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Chromate reductase activity: characterization of Cr(VI) to Cr(III) conversion

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Abstract. In order to understand chromate reductase activity (CRA), related to cellular carcinogenecity and metal toxicity in ecological systems, we began studying the reduction of chromium(VI) salts using cysteine, ascorbic acid and several monosaccharides mainly through EPR and absorption spectroscopic methods. The present study indicates Cr(V) as a definite intermediate and also shows the relative trends of reducing capabilities among various reducing agents employed. Considerable progress has been achieved in the isolation of intermediates and final products as well.

Keywords. Chromate reductase activity; chromium(VI) reduction; chromium(V) species; EPR spectroscopy; absorption spectroscopy.

1. Introduction

Various chromium(VI) compounds such as chromate and dichromate are known to be carcinogenic to cells including those of mammals. This has been well-demonstrated based on epidemiological studies conducted on workers in Cr(VI) industries and also on various test animals. While Cr(VI) compounds were mutagenic in bacteria and mammalian cell systems, Cr(III) compounds were not active mutagens in the same test systems. This then has led to the belief that tetrahedral chromate salts, having charge, size and geometry similar to that of sulphate (SO_4^{2-}), utilize anionic channels in order to cross the membrane barrier and enter the cell. Following this, various small and large molecular weight cellular components reduce Cr(VI) to generate some reactive intermediates and ultimately form covalent Cr(III) linkages between the cellular components and thereby impair normal cellular functions (Connett and Wetterhahn 1983; Tsapakos and Wetterhahn 1983). This is known as *chromate reductase activity* (figure 1).

Although in the literature chromate reductase activity was related to the reductive capacity of certain cellular components, recent studies including ours clearly demonstrate the role of soil components in such phenomenon. In view of this, we suggest that the latter be categorized under the so-called chromate reductase activity or the phenomenon may be renamed as biological chromate [Cr(VI)] reductors in order to eliminate the confusion associated with the term 'reductase activity' assigned to only cellular components. During the last couple of years, there have been intensive

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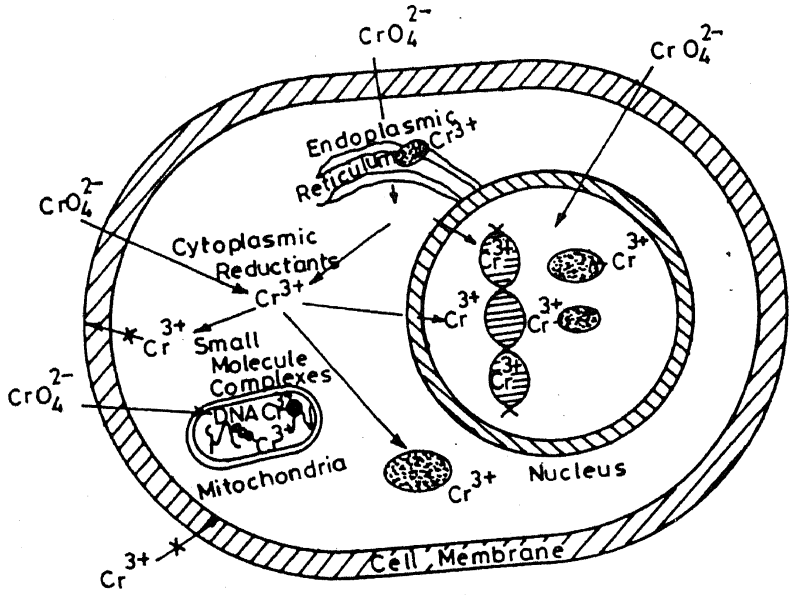

Figure 1. The uptake-reduction model for chromate carcinogenicity (taken from Connett and Wetterhahn 1983).

efforts regarding Cr(VI) reduction by both cellular and soil components (O'Brien *et al* 1985; Boyko and Goodgame 1986; Goodgame and Roy 1987; Branca *et al* 1988; Kitagawa *et al* 1988). The literature is mainly related to the identification of Cr(V) through spectral studies, particularly that of EPR. However all these studies are primarily in the solution state and are not made quantitative. It is rather important to compare the relative abilities of the reduction of these cellular and soil components through establishing their mechanistic aspects. While the former is expected to unravel aspects regarding chromate dermatitis, the latter is important from the point of view of the environmental pollution and toxicity as it relates to the ecological and biological cycles. Further, detoxification mechanisms are largely unexplored.

In view of these facts, we have initiated a systematic programme to unravel the mechanistic details of chromate reductase activity with cellular and soil components in solution as well as in the solid state. As a first step to this programme, we wish to report our results, though preliminary as yet, regarding the interaction of various cellular (cysteine and ascorbic acid) and soil reductants (saccharides: glucose, fructose and galactose) with chromate and dichromate both in nonaqueous and aqueous media using EPR and absorption spectroscopic methods. We also report the isolation of intermediate and final products.

2. Experimental

2.1 *Physical measurements*

Aqueous reactions were performed using potassium salts of chromate and dichromate. The corresponding methanol reactions were performed using 18-crown-6 derivatives of these salts. These derivatives were prepared by refluxing the 1:2 mixture of the chromate salt and 18-crown-6 for a few hours, followed by removing the excess methanol and then crystallizing the product by cooling.

