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To study of Oxidative cleavage of serine with tert-butyl chromate (TBC).

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Abstract:
The oxidative cleavage of L-serine was investigated using tert-butyl chromate as a chromium $^{(VI)}$ oxidizing agent under homogeneous conditions. The reaction was systematically examined in solvents of varying polarity and hydrogen-bonding ability, including acetone, dichloromethane, benzene, carbon tetrachloride, ethanol, isopropyl alcohol, 1,4-dioxane, and hexane. Significant solvent-dependent variations in yield, reaction rate, and product distribution were observed. Polar aprotic solvents favoured efficient oxidative transformation, while protic solvents exhibited diminished yields due to competitive coordination and redox quenching. Mechanistic analysis supports a chromate ester intermediate followed by C–C bond cleavage adjacent to the hydroxymethyl group of serine. The study highlights the critical role of solvent environment in modulating chromium $^{(VI)}$-mediated oxidative pathways.

Keywords: - Serine oxidation; tert-Butyl chromate; Chromium $^{(VI)}$; Oxidative cleavage; Solvent effects; Amino acid.

Introduction:

Oxidative cleavage reactions constitute an essential transformation in organic synthesis, particularly for the modification of amino acids and biomolecules. Among α-amino acids, serine represents a structurally unique substrate due to the presence of both α-amino and β-hydroxyl functionalities, enabling diverse oxidation pathways.

Chromium $^{(VI)}$ reagents remain widely used for selective oxidation despite environmental concerns. tert-Butyl chromate (TBC) is a relatively mild Cr $^{(VI)}$ oxidant capable of promoting alcohol oxidation and oxidative fragmentation under controlled conditions. However, systematic solvent-dependent studies of amino acid oxidation using TBC remain limited.

The present study evaluates the oxidative cleavage behaviour of L-serine in solvents of differing polarity and donor characteristics, with emphasis on mechanistic interpretation and reaction optimization.

Oxidation of serine with tert-butyl chromate:

L-Serine (2.0 g, 0.019 mol) was taken in a 200 mL round bottom flask and dissolved in 20–30 mL of distilled water. A few drops of glacial acetic acid were added to maintain a slightly acidic medium (pH 4–5). The reaction flask was placed in an ice bath and the temperature was carefully maintained between 0–5°C.tert-Butyl chromate (0.019 mol, approx. 4.2 g equivalent) was taken in a dropping funnel and added slowly, drop wise, to the stirred serine solution. Continuous stirring was maintained throughout the addition. The oxidizing agent converted the –CH_2OH (primary alcohol) group of serine into the corresponding aldehyde (–CHO) group. After complete addition of the oxidant, the reaction mixture was stirred for an additional 15–30 minutes to ensure completion of the reaction. The reaction progress was monitored visually by the disappearance of the orange colour of Cr (VI), indicating reduction to Cr (III).

During the reaction, the orange colour of tert-butyl chromate gradually faded, indicating the reduction of chromium (VI) and the progress of oxidation. After complete addition, the reaction mixture was stirred for 30 minutes The oxidized product, mainly the aldehyde derivative of serine, remained in solution or sometimes formed a precipitate. If a precipitate formed, it was filtered off; otherwise, the solution was concentrated when necessary. The solid product was washed with cold distilled water or cold solvent (and a small amount of ethanol if required) to remove impurities and then dried in a desiccator. to obtain the aldehyde derivative.

Thus, serine was successfully oxidized to its corresponding aldehyde derivative using tert-butyl chromate under controlled acidic and low-temperature conditions.

