



Study of Oxidative cleavage of L-cysteine with tert-butyl chromate (TBC)

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Abstract:

The oxidative cleavage of L-cysteine, a biologically important sulfur-containing amino acid, was investigated using tert-butyl chromate (TBC) as a chromium (^{VI})-based oxidizing agent under homogeneous conditions. The study examined the influence of solvent environment on oxidation efficiency, reaction rate, and product formation. Reactions were carried out in dichloromethane, acetone, benzene, n-hexane, 1,4-dioxane, ethanol, and isopropyl alcohol. The proposed oxidation pathway involves initial electron transfer from the thiol group to Cr (^{VI}), followed by formation of sulfur-oxidized intermediates and subsequent cleavage. Polar aprotic solvents, particularly acetone and dichloromethane, generally promoted efficient oxidation, whereas non-polar solvents showed comparatively lower reactivity. Reaction progress and products were evaluated using TLC, UV-Visible, FTIR, NMR, and GC-MS techniques. The findings demonstrate that solvent polarity and solute-solvent interactions significantly influence TBC-mediated oxidative cleavage and provide useful mechanistic insight into chromium (^{VI})-induced oxidation of sulfur-containing biomolecules.

Keywords: L-Cysteine, Oxidative Cleavage, tert-Butyl Chromate, Chromium (^{VI}) Oxidation, Solvent Effect, Dichloromethane, Acetone, Benzene, n-Hexane, Sulphur-Containing Amino Acids, Reaction Mechanism.

Introduction to the Oxidative Cleavage of L-Cysteine:

Amino acids are essential biomolecules involved in numerous physiological, biochemical, and metabolic processes. Among them, L-cysteine is unique because it contains a highly reactive thiol (–SH) group, which plays an important role in protein structure, enzymatic activity, cellular redox regulation, and antioxidant defense. The susceptibility of the thiol group toward oxidation makes L-cysteine an important substrate for studying oxidation reactions involving sulfur-containing biomolecules. Oxidative cleavage is an important chemical transformation involving the oxidation and subsequent rupture or modification of specific bonds within an organic molecule. Chromium (^{VI})-based oxidizing agents are widely employed for such transformations because of their strong electron-accepting ability. Tert-butyl chromate (TBC) is a chromium (^{VI}) oxidant capable of oxidizing suitable organic substrates under appropriate reaction conditions. Its reactivity can be significantly affected by the solvent environment, particularly solvent polarity, dielectric properties, and hydrogen-bonding ability. The present study investigates the oxidative cleavage of L-cysteine using TBC in different solvents, including dichloromethane, acetone, benzene, n-hexane, 1,4-dioxane, ethanol, and isopropyl alcohol. Particular attention is given to reaction rate, oxidation efficiency, product formation, and solvent effects. TLC, UV-Visible, FTIR, NMR, and GC-MS techniques are employed for monitoring and characterization, while a plausible chromium (^{VI})-mediated oxidation pathway is proposed based on the experimental observations.

Experimental work:

Oxidation of L-cysteine with tert-butyl chromate: First, 2.0 g of L-cysteine is taken in a 200 mL round bottom flask and dissolved in 20–30 mL of distilled water. A few drops of glacial acetic acid are then added to maintain a slightly acidic medium (pH 4–5). The reaction flask is placed in an ice bath, and the temperature is carefully maintained between 0–5°C throughout the reaction. A dropping funnel is filled with tert-butyl chromate, which is added slowly and dropwise to the cysteine solution with continuous stirring. During the addition, the temperature is kept below 5°C to avoid side reactions. As the reaction proceeds, the orange colour of Cr⁶⁺ gradually changes to green due to the formation of Cr³⁺. After complete addition of the oxidizing agent, the reaction mixture is stirred for an additional 15–30 minutes to ensure completion. The progress of the reaction is monitored by TLC using the solvent system n-butanol: acetic acid: water (4:1:1), and completion is confirmed by the disappearance of the cysteine spot. During the reaction, a white precipitate of cystine forms. The precipitate is filtered using a Buchner funnel under vacuum, washed with cold distilled water (and a small amount of ethanol if required), and then dried in air or in a desiccator to obtain the final product. As a result, cysteine is oxidized to cystine through the formation of a disulfide (–S–S–) bond under mild acidic and low-temperature conditions.

Table – 1

Solvent variation Data with reaction nature and colour change.