X-band EPR spectra of the reaction solutions were measured on a Varian E-112 ESR spectrometer, using a flat quartz cell with a final metal concentration of 0·05 M in the mixture along with the calculated ratio of the ligands. Tetracyanoethylene anion was used as an internal standard field marker ($g = 2{\cdot}0027$).

Absorption spectra were measured using a Shimadzu UV/Vis-265 spectrophotometer at two different metal concentrations (0·0005 M and 0·005 M) for each system with 1 cm quartz solution cells.

2.2 *Synthetic reactions*

These reactions were carried out in a dry nitrogen atmosphere (glove box) using anhydrous solvents in order to isolate the Cr(V) and Cr(III) species in the solid state.

2.2a *Cr(V)-D-glucose complex*: One m mol of crown-chromate and 1 m mol of D-glucose were taken in 20 ml of methanol and stirred for about 60 h and then the volume of the mixture was reduced. Upon addition of dry ether, the reaction mixture yielded a green precipitate which was subsequently washed several times with a mehanol–ether mixture and dried.

Using the same method, Cr(V)-D-galactose and Cr(V)-D-fructose complexes were synthesized and isolated.

2.2b *Cr(III)-D-glucose complex*: Crown-chromate and D-glucose were taken in a molar ratio of 1:4 (one m mol metal concentration) in methanol and stirred for 4 days when the total volume was condensed to half of the original and an equal amount of dry ether was added in order to precipitate green solid product. The solid was washed and dried before it was subjected to spectral studies.

Using the same method, Cr(III)-D-galactose and Cr(III)-D-fructose complexes were synthesized and isolated. Only the galactose reaction required more time for completion. Cr(III)-D-glucose complex was also synthesized in aqueous solution using a large excess (around 20 equivalents) of the ligand.

2.2c *Cr(III)-L-cysteine ethyl ester complex*: This complex was prepared by reacting 1:8 molar ratio of crown-chromate and the ligand in methanol and stirring for about two hours. A purple coloured product was obtained by removing the solvent, which was subsequently recrystallized.

3. Results and discussion

3.1 *EPR spectroscopy*

3.1a *Reactions with L-cysteine ethyl ester*: Cysteine is an important reducing component of low and high molecular weight entities of the cell and functions, particularly because of its thiol group, as both reducing and coordinating agent. While deprotonated thiol strengthens the binding between metal and sulphur, the corresponding disulphide product is a very poor coordinating ligand via its S atoms.

(i) *Chromate*: Reactions between crown-chromate and L-cysteine ethyl ester with various metal to ligand ratios such as 1:0·125, 1:0·25, 1:2·0 have indicated very sharp

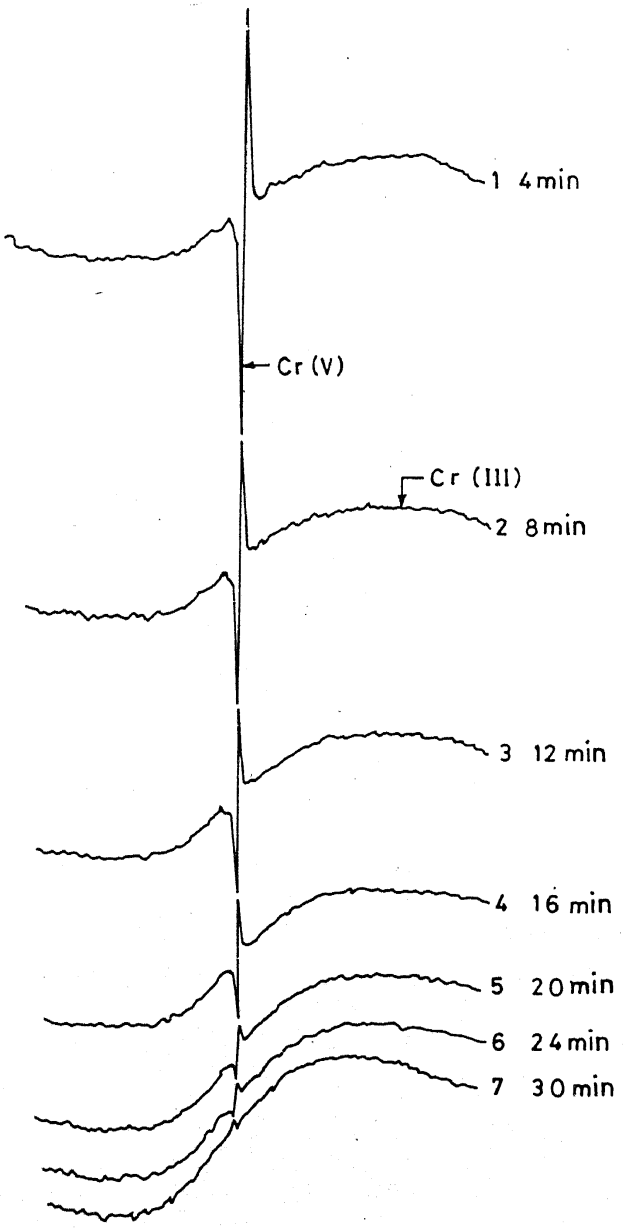

Figure 2. Time dependent EPR spectra of crown-chromate and 2 equivalents of cysteine ethyl ester reaction in methanol.