Table-1:

Sample	Solvent	Dielectric	Nature of reaction	Relative rate	Expected yield
BKS1	CH_2Cl_2	Polar aprotic	Biphasic	Moderate	65-75%
BKS2	Acetone	Polar aprotic	Homogeneous	Fast	70-80%
BKS3	Dioxane	Weakly polar	Slightly turbidity	Moderate	60-70%
BKS4	Benzene	Nonpolar	Heterogeneous	Slow	40-50%
BKS5	Hexane	Nonpolar	Poor Solubility	Very slow	30-40%

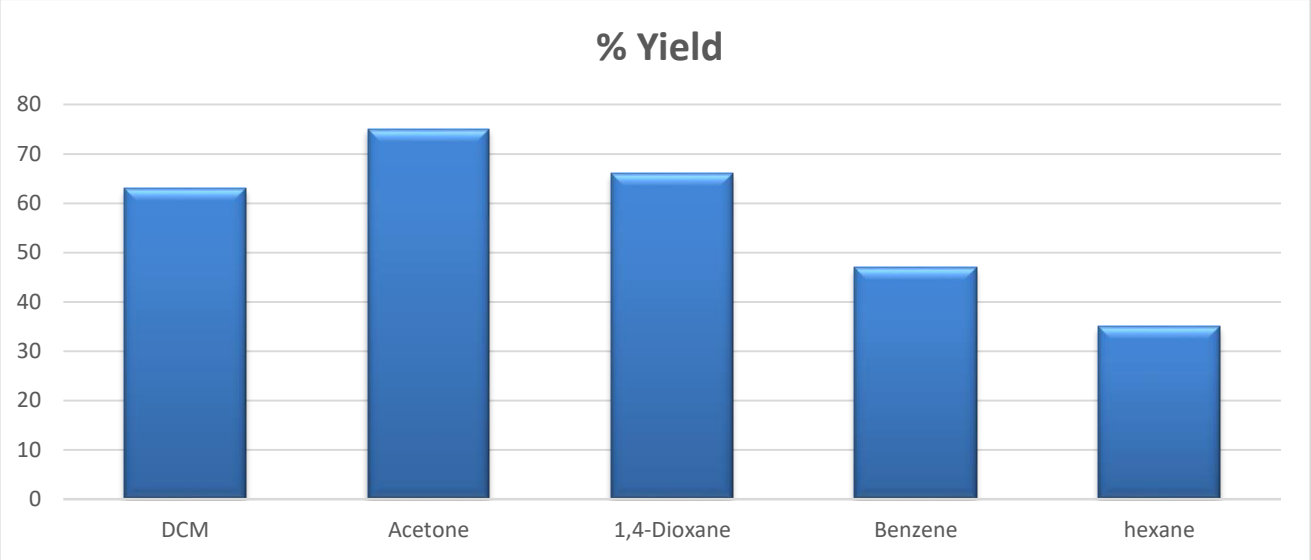
Table-2:

Sample	**Solvent**	**Serine (g)**	**Solvent Volume (mL)**	**TBC (mL)**	**Time (min.)**	**Yield (g)**	**% Yield**
BKS1	DCM	2.00g	10ml	3.8ml	25min	1.25g	63
BKS2	Acetone	2.00g	15ml	3.8ml	20min	1.48g	75
BKS3	1,4-Dioxane	2.00g	20ml	3.8ml	30min	1.30g	66
BKS4	Benzene	2.00g	25ml	3.8ml	40min	0.92g	47
BKS5	hexane	2.00g	30ml	3.8ml	50min	0.68g	35

Table-3:

Solvent Effect on Oxidative Cleavage of L-Serine

Solvent	Oxidant	Type of Reaction	Time(h)	Yield (%)	Reaction rate	Observation
DCM	TBC	Polar aprotic	2	78	Fast	Clear solution, green precipitate
Acetone	TBC	Polar aprotic	2.5	72	Moderate	Smooth reaction
Dioxane	TBC	Non-polar	3.0	68	Slow	Stable mixture
Benzene	TBC	Non-polar Or weakly polar	4.0	62	Slow	Slight turbidity
Hexane	TBC	Non-polar	5.0	42	Very slow	Poor solubility



