

Insights into the Viscoelastic Peculiarities of Cyanobacterial Extracellular Polymeric Substance (EPS)

Hatice Kaplan Can

Hacettepe University: Hacettepe Universitesi

Serap Kavlak

Hacettepe University: Hacettepe Universitesi

Fatma Gurbuz (✉ fatma_gurbuz@yahoo.com)

Aksaray University <https://orcid.org/0000-0003-3561-3889>

Mehmet Odabaşı

Aksaray University

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Abstract

Extracellular polymeric substances (EPSs) can be defined as renewable, high molecular weight polymeric materials produced by bacteria and microorganisms. EPSs are composed of primarily polysaccharides and proteins, with minor amounts of nucleic acids, lipids, and humic substances. Cyanobacterial extracellular polymeric substances have a significant physiological effect on bloom formation and stress tolerance in adverse conditions. Therefore, cyanobacterial EPS has an important factor for aquatic life, environment and human life. For these reasons, determining the structure and structure-property relationships of cyanobacterial EPS is important for understanding its behavior and performance. In this study, the identification of the structure-property relationships, thermal and viscoelastic properties of cyanobacterial extracellular polymeric substance, X-ray diffraction (XRD) analysis, differential thermal analysis (DTA) and dynamic mechanical analysis (DMA) have been performed. Viscoelastic properties of the polymeric materials have been interpreted by certain DMA parameters at a fixed frequency depending on the temperature to understand the performance of cyanobacterial EPS.

1. Introduction

Extracellular polymeric substances (EPSs) are an important high molecular weight carbon rich polymeric materials obtained from microorganisms by bacteria, microalgae with microbe excretion, cell lysis and hydrolysis and sorption in waste water treatment plants [1, 2]. EPSs consist mainly of polysaccharides and proteins (75-90%), as well as minor amounts of nucleic acids, DNA, amphiphilic molecules, other micromolecules and humic substances [3–5]. EPSs have different applications as a potential bioflocculant in a wastewater treatment, bioremediation and sludge dewatering because of the renewable, biodegradable and ecofriendly properties [6].

In biological wastewater treatments, especially in biofilm formation and stability processes, EPSs can affect system performance by changing microbial aggregate properties [7]. In recent years, there has been an increasing demand for EPSs extraction and structural characterization, especially due to environmental awareness and sustainability [8]. Physical, structural, mechanical and rheological properties provide very important information for EPSs control mechanism, performance and strategy. Adsorption, biodegradability, hydrophilic and hydrophobic property, rheological properties, viscoelastic response and environmental applications of EPSs matrix were also investigated due to the structural properties [6, 9–12].

The generation of EPSs by bacteria for example cyanobacteria is one of the important production methods [13]. Cyanobacterial bloom formations can adversely affect ecosystem services, water quality and public health [14]. EPS has an important role in the formation of blooms as it is involved in the formation of aggregates through adsorption and stickiness. Therefore, understanding the structure and properties of EPS may be an important key to prevent the bloom formation of cyanobacteria [15, 16]. The important feature of EPSs is that it contains different groups in its structure. The specific properties of cyanobacterial extracellular polymeric substance is that it contains two different uronic acids, sulphate

groups and monosaccharides with a high number of different groups [17]. Several studies have been reported on cyanobacterial EPS due to these properties [18–20]. For example Xu et al. [21] have been investigated influence of molecular weight on adsorption behavior of cyanobacterial EPS on colloidal particle. Viscoelastic behavior of aqueous solutions of an exocellular polysaccharide from cyanobacteria and the rheological behavior of the aqueous solutions of an extracellular polysaccharide from cyanobacterium have been investigated by Navarini et al. [22, 23] over a wide range of polymer concentrations.

In this study, X-ray diffraction (XRD) analysis, differential thermal analysis (DTA) and dynamic mechanical analysis (DMA) of cyanobacterial extracellular polymeric substance have been carried out to identify the structure-property relationships, thermal and viscoelastic properties. Dynamic mechanical behavior and viscoelastic properties of the EPS were characterized by parameters such as storage modulus (SM), loss modulus (LM), tan delta ($\tan \delta$), dynamic force (DF), complex modulus (CM), dynamic viscosity (DV) and complex viscosity (CV) depending on temperature at a constant frequency to understand the performance of cyanobacterial EPS.

2. Materials And Methods

2.1. Cyanobacteria and Growth Conditions

Cultures of cyanobacteria (*Arthrospira maxima*) were grown under a continuous light regime (fluorescent white light, irradiance on growth flask surfaces, about $25 \mu\text{Em}^{-2} \text{s}^{-1}$ at 20–24°C; in Spirulina medium containing mineral salts [24]. The soluble and bound EPS can be separated using physical and chemical methods [25] while the soluble EPS can be extracted by centrifugation alone, the bound EPS require additional chemical treatment for their extraction. Comte et al. [26] have suggested that not only the EPS yield, but also the chemical composition of EPS depends on the extraction method. EPSs were extracted by ultrasonication, and this was followed by centrifuging]. The cell suspension centrifuged (1500 rpm, 2 min) to remove the culture medium then ultrasonicated in Mili-Q water. EPS was extracted by second centrifugation.