Cr(V) ($g = 1{\cdot}9725$, $\Delta H_{1/2} = 15$–30 G) and very broad Cr(III) ($g = 1{\cdot}9671$, $\Delta H_{1/2} = 380$ G) EPR signals, both varying in their intensities as a function of time (figure 2). The assignment of these signals is consistent with the literature (Goodgame and Roy 1986). While the Cr(V) peak decreases in its intensity with time, the intensity of the Cr(III) peak shows a concomitant increase and approaches saturation value after various time periods depending upon the ligand to metal ratio, the saturation value being higher at high ligand concentrations. This means that higher proportions of Cr(III) species are formed at high ligand to metal ratios, however, with some limiting values beyond a particular ligand to metal ratio. The intensity of the Cr(III) peak increases, under the same instrumental conditions even when the Cr(V) peak is not present, and sometimes after longer time intervals also. Variation in the peak

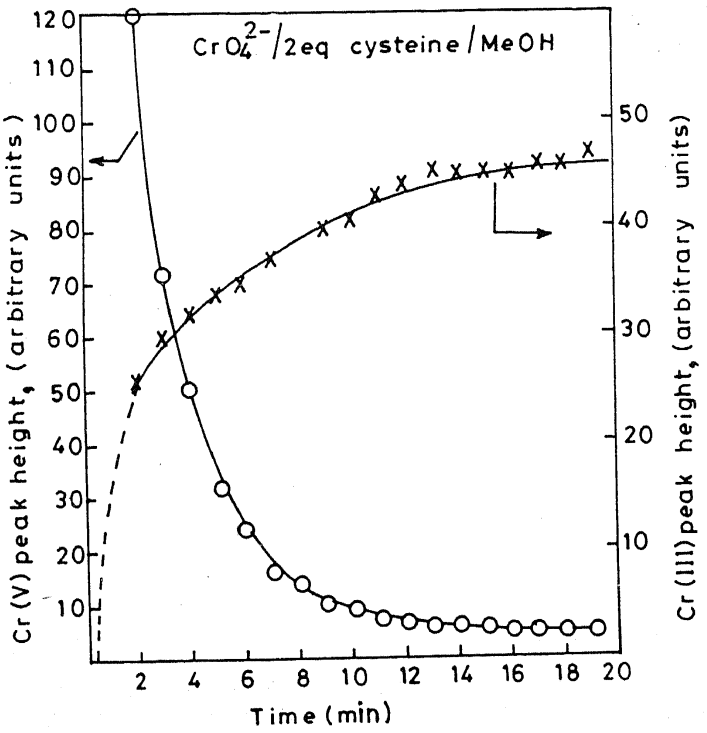

Figure 3. Time vs. EPR peak heights for Cr(V) and Cr(III) species for the reaction shown in figure 2.

intensities of Cr(V) and Cr(III) with respect to time is indicated in figure 3 for a chromate to cysteine ratio of 1:2 in methanol. Plots indicate a trend close to an exponential decay for the Cr(V) species and an opposite trend for the Cr(III) species. The half-life times ($t_{1/2}$) for the Cr(V) species as obtained from the best straight line fits of the corresponding logarithmic plots are 3·0, 2·4 and 2·2 min respectively for 0·125:1, 0·25:1 and 2:1 ligand to metal ratios, therefore, the rate of Cr(V) conversion is higher at high ratios of cysteine ester. Reactions with high ligand to metal ratios such as 4:1 and 8:1 indicate only broad Cr(III) signals in the EPR spectra even when the measurements are made immediately after mixing (< 1 min) the components. The Cr(III) peak intensities increase as a function of time and then approach a saturation value. The final saturation value suggests that the ligand concentration needed for the complete conversion of Cr(VI) to Cr(III) is less than or equal to 4 equivalents. Thus the reaction is assumed to consist of mainly two steps, namely, the conversion of initial Cr(VI) to the intermediate Cr(V) and subsequent reduction to the final Cr(III) species at room temperature. However, the observed increase in Cr(III) signal intensity even after the disappearance of the Cr(V) signal indicates a possible hidden step involved in the conversion process.

(ii) *Dichromate*: Similar observations are noted even in the case of cysteine ester–crown-dichromate at ratios of 0·5:1, 1:1 and 2:1 in methanol. The corresponding half-life values for Cr(V) species are 2·2, 1·6 and 1·0 min respectively.

From the plots of crown-chromate/crown-dichromate with cysteine ethyl ester in methanol it is evident that the $t_{1/2}$ values for the formation of Cr(III) are much larger than those for the decay of the Cr(V) species. Both the EPR and absorption studies indicate that the Cr(III) centre is in an octahedral environment. Therefore, the required coordination-sphere expansion step in the conversion of Cr(V) to Cr(III) is

easy to achieve by the reactive Cr(V) intermediate. Perhaps this process may explain the observed cross-linking during the cellular toxification by Cr(VI) salts.