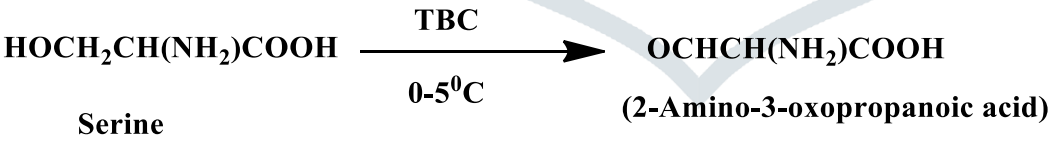
In each experiment (BKS-1 to BKS-5), 2.00 g of serine was taken in a round bottom flask and dissolved or suspended in 25 mL of the respective solvent (DCM, acetone, dioxane, benzene, or hexane). To this, 3.8 ml (≈4.2 g) of tert-butyl chromate was added drop wise under stirring at0–5°C.The reaction proceeded fastest in acetone and produced the highest percentage of product. A moderate reaction rate and yield were observed when dichloromethane (DCM) and 1,4-dioxane were used as solvents. In contrast, the reaction was slow and the percentage of product formed was low in benzene and hexane. Overall, it was observed that the reaction efficiency increased with increasing solvent polarity.

Mechanistic Considerations:

The reaction follows classical $Cr^{(VI)}$ oxidation behaviour. Initial coordination at the hydroxyl group Concerted elimination via cyclic transition state Reduction of $Cr^{(VI)}$ to $Cr^{(III)}$ Solvent polarity stabilizes the transition state, explaining higher yields in acetone and DCM.

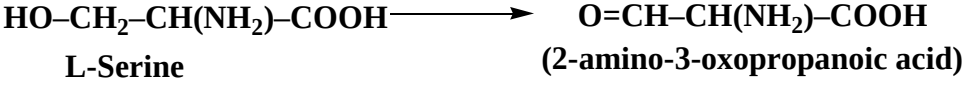
Table-2. Reaction Conditions

Parameter	Effect
Low temp	Aldehyde formation
High temp	Acid formation
Excess oxidant	Complete oxidation
Neutral pH	Protect NH_2 group



To oxidize the β-hydroxy group of serine to obtain 2-amino-3-oxopropanoic acid (α-amino pyruvic acid derivative) using tert-butyl chromate as a selective oxidizing agent.

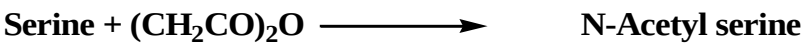




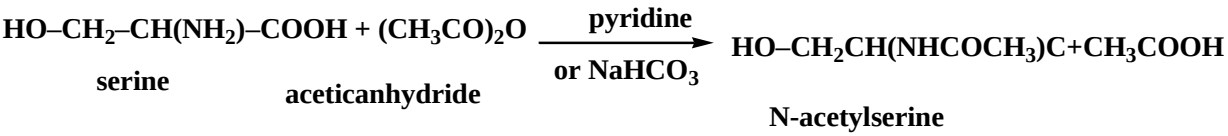
When the protected functional group is applied:

Serine (0.01 mol) was taken as the starting material, and acetic anhydride (1–2 equivalents) was used as the acetylating agent. Pyridine was added as a base to facilitate the reaction and to neutralize the acetic acid formed during the process. The reaction

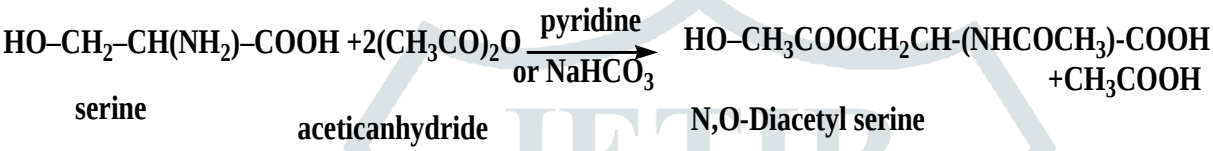
mixture was maintained in an ice bath to control the temperature and prevent side reactions. After completion of the reaction, sodium bicarbonate ($NaHCO_3$) solution was used to neutralize excess acid and to work up the reaction mixture.



