

COMMONWEALTH DEPARTMENT OF HEALTH



# Australian Radiation Laboratory

Quality Assurance of Chromium ( $^{51}\text{Cr}$ ) - EDTA

by

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## ABSTRACT

An efficient method for the quality assurance of chromium ( $^{51}\text{Cr}$ ) - EDTA is described. The method differentiates between the EDTA complex and two possible contaminants:- chromate and chromic ions.

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Methods adopted for the Quality Assurance of Chromium (<sup>51</sup>Cr) - EDTA.

## 1. INTRODUCTION

Chromium ( $^{51}\text{Cr}$ ) - EDTA injection is used in nuclear medicine for the determination of glomerular filtration rate. This report outlines the methods used at the Australian Radiation Laboratory for the quality assurance testing of this product in the ARL monitoring program and also describes the development of the tests used for radiochemical purity and EDTA content of the preparation.

## 2. METHOD DEVELOPMENT

Testing of the preparation for radionuclide content and identity, radionuclidic purity, particulate matter, pH and the presence of benzyl alcohol was carried out by standard laboratory procedures (ARL TR034). Suitable procedures were required for the determination of radiochemical purity and the measurement of the EDTA content.

### 2.1 Radiochemical Purity

Chromate and chromic ion (possibly hydrolysed) were considered to be the likely contaminants of chromium-EDTA preparations and low voltage electrophoresis (LVE) and paper chromatography were investigated as means of achieving separation of the various components. All electrophoretic and paper chromatographic studies were performed using Whatman No. 1 chromatography paper. Following component separation all paper strips were dried and scanned using a Packard Model 7220/21 Radiochromatogram Scanner. Component peaks could be identified and were then cut out and their activity determined by counting in a Packard Auto-Gamma Scintillation Spectrometer.

LVE using a variety of buffers was found to be unsatisfactory due to difficulties in separating the chromate ion from the Cr-EDTA complex. The most promising buffer for LVE was 0.05M barbital pH 8.6 (30 minutes at 400V). With this system the chromic ion remained at the point of application while chromate ion and Cr-EDTA migrated 9cm and 6.5cm respectively.

Several paper chromatographic system were examined for the separation of  $\text{Cr}^{3+}$ ,  $\text{CrO}_4^{2-}$  and Cr-EDTA. While separation of chromate and Cr-EDTA from chromic ion could be readily achieved, it was difficult to obtain a clear cut separation of  $\text{CrO}_4^{2-}$  and the Cr-EDTA complex which tend to run close

together. The introduction of the lead acetate band on the paper strip overcame this problem by retarding  $\text{CrO}_4^{2-}$  as the highly insoluble lead chromate. In the method finally adopted, a lead acetate band was prepared about 5 cm from the origin of the paper chromatogram which was developed with 95% ethanol: water: concentrated ammonia solution (2:5:1). Chromic ion remains at the origin while chromic - EDTA moves with the solvent front. Chromate ion is retained by the lead acetate band.

In order to confirm the accuracy of the proposed method, standards were prepared in which known amounts of sodium chromate ( $^{51}\text{Cr}$ ) and chromic ( $^{51}\text{Cr}$ ) chloride were added to both AAEC and RCC chromium-EDTA. The radiochemical composition of these standards was then measured using the proposed method. Results are shown in Tables 1 and 2.

Sample No.	$^{51}\text{Cr}$ Chromate added	$^{51}\text{Cr}$ Chromate found	Sample No.	$^{51}\text{Cr}$ Chromic added	$^{51}\text{Cr}$ Chromic found
A1	26.7	25.2	A2	23.6	5.2
B1	4.8	3.7	B2	3.2	0.7
C1	3.2	3.3	C2	1.7	0.5

Table 1. Radiochemical composition of mock standards prepared by adding  $^{51}\text{CrCl}_3$  and  $\text{Na}_2^{51}\text{CrO}_4$  to AAEC Cr-EDTA.

Sample No.	$^{51}\text{Cr}$ Chromate added	$^{51}\text{Cr}$ Chromate found	Sample No.	$^{51}\text{Cr}$ Chromic added	$^{51}\text{Cr}$ Chromic found
D1	28.4	6.4	D2	26.1	15.2
E1	4.4	0.7	E2	4.0	1.8
F1	2.6	0.8	F2	2.3	1.3

Table 2. Radiochemical composition of mock standards prepared by adding  $^{51}\text{CrCl}_3$  and  $\text{Na}_2^{51}\text{CrO}_4$  to RCC Cr-EDTA.