3.1b *Reactions with L-ascorbic acid*: This is also an equally important component of cellular reducing agents. Ascorbic acid is a two-electron reductant and the corresponding one- and two-electron oxidized products are good chelating agents. Hence it is expected that the stability of Cr(V) species coordinated to this ligand is increased.

Reaction between the 18-crown-6 derivative of dichromate and ascorbic acid in water-methanol mixture exhibited a very sharp ($\Delta H_{1/2} = 1{\cdot}5$ G) signal at $g = 1{\cdot}9796$ assignable to Cr(V) where the intensity of this signal varied with time. An isotope of chromium, ^{53}Cr, having an I value of 3/2 and natural abundance of 9·55%, exhibited four small satellite bands with a hyperfine spacing of 17·6 gauss which is in agreement with the literature values for an oxygen environment (Goodgame and Roy 1987a; Krumple *et al* 1978). While the decrease in Cr(V) peak intensities was found with reaction mixtures of all ligand to metal ratios, the increasing Cr(V) peak intensity zone was found only with low ligand to metal ratios (1:1 and 1·5:1). The $t_{1/2}$ values of the decreasing portions of Cr(V) peak intensities were found to be 4·2, 11·6 and 13·9 min, respectively, for 2:1, 1·5:1 and 1:1 ascorbic acid to dichromate (figure 4) ratios. This indicated a rapid conversion of Cr(V) species and consequently a quicker Cr(VI) to Cr(III) reaction at higher ligand concentrations. Comparison of $t_{1/2}$ values for the Cr(V) signal between this case and cysteine reaction indicate that the rate of

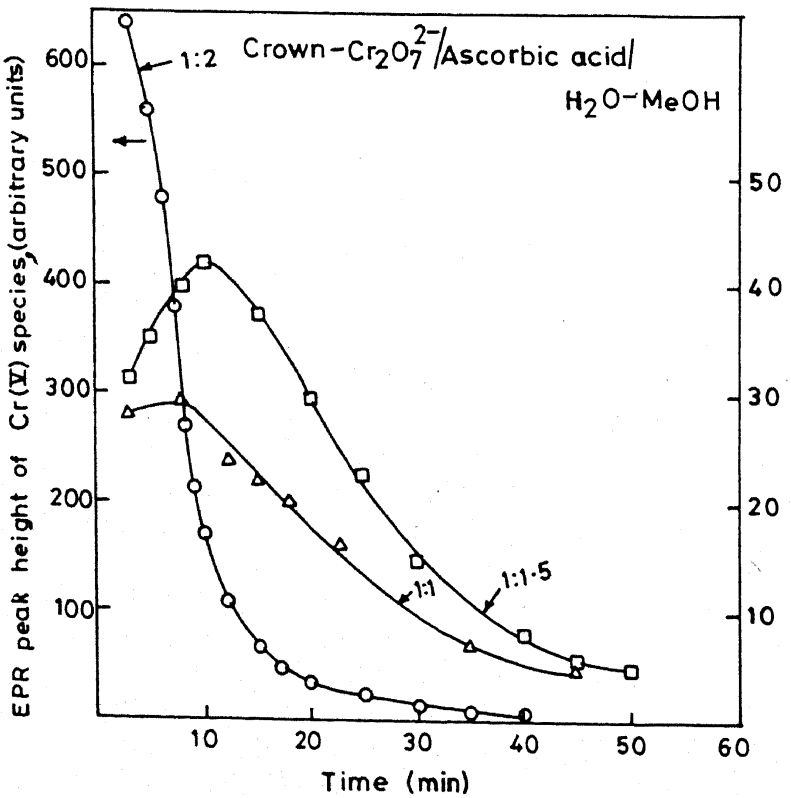

Figure 4. Time vs. EPR peak heights for Cr(V) species of crown-dichromate – ascorbic acid system in water – methanol mixture at different metal to ligand ratios.

conversion is slower with the former and this is favoured by the trend of reduction potentials (E° for cysteine = $-0{\cdot}32$ V and E° for ascorbic acid = $+0{\cdot}08$ V).

3.1c *Reactions with saccharides*: Saccharides and saccharide-containing molecules are abundant in soil and plant materials. Further, these are expected to chelate metals strongly through their oxygen atoms owing to their multihydroxy functions. These may be intermediates in the incorporation of such substances into ecological and biological cycles.

The striking difference between the chromate–cysteine and chromate–saccharide reactions is the time taken for the conversion of Cr(VI) to Cr(III). While this is of the order of weeks in the latter case, in the former, it occurs within a few hours or in about a day or so.