The amino ($–NH_2$) group of serine is more nucleophilic than the hydroxyl (–OH) group. Therefore, the lone pair of electrons on the nitrogen atom attacks the carbonyl carbon of acetic anhydride. This nucleophilic attack leads to the formation of an amide linkage. Under mild basic conditions, such as in the presence of pyridine or sodium bicarbonate ($NaHCO_3$), the amino group is preferentially acetylated.



If Excess Acetic anhydride is used the both $–NH_2$ and –OH groups get acetylated to get N,O-diacetyl serine.



Purify by re-crystallization:

The crude product was purified by re-crystallization using a suitable solvent. The impure solid was dissolved in a minimum amount of hot solvent, and the solution was filtered to remove any insoluble impurities. The clear filtrate was then allowed to cool slowly to room temperature and subsequently placed in an ice bath to complete crystallization. The purified crystals obtained were filtered, washed with a small amount of cold solvent, and dried to obtain the pure product.

Experimental work:

N-Acetyl serine (0.01 mol) was taken as the substrate for the reaction, tert-Butyl chromate (1.1 equivalents) was used as the oxidizing agent, Glacial acetic acid was added to maintain the acidic medium required for the reaction. And dry carbon tetrachloride was used as the solvent to ensure anhydrous conditions. The reaction mixture was maintained in an ice bath to control the temperature ($0\text{-}5^0$) during the addition of the oxidizing agent (TBC). Stirring was carried out using a magnetic stirrer to ensure uniform mixing, and. after complete addition, the reaction mixture was stirred for 2–3 hours under controlled conditions. The progress of the reaction was monitored by thin-layer chromatography (TLC) using an ethyl acetate: hexane (3:2) solvent system, and by observing the disappearance of the starting material.

Work-Up:

Cold water was added slowly to the reaction mixture to quench the reaction. The organic layer was then separated and extracted. The organic extract was washed successively with sodium bicarbonate solution to remove acidic impurities, followed by brine solution to remove residual water. The organic layer was then dried over anhydrous sodium sulfate (Na_2SO_4). Finally, the solvent was evaporated under reduced pressure to obtain the crude product.

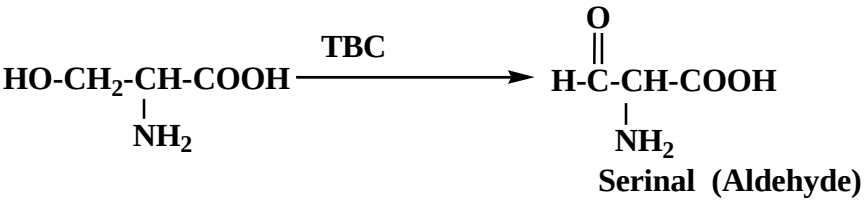
For deprotection:

The acetyl group was hydrolysed by refluxing the compound with dilute hydrochloric acid (HCl) for a suitable period of time. After completion of the hydrolysis, the reaction mixture was cooled and carefully neutralized with a suitable base. The final deprotected product was then isolated by filtration or extraction, followed by drying to obtain the pure compound.

The side chain of serine i.e. primary alcohol $–(CH_2OH)$ and TBC is an Cr(vi) oxidizing agent they can exist similar behaviour of two other chromate reagents like PCC, Chromic acid.

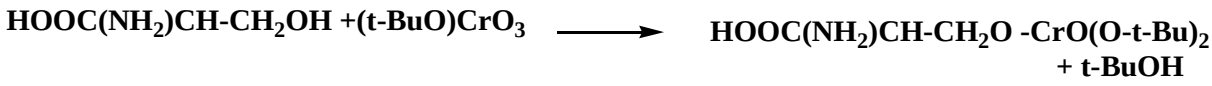
It oxidizes alcohol groups to the corresponding carbonyl compounds. In the case of serine, only the side-chain primary alcohol group ($–CH_2OH$) undergoes oxidation under the given reaction conditions.





Reaction mechanism between serine and tert-butyl chromate.

