

# Synthesis and characterization of modified chitosan as a promising material for enterosorption of heavy metal ions

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## ABSTRACT

Currently, an important ecological problem is environmental pollution and its negative impact on living organisms, the consequences of which are deterioration in general health and the manifestation of various diseases, poisoning, endo- and exotoxicosis. Enterosorption method was proposed as a promising method for removing toxic substances from the living organisms using enterosorbents which can absorb various toxic substances of endogenous and exogenous nature in the lumen of the gastrointestinal tract. It has been proposed to use polymer-containing enterosorbents for eliminating of heavy metals from the organism. The purpose of this research was to synthesize a quaternized derivative of chitosan, specifically N-(2-hydroxybenzyl)-N-ethyl-N-methyl chitosan chloride (Q-CHS). The synthesis of Q-CHS involved the formation of a Schiff base, followed by the quaternization of the amino group of chitosan (CHS). The structures of both pure CHS and quaternized CHS were studied using various physico-chemical methods, including FTIR, NMR, XRD, SEM, DSC and TGA analyses in order to determine the structure and confirm the formation of the final product.

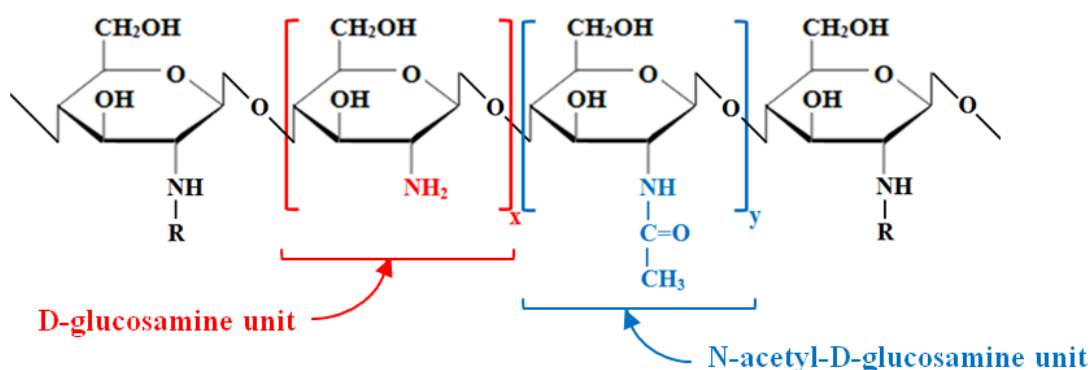
**Keywords:** Synthesis, Characterization, Chitosan, Chemical modification, Quaternization, Structure, Functional group

## 1. Introduction

Deterioration of the environmental situation in the world is directly related to human activities. The development of industry, the construction of factories, greenhouse gas emissions, the treatment of agricultural plants with pesticides lead to destructive processes on the planet and deterioration of the environment. Atmosphere, soil, and water are polluted, resulting in depletion of the ozone layer, death of flora and fauna, major epidemics and serious diseases. Due to global warming, weather conditions have changed, extreme weather phenomena and natural disasters (excessive rains and floods, forest fires and droughts, hurricanes, earthquakes, tsunamis) becoming more common. All this, of course, affects the environmental situation on the planet, human health and concerns every inhabitant of the planet [1].

The direct impact of the negative state of the environment on a man leads to intoxication of the human organism. A number of harmful substances enter the human organism, including heavy metals, a decrease in immunity and, as a consequence, an increase in morbidity, are observed. Considering the intake of heavy metals through food and to prevent serious risks to human health, the development of effective methods for removing heavy metals and eliminating the toxicity of these metals in air, soil and water is of great importance [2-4]. The toxicity of metals is associated with their ability to damage cell membranes, penetrate and destroy biological barriers, bind to proteins, and block enzymes, which leads to pathological changes in the organism and the occurrence of diseases [5].

Recent scientific publications highlight enterosorption as an effective method for removing various toxic substances from the organism. This process involves the oral administration of drugs, known as enterosorbents, which can absorb toxic substances of both endogenous and exogenous origin within the gastrointestinal tract [6]. The use of enterosorbents leads to a decrease in the metabolic load on the detoxification and excretory organs (liver, kidneys, etc.), helps to normalize the motor, defecation and digestive functions of the gastrointestinal tract, and increases the clinical effectiveness of treatment against the background of ongoing antibacterial or antiviral therapy. Currently, to prevent exogenous intoxication and for prophylaxis of toxic-allergic reactions, these sorbents are in demand in gastroenterology, toxicology, allergology, dermatology, surgery, narcology, and oncology [7]. Should be noted important characteristics of enterosorbents, such as chemical resistance, stability of chemical composition, minimal traumatic effect on biological tissues, mechanical strength, selective adsorption, optimal sorption capacity, lack of toxicity, complete elimination from the gastrointestinal tract [8]. In this regard, the search and development of new effective, easily accessible, harmless and improved compositions of these drugs is a topical question today. In order to prepare such sorbents, polymeric materials of plant and animal origin, in particular those obtained from natural CHS, can be used [9].



where R = H or COCH<sub>3</sub>

**Fig. 1.** Chemical structure of CHS.

CHS is a polysaccharide (Fig. 1) consisted of two monosaccharide units: 2-amino-2-deoxy- $\beta$ -D-glucopyranose and 2-acetamido-2-deoxy- $\beta$ -D-glucopyranose linked by  $\beta$ -(1-4) glycosidic bonds [10]. Advantages of CHS and its derivatives compared to other polymers are presented in literature data [11,12]. Antibacterial [13], anti-inflammatory, hemostatic and regenerative effects on the skin [14];

excellent biocompatibility and biodegradability [15]; good water absorption and retention properties [16]; as well as the presence of reactive amino (-NH<sub>2</sub>) and hydroxyl (-OH) groups in the composition, which allow the grafting of other functional groups and moieties in order to enhance certain biological functions [17] are discussed in many scientific publications. Chitosan derivatives are obtained by modification of amino groups, hydroxyl groups at C<sub>3</sub> and C<sub>6</sub> positions via substitution, crosslinking, grafting and other reactions [10]. Chemical modification makes it possible to expand the scope of application of CHS and its derivatives, and improve their characteristic properties [18]. The aim of chemical modification is also to enhance sorption capacity of the chitosan-based sorbents by increasing internal sorption surface and amount of sorption-active centers [19].

In this regard, the given paper is dedicated to modification and quaternization of CHS with the purpose further to use it as an enterosorbent for removal of excess amount of heavy metal ions from the organism. It is planned to develop and apply cross-linked systems based on chitosan and its derivatives with a “memory effect” in relation to heavy metal ions for the purpose of further selective sorption and removal of heavy metal ions from a living organism. The use of polymer memory involves preliminary adjustment of the active centers of a polymer sample to sorbed metals, followed by their fixation by intermolecular cross-linking. The results of studying sorption properties of CHS and its derivatives towards the heavy metal ions will be reflected in future publications. One of the CHS derivatives, specifically N-(2-hydroxybenzyl)-N-ethyl-N-methyl chitosan chloride was synthesized via formation of Schiff base. In this study, the synthesized samples were characterized using various physico-chemical methods including FTIR, <sup>1</sup>H NMR, XRD, SEM and thermal analyses (DSC/TGA).

## 2. Experimental section

### 2.1. Materials and chemicals

Medium-molecular-weight (MMW) of CHS (190-310 kDa, ≥75% deacetylated, viscosity of 200–800 cP, product number: 448877; CAS-No.: 9012-76-4) was purchased from Sigma–Aldrich. All other used reagents (glacial acetic acid (CH<sub>3</sub>COOH), sodium hydroxide (NaOH), salicylaldehyde (C<sub>7</sub>H<sub>6</sub>O<sub>2</sub>), ethyl alcohol (C<sub>2</sub>H<sub>5</sub>OH), sodium borohydride (NaBH<sub>4</sub>), methyl iodide (CH<sub>3</sub>I), sodium chloride (NaCl)) were of analytical grade. All materials were used without preliminary purification. During the experiments aqueous solutions were prepared using distilled water.