With both the AAEC preparation and the RCC preparation, the % chromic ion found was less than that calculated from the chromic chloride added. This result is to be expected as both preparations contain uncomplexed EDTA which would react with free chromic ion to form Cr-EDTA. Formation of Cr-EDTA from hydrolysed chromic ion would be unfavourable kinetically.

Results obtained for chromate added to the RCC preparation however were far lower than expected. It thus appears that the RCC formulation is capable of reducing chromate ion to chromic ion which is subsequently complexed by the excess EDTA present. If sodium chromate carrier is added to the sodium chromate ( $^{51}\text{Cr}$ ) solution prior to the preparation of the standards, then the values found agree with those calculated (Table 3).

Sample No.	% Chromate Added	% Chromate found
G1	30.3	29.5
H1	5.4	5.4
K1	3.2	3.2
L1	1.1	1.2

Table 3. Radiochemical composition of mock standards prepared by adding  $^{51}\text{CrO}_4$  containing 5mg/mL  $\text{Na}_2\text{CrO}_4$  to RCC  $^{51}\text{Cr}$ -EDTA.

Table 4 gives the results for the radiochemical purity of several batches of chromium-EDTA ( $^{51}\text{Cr}$ ) obtained from different sources. It can be seen that the method gives a good separation of the three ions localising all chromic ion activity at the origin and all chromate ion activity at the lead acetate strip. Chromium - EDTA moves to the solvent front and is well separated from these contaminants.

Sample	% Activity at origin	% Activity at Lead Acetate strip	% Activity at Solvent Front
Chromic ( $^{51}\text{Cr}$ ) chloride	99.5	0.5	0
Sodium chromate ( $^{51}\text{Cr}$ )	0.6	99.2	0.2
Chromium-EDTA ( $^{51}\text{Cr}$ ) AAEC - Batch 10	0.3	2.6	97.2
Chromium-EDTA ( $^{51}\text{Cr}$ ) RCC - Batch 8	0.5	1.1	98.4
Chromium-EDTA ( $^{51}\text{Cr}$ ) RCC - Batch 9	1.0	0.5	97.9
Chromium-EDTA ( $^{51}\text{Cr}$ ) AAEC - Batch 11	0.1	0.6	99.2

Table 4. Distribution of radioactivity of various radiopharmaceuticals on Whatman 1 chromatography paper containing a lead acetate strip.

Solvent:- 95% Ethanol: Water: concentrated ammonium hydroxide (2:5:1).  
All results are the mean of three papers.

## 2.2. Total EDTA and Uncomplexed EDTA by UV Spectrophotometry

Copper and cobalt were examined as possible agents for the measurement of EDTA content because of their high stability constants and the ease with which they form EDTA complexes. However, use of these agents was ruled out because of possible interference from benzyl alcohol which absorbs strongly in the region of their absorption maxima. Chromium - EDTA which has a high stability constant and has an UV absorption maximum (560 nm) removed from benzyl alcohol, was investigated and found to be a suitable complex for the determination of EDTA content. While its use required heating to produce the Cr-EDTA complex, it did have the advantage of also enabling the simultaneous measurement of the chemical content of Cr-EDTA in the preparation.

UV absorption spectra for Cr-EDTA and  $\text{Cr}^{3+}$  in pH 5.0 acetate buffer are shown in figure 1. Standard curves were drawn for wavelengths varying from 490-520 nm (Figure 2). The wavelength of 520 nm was selected for EDTA determination since at this wavelength hydrated chromic ions ( $\lambda_{\text{max}}$  575 nm) have minimal absorption while the Cr-EDTA complex ( $\lambda_{\text{max}}$  560 nm) still absorbs strongly enough to produce a satisfactory calibration line.

The spectrophotometric determination of total EDTA and uncomplexed EDTA in two commercial preparations is illustrated in Table 5. From the standard curve obtained at 520 nm (Figure 3) the complexed and uncomplexed EDTA are calculated to be as shown in Table 6.

These results may be compared with the values calculated from product specifications given by the manufacturer.

Manufacturer	Total EDTA (mg/mL)	Complexed EDTA (mg/mL)
AAEC	8.5	1.4
RCC	Not given	0.56

The RCC preparation must thus contain about 1.6 mg/mL EDTA in excess.