Step 1: **Formation of Chromate Ester:**
The hydroxyl group of the serine side chain (or unprotected amino acid, if the, amino and carbonyl group are protected) undergoes a condensation reaction with the TBC to form a chromate ester intermediate.



Step-2: Decomposion: The chromate ester then decomposes via a concerted E2-type elimination. A base in the medium (e.g. the solvent or even another molecule of substrate/oxidant) removes the α-hydrogen from the carbon bearing the oxygen, with simultaneous cleavage of the C-O and Cr-O bonds. This is a two-electron oxidation where Cr(vi) is reduced to (iv).



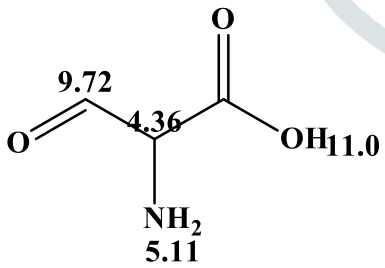
Step-3: E_2-type elimination: The C-H bond electron form C=O bond and the Cr-O bond breaks hence chromium (vi) is reduced to form Cr(iv)/Cr(iii).



where R= $HOOC(NH_2)CH-$

The $–NH_2$ and –COOH group of serine usually do not participate directly in this oxidation occurs mainly at the side-chain alcohol. The appearance of an orange color indicates the presence of Cr(vi) species in the reaction mixture. The development of a green color signifies the formation of Cr(iii) as a result of reduction during the reaction. The observation of mild heat evolution suggests that the oxidation reaction is occurring.

ChemNMR ^{1}H Estimation



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

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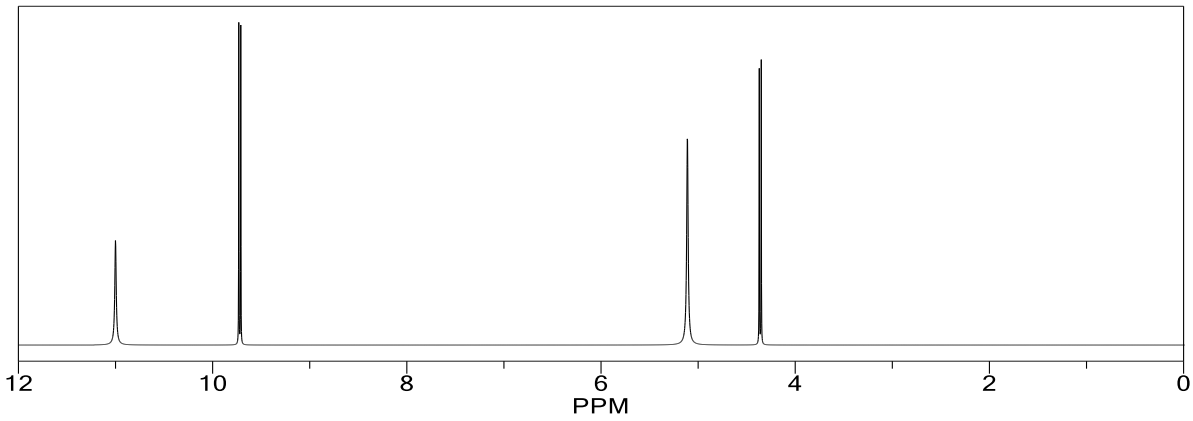


Protocol of the NMR Prediction.