### 2.2. Synthesis of N-substituted quaternized CHS

N-substituted CHS (N-(2-hydroxybenzyl)-N-ethyl-N-methyl CHS chloride) (Q-CHS) was prepared (Fig. 2) according to the procedure [20] with some modifications. 100 ml of 1% solution of acetic acid was added to 2 g (12 mmol) of CHS and mixed using magnet stirrer approximately 5 hours at 35 °C up to complete dissolution of CHS. After dissolving, 2.5 mL salicylaldehyde was added into the CHS solution and stirred for 5 hours at room temperature. At this time, the solution turns yellow.



**Fig. 2.** External appearance of CHS (left) and Q-CHS (right) samples.

Using 1M solution of NaOH pH of yellowish viscous solution was adjusted to 4. Then, 0.01 g of NaBH<sub>4</sub> was dissolved in 30 mL of distilled water (dH<sub>2</sub>O) and gradually added to the mixture and continue stirring at room temperature. After stirring during 12 hours, pH of solution was adjusted to 10, and N-(2-hydroxybenzyl) CHS was prepared. Obtained sample was filtered and washed by dH<sub>2</sub>O, ethanol, acetone, diethyl ether and dried at 40 °C for a day. Next stages (ethylation and quaternization) proceed according to the procedure [21]. 50 mL 1% solution of acetic acid was added to 1 g of N-(2-hydroxybenzyl) CHS and stirred for 3 hours, after mixing 0.33 mL of acetaldehyde was added to this mixture. After stirring 10 hours, 30 mL of 0.01 mol/L NaBH<sub>4</sub> solution gradually added to the mixture and continue to stir at the room temperature for 12 hours. The pH of the solution was adjusted to 10 by adding a 1M NaOH solution, resulting in the formation of N-(2-hydroxybenzyl)-N-ethyl chitosan. Prepared sample was filtered and washed by dH<sub>2</sub>O, ethanol, acetone, diethyl ether and dried at 40 °C.

Last stage, quaternization, was carried out in acetonitrile medium. For this stage 0.7 g of N-(2-hydroxybenzyl)-N-ethyl CHS is mixed in 20 mL acetonitrile and stirred for 30 minutes at 35 °C. Then 0.26 mL methyl iodide, CH<sub>3</sub>I, was added drop by drop into the mixture and continues to mix at 35 °C during 30 hours. The obtained sample N-(2-hydroxybenzyl)-N-ethyl-N-methyl CHS iodide was filtered and washed by dH<sub>2</sub>O, ethanol, acetone, diethyl ether and dried at 40 °C for a day. Then, N-(2-hydroxybenzyl)-N-ethyl-N-methyl CHS iodide was subjected to ion exchange reaction with 10% NaCl aqueous solution for one day and finally N-(2-hydroxybenzyl)-N-ethyl-N-methyl CHS chloride was prepared. The synthesized light orange product was filtered and washed with alcohol, acetone, and diethyl ether. It was then dried at 40 °C for 24 hours until a constant weight was achieved and stored in a desiccator until further use.

### **2.3. Instrumentation**

#### **2.3.1. Attenuated Total Reflectance-Fourier-transform infrared spectroscopy (ATR-FTIR)**

FTIR spectra were acquired through attenuated total reflection (ATR) methodology, using a Perkin-Elmer spectrometer (Norwalk, CT, USA) equipped with universal-ATR accessory, incorporating a diamond optical element and ZnSe focusing elements. The measurements were conducted in the range 4000 - 600 cm<sup>-1</sup> at a resolution of 2 cm<sup>-1</sup>, 32 scans were performed at 25 °C. Recorded spectra were subjected to ATR correction and normalization.

### **2.3.2. Nuclear Magnetic Resonance spectroscopy (NMR)**

<sup>1</sup>H NMR spectra of CHS and Q-CHS were recorded on a Bruker Avance III 400 MHz spectrometer. A dilute solution (around 10 mg mL<sup>-1</sup>) of both CHS samples was prepared using a 1% deuterated acetic acid, CD<sub>3</sub>COOD/D<sub>2</sub>O. Solution spectra were analyzed at room temperature under the following measurements conditions: 90° pulse width 7.5 μs, relaxation delay 1 s, acquisition time 1.4 s, and 16 scans.

### **2.3.3. X-ray diffraction analysis (XRD)**

XRD tests of the powder samples of CHS and its derivatives were performed using Rigaku model ULTIMA XRD diffractometer (Tokyo, Japan) with Cu Kα radiation in the scattering range 5–50° ( $\lambda = 0.154$  nm, 40 kV, 20 mA, scanning rate 4 °C/min) in a fixed time mode at room temperature.

### **2.3.4. Scanning Electron Microscopy (SEM)**

Surface morphology of both CHS and N-(2-hydroxybenzyl)-N-ethyl-N-methyl CHS chloride samples was observed using scanning electron microscope (SEM) S-3400N (Hitachi, Japan), which was taken at 50, 100 and 250 magnifications at acceleration voltage of 1.50 kV and a work distance of about 7 mm, without sputtering.

### **2.3.5. Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA)**

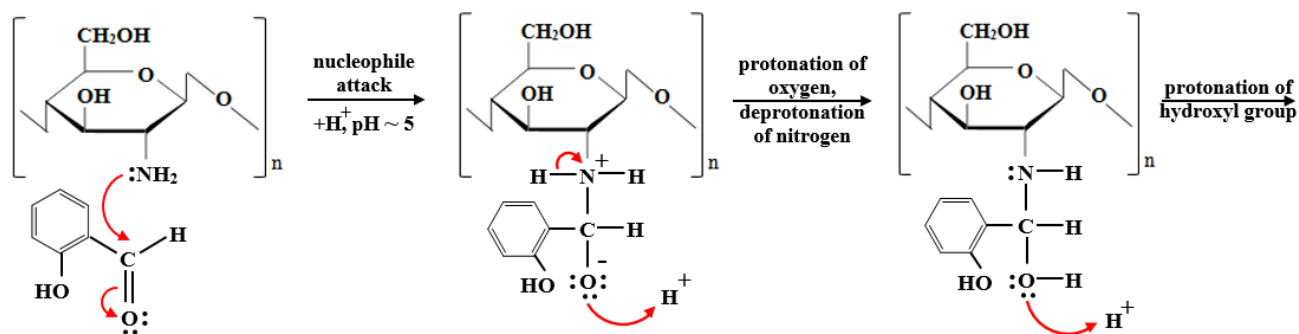
Thermal properties of polymers were assessed using a TA DSCQ2000 differential scanning calorimeter, equipped with a TA Instruments DSC cooling system. During the measurements and thermal treatments, dry nitrogen gas was purged through the cell at a flow rate of 20 mL/min. Samples of approximately 7 mg were equilibrated at 25 °C, heated to 110 °C and kept at this temperature for 15 min, in order to remove all the water adsorbed in the sample. Then, they were cooled from 110 to 25 °C, kept at this temperature for 3 min and re-heated from 25 to 400 °C. Heating/cooling rate was fixed to 20 °C/min in all the experiments.

The thermogravimetric analysis (TGA) was carried out to evaluate the thermal stability and thermal decomposition of polymers with increasing temperature. The TGA was performed using a Perkin-Elmer Pyris Diamond TG-DTA apparatus in a nitrogen atmosphere, in order to prevent thermal oxidation. Samples of about 10 mg were heated from 25 to 700 °C at 10 °C/min with a nominal gas flow of 30 mL/min. TGA curves allowed to evaluate the thermal decomposition profile of polymers, whereas DTG curves were employed to accurately determine the temperature at which both the water removal and the polymer decomposition occurred.

