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Novel Synthesis of Diketo-Acid Chondroitin-4-sulfate as Coordination Biopolymer Precursor through Oxidation of Chondroitin-4-sulfate by Alkaline Permanganate

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Abstract: Coordination biopolymer diketo-acid derivative of chondroitin-4-sulfate (DKA-CS) was prepared by the oxidation of CS with potassium permanganate in alkaline solution at pH's > 12. The chemical structure of the synthesized heterocyclic macromolecule (DKA-CS) has been characterized by elemental analysis and IR spectroscopy. The tendency of DKA-CS as a coordination biopolymer chelating agent for some metal ions has been examined. A tentative mechanism consistent with the experimental results for oxidation is suggested.

Introduction

Chondroitin-4-sulfate, whose repeating units composed of N-acetyl-D-galactosamine-4-sulfate with D-glucuronic acid is a water soluble anionic polyelectrolyte macromolecule [1].

A much work has been done on the kinetics of permanganate oxidation of polysaccharides (PS) that containing functional alcoholic groups by acidic [2-4] or alkaline permanganate [4-9]. The oxidation of pectates [2], carrageenans [3] and carboxymethyl cellulose [4] by acidic permanganate showed sigmoidal curves of S-shapes consisting of two stages for pseudo-first-order plots. The first stage was relatively slow involving the formation of Mn^{3+} and / or Mn^{4+} short lived intermediates, followed by an autoaccelerated period to give rise to the oxidation products at the second stage. One-electron transfer mechanism via free-radical intervention of inner-sphere nature was postulated in these redox systems. On the other hand, the kinetics of oxidation of alginates [5], pectates [6], carboxmethyl cellulose [7], methyl cellulose [8] and carrageenans [9] by permanganate ion in alkaline solutions of higher pH`s was found to proceed through two distinct stages with respect to absorbance-time curves. The first stage was found to be relatively fast involving the formation of detectable transient coordination biopolymeric intermediates [CS, $Mn^{VI}O_4^{2-}$] and / or [CS, $Mn^{V}O_4^{3-}$] involving green manganate (VI) or blue hypomangante (V) short-lived species, followed by slow decomposition of these intermediates to give rise to the reaction product at the second stage. Inner-sphere mechanisms involving one- or two-electron transfer processes of non-free radical intervention were suggested in these redox reactions. Both spectrophotometric and kinetic evidences for formation of such intermediate complexes have been revealed.

Therefore, the present work of permanganate oxidation of chondroitin-4-sulfate as a natural polymer containing both primary and secondary alcoholic groups seems to be of great significant and merit an investigation in order to gain further information on the nature of the products as well as on the interaction of these macromolecule in aqueous alkaline solutions with a special sight on the influence of the nature of the functional groups on the mechanisms and kinetics in these redox systems. In addition, this work aims to synthesize keto-acid derivatives as coordination biopolymer precursors. This biopolymer could be used to encapsulate, protect and deliver bioactive or functional components such as minerals, peptides, proteins, enzymes, drugs, lipids or dietary fibers. It is also useful as precursors for synthesis of new biopolymers as selective biochelating agents for polyvalent cations through formation of their corresponding coordination biopolymeric complexes. These

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complexes would be useful as conductors, selective cation sieves, semi- permeable membranes, biocatalysts and cation exchange resins.

Experimental

All materials used were of analytical grade. Doubly distilled water was redistilled from alkaline permanganate and degassed by bubbling through nitrogen, boiling and cooling under atmosphere [10].

Stock solutions of chondroitin-4-sulfate (ICN Biomedicals, Inc.) were prepared by stepwise addition of the reagent powder to doubly distilled water whilst rapidly stirring the solution to avoid the formation of aggregates which swell with difficulty.

A stock solution of $KMnO_4$ was prepared and standardized by the conventional methods described elsewhere [10,11]. Then, the stock solution was stored in a dark bottle away from light. All other reagents were prepared by dissolving the requisite amounts of the sample in doubly distilled water.

The ionic strength was controlled by addition of $NaClO_4$ as a non-complexing agent. The temperature was controlled within ± 0.05°C.

Polymerization test

The possibility of formation of free radicals was examined by adding acrylonitrile to the partially oxidized reaction mixture; no polymerization was observed indicating that the oxidation reaction probably does not proceed via a free–radical intervention mechanism.

Results and discussion

The experimental results indicated that one mole of CS was consumed 2.7± 0.1 mole of permanganate ion. Hence, such a result for oxidation of chondroitin-4-sulfate by potassium conforms to the following stoichiometric equation,

$3(C_{14}H_{21}NO_{14}S^-)_n+8MnO_4^-=3(C_{14}H_{15}NO_{15}S^-)_n+8MnO_2+8OH^-+5H_2O$ (1)

where $C_{14}H_{21}NO_{14}S^-$ and $C_{14}H_{15}NO_{15}S^-$ represent the chondroitin-4-sulfate and its corresponding keto-acid derivative, respectively.

Preparation of diketo-acid chondroitin-4-sulfate (DKA-CS)

A stoichiometric molar ratio of chondroitin-4-sulfate powder was dissolved in 250 cm^3 of deionized water whose pH was previously adjusted to pH ≥ 12 using sodium hydroxide. This process was performed by stepwise addition of the powder CS to the solution while stirring rapidly and continuously to avoid the formation of aggregates. A 250 cm^3 solution containing the stoichiometric molar ratios of potassium permanganate and sodium fluoride were then added stepwise over 2 h to the CS solution. The reaction mixture was stirred for 48 h at room temperature, the formed MnF_4 was filtered off, and the solution was concentrated to one-fifth of the original solution using a rotary evaporator. A portion of this concentrated solution was acidified using dilute acetic acid to a pH of ca. 5-6. The resultant solution dried under vacuum, and then subjected to elemental analysis and IR spectroscopy. The dikotone were identified by 2,4-dinitrophenylhydrazone and hydroxylamine as described elsewhere [12-15].

