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Osmium as Reagent for Quantitative Analysis of Anti-Cancer Drug Mesna as Trithiocarbonate Derivative

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KEYWORDS

Anti-cancer drug, Mesna, Osmium, Quantitative analysis

ABSTRACT:

A sensitive and rapid spectrophotometric method was used to determine Mesna trithiocarbonate, using osmium chloride. Effect of temperature and pH on the complexation of osmium (II) with sodium2-mercaptoethanesulphonate trithiocarbonate was studied. A plot between absorbance and mole ratio of reagent shows that metal has been consumed completely at mole ratio one and further addition of ligand produces no more complexation. A good linear relationship was observed over the range 2.78x10-1 □g/ml to 5.57 □g/ml of mesna trithiocarbonate in calibration curve. The effective molar absorptivity was calculated from the slope of the calibration graph to be found 1.6x102 ml mol-1 cm-1. The relative standard deviation (RSD%) was found to be □1.16 %. The correlation coefficient value close to +1 indicates a strong direct relationship between two variables. The relationship between absorbance and concentration is very close and direct. Hence, the developed method can be utilized successfully to determine the concentration of sodium 2-mercaptoethanesulphonate trithiocarbonate and drug mesna in pharmaceutical samples by simply knowing its absorbance.

1.Introduction:

Mesna (2-mercaptoethane sulfonate) is a chemoprotectant used to prevent urothelial toxicity in patients receiving antineoplastics like ifosfamide or cyclophosphamide. Mesna has several roles be related to its use in preventing bladder toxicity during oxazaphosphorine chemotherapy. It is an important bacterial co-enzyme and has been used as a mucolytic agent. [1].Its importance lies in the high consumption of these drugs, making it crucial to detect Mesna in biological samples.[2,3,4] Osmium tetroxide (OsO4) is a powerful oxidizing agent widely used in organic synthesis and analytical chemistry[5]. Its ability to react selectively with certain functional groups makes it valuable in quantitative analysis. Mesna contains a thiol (-SH) functional group, which renders it susceptible to oxidation reactions. When Mesna reacts with osmium tetroxide, it undergoes oxidation to form a trithiocarbonate (TTC) derivative, involving the conversion of the thiol group (-SH) to a trithiocarbonate functional group (-SCO_2H). This reaction provides a basis for qualitative analysis, as the formation of the trithiocarbonate product can be detected and characterized using various analytical techniques.[6] Osmium tetroxide's selectivity in reacting with Mesna allows for specific detection even in the presence of other compounds, making it a valuable tool in pharmaceutical analysis and biomedical research related to cancer chemotherapy. However, it's essential to note that osmium tetroxide is toxic and requires careful handling and disposal procedures [7].Overall, the use of osmium tetroxide as a reagent for qualitative analysis of Mesna as trithiocarbonate offers a reliable and selective method for detecting and characterizing this anti-cancer drug, contributing to advancements in chemotherapy optimization and patient care.

It is primarily employed to reduce the toxicity of certain chemotherapeutic drugs, particularly ifosfamide and cyclophosphamide, which are known to cause bladder toxicity[8-12]. Mesna works by detoxifying the metabolites of these drugs in the urinary tract, thereby preventing bladder irritation and damage. Mesna is administered intravenously or orally in conjunction with chemotherapy drugs to minimize side effects and

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improve patient outcomes. It is well-tolerated and has been shown to effectively reduce the incidence and severity of chemotherapy-induced bladder toxicity. Overall, Mesna plays a crucial role in cancer treatment by enhancing the safety and tolerability of chemotherapy regimens, allowing patients to receive potentially life-saving treatments with reduced risk of adverse effects. The literature review involved a few analytical procedures such as spectrophotometric methods [13-14] for quantitative estimation of mesna. Mesnatrithiocarbonate (MTTC) on reacting with Os(Vlll) forms the mesnatrithiocarbonate osmium (MTTCO) complex. The developed Spectrophotometric techniques are straightforward, selective, and sensitive with no sample preparation compared to each and every documented spectrophotometric technique [15-23].