### 3. Results and discussion

#### 3.1. Synthesis of *N*-substituted quaternized CHS (Q-CHS)

Synthesis process of Q-CHS is described by nucleophilic addition, elimination and nucleophilic substitution mechanisms and occurs via arylation, alkylation, reduction and quaternization (formation of quaternary ammonium salts) reactions (Fig. 3). According to the given mechanisms [22,23], introducing an aromatic group into the amine group of CHS was carried out, thus addition of nitrogen atom of amino group (as a nucleophile) of CHS to the carbon atom (as an electrophile) of carbonyl group of aldehyde leads to the shift of electronic density to oxygen of carbonyl group, then protonation of oxygen atom and at the same time deprotonation of nitrogen atom occurred. Next reaction is protonation of hydroxyl group and leaving of water molecule followed at the same time formation of double bond between nitrogen and carbon atoms (resonance-stabilized cation). Chemical reaction was occurred through formation of Schiff base intermediate [24]. Reduction reaction was performed with participation of NaBH<sub>4</sub> as a strong nucleophile providing hydride ion (H<sup>-</sup>). Quaternization (methylation) reaction occurs via nucleophilic substitution mechanism S<sub>N</sub>2 by using methyl iodide in the medium of acetonitrile with formation of quaternized CHS: N-(2-hydroxybenzyl)-N-ethyl-N-methyl CHS iodide. Last stage was ion exchange occurred with participation of aqueous solution of sodium chloride for substitution of iodide ions. This process promotes a better aqueous solubility of N-aromatic CHS derivatives over a wide pH range [25]. The chemical structures of the initial and final products were characterized using various physico-chemical methods to confirm the formation of the final product.



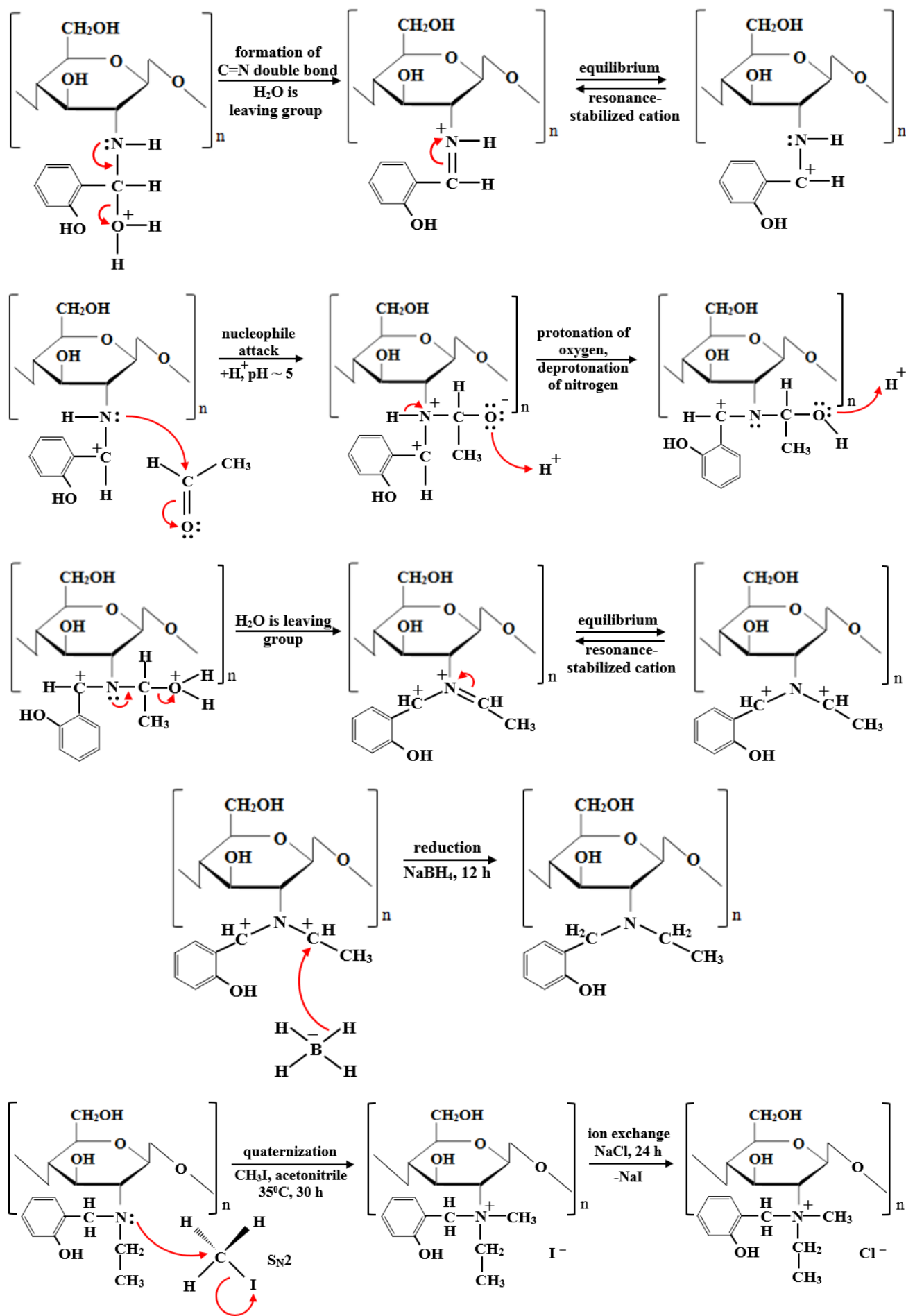


Fig. 3. Probable mechanism of the reaction for obtaining Q-CHS.

## 3.2. Characterization of pristine and quaternized CHS

### 3.2.1. ATR-FTIR spectroscopy analysis

ATR-FTIR spectroscopy is used to study molecular structures, identifying functional groups of the samples. ATR-FTIR spectra of CHS, Schiff base and Q-CHS samples are shown in Fig. 4.