Under our experimental conditions, the diketoacid chondroitin-4-sulfate was found to be in good agreement with the obtained results. This diketoderivative gave satisfactory elemental analysis and broad IR absorption bands at 1690-1650 cm^{-1} (broad) that characterize the carbonyl group of α-diketone [16]. The disappearance of the absorption band of the OH group in the IR spectra indicated the complete oxidation of both OH groups in CS to the corresponding ketone. This product was also reacted with 2,4-dinitrophenyl hydrazine and hydroxylamine to afford the corresponding bis-2,4-dinitrophenyl hydrazone and dioxime derivatives, which gave satisfactory elemental analysis and spectroscopic data as shown in Fig. 1. The yield was 95 %. It was found that the product has a high tendency to chelate with many metal cations such as Ag^+, Ca^{2+}, Ni^{2+}, Pb^{2+}, Cd^{2+}, Ce^{4+}….., etc. as shown in Scheme (I) and in Fig.1. The characterstics and geometrical configuration of these complexes are in progress in our laboratory

IR-spectra

The IR-spectra were scanned on a Pyc Unicum Sp 3100 spectrophotometer using the KBr disc technique (4000-200 cm^{-1}). The FTIR-spectra were scanned ANAL: Diketoacid chondroitin-4-sulfate (DKA-CS) C14H15NO15S FTIR: 3430 (OH of COOH group); 1795-1730 (broad) (C = O of -diketone); 1639 (C = O of COOH, γas OCO); 1418 (C = O of COOH, γs OCO) and 1338 cm^{-1} (C — O — C of CS) [12

2,4-Dinitrophenyl hydrazone derivative

ANAL: $C_{26}H_{23}N_9O_{21}S$ (829): Cald (Found) : C, 37.64 (37.45); H, 2.77 (2.66); N, 15.20 (15.10).

Dioxime derivative

ANAL: $C_{14}H_{17}N_3O_{15}S$(499):Cald (Found):C, 33.67 (33.62); H, 3.41 (3.39); N, 8.42 (8.40)

The most suitable reaction mechanism which may be suggested for oxidation in the present work involves a fast deprotonation of chondroitin-4-sulfate substrate by the alkali to form the corresponding alkoxide, followed by the attack of permanganate ion on the alkoxide to form a more reactive transient species prior to the formation of the green intermediate complex [CS-$Mn^{VI}O_4^{2-}$] in the rate-determining step of the initial fast stage. Also,

the formed $Mn^{VI}O_4^{2-}$ is capable to oxidize the formed alkoxide ion, but the reaction is of several orders of magnitude slower than permanganate oxidation owing to its lower reactivity [5-9, 17] and, hence, would not influence the kinetics of the initial stage reaction. The spectral changes during the progress of oxidation reaction for the formation of the coordination biopolymer complex are shown in Figs. 2 and 3, respectively. Again, the oxidation mechanisms for formation and decomposition for coordination biopolymer complex are illustrated in Schemes (I) and (II), respectively.

An alternative reaction mechanism based on the presence of two competitive reactions in the rate-determining step [7] may also be suggested. The first step corresponds to the removal of protons from the alcoholic group by alkali to give the reactive alkoxide form. This removal is followed by the attack of MnO_4^- to the alkoxide center which facilitated by the polarization of the Mn-O bond or $NaMnO_4$ forming the reactive transient species C_1 and C_2 prior to the formation of stable [CS, $Mn^{VI}O_4^{2-}$] and/or [CS, $Mn^{V}O_4^{3-}$] intermediate complexes. The presence of Na^+ cations may facilitate the oxidant attack since it reduces the net charge of the intermediate complexes formed. The proposal that alkoxide is formed prior to the attack of MnO_4^- ion fits some of the experimental facts such as the dependence of the formation rates of intermediates on [OH^-].