Sample	Solvent	Time (min.)	Nature of Oxidation	Colour Change	Product Yield	Yield%
BKC1	DCM	30	Moderate oxidation	Orange → Light Green	1.49 g	82%
BKC2	Acetone	45	Fast & smooth oxidation	Orange → Green (rapid)	1.64 g	90%
BKC3	Dioxane	40	Moderate, controlled	Orange → Pale Green	1.42 g	78%
BKC4	Benzene	50	Slow oxidation	Orange → Slight Green	1.18 g	65%
BKC5	hexane	70	Slower oxidation	Orange/red brown → Greenish/ Green	0.95 g	52%

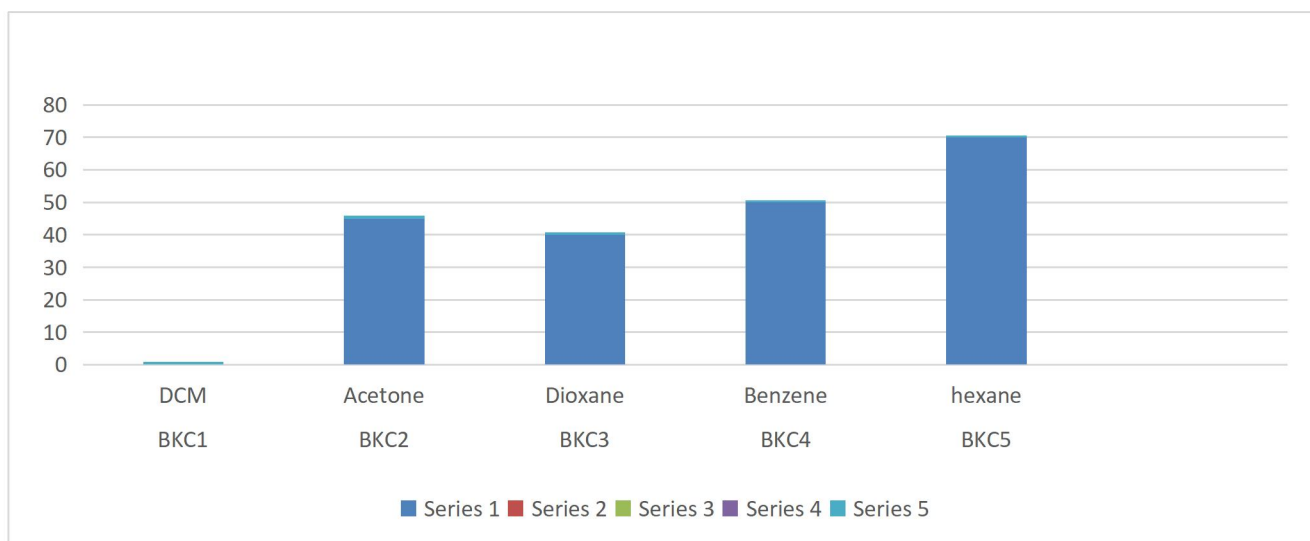
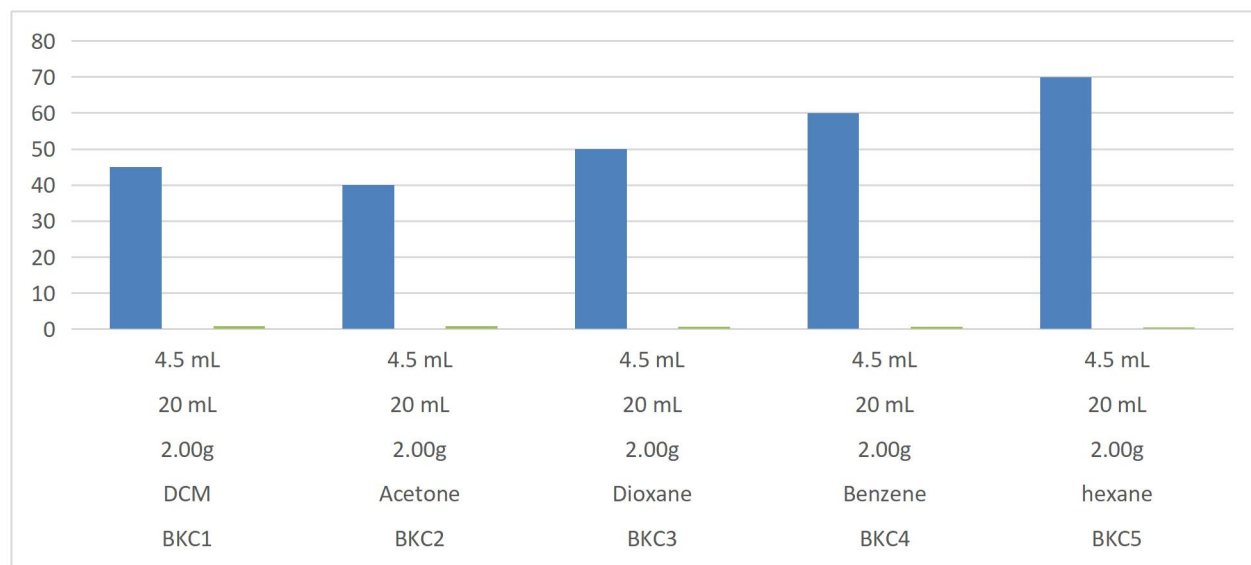