Reactions between chromate and fructose, with ligand to metal ratios of 1:1 (MeOH), 4:1 (H_2O) and 6:1 (H_2O), show increasing intensities of Cr(V) signals for 2–4 days, which then start decreasing and completely disappear after 8 to 30 days depending upon the ratio of the ligand to metal. Increasing amounts of Cr(III) are also found as the time period increases and this approaches a saturation value around 14–16 days in the case of 4:1 and 6:1 ligand to metal ratios. The peak intensities are higher for high fructose concentrations and the study indicates that more than 4 equivalents of fructose are needed to completely reduce and complex the chromate in its final Cr(III) form. While the $t_{1/2}$ value for the Cr(V) signal intensity (in the

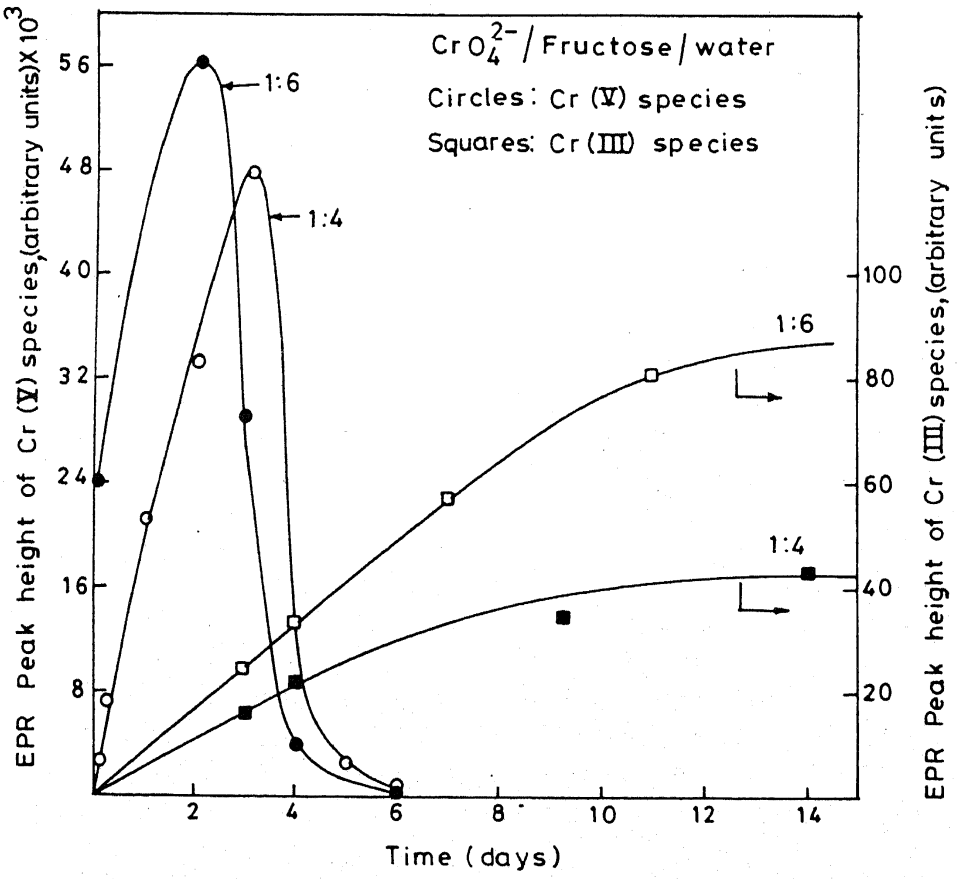

Figure 5. Time vs. EPR peak heights for Cr(V) and Cr(III) species of aqueous solutions of D-fructose – chromate at 4:1 and 8:1 ligand to metal ratios.

decreasing region) is about 11–12 days for 1:1 (in MeOH) and 0·5 days for the other two ratios, the corresponding $t_{1/2}$ values for the Cr(III) signal in the increasing region are 7·6 and 4·8 days, respectively, for 4:1 and 6:1 fructose to chromate ratios (figure 5). The results again emphasize the fact that the Cr(VI) to Cr(III) reduction is quicker at higher ligand concentrations.

Similar trends were obtained between crown-chromate and glucose in methanol at 1:1 and 4:1 ligand to metal ratios. The Cr(V) peak heights are at least 20-fold higher with fructose as compared to that with glucose at any given time whereas the $t_{1/2}$ values for the decay of Cr(V) species are at least 2–3 times larger for the glucose as compared to fructose reactions. The total completion time of the Cr(VI) to Cr(III) reaction indicates that glucose needs longer time periods as compared to fructose at the same ligand to metal concentrations. This means that the fructose portions of soil components can more efficiently solubilize Cr(VI) salts as compared to their glucose counterparts. Of course the ability to transport the metal ions will depend upon the stability of the active and soluble Cr(V) species and the rate of conversion of the reaction depends upon the lability of this complex. Therefore while the lability of the intermediate species is the key aspect in chromate reductase activity related to cellular systems, the stability of the intermediate species is important from the environmental pollution and toxicity aspects. The observed $t_{1/2}$ values of the Cr(V) species with saccharide ligands are large when compared to the cysteine reactions and this strongly suggests that the slow step in the former is the conversion of the Cr(V) species to the final Cr(III) product.

The logarithmic plots in all the cases deviate from linearity at longer time periods, therefore, the $t_{1/2}$ values presented here are from the best straight line fit obtained after excluding such points. However, the nonlinearity found at longer time periods indicates that the decay process is more complex than simple first order.