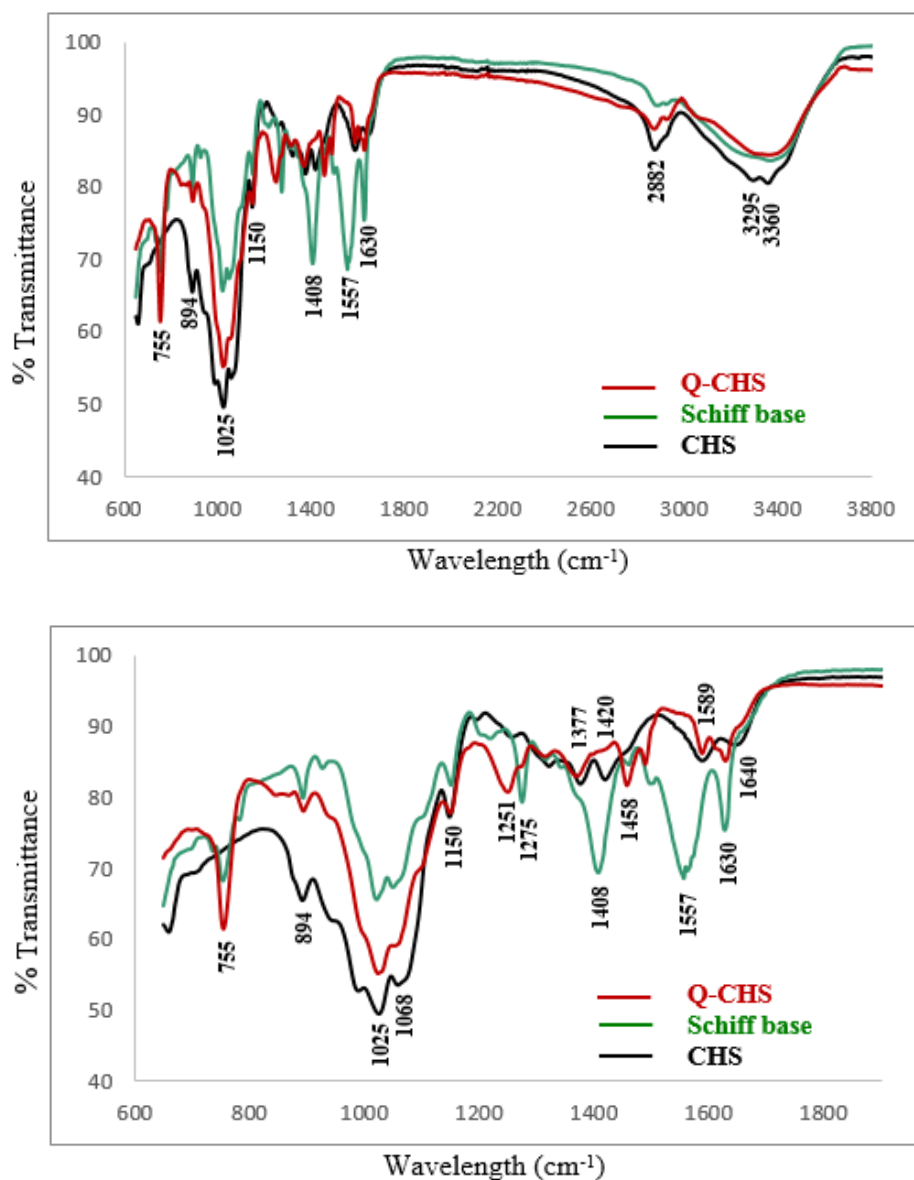


Fig. 4. FTIR spectra of CHS, Schiff base and Q-CHS samples.

All three spectra showed C–O antisymmetric  $\beta$ -(1→4) glycosidic bonds at  $1150\text{ cm}^{-1}$  and characteristic polysaccharide peaks at  $1025$  and  $1068\text{ cm}^{-1}$ . C–N axial deformation of amine groups in CHS at  $1420\text{ cm}^{-1}$  is not observed in other samples; C–H stretching at  $2882\text{ cm}^{-1}$  shifted to  $2899\text{ cm}^{-1}$ ; and both O–H and N–H stretching overlapped at  $3295\text{--}3360\text{ cm}^{-1}$  are presented as a broad band due to the hydrogen bond interactions. The modification of CHS with salicylaldehyde was indicated by the resulting peaks at  $755\text{ cm}^{-1}$ ,  $1251\text{ cm}^{-1}$  and  $1458\text{ cm}^{-1}$ , related to C–H out of plane bending of the aromatic ring, C–O phenolic stretching and C=C stretching of the aromatic groups, respectively [26].



The imine band appeared at  $1557\text{ cm}^{-1}$  confirmed the formation of Schiff base. Appearance of this band at  $1557\text{ cm}^{-1}$  can be explained by occurring the crosslinking reaction between CHS and aldehyde [27]. The spectrum of quaternized CHS shows a peak at  $1589\text{ cm}^{-1}$ , representing the C=N elongation vibrations of the residual imine bonds. Also, the characteristic peak ascribed to  $\text{NH}_2$  deformation vibration and amide II band in CHS at  $1589\text{ cm}^{-1}$  [28] was weakened in the spectrum of Q-CHS and disappeared in the spectrum of Schiff base. Weak absorption band at  $1648\text{ cm}^{-1}$  is observed in spectra due to the amide group of acetylated moiety of CHS [29]. The results showed that the chemical modification of CHS with salicylaldehyde occurred at the C-2 position, indicating the stretching C=N vibrations characteristic of Schiff's base imines and N-H bending vibrations at  $1630\text{ cm}^{-1}$  [30-33]. In FTIR spectra of Schiff base and Q-CHS peaks at about  $1490\text{ cm}^{-1}$  belong to C=C stretching and C-H deformation of the aromatic groups, and C-H bending of  $^+\text{N}$  (2-hydroxybenzyl, ethyl, methyl) group, respectively [34,25] (Table 1).

**Table 1**

Interpretation of FTIR spectra data of the obtained samples.

Wavenumber, $\text{cm}^{-1}$	Functional groups	Ref.
3295-3360	O-H and N-H stretching	Matias [26]
1630, 1557	C=N vibrations characteristic of Schiff's base imines	Ismiyarto [30]
1500-1490	C-C stretching of aromatic ring	Jiao [31]
1490	C=C stretching and C-H (out of plane) deformation of the aromatic group, C-H bending of $^+\text{N}(\text{R})_3$ group	Santos [34]
1458	$\text{CH}_2$ , $\text{CH}_3$ bending, C=C stretching of the aromatic groups	Piras [35]
1251	C-O phenolic stretching, C-C bending vibrations of aromatic ring	Saeed [39]
1150	C-O antisymmetric $\beta$ -(1 $\rightarrow$ 4) glycosidic bonds	Bhandari [37]
1068	C-OH stretching of hydroxyl group	Yongping [25]
1025	characteristic polysaccharide peaks	Alli [40]
894	glucopyranose ring	Chopra [38]
755	C-H out of plane bending of the aromatic ring	Piras [35]

An increase in the intensity of the peaks at  $755\text{ cm}^{-1}$  ( $-\text{CH}_2$ ) is evident in the spectra of both the Schiff base and Q-CHS. The appearance of a peak at about  $1458\text{ cm}^{-1}$  ( $-\text{CH}_2$ ,  $-\text{CH}_3$  bending) in Schiff base and Q-CHS, which is completely absent on the spectrum of CTS, indicates that alkyl groups have been introduced into the N-aromatic CHS derivatives, illustrating the bending of the alkyl groups attached to the quaternary ammonium [35]. Additionally, the presence of a weak peak at  $1640\text{ cm}^{-1}$  indicates the vibrations of the remaining absorbed water [36]. Carbohydrates have a chemical skeleton made up of C-O, C-C, and C-H, which causes stretching vibrations and bending of the C-H group to occur in the fingerprint region between  $1450$  and  $700\text{ cm}^{-1}$ . Glucopyranose ring corresponded to the peak at roughly  $894\text{ cm}^{-1}$ , while the bending vibration of the C-H group is reflected in the absorption peak at  $1420\text{ cm}^{-1}$  [37,38]. Additionally, C-C bonds stretching and bending vibrations on the aromatic ring of the aldehyde appeared at  $1500$ - $1490$  and  $1251\text{ cm}^{-1}$ , respectively. There is no detectable residual of free Sal, as evidenced by the absence of the salicylaldehyde characteristic band in the area  $1660$ - $1730\text{ cm}^{-1}$  in the Schiff base spectrum [39]. The characteristic bands of primary and secondary