Table – 2

xSample	Solvent	cysteine (g)	Solvent Volume (mL)	TBC (ml)	Time (min)	Yield(g)	Yield%
BKC1	DCM	2.00g	20 mL	4.5 mL	45	1.49 g	82%
BKC2	Acetone	2.00g	20 mL	4.5 mL	40	1.64 g	90%
BKC3	Dioxane	2.00g	20 mL	4.5 mL	50	1.42 g	78%
BKC4	Benzene	2.00g	20 mL	4.5 mL	60	1.18 g	65%
BKC5	hexane	2.00g	20 mL	4.5 mL	70	0.95 g	52%



Mechanistic explanation

1. Reactive sites in L-cysteine.

L-Cysteine contains three i.e. -SH, -NH₂ and -COOH group are important functional groups:



The thiol group (-SH) is the principal oxidation-sensitive site. Sulfur has a high polarizability and readily undergoes electron transfer toward an electrophilic Cr (VI) Centre.

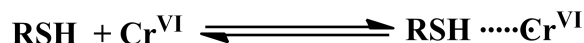
The reaction may therefore be represented as:



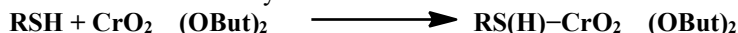
Where -R=CH₂-CH(NH₂)-COOH

2. Initial interaction between cysteine and TBC.

The tert-butyl chromate contains chromium in the +6-oxidation state. The Cr (VI) Centre is strongly electrophilic.

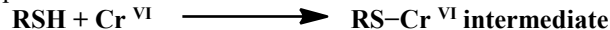


The sulfur atom of cysteine donates electron density toward chromium:



This initial association can be regarded as a cysteine-chromium precursor complex.

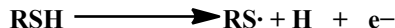
A more chemically meaningful representation is:



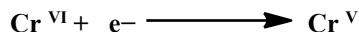
This intermediate facilitates transfer of electron density from sulfur to chromium.

3. Formation of a sulfur-chromium intermediate.

The oxidation can proceed through formation of an S-Cr interaction:

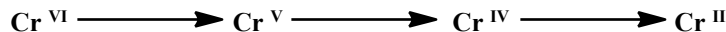


Followed

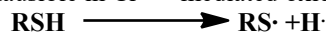


The important mechanistic feature is that sulfur is oxidized while chromium is reduced.

Electron transfer can be represented as:



Thus, a Cr(V) intermediate is mechanistically plausible in Cr(VI)-mediated oxidation.



Therefore:

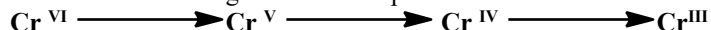


For cysteine:



4. Successive electron-transfer process.

Further electron transfer from the sulfur-containing substrate can produce additional reduction of chromium.



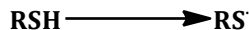
The exact sequence and lifetime of these oxidation states depend upon solvent, substrate concentration, acidity and TBC concentration.

The oxidation is proposed to involve successive one-electron and/or two-electron transfer steps between the thiol sulfur of cysteine and the Cr^(VI) oxidant, with transient Cr^(V) and/or Cr^(IV) species potentially participating before formation of the thermodynamically more stable Cr^(III) species.

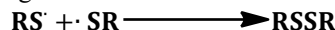
5. Formation of cysteine-derived sulfur intermediates.

After the initial electron-transfer step, cysteine can form sulfur-Cent red intermediates.

A simplified sequence is:



Two sulfur-centred species can undergo coupling:



Therefore.



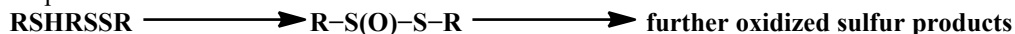
For cysteine.



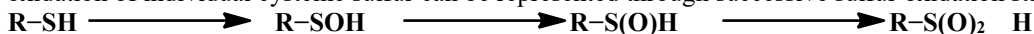
The disulfide formed is cystine.

6. Further oxidation under excess TBC.

An important research consideration is that cystine need not necessarily be the final oxidation product. If the reaction contains excess TBC or prolonged reaction time, the sulfur-containing product can undergo further oxidation. A simplified sequence can be represented as:



Alternatively, oxidation of individual cysteine sulfur can be represented through successive sulfur oxidation states:



corresponding broadly to sulfenic-, sulfinic-, and sulfonic-acid-level oxidation.

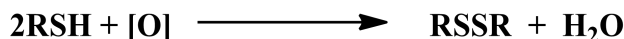
Thus, the final product distribution depends strongly on the oxidant/substrate ratio and reaction conditions.