3.2 *Absorption spectroscopy*

3.2a *Cysteine–ethyl ester reactions*: Spectra were taken for the reactions with crown-chromate and crown-dichromate at different ligand to metal ratios over one to three weeks. The trends observed are similar in nature. The spectral changes, in methanol solutions, include the disappearance of the 265, 350 and 440–450 nm bands which are originally present in crown Cr(VI) salts and the appearance of a new 575–580 nm broad band with high frequency shoulders around 650 and 695 nm. Representative spectra are shown in figure 6 for crown-chromate and cysteine ester at 1:1 ratio.in methanol. The low intensity, high wavelength bands (400–900 nm) were measured at 10-fold higher concentrations than in the corresponding low wavelength region and these are shown in the inset (figure 6). The shoulder observed around 430 nm in crown-chromate has shifted to 415–420 nm and become more pronounced with a large decrease in its optical density due to the cysteine reaction. This shoulder, in fact, is present even in the spectra of Cr(III)-cysteine complex isolated from a synthetic reaction. This and the appearance of the new 575–80 nm broad band seem to be characteristic of a distorted octahedral Cr(III) complex with a rather unsymmetrical coordination sphere. The intensity of the 350 nm band decreases with time and finally vanishes. The $t_{1/2}$ values for this band are found to be smaller for high ligand to metal concentrations. These values are 9·3, 4·8 and < 1 min for cysteine to chromate ratios of 0·5:1, 1:1 and 2:1 respectively. Further, the dichromate–cysteine reactions

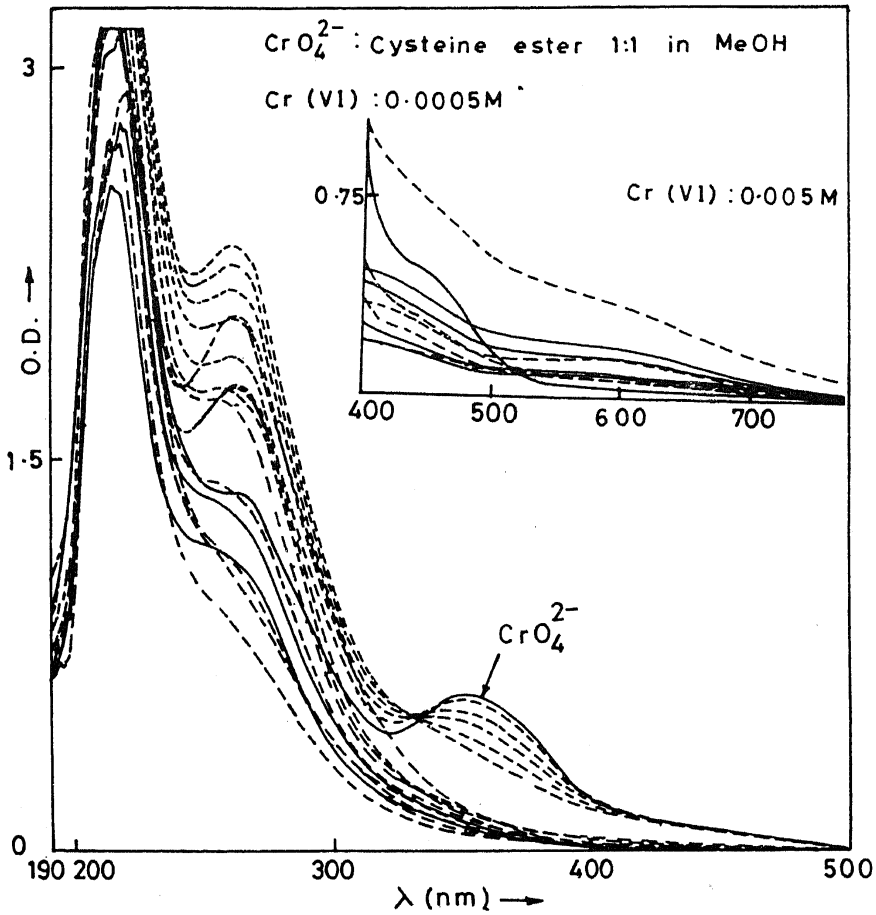

Figure 6. Absorption spectra of 1:1 methanol solution of cysteine ester and crown-chromate as a function of time. Main: Cr(VI) concentration of 0·0005 M, 190 to 500 nm; Inset: Cr(VI) concentration of 0·005 M, 400 to 800 nm.

indicated $t_{1/2}$ values of 13·5 h, 9·4 h, 22·4 min and 5·8 min, respectively, for 1:1, 2:1, 4:1 and 8:1 ratios. The rather long life times observed with dichromate indicates the presence of two chromium centres. At high ligand concentrations, the decrease in the intensity of 350 nm band parallels the increase in the intensity of the newly formed 580 nm band. Based on the features of this 350 nm band, we were able to determine the optimal ligand concentration to be more than 2 equivalents in the case of chromate and over 4 equivalents in the case of dichromate reactions. This suggests that the cysteine ligand acts at least as a bidentate one where coordination occurs through both the S and the N atoms. Similar spectral characteristics have been found by Hojo and coworkers in case of reaction of sodium chromate with penicillamine (Hojo *et al* 1977).