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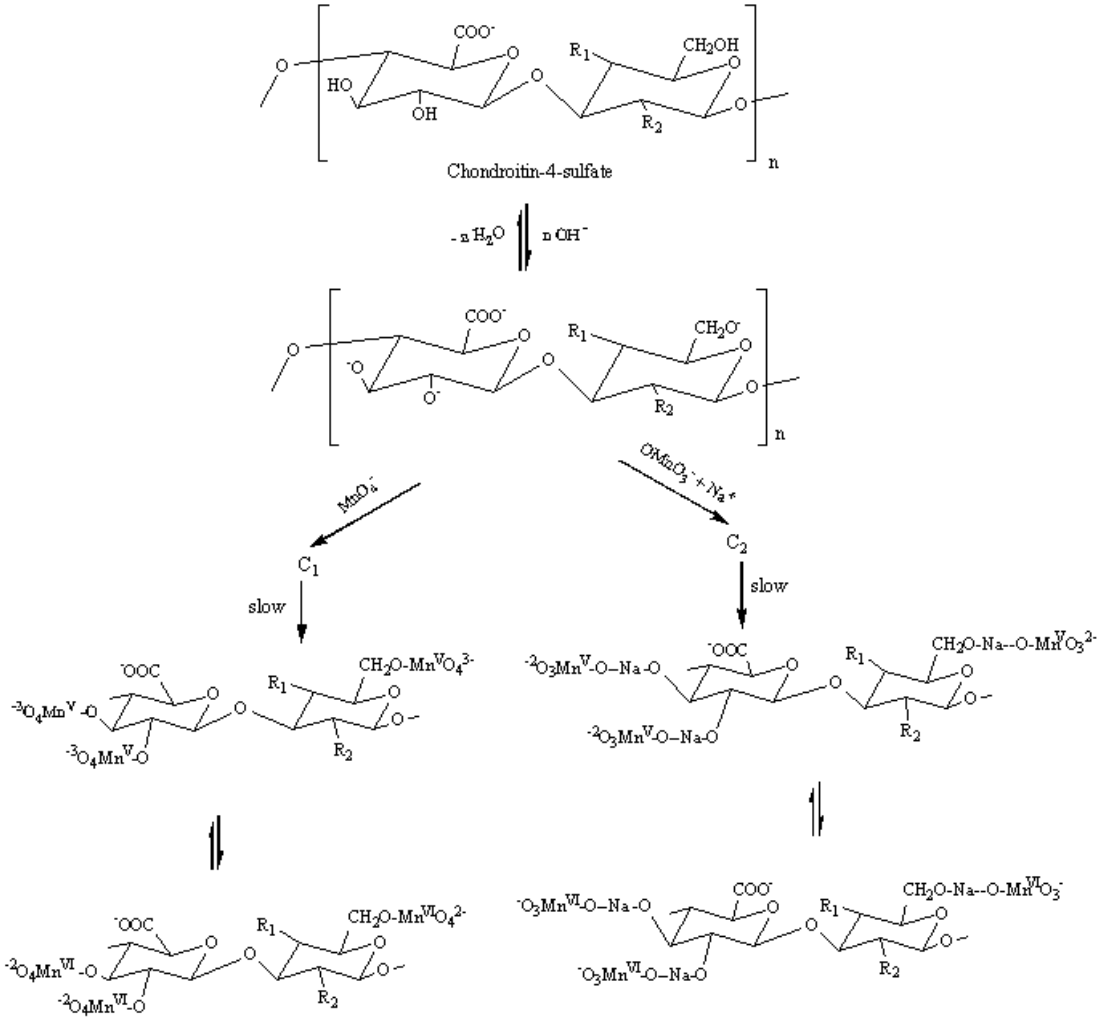
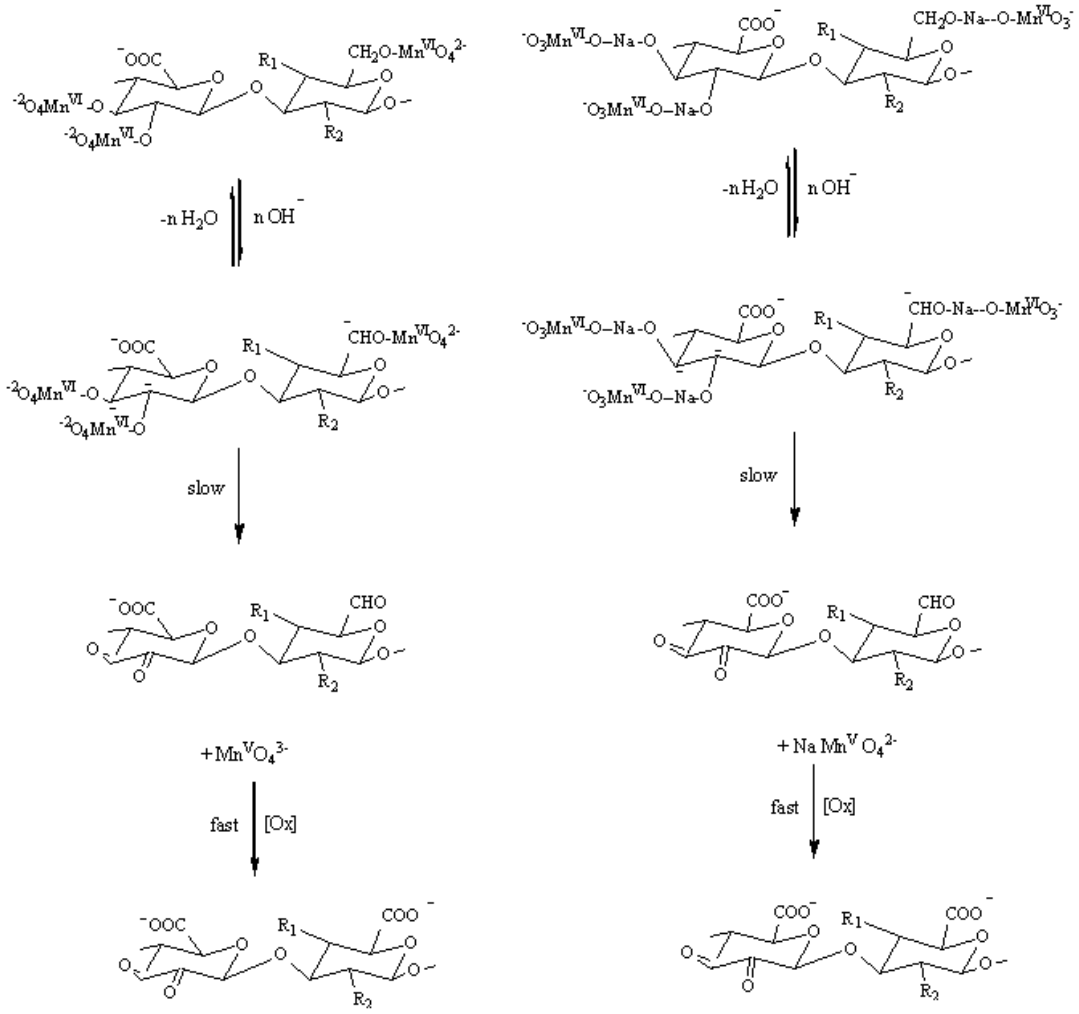
Formation