7. Proposed overall redox process.

The central redox transformation can be summarized as:



For disulfide formation:



where [O] represents the effective oxidizing equivalent supplied by TBC.

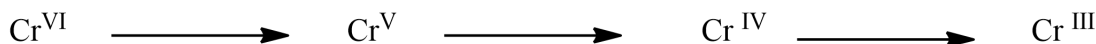
For cysteine:



The useful research correlation is:

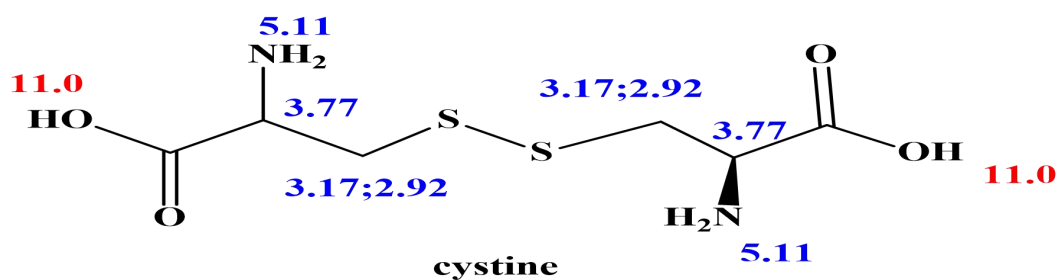


with concurrent chromium reduction:

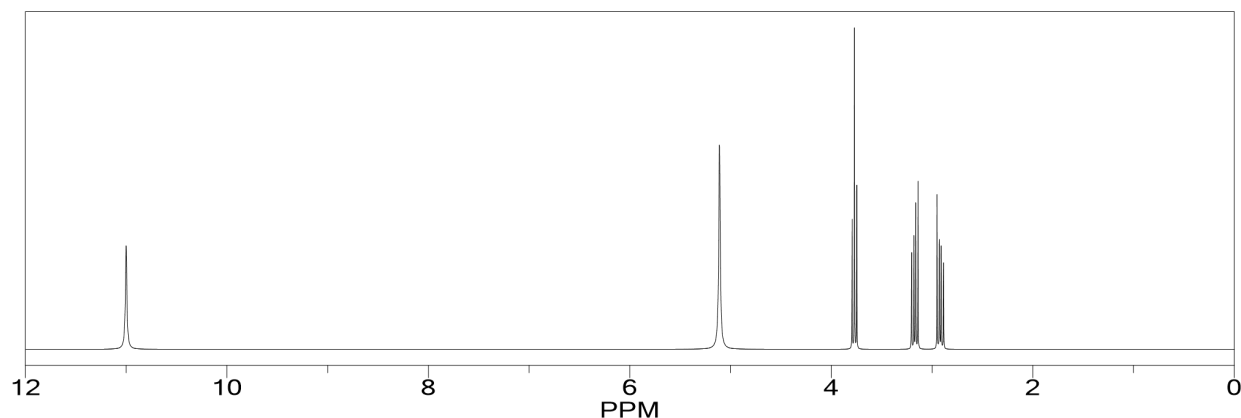


and, under stronger oxidation:



ChemNMR ^1H Estimation

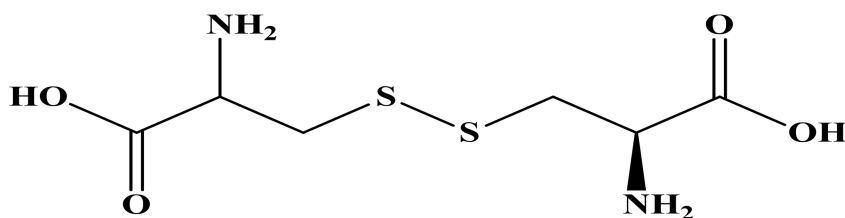
Estimation quality is indicated by color: **good**, **medium**, **rough**.

**Protocol of the 1H-1H NMR Prediction**

Node	Shift	Base	Inc.	Comment (ppm rel. to TMS)
OH	11.0	11.00		carboxylic acid
OH	11.0	11.00		carboxylic acid
NH ₂	5.11	2.00		amine
		3.11		general corrections
NH ₂	5.11	2.00		amine
		3.11		general corrections
CH ₂	3.05	1.37		methylene
		1.23		1 alpha S
		0.23		1 beta N
		0.22		1 beta C(=O) O
CH ₂	3.05	1.37		methylene
		1.23		1 alpha S
		0.23		1 beta N
		0.22		1 beta C(=O) O
CH	3.77	1.50		methine
		1.13		1 alpha N
		0.87		1 alpha C(=O) O
		0.27		1 beta SR
CH	3.77	1.50		methine
		1.13		1 alpha N
		0.87		1 alpha C(=O) O
		0.27		1 beta SR