During the course of the cysteine–ethyl ester reaction, the intensity of the 265 nm band initially increases for a short period and later decreases. During this decrease, the band shows a blue shift and becomes a shoulder to the 215 nm UV band and thereby complicates the quantitative analysis. Further, these changes seem to be limited to only cysteine. Ascorbic acid or saccharide reactions do not show any changes thus indicating the specific reactivity of the thiol group. The peak optical density value is large for high ligand equivalents and the crude decay values of this band (as it shifts in position and also becomes a shoulder) indicate that as the ligand concentration increases the $t_{1/2}$ values for this band decrease both in methanol and water. Also, it is

noticed that the intermediate species responsible for this band are less stable in water than in methanol. Thus the observations indicate that the intermediate species may have a thiol ligand in its coordination sphere.

3.2b *Reactions with ascorbic acid*: Reactions with ascorbic acid are conducted in water at ligand to metal ratios of 0·5:1, 1:1 and 2:1 with potassium chromate. Both the 270 and 370 nm bands decrease in intensity at roughly similar rates as can be seen from the $t_{1/2}$ values. These are 15·3, 5·0 and 2·3 h for the 270 nm band and 16·2, 5·4 and 2·8 h for the 370 nm band, respectively, for the same ligand to metal ratios. A well-pronounced band around 420 nm exists in the final spectra, at all ligand concentrations, and can be obtained relatively easily, in addition to the new 570 nm band, in spectra recorded at 0·005 M metal concentration. The latter band is broad in nature and is associated with a high frequency shoulder around 660 nm and all of this is indicative of octahedral coordination. Actually the increase in the intensity of the 570 nm band parallels the decrease in the intensities of the 270 and 370 nm bands. The data indicate that more than two equivalents of ascorbic acid are needed for the complete conversion and chelation of Cr(VI) in its final Cr(III) form. The absorption spectra measured with ascorbic acid do not indicate clearly any peak that can be attributed to the Cr(V) species, which was noted by us based on EPR spectroscopy.

3.2c *Reactions with sodium ascorbate*: The reactions conducted between potassium chromate and sodium ascorbate in water exhibit absorption spectral trends which are parallel to those of the ascorbic acid reactions. The $t_{1/2}$ values for the 370 nm band are 2·3 h, 5·8 min and < 1 min while for the 270 nm band they are 2·2, 1·4 and 0·6 h, respectively, for ascorbate to chromate ratios of 2:1, 4:1 and 8:1. Based on the data from the 370 nm band, the optimal ligand concentration for the completion of the reaction is estimated to be around 4 equivalents. The new band is observed around 575 nm in this case.

3.2d *Reactions with saccharides*: Crown-chromate reactions with fructose in methanol exhibit a 580 nm band for the Cr(III) product with similar characteristics as those found in other cases. Characteristic spectra are shown in figure 7 for the reaction in methanol of crown-chromate and fructose in 1:4 ratio. The inset shows the spectra at high concentration. The quantitative trend of the 580 nm band intensity suggests that the complete conversion of Cr(VI) to Cr(III) in methanol can be achieved with about four fructose equivalents. Crown-chromate reactions with D-glucose and D-galactose exhibit similar spectral changes except that the rate of conversion is rather low. Further the reactions carried out with crown-dichromate and saccharides in methanol show that a concentration of roughly double the number of equivalents of ligand is needed for the completion of the reaction as compared to that for the corresponding crown-chromate reactions. Thus this result is consistent with the presence of two chromium centres in crown-dichromate.

3.3 *Isolation and characterization of species*

3.3a *Cr(V)-saccharide complexes*: The Cr(V)-saccharide solid complexes isolated from the reactions of chromate-crown and saccharides (D-glucose, D-galactose and D-fructose) in 1:1 ratio exhibit characteristic Cr(V) EPR signals ($g = 1{\cdot}977$ and a