2.2. The X-ray Diffraction (XRD) Analysis

The X-ray diffraction, (XRD) pattern was obtained from a Rigaku D-Max 2200 powder diffractometer. The XRD diffractogram was measured at 2θ , in the range 2–70°, using a Cu-K α incident beam ($\lambda = 1.54059 \text{ \AA}$), monochromated by a Ni-filter. The scanning speed was 1°/min, and the voltage and current of the X-ray tubes were 40 kV and 30 mA, respectively. The Bragg equation was used to calculate the interlayer spacing (d), $n\lambda = (2d \sin\theta)$ where n is the order of reflection, and θ is the angle of reflection. Crystallinity of the extracellular polymeric substance was calculated using the Equations 1 and 2

where s is the magnitude of the reciprocal-lattice vector which is given by $s = (2\sin\theta)/\lambda$ (θ is one-half the angle of deviation of the diffracted rays from the incident X-rays and λ is the wavelength); $I(s)$ and $I_c(s)$

are the intensities of coherent X-ray scattering from both crystalline and amorphous regions and from only crystalline region of EPS sample, respectively.

where W_c and W_a are the areas of the crystalline and amorphous portions in the X-ray patterns, respectively.

2.3. Differential Thermal Analysis (DTA)

Differential Thermal Analysis (DTA) of the EPS sample was recorded using Shimadzu DTG-60H thermal analyzer in dynamic nitrogen atmosphere (100 mL/min) at a heating rate of 10°C/min, 10 mg sample in platinum crucibles as sample vessel, using α -Al₂O₃ as reference.

2.4. Dynamic Mechanical Analysis (DMA)

Dynamic mechanical behavior of EPS was performed by TA Instruments, Q800 Dynamic Mechanic Analyzer (DMA). Instrument was calibrated according to manufacturer's instruction before analysis. Mixtures of polymer and Al₂O₃ (50:50 wt %) were prepared and these powdered mixtures were loaded into the DMA using a novel powder-holder, which consisted of upper and lower tray of stainless steel. The dimensions were measured. The holder was clamped directly into the DMA dual cantilever rig and tightened 6 PSI torque using the torque tool. The temperature dependence of polymers was measured at a constant frequency ($\omega = 1\text{Hz}$) using a standard temperature sweep (3 °C/min) from 30 to 250 °C.

3. Results And Discussions

3.1. X-Ray Diffraction Analysis of Extracellular Polymeric Substance

X-ray diffraction (XRD) is a rapid analytical and spectroscopic technique most widely used for phase identification of a crystalline material. A polymeric materials can be considered partly crystalline and partly amorphous. Extracellular polymeric substances (EPS) are the prolongable, green and the important group of polymeric materials. These type of biological polymers have a wide range of high molecular weight molecules like polysaccharides, proteins, nucleic acids, phospholipids, uronic acids, humic substances, lipids, etc [27]. Due these functional groups intra- and intermolecular H bonding can be important role in EPS and formation of crystalline forms.

XRD analysis is used for the structural analysis of EPS to determine the crystallinity degree and crystalline/amorphous character of the substance. Fig. 1 showed the XRD pattern of the cyanobacterial extracellular polymeric substance in the range of 2–70°. The XRD diffractogram of EPS exhibited the characteristic mainly diffraction peaks at 9.12, 18.2, 29.1, 31.8, 37.7, 45.5 and 56.5° with inter-planar spacing (d -spacing) 9.719, 4.927, 3.164, 2.920, 2.521, 2.159 and 1.847 Å, respectively (Fig. 1). XRD pattern also predicts that EPS is just about the amorphous in nature with %13.47 percent crystallinity. In the diffractogram, crystalline parts can be recognized as sharp narrow peaks while amorphous phase

gives a broad peak with low-crystalline structure. It could be difficult to interpretation of broad peaks of several amorphous polymers in X-ray scattering diffractogram and therefore the ratio between crystalline and amorphous intensities is used to calculate the amount of crystallinity in the material.

3.2. Differential Thermal Analysis (DTA) of Extracellular Polymeric Substance

Thermal stability and weight loss of EPS with Thermogravimetric Analysis (TGA) was analysed and publish our previous study [16]. EPS of cyanobacterial origin showed the degradation in three steps and with the temperature range 30 and 800 °C. Thermal degradation of EPS of cyanobacterial can be identified as in three main steps and with in the temperature range 30 and 800 °C. Thermal stability can be ascribed to the existence of sulfate groups, uronic acids and calcite crystal that prevent whole of decomposition. Degradation rates arranging in a decreased order from amide I, amide II and polysaccharides-like substances to polysaccharide can be given [16]. Tg is the glass-rubber transition that occurs in amorphous polymers and Tm (melting point) occurs when the ordered regions of a polymer fall apart upon heating can be seen From the DTA curve. When the temperature increases of macromolecule, an amorphous solid will become less viscous. Then a particular temperature the molecules obtain enough freedom of motion to spontaneously arrange themselves into a crystalline state [28]. This transition from amorphous solid to crystalline solid is an exothermic process and DTA analysis showed a significant thermal transition of EPSs (Fig. 2).