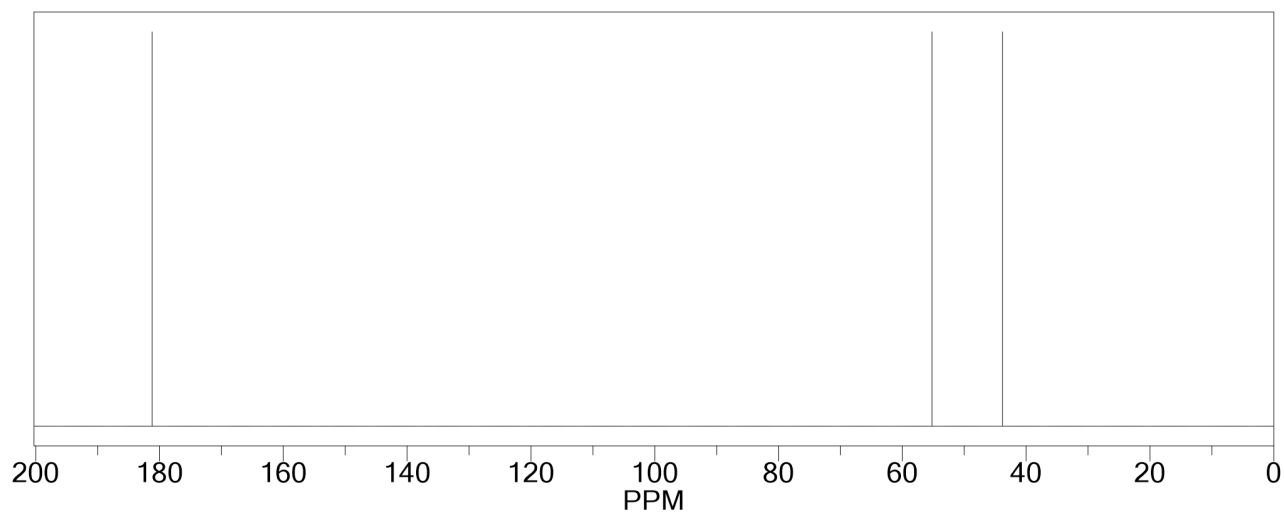
¹H NMR Coupling Constant Prediction

shift	atom index	coupling partner	constant and vector
11.0	13		
11.0	14		
5.11	3		
5.11	9		
3.05	4	diastereotopic	13.4 H-C-H
	8		7.0 H-C-CH-H
3.05	7	diastereotopic	13.4 H-C-H
	8		7.0 H-C-CH-H
3.77	3		
	4		7.0 H-C-CH-H
3.77	8		
	7		7.0 H-C-CH-H

Chem NMR ¹³C Estimation.**Cystine**

Estimation quality is indicated

by color: good, medium, rough.

**Protocol of the C-13 NMR Prediction.**

Node	Shift	Base + Inc.	Comment (ppm rel. to TMS)
CH₂	43.8	-2.3	aliphatic
		9.1	1 alpha -C
		11.9	1 alpha -S
		2.0	1 beta -C(=O)-O
		11.3	1 beta -N
		11.4	1 beta -S
		-2.5	1 gamma -C
		0.3	1 delta -C
		2.6	general corrections
CH₂	43.8	-2.3	aliphatic