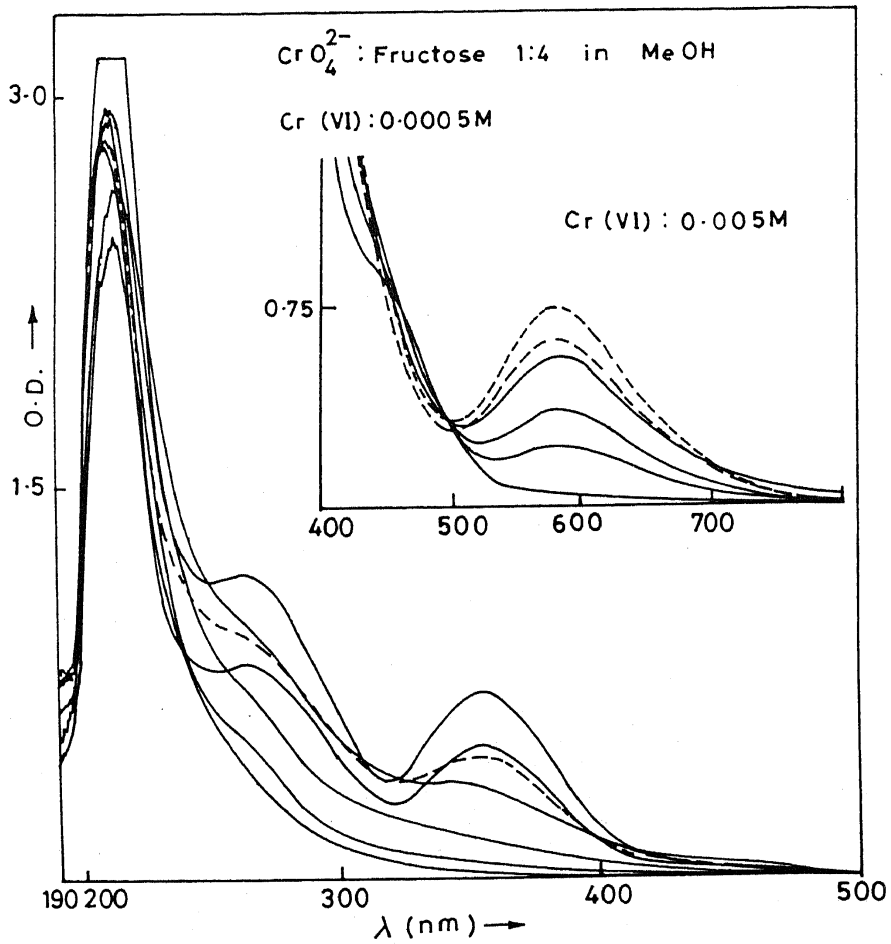

Figure 7. Reaction between fructose and crown-chromate in 4:1 ratio in methanol. Other details are same as in figure 6.

hyperfine spacing of 17·5–18 G) both in the solid state and in methanol solution. When these solids are stored at 0°C over a month, EPR signals from Cr(V) are still found and hence the complexes are stable. The absorption spectra also confirm this result.

3.3b *Cr(III)-saccharide complexes*: The Cr(III)-saccharide complexes isolated from reactions between crown-chromate and saccharide (D-glucose, D-galactose and D-fructose) in ratios of 1:4 show characteristic broad Cr(III) EPR spectra. The absorption spectra are fully consistent with these observations. Those bands are quite different from those of the Cr(III) aquo species. Further, these synthetic reactions indicate that less than four equivalents of saccharide are sufficient to completely convert the chromate salt to the Cr(III)-saccharide complex. The Cr(III)–D-glucose complex isolated from aqueous reaction mixtures containing 20 equivalents of ligand also exhibit similar spectral characteristics.

3.3c *Cr(III)–L-cysteine-ethyl ester complex*: The purple solid that is isolated from a crown-chromate to cysteine (1:8) reaction shows broad EPR spectra consistent with the Cr(III)–cysteine reaction spectrum. The absorption spectra indicate complete reduction to Cr(III) and also show characteristic Cr(III)-cysteine bands suggesting an octahedral environment in the final complex.

4. Conclusions

EPR spectra used in this study to understand Cr(VI) to Cr(III) conversion provide characteristic and conclusive evidence for the presence of Cr(V) and Cr(III) species in all the reactions carried out. Absorption spectra support this inference. The study provides optimal ligand concentrations for reducing Cr(VI) and complexing the final Cr(III) product. The reducing capabilities of different agents follow the trend: cysteine > ascorbic acid > saccharides. Among the saccharides studied, D-fructose is the most efficient reductant. The reductions are found to be slow in aqueous solutions as compared to the corresponding reactions in methanol. The stability of the Cr(V) species is dependent upon the complexing and chelating capacities of the reducing agents. Therefore, the Cr(V) species formed in the presence of cysteine ester are the least stable and those formed in the presence of saccharides are the most stable.

The effective reducing capabilities found in the present studies with cysteine and ascorbic acid indicate the reasons for long standing clinical observations of chromate dermatitis caused by these reducing agents. Further, the long life-times observed for the Cr(V) species complexed with saccharides may explain their role in the transportation of metals through such active species and their entry into the ecological and biological cycles. The intermediate- and product-isolation aspects are encouraging and well-supported through the present study. Thus, the present study provides positive scope for a thorough understanding of chromate reductase activity and its detoxification aspects.

Acknowledgements

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References

Branca M, Micera G and Dessi A 1988 *Inorg. Chim. Acta* **153** 61
Boyko S L and Goodgame D M L 1986 *Inorg. Chim. Acta* **123** 189
Connett P H and Wetterhahn K E 1983 *Struct. Bonding (Berlin)* **54** 93
Goodgame D M L and Roy A M 1986 *J. Inorg. Biochem.* **26** 219
Goodgame D M L and Roy A M 1987a *Inorg. Chim. Acta* **135** 115
Goodgame D M L and Roy A M 1987b *Inorg. Chim. Acta* **135** L5
Hojo Y, Suguira Y and Tanaka H 1977 *J. Inorg. Nucl. Chem.* **39** 1859
Kitagawa S, Seki H, Kametani F and Sakurai 1988 *Inorg. Chim. Acta* **152** 251
Krumple M, DeBoer B G and Rocek J 1978 *J. Am. Chem. Soc.* **100** 145
O'Brien P, Barret J and Swanson F 1985 *Inorg. Chim. Acta* **108** L19
Tsapakos M J and Wetterhahn K E 1983 *Chem. Biol. Interactions* **46** 265