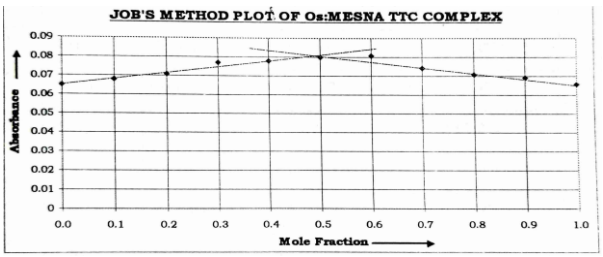
Qualitative and quantitative determination with preseperation of drug sample from excipients is not much investigated. In chemotherapy mostly formulation having combination of anticancer drugs are given to the patients. Not much analytical methods are yet available for determination of drug in combination with other drugs. Literature survey reveals that only few publications are available which are not directly linked with stated determinations [24-27]. Development of such quality test experiments which are economical, can be carried out using simple equipment, fast and result in formation of stable complex with transition metal is still a challenge. With the advancement in the field of inorganic chemistry the role of transition metal complexes as therapeutic compounds is becoming increasingly important [28-30].

2. Experimental

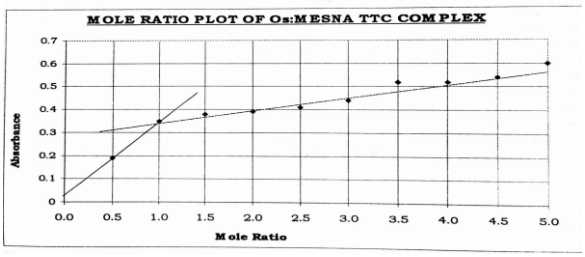
2.1 Instruments and reagents - An Elico SL-159 double-beam spectrophotometer was utilized for all UV-visible spectrophotometric measurements**.** No additional purification was done to the chemicals used for analytical quality. The FTIR spectra were captured using KBr pellets, in the 4000-400cm-1 range with the help of a Nicolet FTIR spectrometer. Tetracyanoethylene was used as a marker, and the ESR spectra were captured within the 2000 gauss scan range in a Varian ESR spectrometer. The 1H NMR spectra were captured using deuterium oxide as a solvent, on a Varian-300MHz spectrometer. Thermogravimetric analysis was done using the Dupont thermal analysis system was employed under a nitrogen atmosphere at a rate of 15 °C/min (from 0 °C to 800°C)., sodium 2-mercaptoethanesulphonate and potassium hydroxide were mixed for the preparation of MTTC equimolar amount of carbon disulphide at 0°C till a light yellow-coloured semisolid mass is obtained, then dissolved in distilled water to get a standard solution. OsO_4 (E. Merck) was dissolved in 40 ml of 0.5M Sulphuric acid in double distilled water to prepare Osmium solution. To prepare solutions of lower concentration, portions of the original standard stock solution were diluted.

2.2 Preparation of MTTCO complex – A dark brown coloured complex is created when MTTC solution at 30°C is mixed with Osmium tetroxide solution. For optimal colour development and full complex formation, 60 minutes is the minimum amount of time needed, thereafter the intensity of the colour remains constant for 23 hours. The λmax of the MTTCO complex is 505 nm.

2.3. Composition of MTTCO complex: The complex's composition was found to be 1:1 for Os(II): sodium 2-mercaptoethanesulphonate trithiocarbonate complex by the Job's approach of continuous variance and the mole ratio method. The graph was plotted and is shown in figure 1:



(a)



(b)

Figure 1: (a) Mole ratio plot and (b) Job's method of

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continuous variation plot for MTTC- Os(Vlll) complex

2.4 Procedure:

Different aliquots containing MTTC at concentrations ranging from $2.78x10^{-1}$ µg/ml to 5.57 µg/ml were prepared. An excess of a molarity-based palladium chloride solution (4.0157M) was added to each aliquot while maintaining the volume constant using double distilled water. The temperature and pH conditions were kept constant while the absorbance of each set was measured at the complex's λ max, which is 505 nm. A Beer-Lambert plot was obtained to determine the unknown concentration and validate the method.

3.Results and discussion:

The calibration curve for the MTTCO complex shows a strong linear connection across the range 2.78µg/ml to 5.57µg/ml of MTTC with ε value $1.6X\ 10^2$ ml mol^{-1} cm^{-1} and relative standard deviation (RSD%) 1.16 % as given in table 1. The regression equation has an 'a' value of 0.38 X 10-1 and a 'b' value of $1.32\ X\ 10^{-2}$. Thus the regression equation for the MTTCO complex is

$$Y = 0.38 \times 10^{-1} + 1.32 \times 10^{-2} X.$$

Also, the correlation coefficient (r) as calculated was found to be 0.97. The correlation coefficient illustrates how much of a reciprocal reliance exists between concentration and absorbance. As a result, using the suggested approach and knowing its absorbance, one may determine the concentration of MTTC.