R_1= -OSO_3^- and R_2 = -NH-CO-CH_3

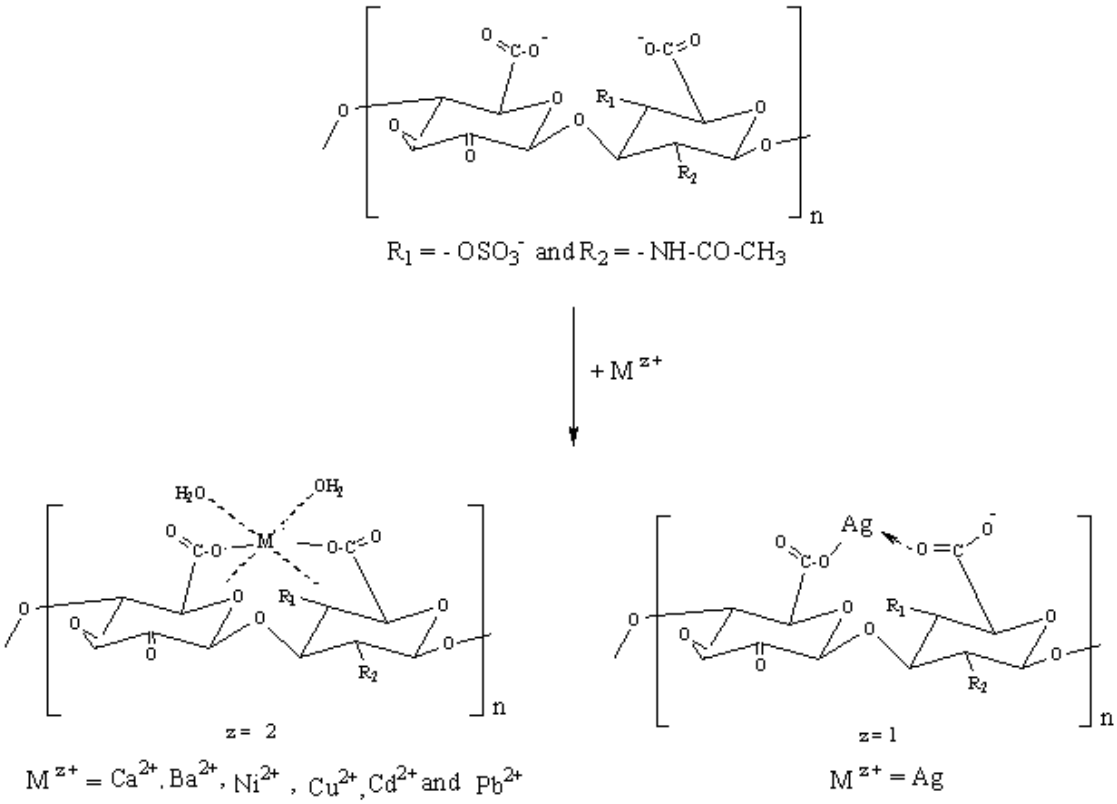
Scheme I

Decomposition

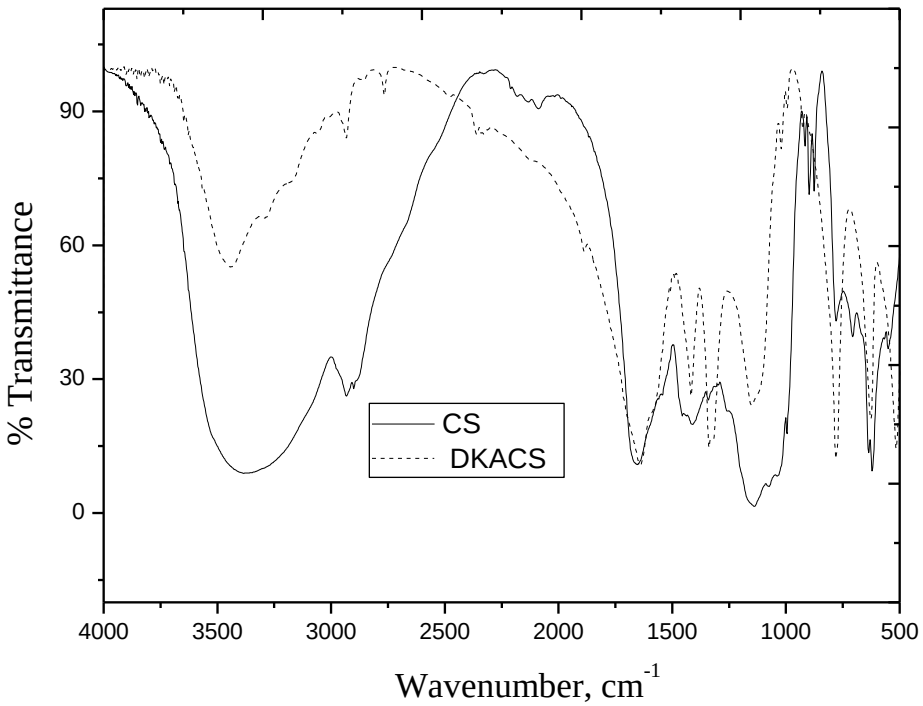


R_1=-OSO_3^- and R_2 = -NH-CO-CH_3

Scheme II


R1 = - OSO3- and R2 = - NH-CO-CH3
+ M z+
z = 2
M z+ = Ca2+, Ba2+, Ni2+ , Cu2+, Cd2+ and Pb2+
z = 1
M z+ = Ag