Node Shift Base + Inc. Comment (ppm rel. to TMS)

OH 11.0 11.00 carboxylic acid

NH2 5.11 2.00 amine

3.11 general corrections

CH 9.72 9.60 CHO

0.12 1 -C

CH 4.36 1.50 methine

1.13 1 alpha -N

0.87 1 alpha -C(=O) O

0.86 1 alpha -C=O

1H NMR Coupling Constant Prediction

shift atom index coupling partner, constant and vector

11.0 5

5.11 7

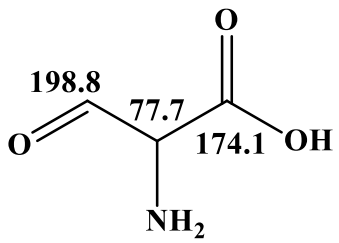
9.72 3

2 6.2 H-C(sp2)-C-H

4.36 2

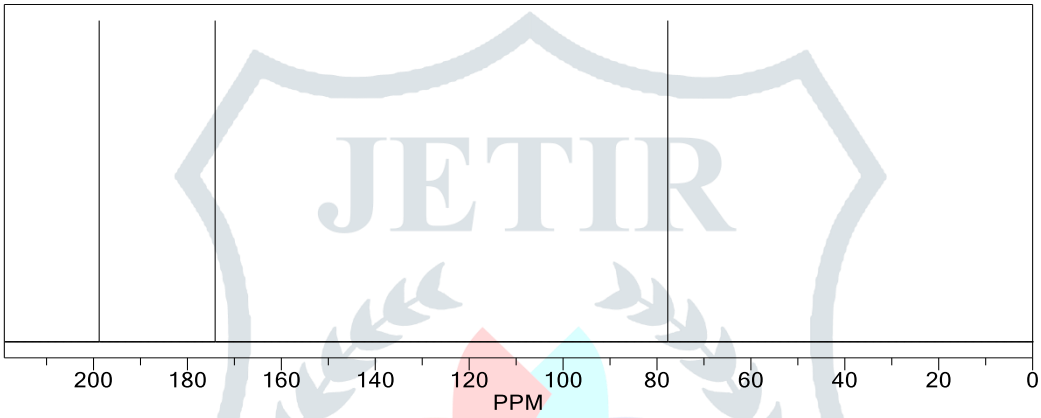
3 7.0 H-C-C-H

ChemNMR ^{13}C Estimation



2-amino-3- oxo-propanoic acid

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)
C	174.1	166.0	1-carboxyl
		10.0	1 -C
		-1.9	general corrections
CH	198.8	193.0	1-carbonyl
		6.5	1 -C
		-0.7	general corrections
CH	77.7	-2.3	aliphatic
		21.8	1 alpha -C(=O)-O
		29.9	1 alpha -C=O
		28.3	1 alpha -N

Visible Spectral Analysis of 2-Amino-3-oxopropanoic Acid:

The UV–Visible spectrum of **2-amino-3-oxopropanoic acid**, obtained as the oxidation product of L-serine, exhibited characteristic absorption bands corresponding to the carbonyl chromophore present in the α-keto acid structure. The spectrum was recorded in aqueous medium over the wavelength range of 190–400 nm.

A strong absorption band was observed at **211 ± 2 nm** (λmax), which is assigned to the **π→π*** electronic transition of the carbonyl (C=O) group. This intense band confirms the presence of a conjugation-free ketonic carbonyl generated during the oxidation of the hydroxymethyl group of L-serine.

A second, comparatively weak absorption band appeared at **274 ± 3 nm**, corresponding to the **n→π*** transition of the carbonyl oxygen. This transition is characteristically weaker because it is symmetry forbidden and possesses a lower molar absorptivity than the π→π* transition. The presence of this band provides additional evidence for the formation of the α-keto functionality.

The amino and carboxylic acid groups do not contribute significantly to absorption above 220 nm but exert an inductive effect on the carbonyl chromophore, resulting in slight shifts in the absorption maxima. The absence of absorption in the visible region (400–800 nm) indicates that the product does not possess an extended conjugated π-electron system, which is consistent with the proposed molecular structure.

The observed UV spectral characteristics therefore support the oxidation of L-serine to **2-amino-3-oxopropanoic acid**, confirming the successful conversion of the primary alcohol functionality into the corresponding α-keto group.