S.No.	Weight taken in µg	Weight found in µg	Coeff of varience	RSD %	Reg Eq. Startial data*
1	2.78×10^{-1}	2.69×10^{-1}	1.26	1.16	
2	5.57×10^{-1}	5.57×10^{-1}	0.00	0.00	Slope b = 1.32
3	8.35×10^{-1}	8.32×10^{-1}	1.35	1.25	
4	1.11×10^{-2}	1.18×10^{-2}	0.88	0.62	
5	1.39×10^{-2}	1.41×10^{-2}	0.71	0.50	
6	1.67×10^{-2}	1.72×10^{-2}	0.47	0.33	
7	1.95×10^{-2}	2.01×10^{-2}	0.64	0.57	
8	2.23×10^{-2}	2.24×10^{-2}	0.63	0.50	Intercept a= 0.38
9	2.51×10^{-2}	2.55×10^{-2}	0.39	0.28	
10	2.78×10^{-2}	2.82×10^{-2}	0.45	0.40	
11	3.06×10^{-2}	3.02×10^{-2}	0.53	0.45	
12	3.34×10^{-2}	3.35×10^{-2}	0.06	0.42	
13	3.62×10^{-2}	3.68×10^{-2}	0.27	0.19	
14	3.90×10^{-2}	3.87×10^{-2}	0.15	0.10	Correlation coeffi r= 0.97
15	4.18×10^{-2}	4.24×10^{-2}	0.24	0.17	
16	4.45×10^{-2}	4.47×10^{-2}	0.38	0.34	
17	4.73×10^{-2}	4.78×10^{-2}	0.22	0.21	
18	5.01×10^{-2}	5.08×10^{-2}	0.19	0.14	
19	5.29×10^{-2}	5.30×10^{-2}	0.20	0.181	
20	5.57×10^{-2}	5.60×10^{-2}	0.17	0.12	

Table 1:Calibration data of MTTC as its Os(Vlll) complex

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The FTIR spectra of trithiocarbonate sodium2-mercaptoethanesulphonate and it's 1:1 Os (VIII) complex in solid state as KBr pellets were recorded in the range 400- 4000 cm^{-1} band at 1133.54 cm^{-1} due to C=S stretching in the spectrum of pure mesna undergoes shifting to 1009.71 cm^{-1} in the spectrum of trithiocarbonate of mesna due to conversion of sulphydryl group to trithiocarbonate group. This band completely disappears in the spectrum of Osmium (VIII) of Mesna trithiocarbonate due to complexation of TTC group with Os(VIII). Also, a stretching band at 658.55 cm^{-1} assigned to C-S stretch is present which gets shifted to 801.45 cm^{-1}after the stoichiometric derivation to trithiocarbonate which confirms the conversion of –SH of sulphydryl group into K^{+}-S-C(S)-S-R. in mesna mesna TTC spectra and the –S=O band acquire more localized double bond character and concequently its frequency changes. This small shift of symmetrical and asymmetrical band indicates that lone pair of electrons on sulphur atom are not involved in bonding with central metal ion.As a result to symmetric and asymmetric stretching of –S=O in Mesna TTC are seen at 1049.58 cm^{-1} and 1187.93 cm^{-1} in the FTIR spectra of Mesna TTC :Os (VIII) complex can be seen in figure 2.