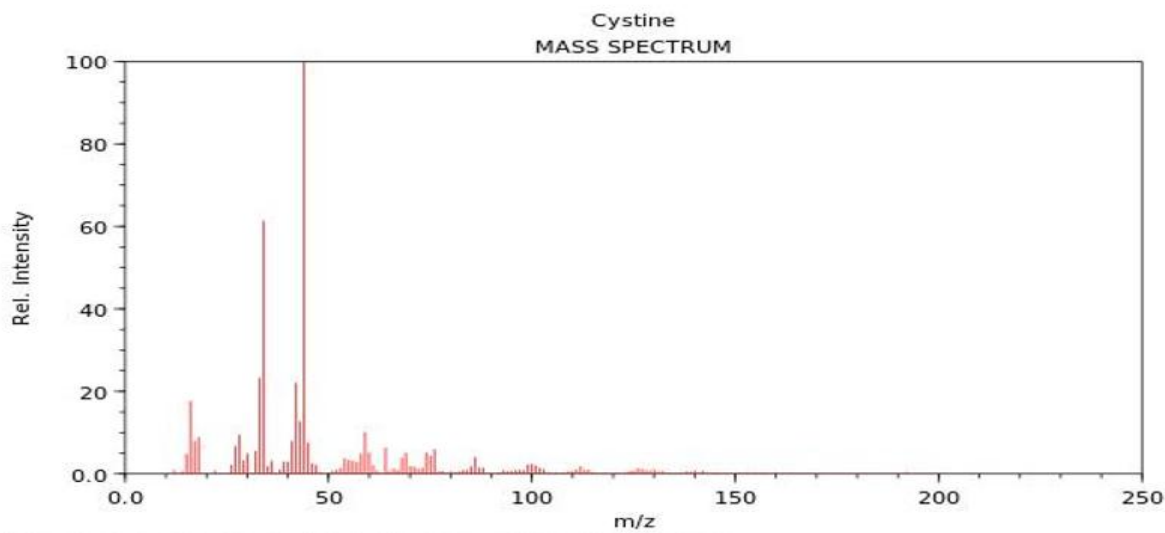
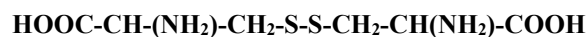
		9.1	1 alpha -C
		11.9	1 alpha -S
		2.0	1 beta -C(=O)-O
		11.3	1 beta -N
		11.4	1 beta -S
		-2.5	1 gamma -C
		0.3	1 delta -C
		2.6	general corrections
C	181.2	166.0	1-carboxyl
		11.0	1 -C-C
		4.2	general corrections
C	181.2	166.0	1-carboxyl
		11.0	1 -C-C
		4.2	general corrections
CH	55.2	-2.3	aliphatic
		21.8	1 alpha -C(=O)-O
		9.1	1 alpha -C
		28.3	1 alpha -N
		11.4	1 beta -S
		-3.6	1 gamma -S
		0.3	1 delta -C
		-9.8	general corrections
CH	55.2	-2.3	aliphatic
		21.8	1 alpha -C(=O)-O
		9.1	1 alpha -C
		28.3	1 alpha -N
		11.4	1 beta -S
		-3.6	1 gamma -S
		0.3	1 delta -C
		-9.8	general corrections

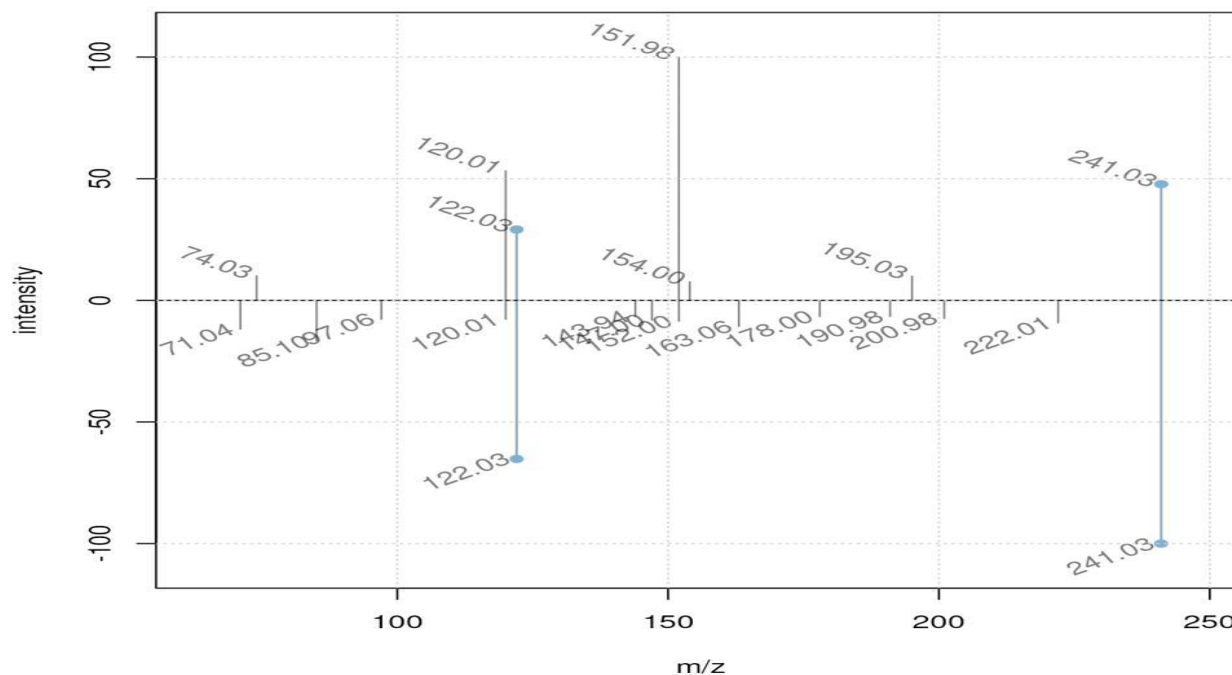
Mass spectrometry (MS) of L-Cystine

L-Cystine molecular formula: $C_6H_{12}N_2O_4S_2$

Molecular weight: $240.30 \text{ g mol}^{-1}$

The molecular structure is:





Expected MS ions.