Sample	Absorbance (520nm)
Standard EDTA 5 mg/mL	0.264
10 mg/mL	0.525
15 mg/mL	1.788
(i) AAEC Cr-EDTA	
With excess chromic chloride	0.431
No added chromic chloride	0.105
(ii) RCC Cr-EDTA	
With excess chromic chloride	0.115
No added chromic chloride	0.037

Table 5. Determination of complexed and uncomplexed EDTA by UV spectrophotometry.

Sample	Total EDTA mg/mL	Complexed EDTA mg/mL
AAEC Cr-EDTA	8.2	2.0
RCC Cr-EDTA	2.2	0.7

Table 6. Complexed and uncomplexed EDTA in AAEC and RCC preparations. Calculated from data in Table 5.

#### ACKNOWLEDGEMENTS

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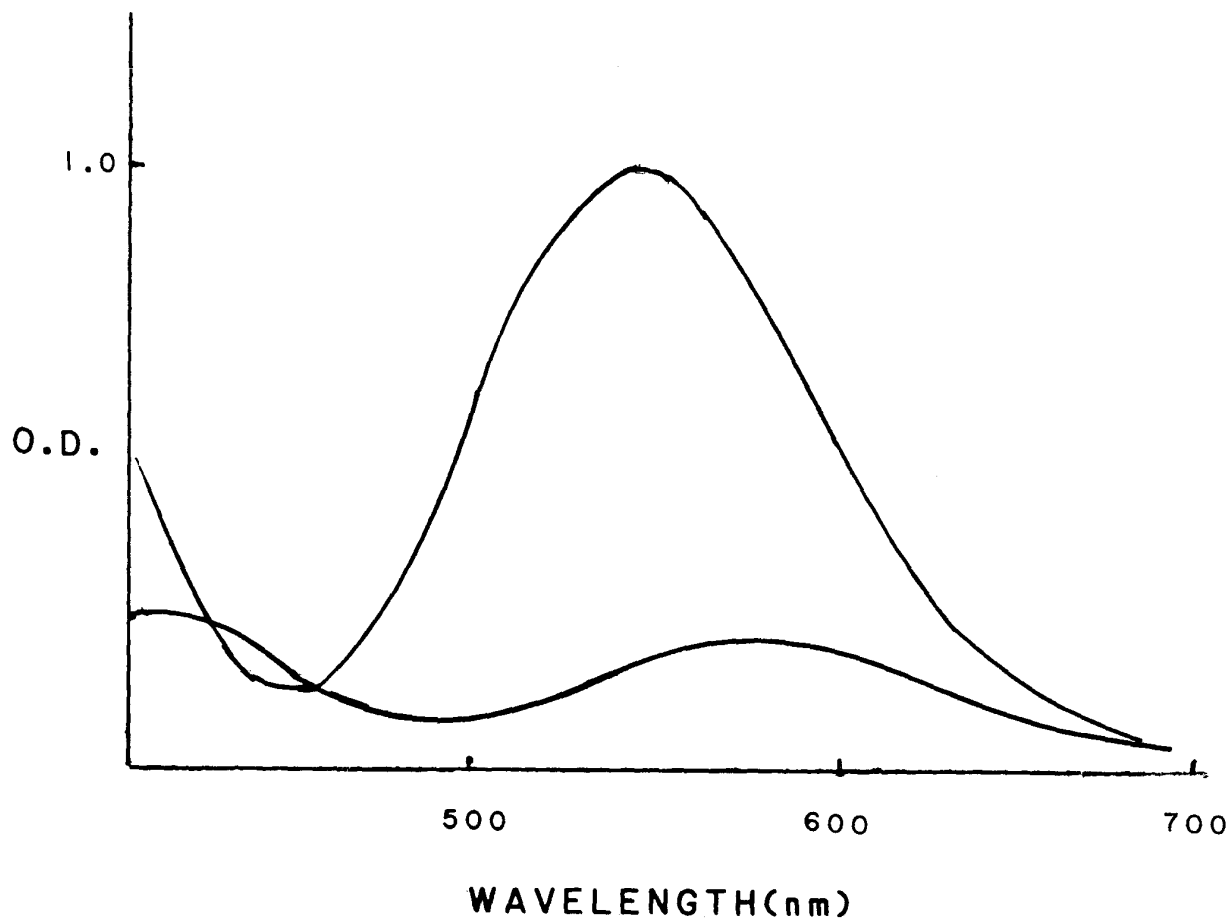