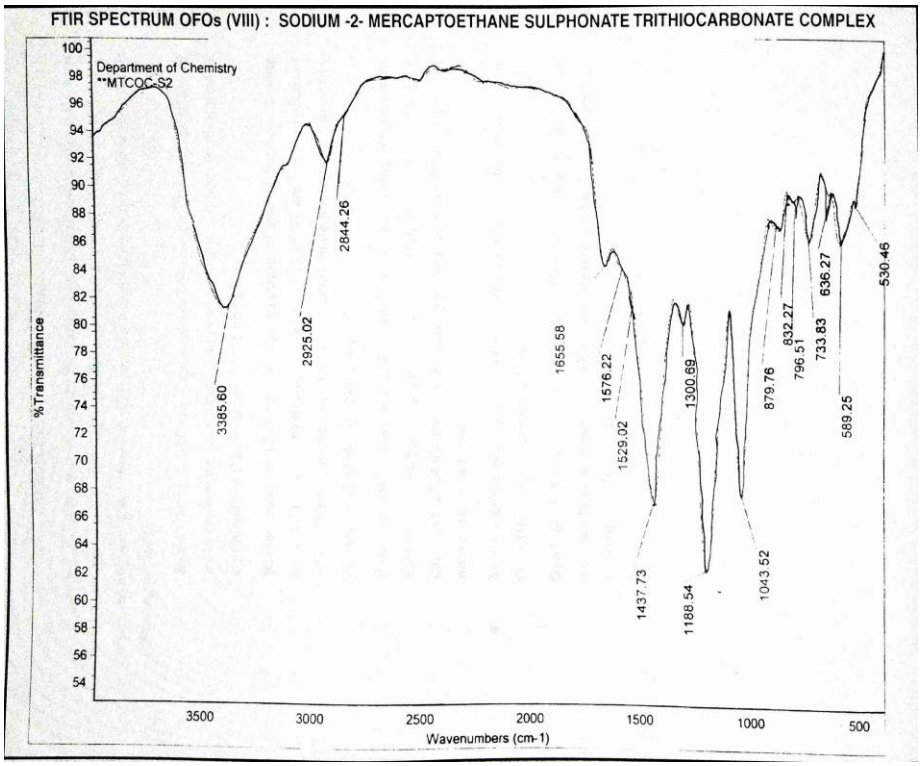

Figure 2:FTIR spectra of MTTCO complex

The ^{1}H NMR spectra of pure sodium sodium2-mercaptoethanesulphonate trithiocarbonate and its 1:1 complex with Os (VIII) recorded at 299.9 MHz in D_2O .MTTC exhibit an NMR signal at 2.77 ppm by S-CH_2 proton which shows a downfield shift MTTCO complex to 3.09 ppm. Chemical shift at 2.6 ppm attributed to the disulphide protons of trithiocarbonate group disappeared completely in its Os (VIII)complex confirming the binding between Osmium (VIII) and Sulphur atom of trithiocarbonate group as can be seen in figure 3.

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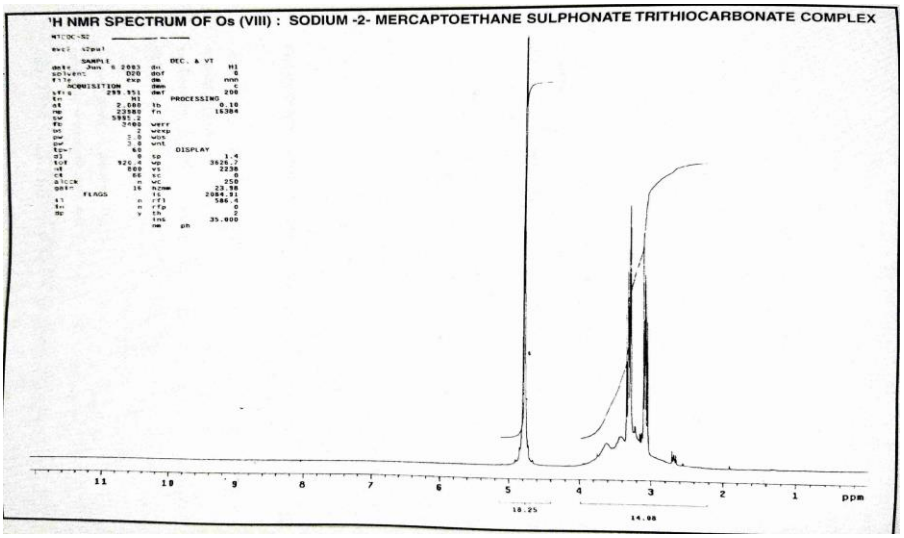

Figure 3: 1H NMR spectra of MTTCO complex

At liquid nitrogen temperature, the electron spin resonance spectra of MTTC and its 1:1 Os(Vlll) complex were recorded . Mesna's diamagnetic nature is confirmed by the absence of a resonance peak in its ESR spectra. But its trithiocarbonate gives an ESR signal at 1400 gauss at liquid nitrogen temperature, which indicates paramagnetism. The Lande's splitting factor "g" of the complex as calculated is 2.0023 indicating the presence of a free electron in the MTTC moiety whereas the MTTCO complex exhibits no ESR signal which confirms its diamagnetic nature.It can be seen in figure 4.