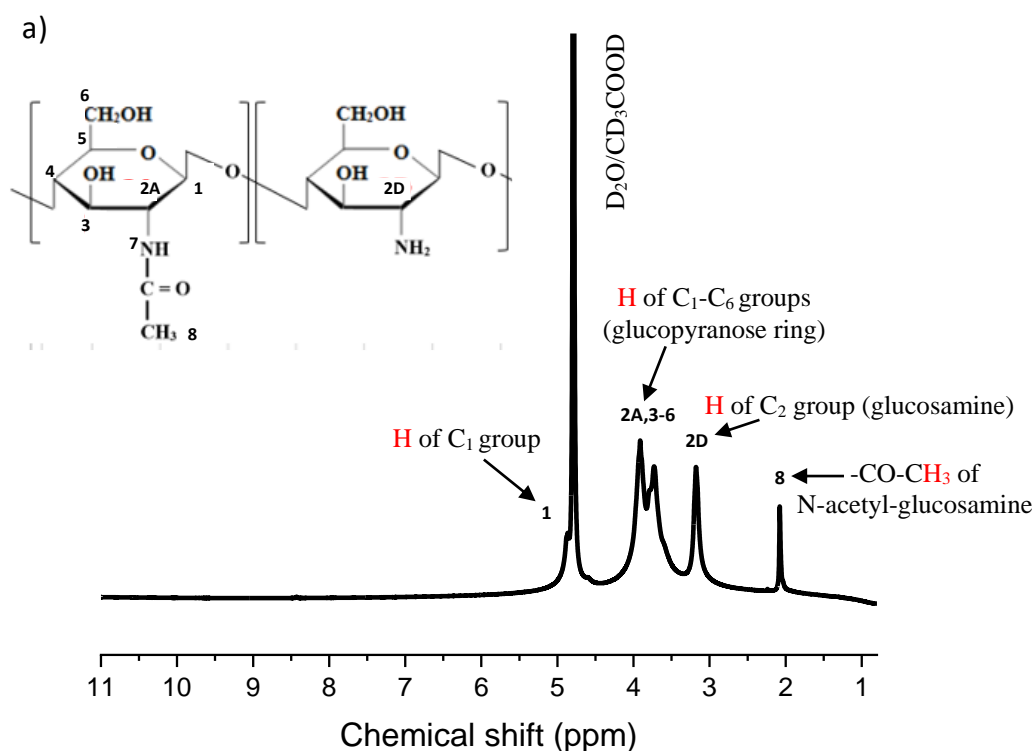
alcohols at 1150 and 1025  $\text{cm}^{-1}$  on CHS spectrum are retained on spectrum of Q-CHS, which signifies that the substitution took place only at amino groups and not at C-3 or C-6 on the CHS [40].

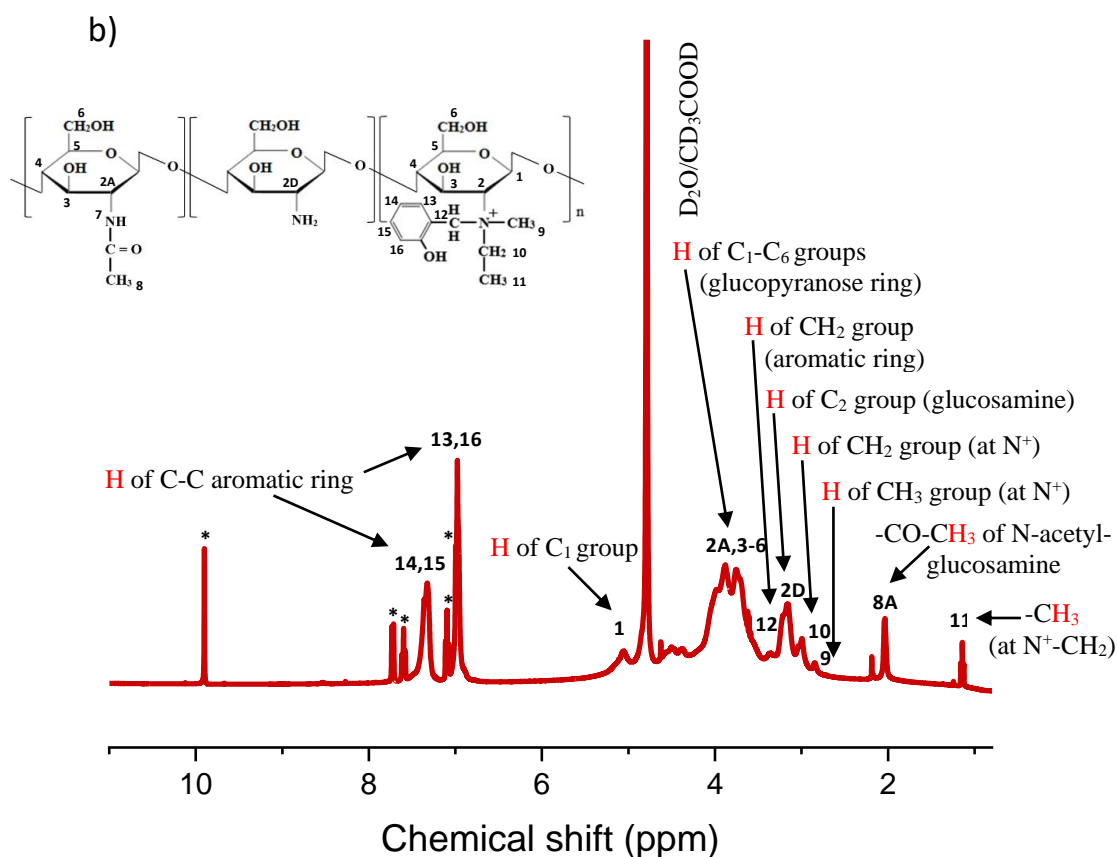
C-O stretching band at 1068  $\text{cm}^{-1}$ , related to primary hydroxyl group, confirmed no reaction had observed into this group of CTS. The amplified intensity of the peak at approximately 1458  $\text{cm}^{-1}$ , attributed to the methyl group in the quaternized N-aromatic CHS sample, signifies the introduction of this functional group into the N-aromatic CHS derivative, leading to the formation of Q-CHS [25]. These all indicate that the amino groups of CHS reacted with the aromatic aldehyde to form a N-substituted Q-CHS.

### 3.2.2. NMR spectroscopy analysis

NMR spectroscopy is an analytical method using for determination of the molecular structure and chemical composition of a substance. This method was used to identify the chemical structure of CHS and Q-CHS.

Fig. 5 shows the  $^1\text{H}$  NMR spectra of CHS and Q-CHS. Signal at 4.8 ppm was attributed to the solvent  $\text{D}_2\text{O}/\text{CD}_3\text{COOD}$ . The signals at 4.9 ppm are related to the proton in  $[\text{C}_1]$ ; multiple signals at 3.6-4.0 ppm are related to protons in  $[\text{C}_1]$ - $[\text{C}_6]$  of glucopyranose ring of CHS [41]. In CHS, the peak at 2.0 ppm was assigned to the three acetyl protons of N-acetyl glucosamine, while the peak at 3.1 ppm corresponded to the proton in  $[\text{C}_2]$  of the glucosamine or N-acetyl glucosamine residue. A decrease in the intensity of the signal at 3.1 ppm is observed. This decrease may be ascribed to the reaction involving amino groups at the  $[\text{C}_2]$  position of modified CHS and methyl iodide, leading to the formation of quaternary amino groups with a positive charge.