Fig. 1

UV ABSORPTION CURVES FOR Cr-EDTA (3 mg/mL) AND CrCl<sub>3</sub> (4 mg/mL) in  
pH 5.0 ACETATE BUFFER

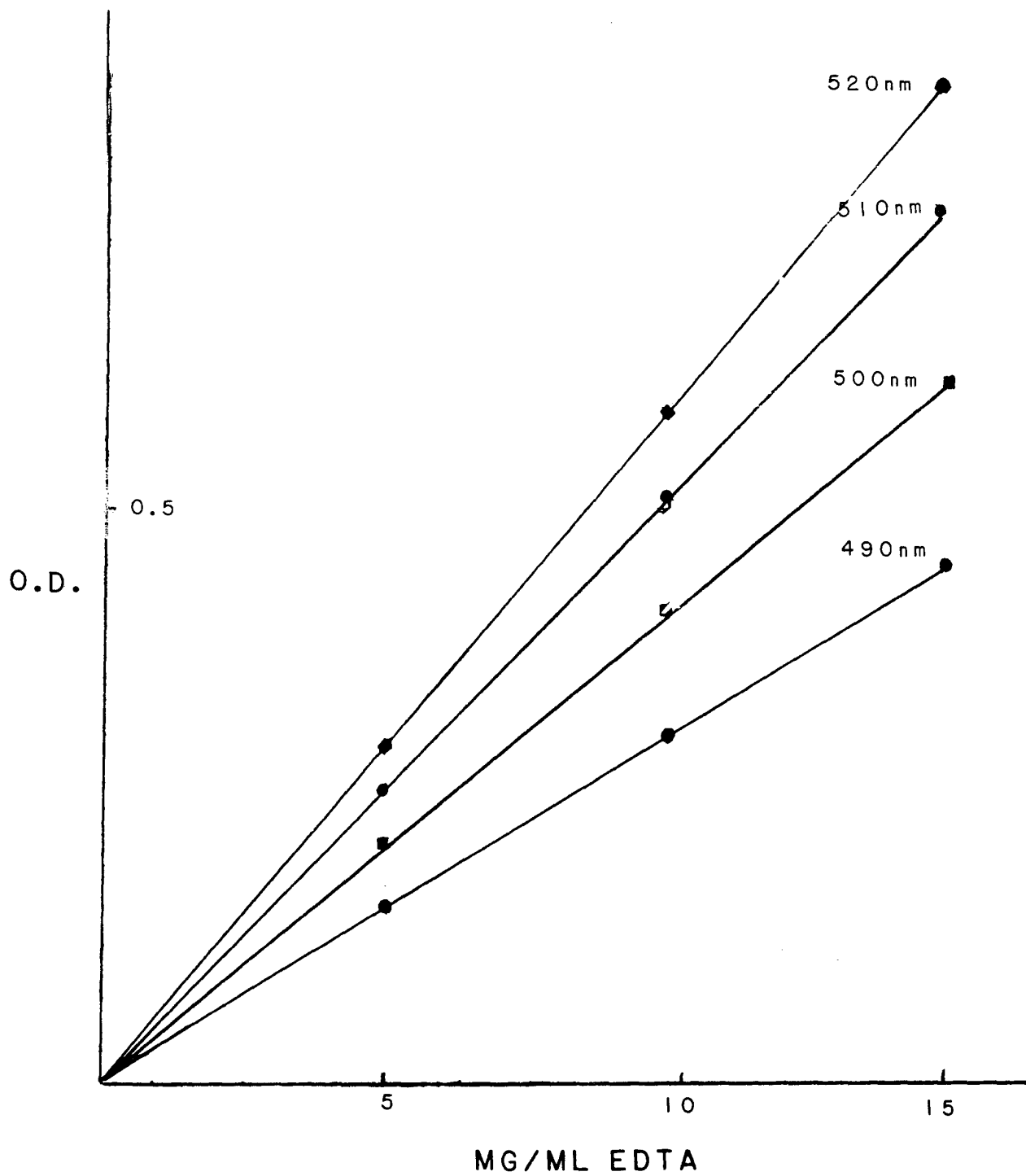


Fig. 2  
STANDARD CURVES FOR SOLUTIONS CONTAINING 0-15 mg/ml Na<sub>2</sub> EDTA AT  
VARYING WAVELENGTHS

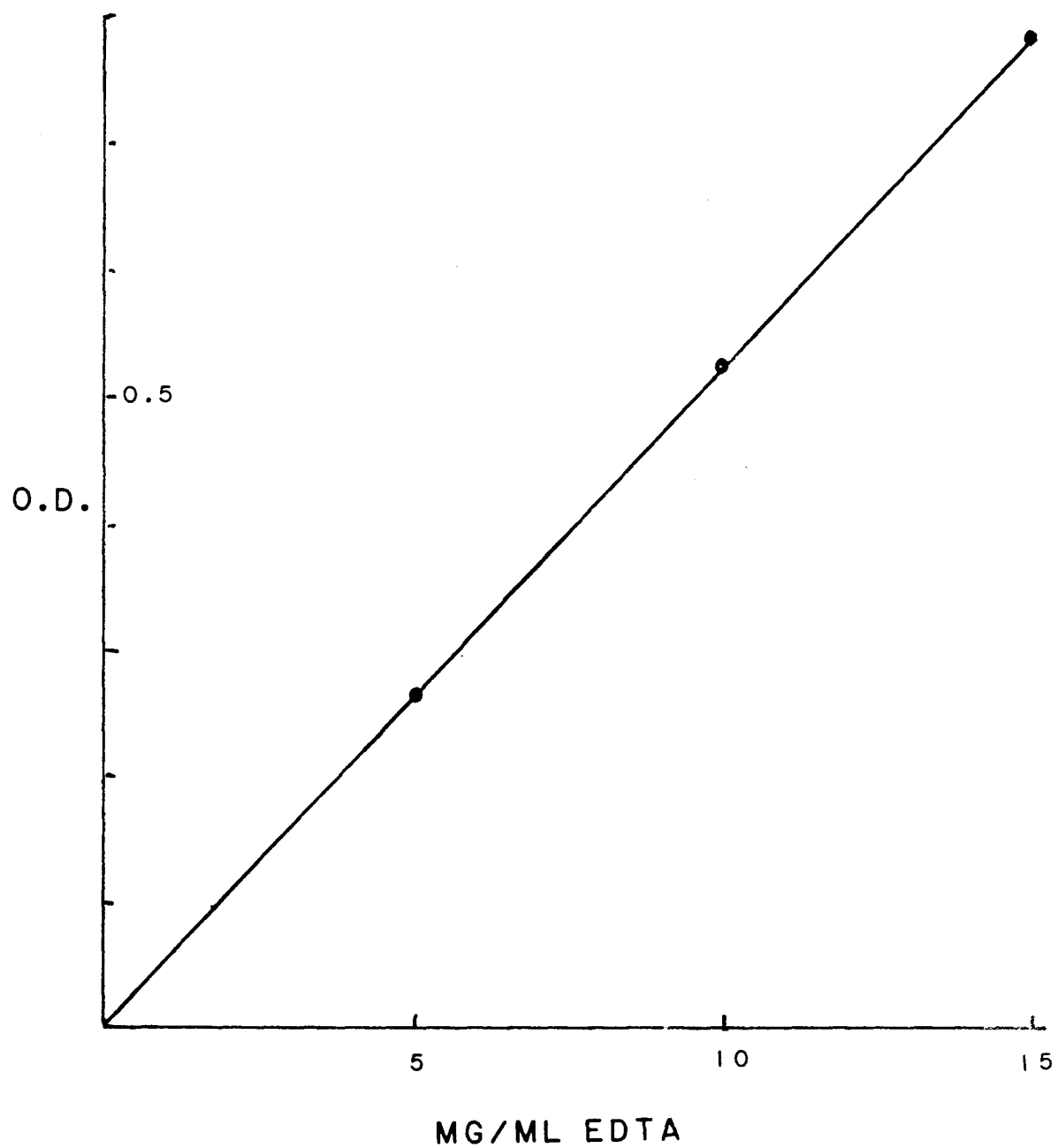


Fig. 3

STANDARD CURVE FOR THE ASSAY OF EDTA AND Cr-EDTA AT 520 NM

## APPENDIX I

### Methods adopted for the Quality Assurance of Chromium ( $^{51}\text{Cr}$ ) – EDTA

#### 1. Radionuclide Content

Measure the radionuclide content of the unopened vial using the CRC-2N Capintec Dose Calibrator.