From the DTA thermogram of the extracellular polymeric substance, the first broad peak can be defined as glass transition temperature, Tg, at around 50.0°C. DTA thermogram also showed characteristic exothermic transition of exopolymer with melting of crystalline regions at 97.8°C followed by Tg transition. This transition peak can be explained as water molecules due to the hydrophilic groups of cyanobacterial EPS such as carboxyl, sulfate and hydroxyl.

Theoretically, water may induce the glass-rubbery transition (Tg) of amorphous regions of EPS before the melting of crystals. A broad endothermic transition at 200°C can be attributed as melting of crystal regions of EPS due to the α -1-4-glucose linkage (C-O-C) of polysaccharides units [16]. Releasing absorbed water and chain conformational change that could be associated with inter- and/or intramolecular interactions of polymer chain give two endothermic processes at 97.8°C and the other at 200°C [29]. Besides to the water release of absorbed water, these endothermic processes also can be resulted from the movements and interactions of lateral groups and associated with movements of the main skeleton at 350°C and 480.4°C, respectively. At the corresponding DTA thermogram (Fig. 2) exhibit endothermic relaxations whose temperature to higher temperatures and the fixed area confirmed the existence of strong inter–intra chain interactions [30].

3.3. Dynamic Mechanical Analysis of Extracellular Polymeric Substance

Dynamic mechanical analysis is one of the most important techniques used to investigate the viscoelastic properties of polymeric materials. In particular, the dynamic mechanical viscoelastic behaviors of biological materials, natural polymers and polysaccharides are very important to describe their basic properties and behaviors. In this technique, sinusoidal oscillating stress is applied and the response of a polymeric material, sinusoidal deformation, is mainly measured at a certain fixed frequency and a temperature range. Viscoelastic properties of the polymeric materials can be described by parameters obtained from the stress response due to the sinusoidal deformation [31].

The dynamic mechanical properties provide information of one of the most fundamental properties of polymers, the glass transition temperature (T_g). The T_g can be obtained by storage modulus (SM) onset, loss modulus peak (LM), or $\tan \delta$ peak.

Figure 3 showed the storage modulus (SM) and derivative storage modulus (dSM) curves of the EPS in the temperature range of 30-250 °C. The storage modulus indicated the elastic nature of the polymeric material and related with the stored energy representing elastic part in a deformation. The storage modulus showed a typical shape for polymeric material that decreased with increasing temperature. Four transition temperatures were observed from the dSM-T curve at 48.00 °C, 91.53 °C, 204.72 °C and 219.85 °C. Up to 48.00 °C the EPS segments were rigid and chain segments were frozen in a fixed positions (glassy region) [32]. The first transition temperature, 48.00 °C were defined as the glass transition temperature for the EPS and obtained from the temperature of the midpoint of the inflectional tangent at SM-T curve [33] and peak minimum at dSM-T curve. The second observed peak at 91.53 °C may be due to the removal of water molecules. Because EPS contains functional groups such as carboxyl, sulfate and hydroxyl, and these groups can be removed from the structure with the increasing temperature. The other two peaks observed at higher temperatures were related with the degradation/decomposition.

Figure 4 showed the loss modulus and $\tan \delta$ plots depending on temperature. The loss modulus (LM) demonstrated the viscous nature of the EPS and related to the loss energy representing the viscous part of molecule. $\tan \delta$ (loss tangent) is another dynamic mechanical parameter measures damping in the polymeric material which is equal to the ratio of the loss modulus to storage modulus. It was observed that the loss modulus and $\tan \delta$ followed the similar behavior with the storage modulus. The loss modulus and $\tan \delta$ curves reached a maximum with the increase in temperature and then decreased due to the energy loss caused by the free movement of the segments [34]. The three peaks were observed from both of these curves with a small shoulder at higher temperature region.

The main transitions were seen at 56.95 °C, 94.50 °C, 225.11 °C and 59.99 °C, 94.50 °C, 226.61 °C from LM-T and $\tan \delta$ -T curves, respectively. All transition temperatures obtained from DMA parameters (SM, LM and $\tan \delta$) were in good agreement with each other. The first peak maximum values at lower temperatures were associated with the glass transition temperature of EPS. The second and the third peak maximum values were originated from the removal of small groups and degradation/decomposition of main chains of the EPS. Although the glass transition temperature is

generally reported as peak in the tan delta in the literature, the glass transition temperatures found from other parameters are also used as long as it is specified from which parameter they are found.

Figure 5 and 6 showed the behavior of dynamic force, complex modulus, dynamic and complex viscosity with increasing temperature at a constant frequency. The complex modulus is the total resistance of a polymeric material to deformation, however, whether the deformation of the polymeric material is elastic or viscous [35]. The dynamic force and complex modulus gradually decreased with increasing temperature up to ≈ 200 °C in two steps and decreased sharply after this temperature region. The dynamic viscosity plots showed three peaks at 55.14 °C, 94.50 °C, 225.11 °C with a shoulder at 200.98 °C. While the complex viscosity initially decreased slightly with increasing temperature in the low temperature region, it decreased sharply after reaching the high temperature. The reason of this behavior can be interpreted as the thermal transition of polymeric EPS from solid state to soft state with increasing temperature.