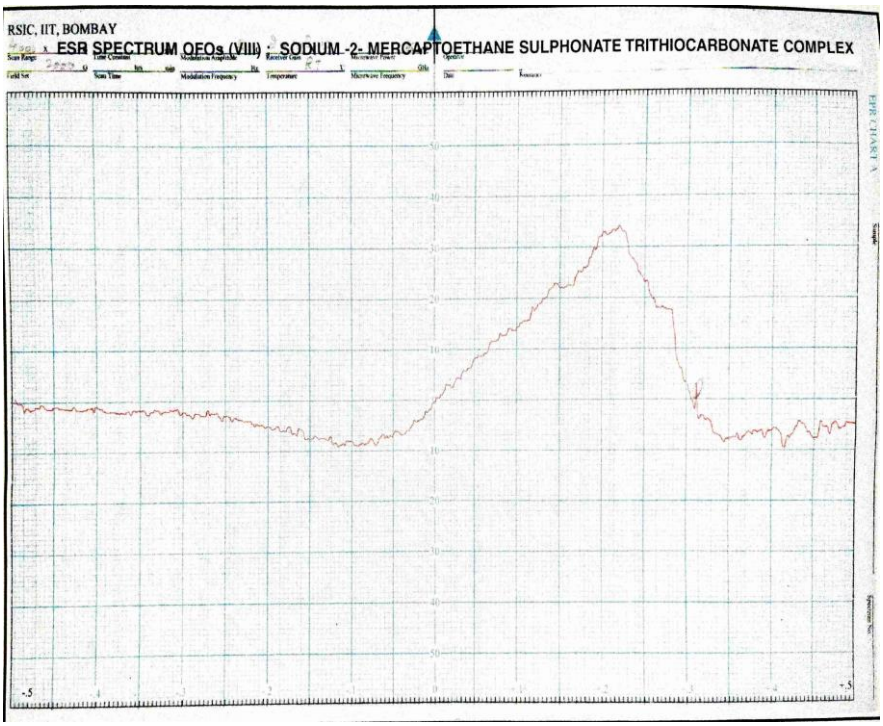

Figure 4: ESR spectra of MTTCO complex

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Thermogravimetric analysis of MTTCO complex conducted at a speed of 15 degrees per minute in a nitrogen atmosphere in the temperature range 0^{o}C to 100^{0}C shows weight loss at 168.32^{0}C due to loss of lattice water molecules. There is no weight loss after 396.44^{0}C and remain constant upto 800^{0}C.This breaking of MTTC can be seen in Figure 5.