Ion / m/z	Assignment	Significance
240	Molecular ion, M^+	Molecular mass of cystine
241	$[M + H]^+$	Protonated cystine in positive ESI
239	$[M - H]^-$	Deprotonated cystine in negative ESI
120	Cysteine-related fragment	Cleavage of the S–S bond / characteristic fragmentation
103–105	Sulfur-containing fragments	Further fragmentation
74–76	Small amino-acid-related fragments	Backbone fragmentation

Important observation

For ESI-MS in positive-ion mode, the most useful peak is generally:

$$m/z \approx 241 = [M + H]^+$$

because cystine has a molecular mass of approximately $240.30 \text{ g mol}^{-1}$.

For negative-ion ESI:

$$m/z \approx 239 = [M - H]^-$$

The S–S bond can undergo cleavage during fragmentation, producing cysteine-related sulfur-containing ions. This is particularly useful when confirming cystine formation in a cysteine oxidation experiment. Mass spectrometric analysis of the isolated product showed a molecular ion consistent with cystine ($\text{C}_6\text{H}_{12}\text{N}_2\text{O}_4\text{S}_2$; molecular mass $240.30 \text{ g mol}^{-1}$). In positive-ion ESI-MS, the protonated molecular ion $[M+H]^+$ is expected at m/z 241, whereas negative-ion analysis gives the deprotonated ion $[M-H]^-$ at m/z 239. Fragmentation involving cleavage of the disulfide (S–S) bond gives characteristic sulphur-containing cysteine-related fragments. The observed molecular-ion and fragment-ion pattern supports the presence of a disulfide-containing amino-acid structure.

Relevance to Fishery Research

From a fisheries perspective, cystine is therefore relevant in several interconnected areas, including:

Fish nutrition – as part of sulphur-containing amino-acid metabolism.

Growth and development – through its relationship with protein synthesis and tissue maintenance.

Antioxidant defense – through conversion to cysteine and subsequent glutathione synthesis.

Stress physiology – through its participation in cellular redox regulation.

Immunity and disease resistance – through maintenance of cellular antioxidant capacity.

Feed formulation – for optimizing sulphur amino-acid balance.

Aquatic toxicology – as a component of investigations into oxidative stress caused by environmental contaminants.

Significance in the Present Research

The relationship between cystine and fisheries becomes particularly relevant when studying the oxidation chemistry of sulphur-containing amino acids. Fish tissues and aquatic organisms contain proteins, peptides, glutathione, and other sulphur-containing biomolecules in which cysteine and cystine participate in redox processes. Consequently, understanding the chemical behaviour of cystine, especially its susceptibility to oxidation and reduction, provides a useful biochemical foundation for interpreting oxidative processes occurring in aquatic organisms.

In the present research, the investigation of cysteine/cystine oxidation can therefore be considered relevant to fisheries and aquaculture at the biochemical level. Knowledge of sulfur-centered oxidation reactions may contribute to a better understanding of oxidative stress, antioxidant metabolism, and biochemical responses of fish to environmental and nutritional factors. This connection provides a scientific basis for relating fundamental oxidation chemistry to fish physiology, aquaculture.

Objective of the Present Investigation

The present work aims to investigate the oxidative cleavage of L-cysteine using tert-butyl chromate under homogeneous conditions in solvents such as dichloromethane, acetone, benzene, n-hexane, dioxane, ethanol, and isopropyl alcohol. Particular emphasis is placed on understanding solvent-dependent variations in reaction behaviour, identifying oxidation products, and proposing a mechanistic pathway for chromium (VI)-induced oxidative cleavage. The study is expected to provide valuable insights into the oxidation chemistry of sulphur-containing amino acids and contribute to the broader understanding of solvent-controlled oxidation processes.

Scope of Work

The present investigation deals with the oxidative cleavage of L-cysteine using tert-butyl chromate (TBC) under homogeneous reaction conditions. The study examines the oxidation behaviour of L-cysteine and evaluates the influence of different solvents, including dichloromethane, acetone, benzene, n-hexane, 1,4-dioxane, ethanol, and isopropyl alcohol. Particular emphasis is placed on the effect of solvent polarity and hydrogen-bonding characteristics on reaction rate, oxidation efficiency, and product formation. The reaction progress and products are investigated using suitable analytical techniques such as TLC, UV-Visible spectroscopy, FTIR, NMR, and GC-MS. Special attention is given to the oxidation of the thiol (-SH) group and the possible formation of sulphur-containing intermediates. Based on experimental observations, a plausible mechanism involving chromium (VI) species, electron-transfer processes, and oxidative cleavage is proposed. The study is expected to provide useful insight into solvent-dependent oxidation of sulphur-containing amino acids.

Importance of Analytical Techniques

The combined application of UV-Visible spectroscopy, FTIR spectroscopy, NMR spectroscopy, GC-MS, and TLC provided complementary information regarding reaction kinetics, functional group transformations, structural characterization, product identification, and mechanistic interpretation. Together, these techniques enabled comprehensive evaluation of the oxidative cleavage of L-cysteine by tert-butyl chromate in different solvent systems.