The storage modulus, complex viscosity and dynamic force values at a certain temperatures (30 °C, 50 °C, 100 °C, 150 °C, 200 °C and 250 °C) were summarized in Table 1 for the cyanobacterial EPS.

All parameters showed slow decreasing behavior up to about 200 °C, and after this temperature they showed a sharp decreasing trend due to the degradation/decomposition of the main chain cleavage of the polysaccharide component in cyanobacterial EPS.

4. Conclusion

In the present study, structural characterization and structure-property relationships of the cyanobacterial EPS were reported. It was observed from the XRD diffractogram of EPS had a low-crystalline character. The degree of crystallinity was found to be %13.47. Thermal characterization was followed by DTA. T_g and melting transitions were defined and associated with hydrophilic unit in polysaccharides. Viscoelastic properties of the cyanobacterial EPS were described by parameters obtained from the stress response due to the sinusoidal deformation using DMA. The storage modulus, dynamic force, complex modulus and complex viscosity parameters demonstrated a typical shape for polymeric material that decreased with increasing temperature due to the polysaccharide components of cyanobacterial EPS. Thermal transitions were obtained from DTA and the storage modulus, loss modulus and tan delta and all transition temperatures obtained from these parameters were in good agreement with each other.

Declarations

Conflict of Interests

The authors of the manuscript solemnly declare that no scientific and/or financial conflicts of interest, exists with other people or institutions.

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Tables

Table 1. Storage Modulus (SM), Complex Viscosity (CV) and Dynamic Force (DF)-Temperature (T) results of extracellular polymeric matrix.

DMA Parameters	T (°C)					
	30	50	100	150	200	250
SM (GPa)	661.8	650.4	613.3	587.1	540.4	394.1
CV (GPa.s)	36.6	35.9	33.9	32.5	29.9	21.8
DF (F)	21.3	21.2	21.1	20.2	18.6	13.6

Figures

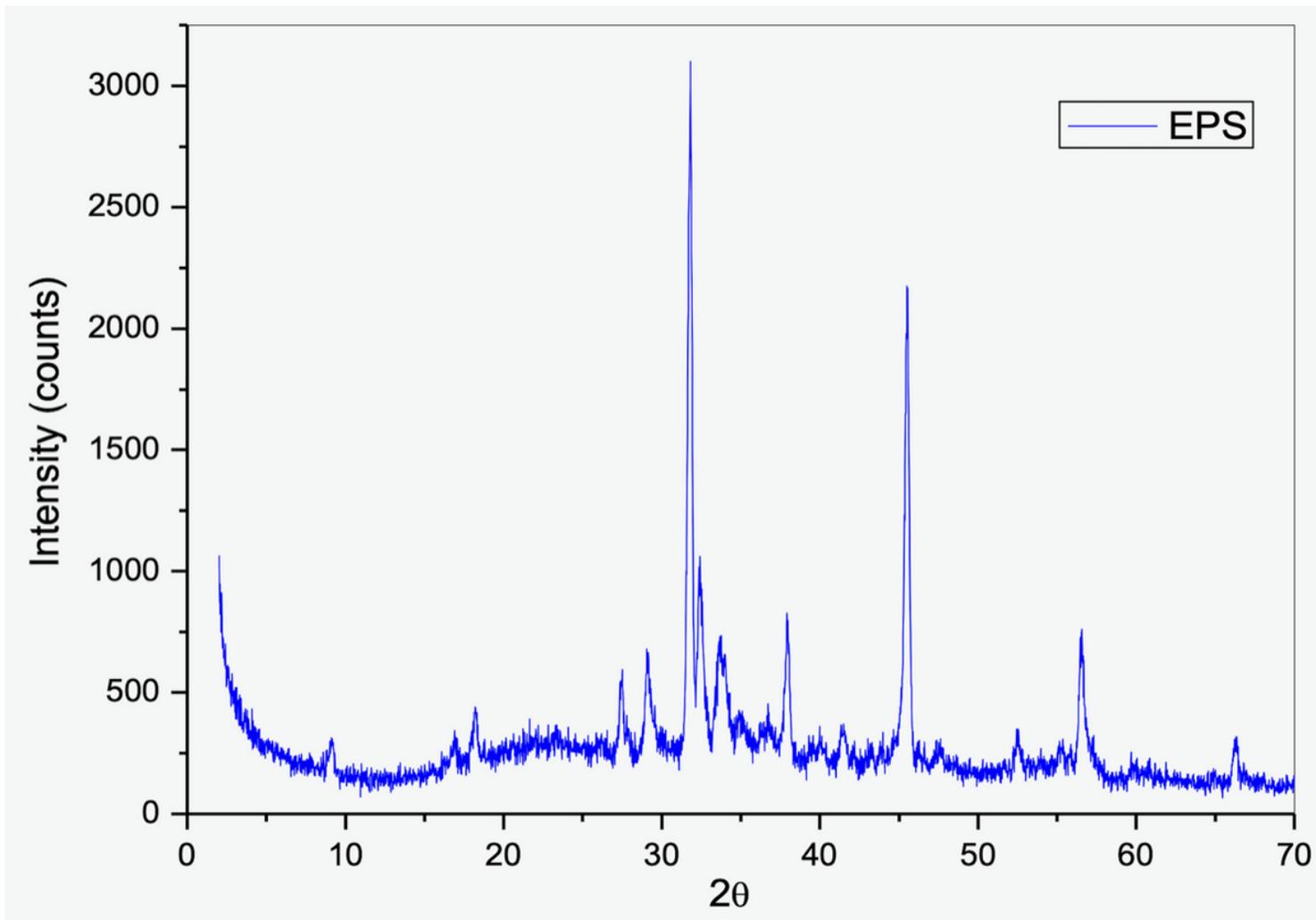


Figure 1

XRD pattern of the extracellular polymeric substance.