Scheme III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Wavenumber, cm-1
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DKACS
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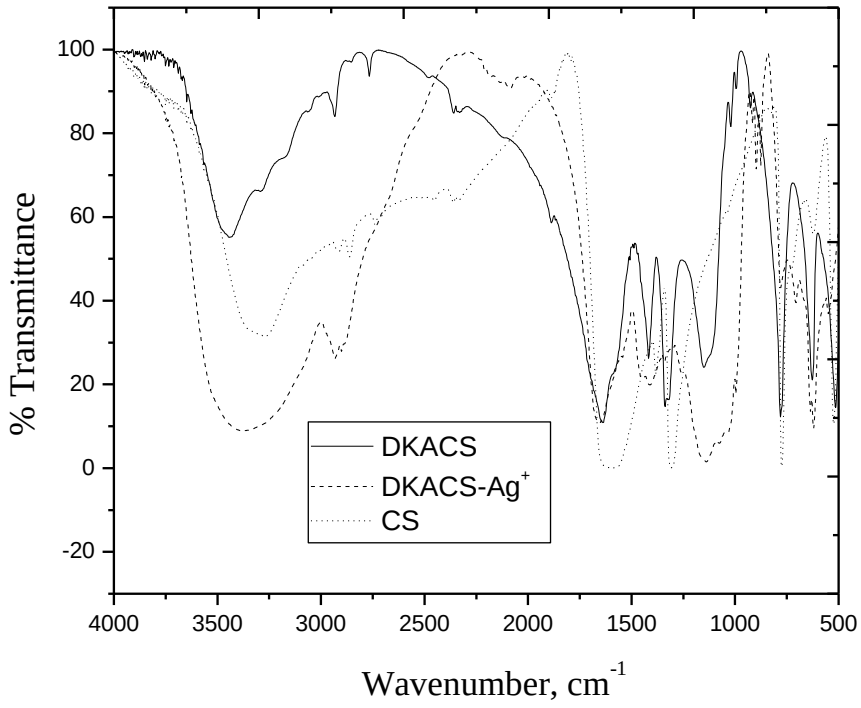
60
30
0
4000
3500
3000
2500
2000
1500
1000
500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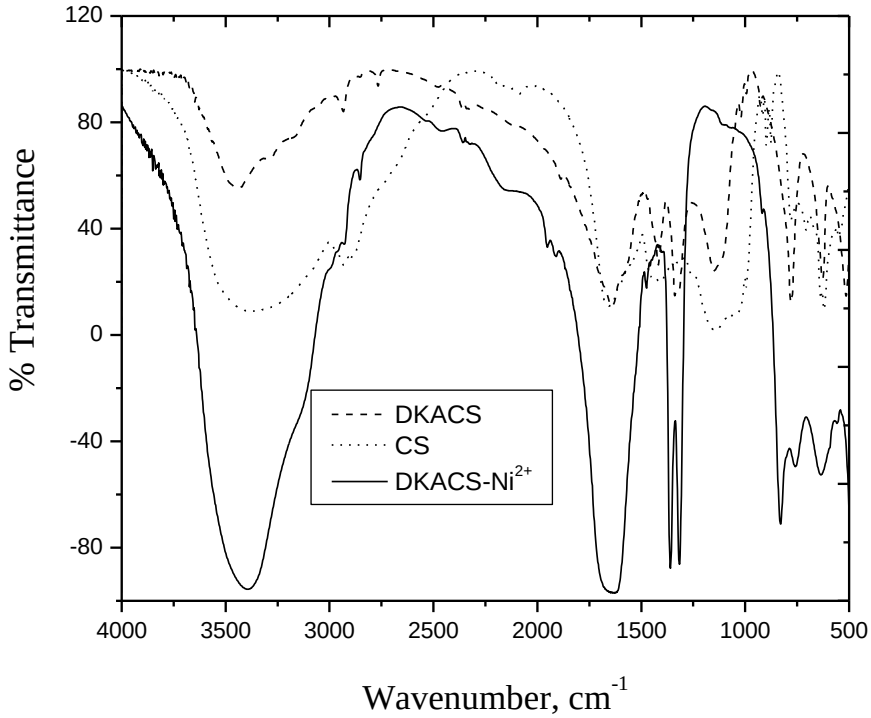
80
60
40
20
0
-20
4000
3500
3000
2500
2000
1500
1000
500
Wavenumber, cm-1
DKACS
DKACS-Ag+
CS


% Transmittance
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80
40
0
-40
-80
4000
3500
3000
2500
2000
1500
1000
500
Wavenumber, cm-1
DKACS
CS
DKACS-Ni2+