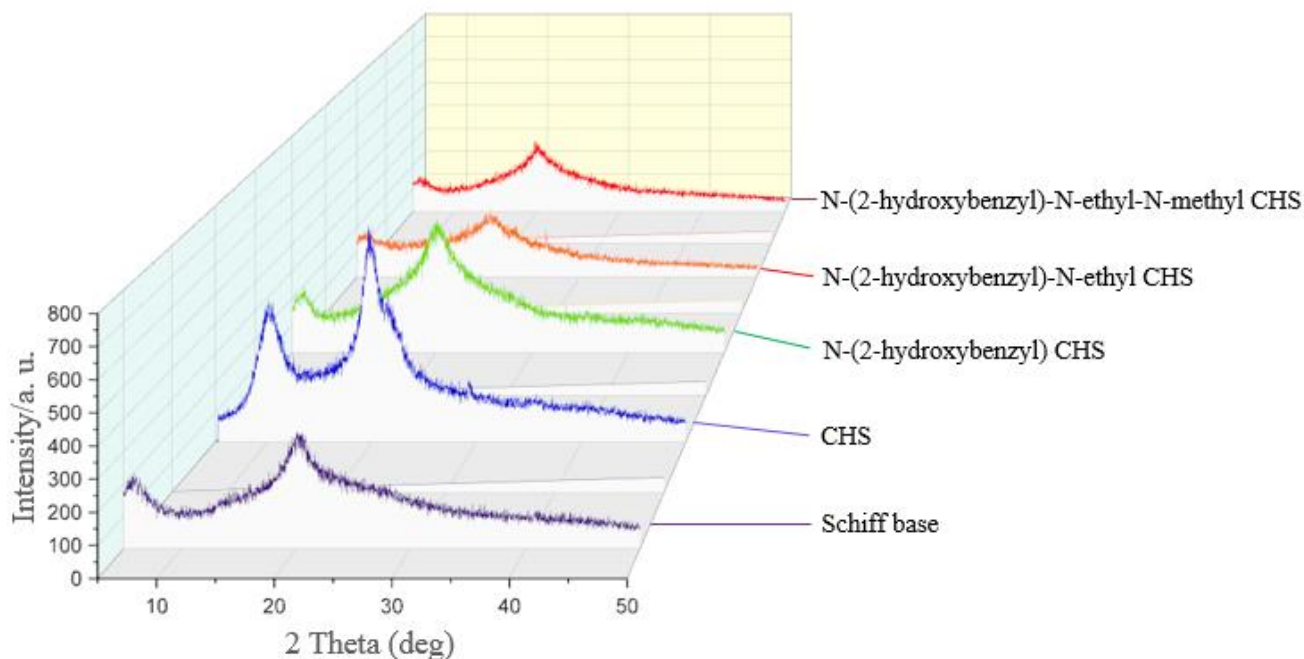


**Fig. 5.** <sup>1</sup>H NMR spectra of (a) CHS and (b) Q-CHS in  $D_2O/CD_3COOD$ .

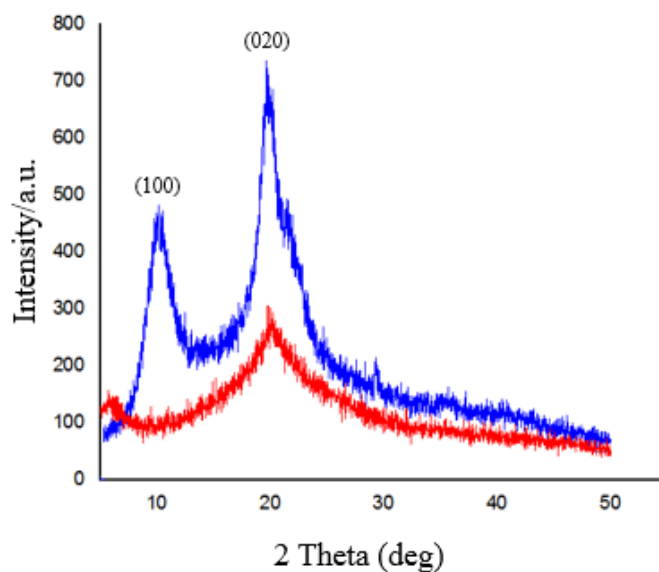
According to the literature data [42] shift of the peak from 3.2 (CHS) to 3.1 (Q-CHS) ppm is assigned to the quaternary amino group. The obtained <sup>1</sup>H NMR spectra revealed some differences in the structure of the two compounds. Peaks characteristic of the three radical groups bonded to the quaternary nitrogen were present in the spectrum of the quaternized CHS but absent in the spectrum of the initial CHS. The signals at 3.1 ppm are assigned to ethyl-methyl-(2-hydroxybenzyl) amino groups, while the peaks at 2.8–3.3 and 1.1 ppm are attributed to the protons of methylene and methyl groups at the quaternized amino groups. Additionally, peaks at 6.9–7.3 ppm are associated with aromatic protons within the composition of quaternized CHS [43,44]. The FTIR and NMR data showed that Q-CHS was successfully synthesized and obtained results were consistent with the literature data.

### 3.2.3. XRD analysis

XRD method of analysis is used for determination of crystal structure, phase, and structural parameters of the substance.



**Fig. 6.** (a) XRD spectra of CHS and its derivatives ( $\lambda = 0.154$  nm, 40 kV, 20 mA, scanning rate 4 °C/min).



**Fig. 6.** (b) XRD spectra of CHS (blue) and Q-CHS (red) ( $\lambda = 0.154$  nm, 40 kV, 20 mA, scanning rate 4 °C/min).

The X-ray diffraction patterns of CHS, its modifications and quaternized form are presented in Fig. 6. As it is noted in literature [45] CHS is related to semicrystalline material, having two expressed peaks at  $2\theta = 10.14^\circ$  and  $19.66^\circ$  due to diffraction on the (100) and (020) planes of the crystal lattice with interplanar spacings of 8.8 Å and 4.5 Å, respectively. The crystalline unit cell of CHS has an orthorhombic structure [46]. As can be seen some differences in peak height, width and position them are observed in diffractograms. In case of quaternized CHS, intensities of peaks became smaller and one of the peaks ( $10.14^\circ$ ) shifted to the left ( $5.7^\circ$ ). The observed changes in XRD patterns are connected with carried out modification and quaternization reactions of initial CHS. Introducing an aromatic

group into the amine group of CHS provides steric hindrance to the folding of the molecular chains of the CHS derivative. This behavior, which is characteristic of CHS derivatives, has been described in the literature [47]. The crystallinity of the obtained samples is dependent of the composition, spacial structure, nature of bondings in the molecules of synthesized compounds [31]. As can be seen from Fig. 6, when comparing the diffraction patterns of the initial CHS, its modified forms, as well as the final product, a decrease in crystallinity is observed in all these compounds. Crystal indices (%) CHS and Q-CHS were determined using the following formula [48,46]:

$$\text{Crystalline index}_{\text{CHS}} (\%) = \frac{(I_{020} - I_{am})}{I_{020}} \times 100 = ((643 - 240) / 643) \times 100 = 62.7 \%$$

$$\text{Crystalline index}_{\text{Q-CHS}} (\%) = \frac{(I_{020} - I_{am})}{I_{020}} \times 100 = ((301 - 156) / 301) \times 100 = 48.2 \%$$

where,  $I_{020}$  is the maximum intensity at  $2\theta \approx 20^\circ$  and  $I_{am}$  is the intensity (amorphous scattering) at  $2\theta \approx 16^\circ$  [49]. Observed alteration in diffractograms, [50] namely, decreasing in the crystallinity index, widening and decreasing in intensities of peaks in presented CHS derivatives show that all derivatives display a less crystalline character compared to initial CHS.