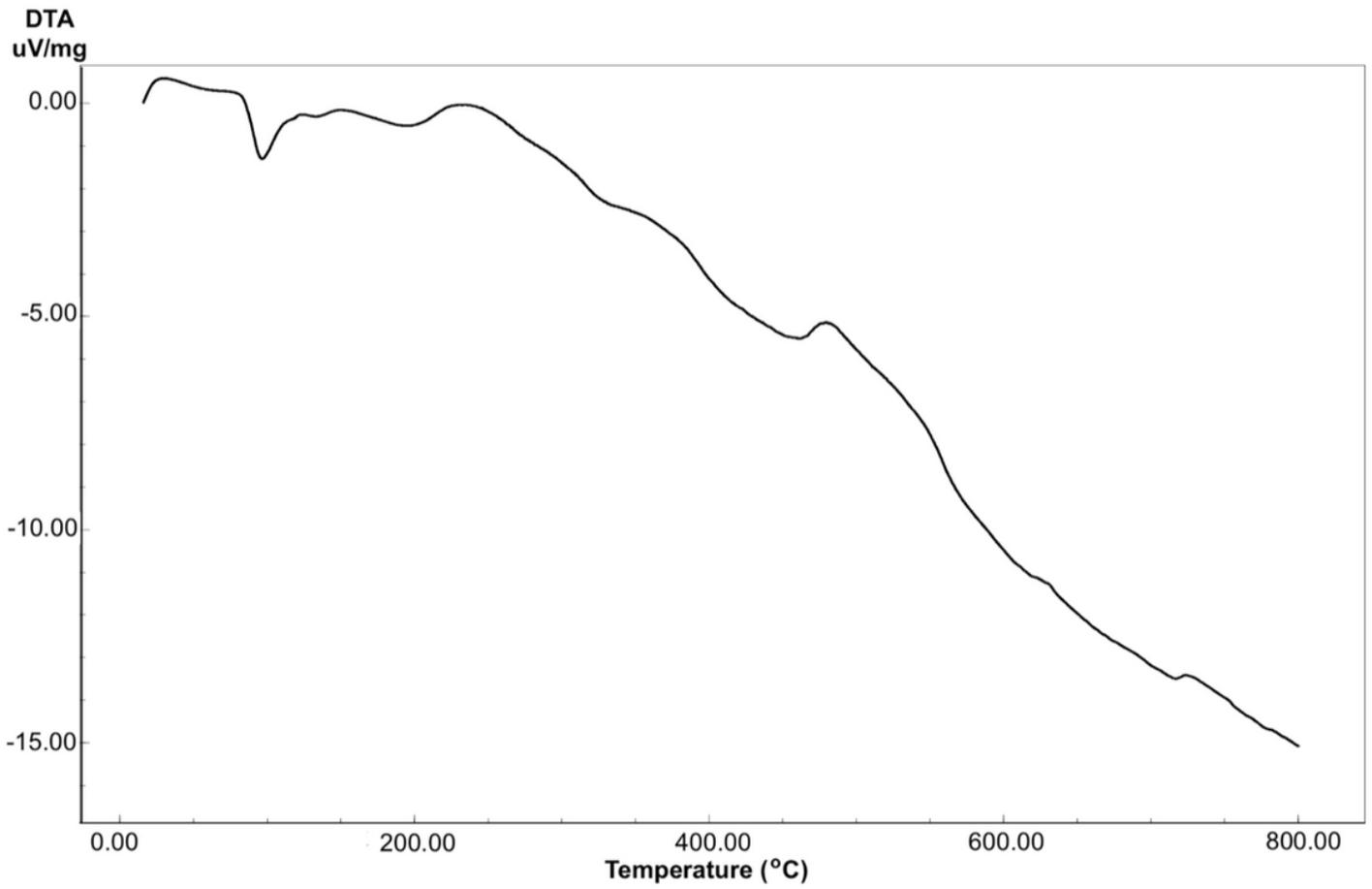


Figure 2

DTA thermogram of the extracellular polymeric substance.