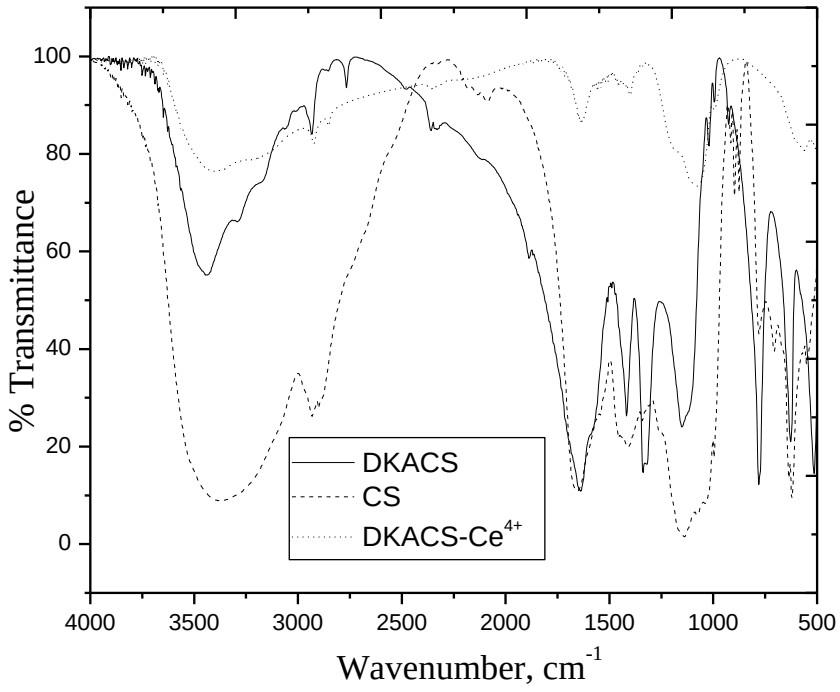


Figure 1. FTIR spectra of chondroitin-4-sulfate, its diketo-acid derivative (II) and metal complexes.

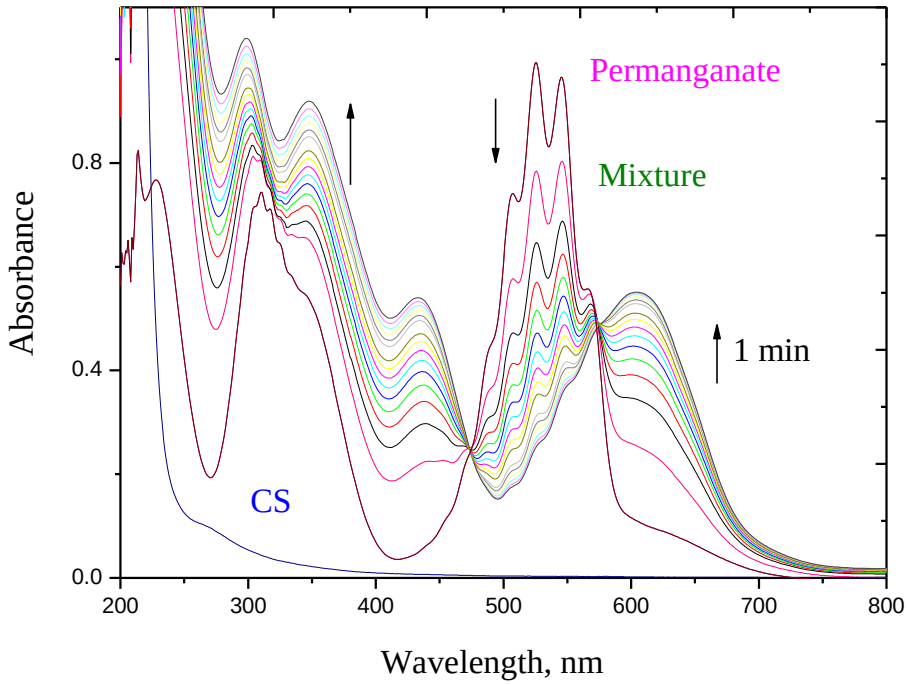


Figure 2. Spectral changes (200-800 nm) during the formation of the intermediate complex in the oxidation of chondroitin-4-sulfate by alkaline permanganate. $[MnO_4^-] = 4x10^{-4}$, [CS] = $5.5x10^{-3}$, $[OH^-] = 0.05$ and I = 0.1 mol dm^{-3} at 25^o C (scanning time intervals = 1 min).