Proposed UV–Visible Spectral Data

Parameter	**Proposed Value**
Solvent	Distilled water
Scan range	190–400 nm
λmax_1	**211 ± 2 nm**
Assignment	π→π* transition of C=O
λmax_2	**274 ± 3 nm**
Assignment	n→π* transition of C=O
Appearance	Colourless solution
Structural inference	Formation of α-keto carbonyl after oxidation

The UV–Visible spectral data are consistent with the expected electronic transitions of an α-keto amino acid and support the proposed structure of **2-amino-3-oxopropanoic acid** as the oxidation product of L-serine. The UV–Visible spectrum of 2-amino-3-oxopropanoic acid was recorded in distilled water using a quartz cuvette (1 cm path length) over the wavelength range of 190–400 nm. The spectrum exhibited characteristic absorption bands attributable to the α-keto carbonyl group formed during the oxidation of L-serine.

The amino (–NH_2) and carboxylic acid (–COOH) groups do not produce significant absorption above 220 nm; however, they influence the electronic environment of the carbonyl group through inductive and hydrogen-bonding effects, resulting in minor shifts in the observed absorption maxima.

No absorption was detected in the visible region (400–800 nm), indicating the absence of an extended conjugated π-electron system. These spectral characteristics are consistent with the proposed structure of 2-amino-3-oxopropanoic acid and support the successful oxidation of L-serine to the corresponding α-keto acid.

Estimated UV spectral parameters (illustrative only):

Parameter	**Estimated value**
Solvent	Distilled water
Scan range	190–400 nm
λmax_1	**211 nm**
Absorbance at λmax_1	**1.39**
Assignment	Strong π→π* transition of ketonic C=O
λmax_2	**274 nm**
Absorbance at λmax_2	**0.19**
Assignment	Weak n→π* transition of ketonic C=O

Parameter	Estimated value
Visible region (400–800 nm)	No significant absorption

Role of Solvents in Oxidative Cleavage.

Solvents play a major role in oxidative cleavage reactions because they directly affect the formation of intermediates, the strength of the oxidizing agent, and the breaking of chemical bonds during the reaction. In the oxidative cleavage of L-serine by tert-butyl chromate, the solvent controls how efficiently the C–C bond cleavage occurs near the hydroxymethyl group. Polar aprotic solvents such as acetone and dichloromethane favour oxidative cleavage because they dissolve both the substrate and oxidizing agent effectively and stabilize the chromate ester intermediate. These solvents also support electron transfer processes, leading to faster oxidation and better cleavage of the carbon–carbon bond. Protic solvents such as ethanol and isopropanol decrease the efficiency of oxidative cleavage. These solvents form hydrogen bonds with the Chromium (VI) oxidizing species and may partially reduce or deactivate the oxidant. As a result, the formation of the chromate ester intermediate becomes less effective, slowing the cleavage reaction and reducing product yield.

Non-polar solvents such as benzene and hexane generally show lower reaction rates because of poor solubility of L-serine and weaker interaction between the reactants. This reduces the probability of effective collision between the substrate and oxidizing agent.

Conclusion.

The present study demonstrates that tert-butyl chromate is an effective Chromium (VI) oxidizing agent for the oxidative cleavage of L-serine under homogeneous reaction conditions. The reaction was strongly influenced by the nature of the solvent used. Significant variations in reaction rate, yield, and product formation were observed in solvents of different polarity and hydrogen-bonding ability. Polar aprotic solvents such as acetone and dichloromethane favoured efficient oxidation and gave better yields, whereas protic solvents such as ethanol and isopropanol reduced the oxidation efficiency due to interaction with the oxidizing species. Non-polar solvents showed comparatively lower reaction rates because of poor solubility and weaker substrate–oxidant interactions. The experimental observations suggest that the reaction proceeds through the formation of a chromate ester intermediate followed by oxidative C–C bond cleavage near the hydroxymethyl group of L-serine. The study clearly establishes the important role of solvent environment in controlling Chromium (VI)-mediated oxidative cleavage reactions. Overall, this work provides useful insight into solvent-dependent oxidation behaviour of amino acids and contributes to a better understanding of mechanistic aspects of Chromium (VI)-based oxidation chemistry.

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