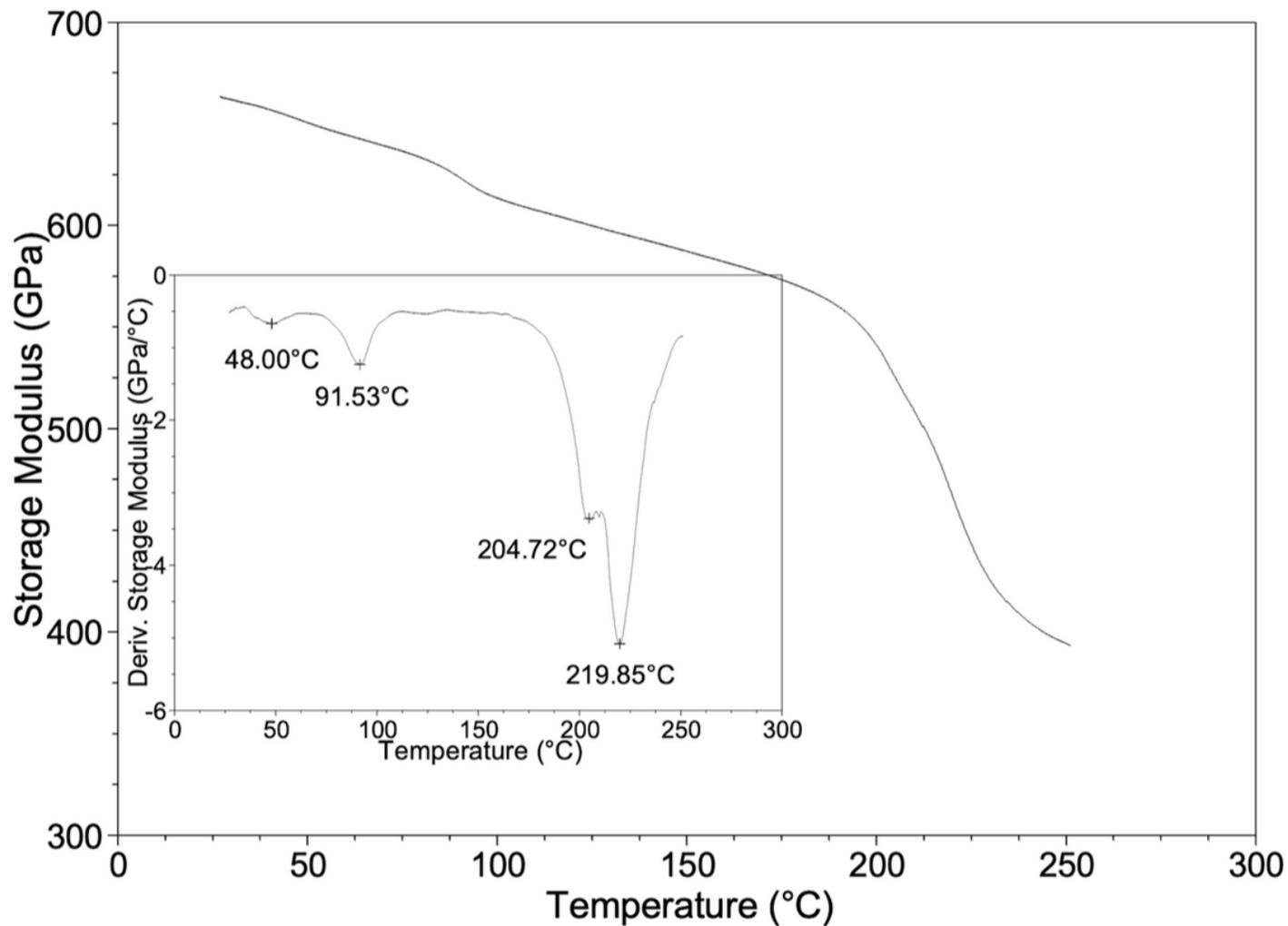


Figure 3

Plots of storage modulus (SM) and derivative storage modulus (dSM) vs. temperature (T) of the extracellular polymeric substance.