#### 2. Radionuclide Identity

Record the gamma spectrum of a 1-2  $\mu\text{Ci}$  sample using either a NaI(Tl) or a Ge(Li) detector. The spectrum is identical to that of a standard Chromium-51 spectrum showing only the 320 keV characteristic gamma ray of chromium-51.

#### 3. Radionuclidic Purity

Determine the radionuclide purity of a 5-10  $\mu\text{Ci}$  sample using a Ge(Li) detector.

#### 4. Particulate Matter

Examine the sample against both black and white backgrounds for the presence of particulate matter. No particles visible to the human eye should be present.

#### 5. pH

Measure the pH of the solution using either a pH meter or narrow range pH paper.

## 6. Benzyl Alcohol

Determine the benzyl alcohol content using high pressure liquid chromatography.

Column -  $\mu$  Bondapak C18  
Solvent - 50 % methanol.

Measure the peak area of the benzyl alcohol peak produced following the injection of a 10–20  $\mu$ l sample. Compare the peak area to that produced by the injection of the same volume of a standard solution of 0.05 % benzyl alcohol in saline.

Calculate the benzyl alcohol content of the preparation.

## 7. Radiochemical Purity

Cut three 40 cm strips of Whatman No. 1 chromatography paper. Mark the origin on each strip and at a distance of 5 cm from the origin, pipette a thin band (<0.5 cm width) of freshly prepared 5% lead acetate. Allow the paper to dry in a drying oven prior to use.

Apply a 2–5  $\mu$ l spot of the solution to be tested on the origin and develop the paper by ascending chromatography using freshly prepared 95 % ethanol: water: concentrated ammonia solution (2:5:1). After the solvent has moved 15–20 cm, remove the paper, mark the solvent front and dry at 50°C. Scan the papers using the Packard 7220/21 Radiochromatogram scanner. Confirm that any impurity peaks are only found either at the origin or at the lead acetate strip. Chromic EDTA moves with the solvent front.

Cut each paper into three strips

- (i) 1 cm on either side of the origin - contains chromic impurity
- (ii) 1 cm on either side of the lead acetate strip - contains chromate impurity
- (iii) 3 cm on either side of the solvent front - contains chromium-EDTA

A/III

Count each segment in the Packard 5912 Auto Gamma scintillation spectrometer and calculate the percentage of the batch activity associated with each segment.

8. Determination of Total EDTA and Uncomplexed EDTA Reagents

(a) 2<sup>0</sup>/o Chromic Chloride

Dissolve 2 g AR chromic chloride in 100 mL distilled water

(b) 1M Sodium Acetate Buffer pH 5.0

Dissolve 41 g AR sodium acetate in 500 mL distilled water and adjust the pH to 5.0 using dilute acetic acid.

(c) Sodium EDTA Standard Solution

Prepare a 100 mg/mL standard EDTA solution by dissolving 12.7 g AR disodium EDTA in distilled water and making up to volume in a 100 mL volumetric flask.

Prepare standard solutions containing 5, 10 and 15 mg/mL EDTA by quantitative dilution

Dispense into individual 10 ml glass test tubes

Tubes 1-3 0.5 mL of 5, 10, 15 mg/mL EDTA standards

Tube 4 0.5 mL water as a reagent blank

Tube 5 0.5 mL of sample for total EDTA determination

Tube 6 0.5 mL of sample for complexed EDTA determination

Add 1 mL of 2<sup>0</sup>/o chromic chloride solution to tubes 1-5. Add 1 mL distilled water to tube 6. Add 2 mL of sodium acetate buffer to all tubes and heat in a boiling water bath for 5 minutes.

Allow the samples to cool and measure the absorbance of each sample at 520 nm in the UV spectrophotometer using the reagent blank in the reference cell. Draw a standard curve from the measurements of tubes 1-3. From the measured absorbance of tube 5, the total EDTA concentration (as free acid) may be read from the curve. Tube 6 gives the EDTA present as Cr-EDTA. The difference between the two values gives the uncomplexed EDTA present.

Total chromium present may be calculated from the complexed EDTA concentration.

$$\text{Cr (mg/mL)} = 0.178 \times \text{Complexed EDTA (mg/mL)}.$$