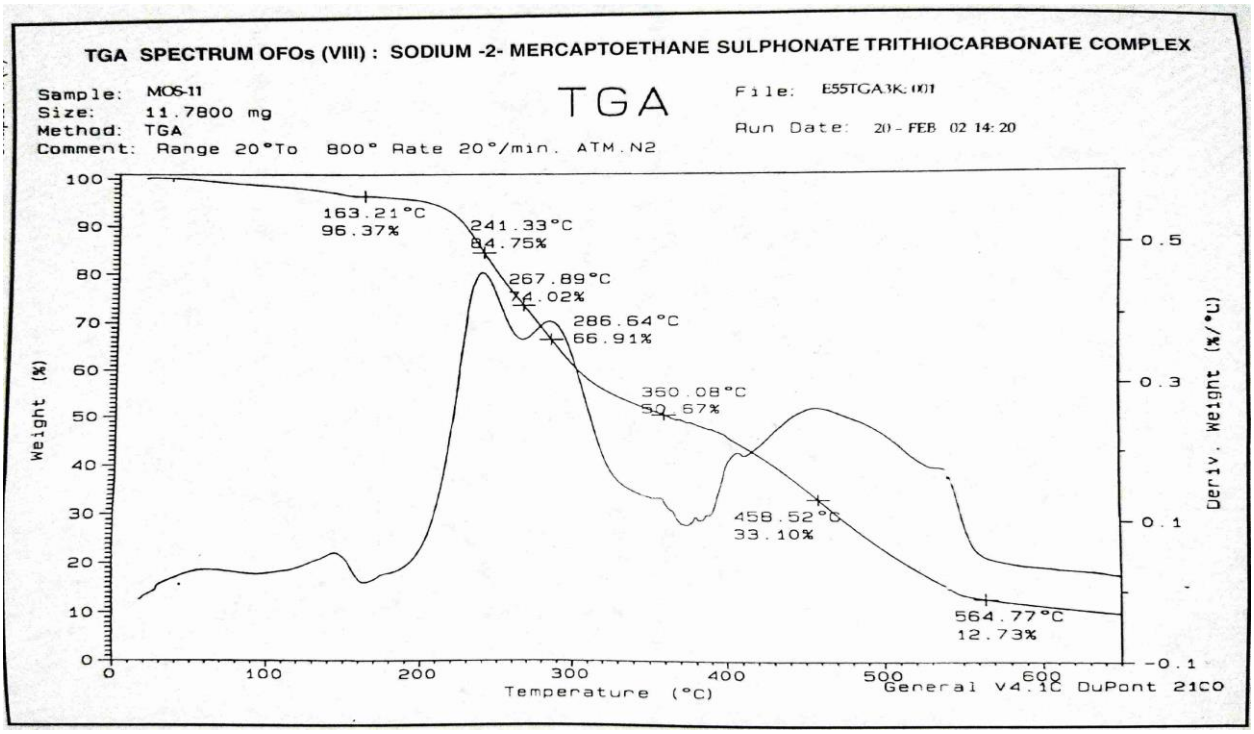

Figure 5. TGA spectra of MTTCO complex

The complex's thermal analysis (TGA) further validates the stoichiometry and the complex's geometry. Based on the aforementioned research, a complex structure has been suggested which can be seen in Figure 6.

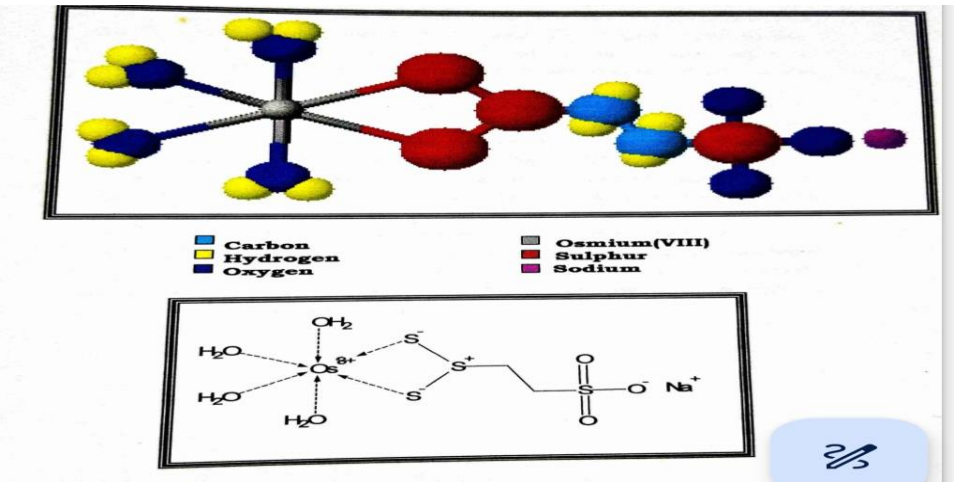

Figure 6: Structure of complex

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4. Conclusions :

An attempt was made to synthesize a trithiocarbonate derivative of commercial drug sample sodium 2-mercaptoethanesulfonate (brand name Mesna) in this research work. The synthesized trithiocarbonate derivative was used as a ligand to prepare the Os(Vlll) complex quantitatively. Calibration data for the MTTCO complex was then determined. The complex's FTIR spectrum shows sulphur atom binding of trithiocarbonate ensemble with Osmium(Vlll). H1 NMR studies of the complex in D_2O further confirm the involvement of the sulphur atom of the trithiocarbonate group in complexation with Os(Vlll). The absence of a signal in the complex's ESR spectrum shows the diamagnetic nature of the complex. TGA study confirms the structure of the MTTCO complex. The synthesized ligand MTTC, according to the results binds to the S, S donor sites of trithiocarbonate in a bidentate manner with metal ions. The proposed method is simple, precise, less time-consuming and economical than earlier proposed methods for quantitative estimation of drug mesna in commercial samples with basic equipment.

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