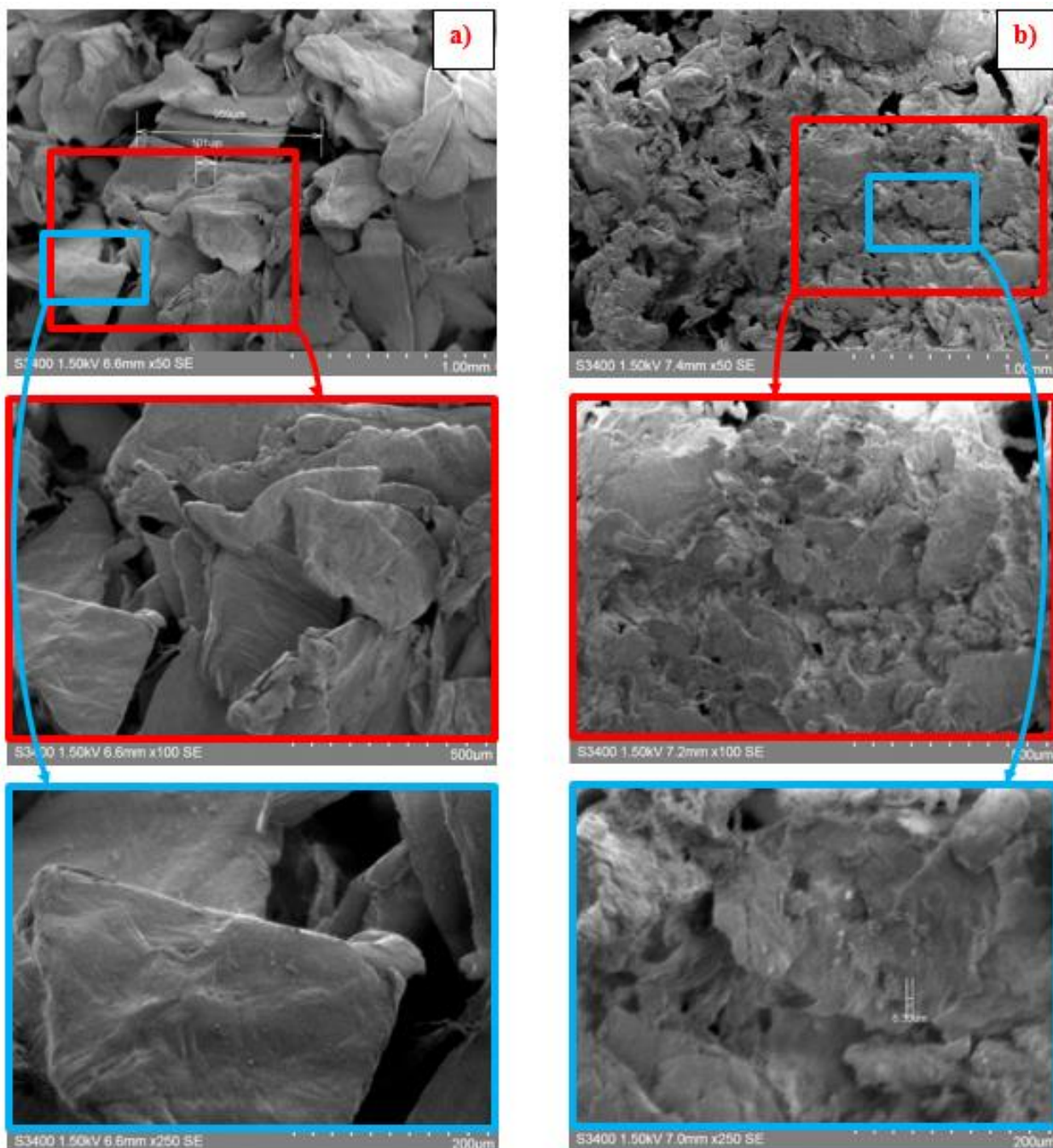
### 3.2.4. SEM analysis

SEM-EDS analysis is used to study morphology (texture), elemental composition, crystalline structure of the solid samples' surface. The morphological structures of CHS and Q-CHS are presented in Fig. 7. Morphology of pure CHS is characterized by irregularly shaped aggregates ranging in size from 101 to 959  $\mu\text{m}$  (Fig. 7a). Modification and quaternization of CHS caused some changes in morphology [43,51]. Thus, the surface of the samples became rougher, the formation of a less dense and porous structure of the samples of CHS derivative is observed, which can facilitate the sorption of metal ions from solutions (Fig. 7b), as well as due to the presence of reactive functional groups, i.e. due to the developed surface containing many active adsorption sites [52]. The results of elemental analysis to identify the elemental identification of the surface of the samples are presented in Table 2.

**Table 2**

Elemental characterization of CHS and quaternized CHS.

Element symbol	CHS		N-(2-hydroxybenzyl)-N-ethyl-N-methyl CHS chloride	
	weight %	atomic %	weight %	atomic %
C	38.19	43.92	43.06	49.34
N	23.65	23.32	17.13	16.84
O	37.55	32.43	38.59	33.20
Na	0.17	0.10	1.22	0.62
Mg	0.10	0.06	-	-
Al	0.08	0.04	-	-
Si	0.26	0.13	-	-
Total	100.00		100.00	



**Fig. 7.** SEM analysis of (a) CHS and (b) quaternized CHS under 50X, 100X and 250X magnifications, respectively (acceleration voltage: 1.50 kV; work distance: about 7 mm; without sputtering).

The elemental composition showed that the weight and atomic amounts of carbon in quaternized CHS sample increased indicating that the CHS had been modified by alkyl and aromatic groups. Elemental analysis represents also the presence of traces of impurities (Mg, Al, Si) on the surface of pure CHS samples. Traces of impurities in quaternized sample disappear.

### 3.2.5. DSC and TGA thermal analyses

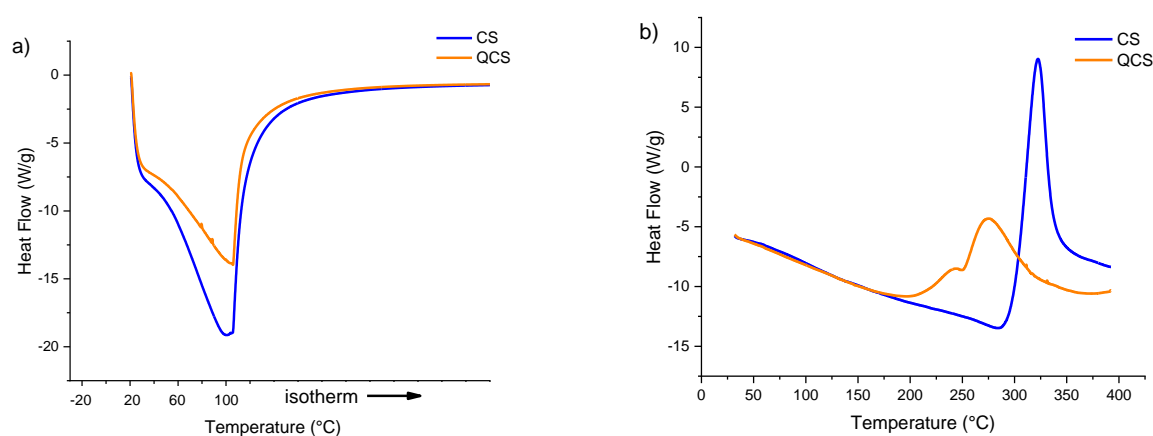
Thermoanalytical techniques, such as thermogravimetry (TGA) and differential scanning calorimetry (DSC), are particularly useful to evaluate the correlation between chemical structure and thermal transitions and stability of both polymers. Fig. 8a and b shows, respectively, the first and

second run of DSC curves for CHS (CS) and quaternized CHS (QCS). Quantitative data are listed in Table 3.

**Table 3**

Thermal characteristics (DSC and TGA) of CHS and Q-CHS.