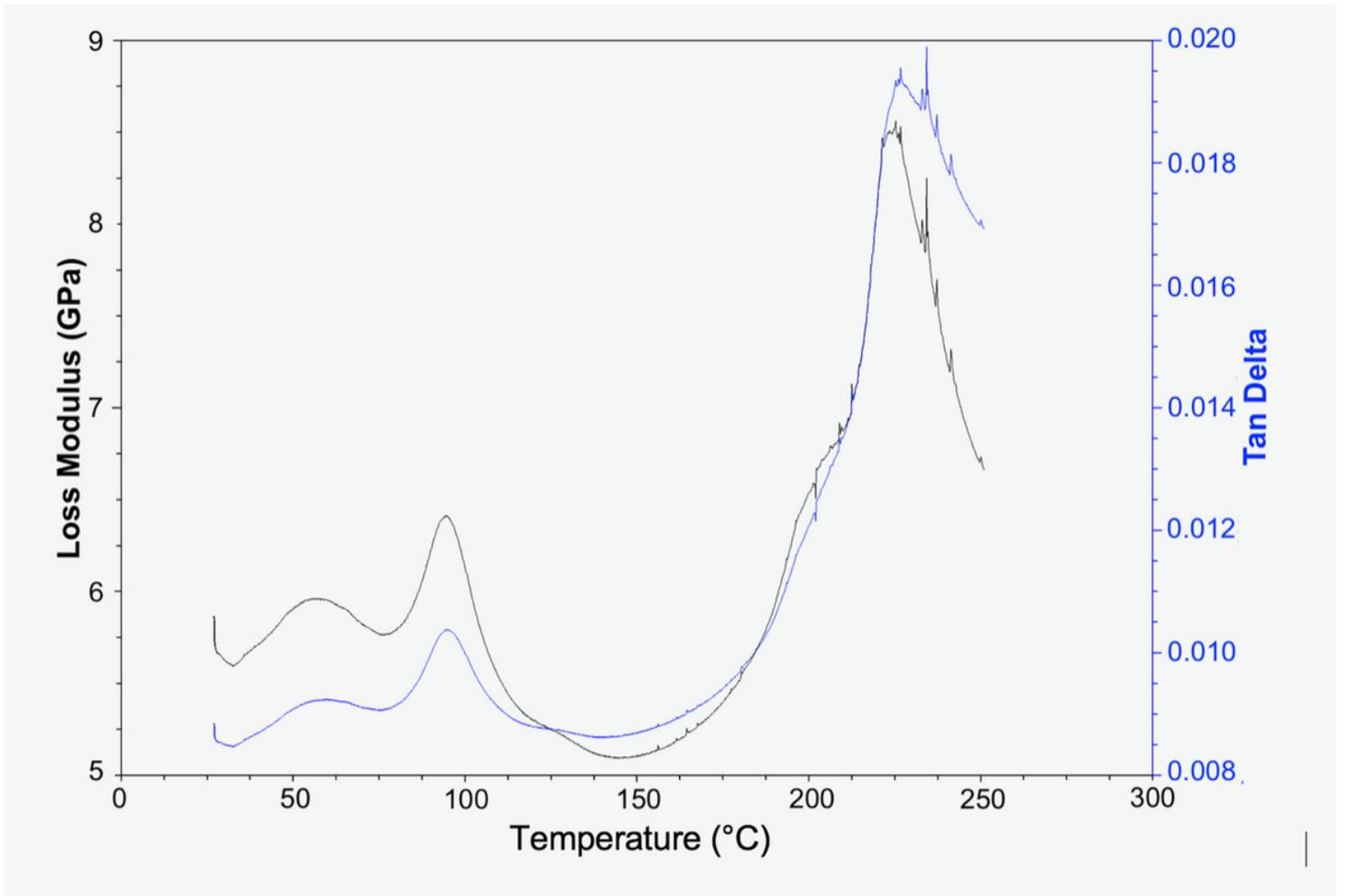


Figure 4

Plots of loss modulus and $\tan \delta$ vs. temperature of the extracellular polymeric substance.