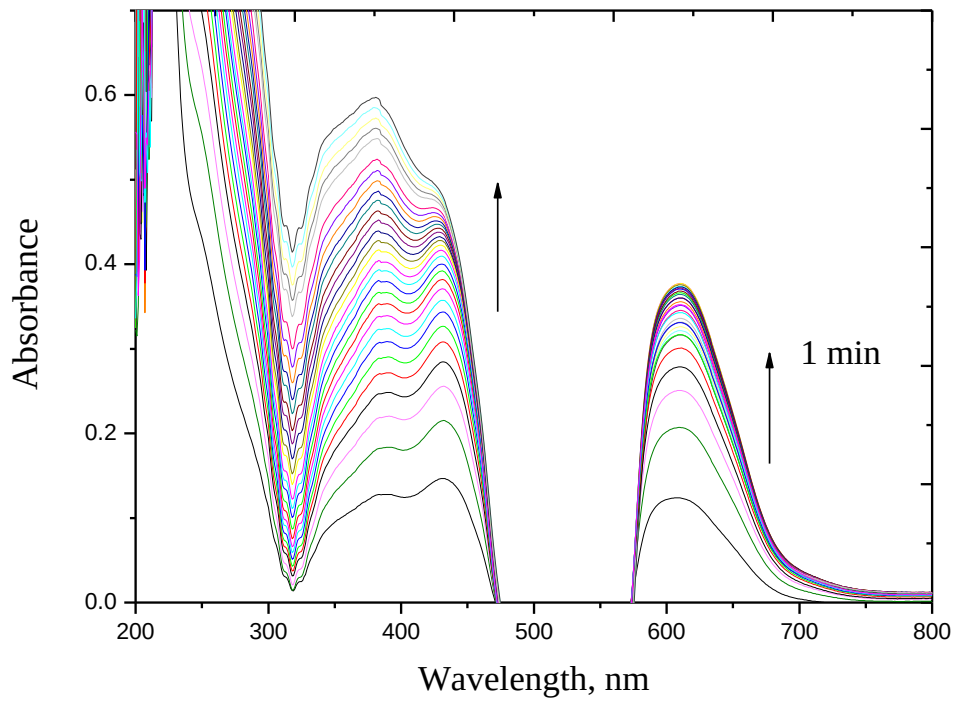
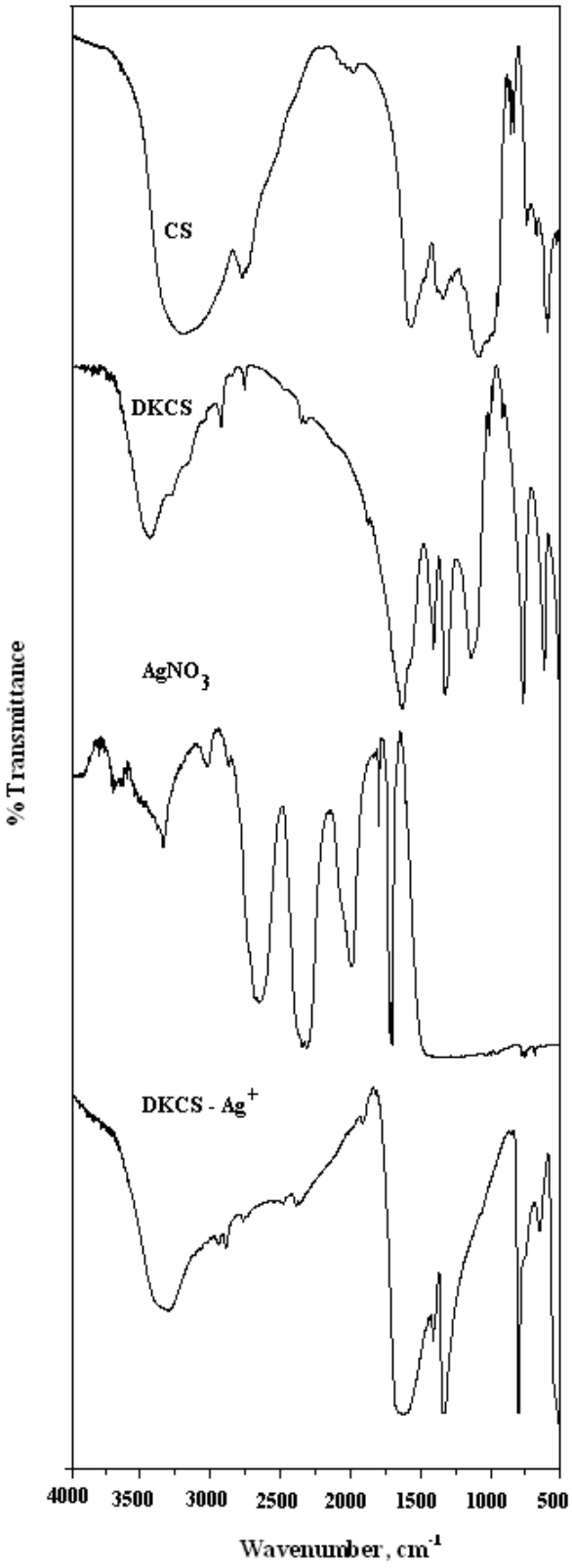
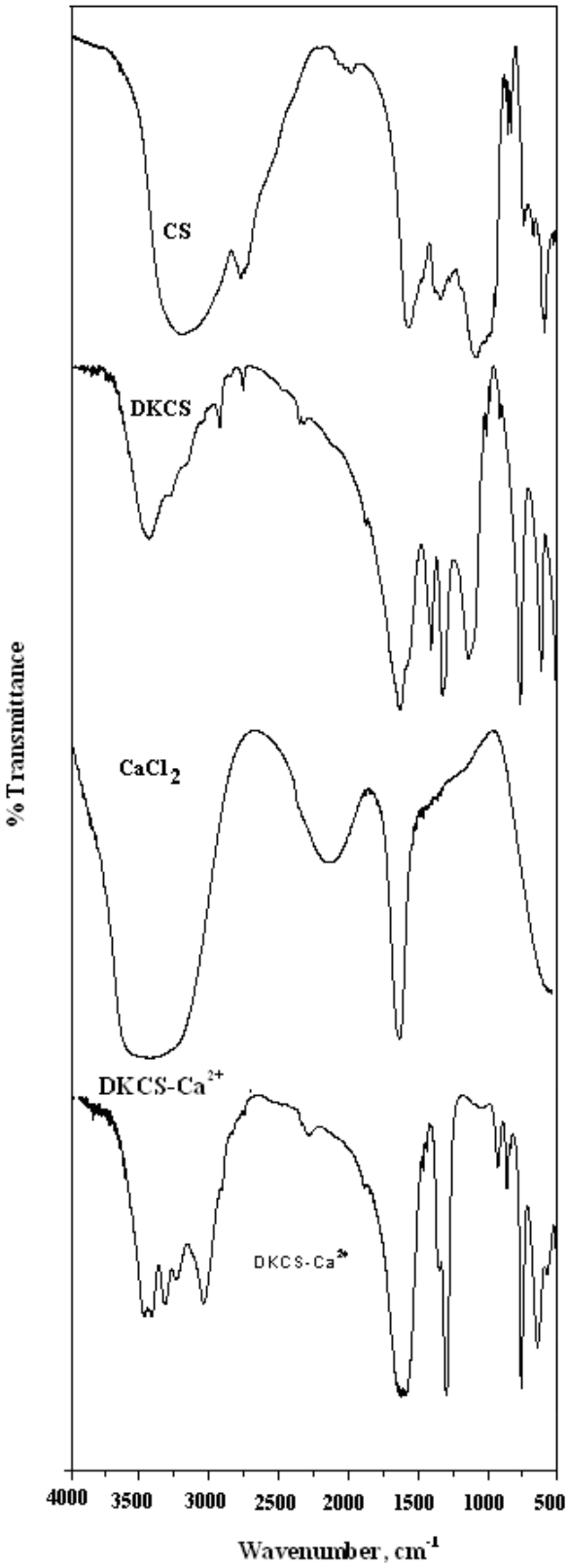
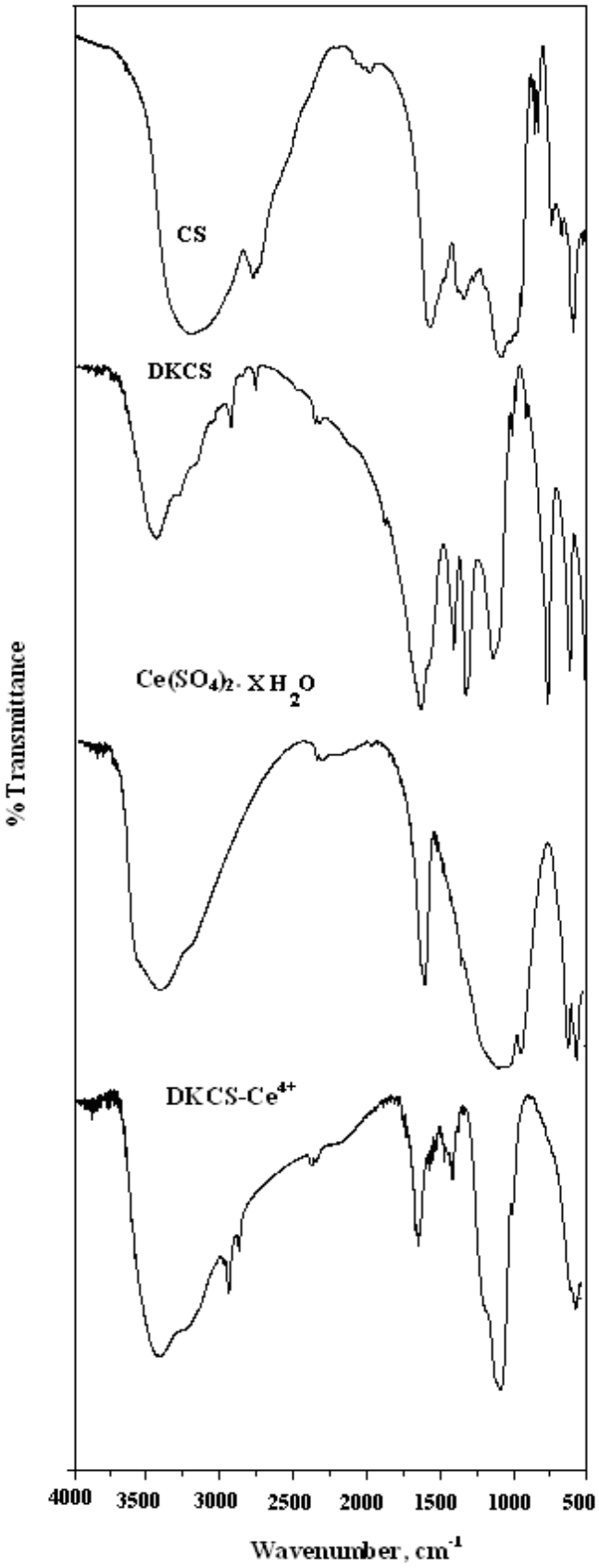


Figure 3. Spectral changes (200-800 nm) during the formation of the intermediate complex in the oxidation of chondroitin-4-sulfate by alkaline permanganate. $[MnO_4^-] = 4x10^{-4}$, $[CS] = 5.5x10^{-3}$, $[OH^-] = 0.05$ and I = 0.1 mol dm^{-3} at 25° C (scanning time intervals = 1 min). Ref. $[MnO_4^-] = 4x10^{-4}$ mol dm^{-3}.


CS
DKCS
AgNO3
DKCS - Ag+
% Transmittance
4000 3500 3000 2500 2000 1500 1000 500
Wavenumber, cm-1


CS
DKCS
CaCl2
DKCS-Ca2+
DKCS-Ca2+
% Transmittance
4000 3500 3000 2500 2000 1500 1000 500
Wavenumber, cm-1


CS
DKCS
Ce(SO4)2·XH2O
DKCS-Ce4+
% Transmittance
4000
3500
3000
2500
2000
1500
1000
500
Wavenumber, cm-1