$ (r = 0.9995) Eq. (7)
$^1D_{590} = 78 C_{Mo} - 0.038$ (r = 0.9950) Eq. (8)
$^1D_{690} = -29 C_{Mo} + 0.006$ (r = 0.9900) Eq. (9)
$^1D_{820} = -1.7 \times 10^2 C_{Mo} + 0.037$ (r = 0.9980) Eq. (10)

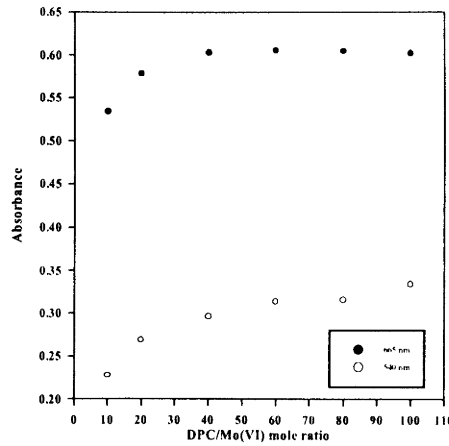

Fig. 3 Reagent-to-Mo^{6+} mole ratio dependence of absorbances at 540 and 665 nm

Due to spectral interference in simultaneous analysis, for chromium determination, the cumulative equations (i.e., Eq. (1) + Eq. (2), and Eq. (5) + Eq. (7) or Eq. (6) + Eq. (8)) were formed in the case of spectrophotometry and first-derivative spectrophotometry, respectively. At first, molybdenum concentration should be calculated by using Eq. (3) in spectrophotometry and by Eq. (10) in first-derivative spectrophotometry, respectively. Eq.(10) was preferred because of the higher extinction coefficient than that of Eq. (9). For the same reason, the cumulative equation of Eq. (6) + Eq. (8) was used in the first-derivative spectrophotometric chromium determination.

Reproducibility and linear range

The relative standard deviation (RSD) of five successive determinations of Cr^{6+} (0.44 mg/l) individually was 0.8%, and in admixture with Mo^{6+} (82.2 mg/l) was 1.5% in spectrophotometry. The RSDs for the first-derivative spectrophotometric analysis of Cr^{6+} (0.44 mg/l) at 590 nm individually and for its admixture with Mo^{6+} (82.2 mg/l) were 1.2 and 2.71%, respectively. The RSDs for Mo^{6+}(82.2 mg/l) were 0.3% and 1.4% for spectrophotometric and first-derivative spectrophotometric analysis, respectively. The linear ranges in both spectrophotometric and the first-derivative spectrophotometric evaluation with reasonable reproducibility were between 2.9x10^{-6}- 2.9x10^{-5} M for Cr^{6+} and 2.9x10^{-4}- 1.3x10^{-3} M for Mo^{6+}.

Application to synthetic samples

The analytical results for synthetic mixtures containing varying Cr^{6+} and Mo^{6+} were depicted in Table 1. These results were satisfactory agreement with added amounts, as shown.

Application to a real sample

The diluted sample solution of dental alloy Wiron 99 spiked with 2.0x10^{-2} M Mo^{6+} solution was analyzed according to the proposed procedure. Chromium was calculated as (21.1±0.38)% using the cumulative equation of Eq. 6 and Eq. 8, and Mo was found as 3.79+0.04 mg/10 ml solution mentioned above (original amount in dental alloy plus spiked Mo) by means of Eq. 10. Results obtained both Cr and Mo were in good agreement with those declared by the producer.

Table 1 Analytical data for synthetic samples

Metal ions (added, μg)		Metal ions (found, μg) (within 95% confidence interval)	
Cr^{6+}	Mo^{6+}	Cr^{6+}	Mo^{6+}
2.10	768	1.98±0.24	760±0.21
3.10	576	3.00±0.28	571±0.26
4.20	384	4.30±0.31	370±0.32

In this study, it was established that the simultaneous determination of Cr^{6+} and Mo^{6+} by spectrophotometry and first-derivative spectrophotometry might be achieved on the condition that Mo^{6+}/Cr^{6+} mole ratio was greater than 40. Also, it was stated in the literature that Mo^{6+} at ≥5 mole-to-mole ratios interferes with the spectrophotometric determination of Cr^{6+} using DPC as ligand[18,19]. For the simultaneous determination of Cr^{6+} and Mo^{6+}, there were two important points to be considered: (i) The concentration of DPC should be sufficient (i.e., DPC/Mo^{6+} mole ratio should lie within 40-100 range). (ii) For optimum color formation, the mixture solution of Cr^{6+}-Mo^{6+}-DPC should be incubated for 60 min at room temperature or for 10 min in a 40°C water bath. The absorbance and ^1D values of molybdenum blue formed during incubation might be used for the determination of molybdenum concentration. Additive absorbance and ^1D values and cumulative equations of Eq. (1) + Eq. (2), and Eq. (6) + Eq. (8) for the spectrophotometry and the first-derivative spectrophotometry, respectively, might be useful to determine chromium concentration in the presence of a calculated amount of molybdenum. On the other hand, in the range of 5-40 Mo^{6+}/Cr^{6+} mole ratios, Cr^{6+} can not be determined in the presence of Mo^{6+} by these procedures. So, the interference effect of Mo^{6+} at high concentrations would be converted to an advantage of simultaneous determination. Also, the proposed method, when compared to the first-derivative spectrophotometric method[19] using an internal standard, was simpler and combined with the advantage of simultaneous analysis at more than 40-fold Mo^{6+} concentrations.

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