Sample	DSC				TGA	
	1 <sup>st</sup> heating run		2 <sup>nd</sup> heating run		Thermal decomposition event	
	$\Delta H_{\text{evap}}$ (J/g)	$T_g$ (°C)	$T_{\text{degr}}$ (°C)	$\Delta H_{\text{degr}}$ (J/g)	$T_{\text{onset}}$ (°C)	$T_{\text{max}}$ (°C)
CHS	374	103	321	245	250	301
Q-CHS	269	128	275	210	211	255/396



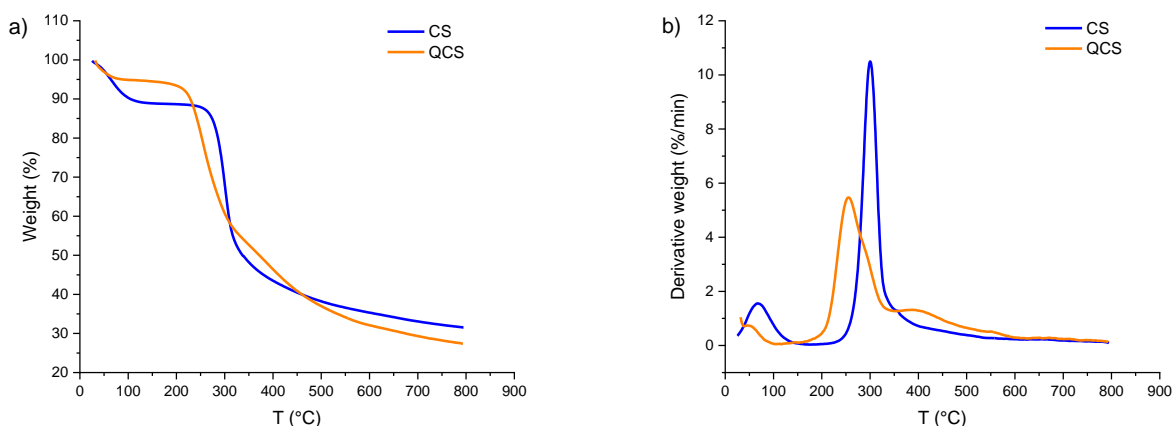
**Fig. 8.** DSC thermograms of CHS (CS) and quaternized CHS (QCS): (a) first and (b) second heating run. Approximately 7 mg samples were heated/cooled at 20 °C/min under dry nitrogen gas flow at a rate of 20 ml/min.

In the first heating run, both thermograms showed a broad endothermic peak, typically ascribed to the loss of adsorbed and bound water [53]. Although there were no discernible shifts in the peak position, there were notable differences in the enthalpy values. These variances suggest alterations in the water holding capacity and the strength of interactions between water molecules of the two polymers. In particular, Q-CHS exhibited a lower enthalpy than CHS (210.25 vs 245.2 J/g) due to the chemical modification of amino groups which reduced its ability to interact with water molecules through hydrogen bonds [54].

In the second heating run, the endothermic peak appeared no longer, since all the moisture was removed during the first run. A baseline step, due to the different heat capacity below and above the  $T_g$ , was detected, but it appeared slight since CHS is a partially crystalline polymer and because of its low specific volume resulting from rigid chain structures [55]. A higher  $T_g$  value was detected for modified CHS (128.4 vs 103.3 °C) due to the steric hindrance of grafted groups which hamper the movement of polymer chains and lead to the formation of a more rigid arrangement compared to unmodified CHS. Furthermore, in both thermograms an intense exothermic peak appeared, due to the

thermal decomposition of the polymer, encompassing both the deacetylation process and the decomposition of the substituted sites in the quaternized polysaccharide [56]. It is noteworthy that the exothermic peak of Q-CHS appeared as a much more complex band and shifted to lower temperature than that relative to CHS. Indeed, the peak maximum was detected around 321 °C for CHS and around 275 °C for functionalized CHS. This evidence suggested that the presence of the quaternary ammonium moiety on polymer chains reduce the polymer thermal stability due to both the weakening of interchain interactions and the reduction of polymer crystallinity with respect to CHS [57,58].

Fig. 9 displays the outcomes from TG and DTG analyses.



**Fig. 9.** TGA and DTG thermograms of (a) CHS and (b) Q-CHS. **Approximately 10 mg samples were heated from 25 to 700 °C at 10 °C/min with a 30 ml/min gas flow.**

A gradual loss in weight occurred up to 200°C for both CHS and quaternized CHS, due to the removal of adsorbed and bound water, with reductions of 10% and 5%, respectively. This results confirmed that water content in CHS was larger compared to Q-CHS, according to DSC results. The chemical modification of CHS amino moieties imparts to the polymer a hydrophobic character, thus reducing the extent of interactions with water molecules. The second loss event was attributed to the thermal decomposition of acetylated units within the polymer chains along with the depolymerisation [57,59]. Q-CHS showed a more complex decomposition band with respect to CHS, and it appeared less thermally stable than CHS, as suggested by its lower  $T_{\text{onset}}$  (Table 3). DTG curves, showed in Fig. 9b confirmed this evidence. Indeed, the temperature of the maximum rate of degradation decreased from 301 to 255 °C, from neat to quaternized CHS, likely due to the functionalization of the amino groups which reduced both the intra-chain interaction strength and the degree of crystallinity of the polymer, thus considerably lowering its thermal stability [60].

#### 4. Conclusion

The unfavorable ecological situation in the world leads to environmental deterioration, which affects the quality of drinking water and food. All this leads to intoxication of living organisms, as a result of which pathological changes are observed, leading to an increase in the number of diseases.



Enterosorption method is proposed as a promising direction in the treatment and prevention of certain diseases, toxic-allergic reactions, a method of cleansing the organism from various toxic compounds using enterosorbents. In this regard, it was proposed to use a polymer material based on CHS as an enterosorbent due to its unique properties, such as non-toxicity, high biological activity, biocompatibility, biodegradability, and high sorption properties. Containing reactive functional (amino and hydroxyl) groups, CHS can be subject to various modifications in order to improve certain properties and thus can find wide application. In this study, Q-CHS was successfully synthesized from initial CHS through arylation, alkylation, reduction, quaternization reactions with the purpose to use this compound in future as an enterosorbent. Initial and obtained Q-CHS were characterized by FTIR, NMR, XRD, SEM, thermal DSC and TGA analyses. Thus, the characteristic bands of primary and secondary alcohols at 1150 and 1025  $\text{cm}^{-1}$  on CHS spectrum are retained on spectrum of Q-CHS, which signifies that the substitution took place at amino groups and the amplified intensity of the peak at approximately 1458  $\text{cm}^{-1}$  indicates formation of Q-CHS. NMR data showed that peak at about 3 ppm is assigned to the quaternary amino group. Observed XRD diffractograms indicate that Q-CHS has a less crystal index (48.2%) compared to initial CHS (62.7%). The elemental composition showed that the weight and atomic amounts of carbon in quaternized CHS sample increased indicating that CHS had been modified by alkyl and aromatic groups. Decrease of the temperature of the maximum degradation rate from 301 °C (CHS) to 255 °C (Q-CHS) is associated with the quaternization process which reduced both the intra-chain interaction strength, and the degree of crystallinity of the polymer, thus considerably lowering its thermal stability. It was found that Q-CHS was successfully synthesized and obtained results were consistent with the literature data. Future studies will include studying of sorption capacity of CHS derivatives towards some heavy metals, excess concentration of which in organism can lead to some pathologies.

### **CRedit authorship contribution statement**

**S.S. Fatullayeva:** Writing – original draft, Visualization, Formal analysis. **D.B. Tagiyev:** Review & editing, Project administration. **N.A. Zeynalov:** Conceptualization, Review & editing, Supervision. **M.G. Raucci:** Review & editing, Supervision, Funding. **E. Amendola:** Project administration, Supervision. **G. Gomez d’Ayala:** Visualization, Investigation, Formal analysis. **A.D. Guliyev:** Methodology, Review & editing. **Sh.D. Tagiyev:** Investigation. **M.R. Marcedula:** Investigation, Methodology. **C. Demitri:** Methodology, Investigation, Formal analysis. **A.R. Guliyeva, & R.H. Suleymanova** – Resources, Investigation.

All authors have read and agreed to the published version of the manuscript.

### **Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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