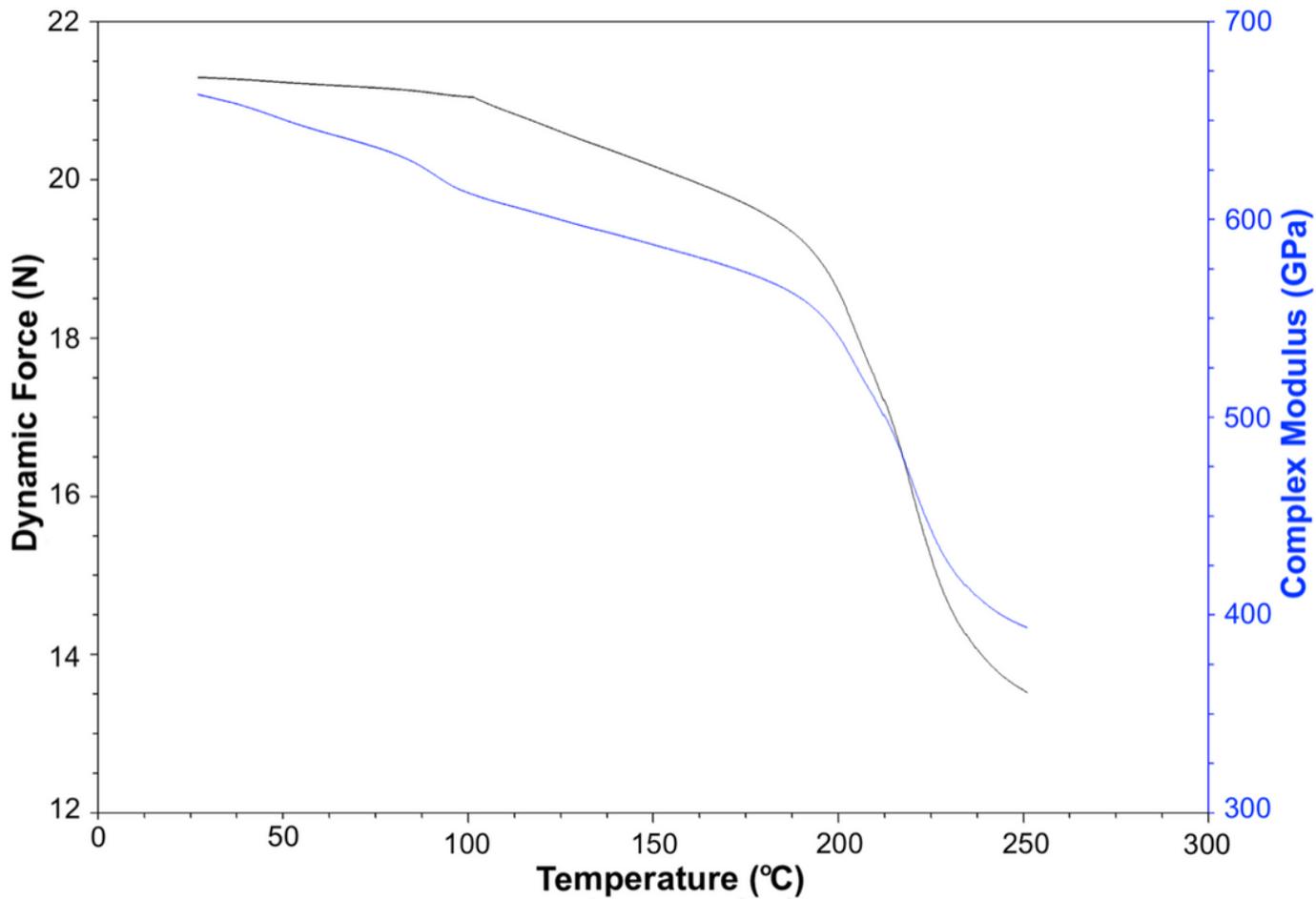


Figure 5

Plots of dynamic force and complex modulus vs. temperature for extracellular polymeric substance.

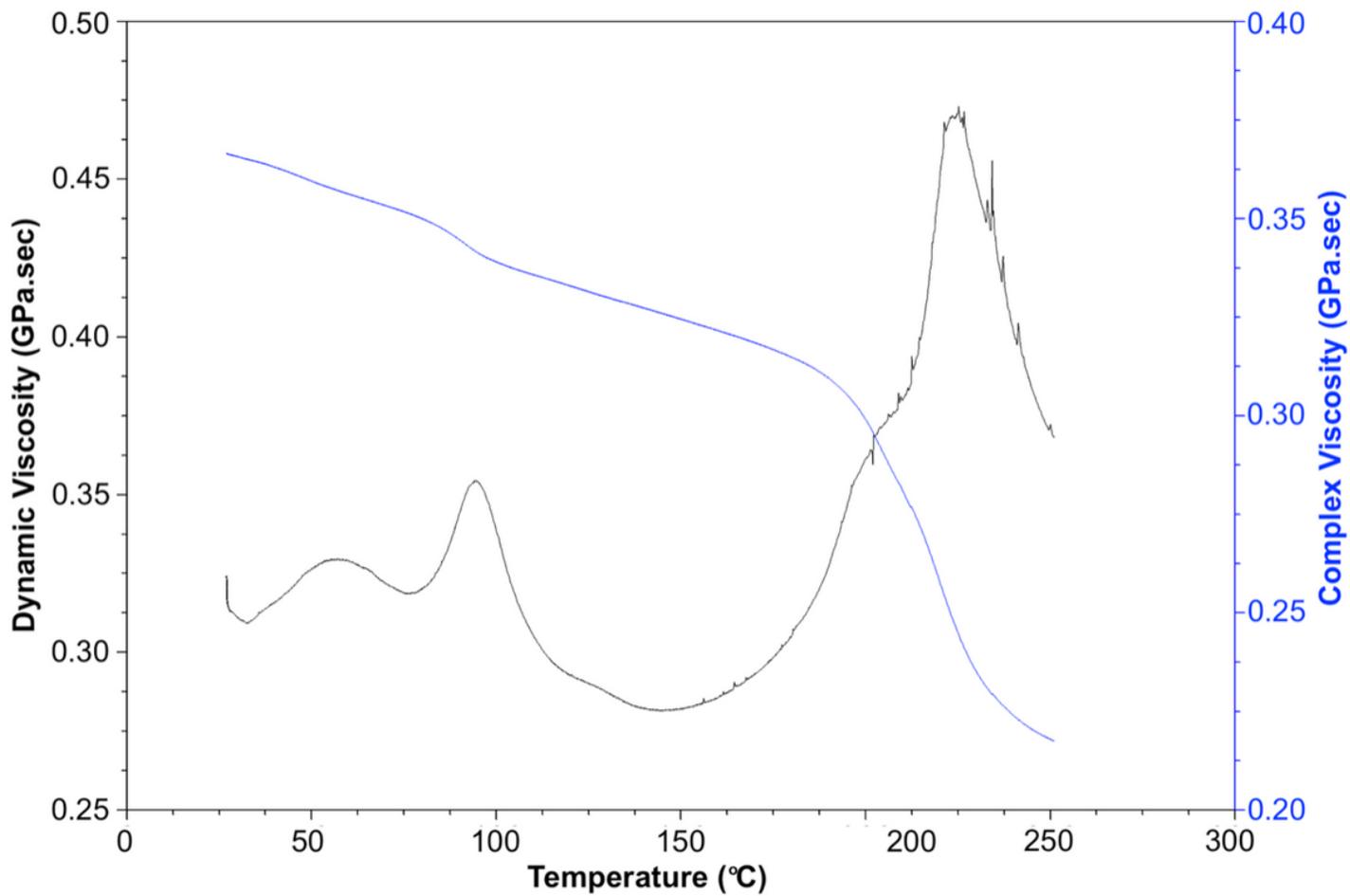


Figure 6

Plots of dynamic viscosity and complex viscosity vs. temperature for extracellular polymeric substance.