Nuclear Magnetic Resonance (NMR) Spectroscopy

Nuclear Magnetic Resonance (NMR) spectroscopy is one of the most powerful analytical techniques for the structural characterization of organic compounds. In the present study, ^1H NMR and ^{13}C NMR spectroscopy were employed to confirm the structures of the oxidation products obtained from the oxidative cleavage of L-cysteine by tert-butyl chromate (TBC).

The purified reaction products were dissolved in suitable deuterated solvents such as deuterated water (D_2O), deuterated dimethyl sulfoxide ($\text{DMSO}-d_6$), or deuterated chloroform (CDCl_3), depending upon their solubility. The NMR spectra were recorded at room temperature using a high-resolution NMR spectrometer operating at appropriate frequencies for proton and carbon nuclei.

^1H NMR Spectroscopy

^1H NMR spectroscopy provides information regarding the number, environment, and connectivity of hydrogen atoms present in a molecule. Chemical shift values (δ , ppm), multiplicity, coupling constants (J values), and signal integration were analyzed to identify structural changes occurring during oxidation. The disappearance of signals corresponding to thiol (-SH) protons and changes in α -CH and CH_2 proton resonances provided evidence for oxidation and cleavage reactions.

^{13}C NMR Spectroscopy

^{13}C NMR spectroscopy was utilized to identify different carbon environments within the oxidation products. Carbonyl carbons generally appeared in the downfield region (160–220 ppm), whereas aliphatic carbons appeared in the up-field region (10–80 ppm). The appearance of new carbonyl signals and the disappearance of characteristic cysteine carbon resonances indicated oxidative degradation of the amino acid framework.

Significance in the Present Study

NMR analysis played a crucial role in confirming the structures of oxidation products formed during the reaction of L-

cysteine with tert-butyl chromate. The technique provided direct evidence for sulphur oxidation, carbon-sulphur bond cleavage, oxidative deamination, and the formation of carbonyl-containing products. Together with FTIR, UV-Visible spectroscopy, GC-MS, and TLC analysis, NMR spectroscopy contributed significantly to elucidating the reaction mechanism and validating the proposed oxidative cleavage pathway.

Expected NMR changes for L-cysteine oxidation

L-Cysteine (starting material):

α -CH proton: ~ 3.8 – 4.2 ppm

CH_2 -S proton: ~ 2.8 – 3.2 ppm

SH proton: ~ 1 – 3 ppm (often broad or absent due to exchange)

Carboxylic carbon: ~ 170 – 175 ppm (^{13}C NMR)

After oxidation by TBC:

SH signal disappears.

New carbonyl signals may appear in ^{13}C NMR (~ 180 – 210 ppm).

CH_2 -S signals decrease or disappear if C-S bond cleavage occurs.

Additional aldehyde proton signals may appear around 9 – 10 ppm if aldehydic products are formed.

Work Done

The present research work was undertaken to investigate the oxidative cleavage of L-cysteine using tert-butyl chromate (TBC) under homogeneous conditions. A literature survey was initially conducted on chromium (VI)-mediated oxidation of sulphur-containing amino acids and solvent effects. Oxidation reactions were subsequently performed in different solvents, including dichloromethane, acetone, benzene, n-hexane, 1,4-dioxane, ethanol, and isopropyl alcohol. Reaction progress was monitored by TLC and UV-Visible spectroscopy, while the isolated products were characterized using FTIR, NMR, and GC-MS techniques. Comparative evaluation of reaction time, conversion, and product yield was carried out. Based on the experimental and spectroscopic observations, a plausible mechanism for TBC-mediated oxidative cleavage of L-cysteine was proposed.

Results

L-Cysteine underwent oxidative cleavage in the presence of tert-butyl chromate.

Solvent properties significantly affected reaction rate and product yield.

Polar aprotic solvents showed the highest oxidation efficiency.

Sulphur oxidation was identified as the initial step of the reaction.

Oxidative cleavage generated lower molecular weight oxidation products.

FTIR, NMR, GC-MS, and TLC analyses supported the proposed reaction pathway.

The study established a strong relationship between solvent polarity and oxidation behaviour.

Acknowledgement

I am highly thankful to Dr. Mohini Gupta, Head of the Department, Nilamber -Pitamber University, Medininagar for providing the necessary laboratory facilities and a conducive research environment required for carrying out this work.

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I am deeply grateful to my friends and fellow researchers for their encouragement, helpful discussions, and moral support throughout the course of this investigation.

Finally, I express my heartfelt gratitude to my parents and family members for their unwavering love, patience, inspiration, and continuous support. Their faith in me has been the foundation of my academic and personal achievements.

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