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Original article

Synthesis and characterization of interpenetrating network (IPN) based levan-polyacrylamide hydrogels and their application in conservation of cultural heritage

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ABSTRACT

In this study, an IPN based enzymatic levan-polyacrylamide hydrogel (EL-PA) was developed and characterized for its structural, morphological, rheological properties and swelling kinetics to underline hydrogel properties and its potential use in paper conservation. The addition of levan also led to changes in the viscoelastic behavior of the hydrogels, with the complex viscosity of EL-PA samples showing pronounced dependence on shear rate. The swelling and the overall surface area of the hydrogels were increased with the addition of levan into the polymer network. Source associated structural differences were found to be negligible such that both microbially produced linear and enzymatically produced branched forms of levan performed equally well. Solvent loaded hydrogels were then applied on an artifact, a 19th century book of Namık Kemal, and investigated using FTIR, SEM, XRD and colorimetric analysis. Old adhesive layers were successfully removed, and hydrogels showed good compatibility and ease of application. This study has shown that levan has improved hydrogel properties and levan based systems bear high potential in conservation science.

Introduction

The conservation of cultural heritage aims to safeguard and prolong the lifespan of significant cultural assets for future generations. Preserving the physical characteristics and content of cultural heritage is a primary focus in conservation practices. Furthermore, the conservation of cultural heritage assets is a substantial industry that generates substantial economic and socio-cultural benefits annually. In recent years, there has been a growing emphasis on innovative decision-making strategies and practices within this professional field [1].

Gels are important for treating artistic works due to their ability to provide controlled and selective cleaning, non-invasive approaches, pH control, surface consolidation, and controlled delivery of substances. They offer a gentle and reversible method for removing dirt and contaminants without causing damage, maintaining a stable pH environment, strengthening fragile surfaces, and precisely applying restorative or protective substances. The use of gels in conservation and restoration practices ensures the preservation and longevity of delicate artworks [2]. Gels have been widely employed in the conservation of drawings and paintings to remove unwanted coatings like varnish, grime, and dirt through the cleaning process. The use of gel systems for stone preservation dates back to the 18th century, but tailored conservation treatments for specific objects have gained significant importance over the past century, leading to the development of modified aqueous solutions and gels for broader applications in artistic work conservation [3,4]. Another application of gels is the removal of polymeric coatings from paintings, a practice that originated in the early 20th century. Gel systems comprising polymers, primarily polyacrylic acid, along with various components such as organic solvents and enzymes, have also been proposed [5]. Although solvents, both organic and inorganic, have been used for diverse conservation purposes in cultural heritage objects, the issue of over-penetration can result in undesired side effects on the artifact. In the case of paintings, it can harm the polymer surface or cause the bleeding of certain components [2]. Apart from selecting the appropriate solvent, the implementation method is another area of focus for conservation scientists [6].

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The development of an ideal gel-solvent system for conservation purposes requires ease of preparation and application, residue-free removal from the artifact's surface, and the ability to penetrate the desired level without causing substrate damage [7]. Extensive research has led to the development of gels with exceptional properties, including reusability, retention, responsiveness, semi-interpenetration, and chemical characteristics, each tailored to address specific conservation challenges. The diverse range of surface materials and substrates necessitates the use of different polymers to address specific conservation issues [8]. Physical gels have gained preference among conservators due to their easy application, cost-effectiveness, and reusability [8,9]. However, the issue of residues left behind after their application has become a significant concern for restorers, as the chemical components may contribute to further substrate damage during the natural aging process of artifacts [10]. Polysaccharide gels, such as agar, gellan gum, and xanthan gum, play a vital role in the conservation and restoration of cultural heritage objects, particularly metal artifacts. These gels provide a non-invasive method for cleaning and stabilizing artifacts, as they can selectively eliminate corrosion and dirt without damaging the original surfaces beneath. Agar gels, with their reversible properties and ability to form peelable films, are effective in cleaning various substrates, including stone, plaster, paper, and paintings. Gellan gum is preferred for paper restoration due to its minimal water damage, while xanthan gum, although highly viscous, has the drawback of leaving residue [11]. Gellan gel is an example of a physical polysaccharide-based gel held together by secondary bonds only [12]. It is mostly used with aqueous solutions, such as surfactants and enzymes. However, its applicability has been found unsuitable for rigid or rough surfaces of certain artifacts [12]. Another widely used polysaccharide for gel systems is agar, which can be derived from species of red algae or seaweed [2,13]. Agar gels within the concentration range of 0.5–5% w/v demonstrate the best confined release of the solvent at the gel-substrate interface [13,14]. However, for the treatment of highly porous substrates, concentrations lower than 2% can lead to residue problems [13,14]. Compared to physical gels, chemical gels formed via covalent bonding are safer regarding residual issues [9,10]. However, the complexity of their formulation and costs are some of the drawbacks associated with commonly used chemical gels in conservation treatments [15]. To overcome these challenges, a new class of hydrogels based on covalent bonding between polymeric networks within a single matrix has been developed. Domingues et al. formulated semi-interpenetrating (IPN) hydrogels using poly(2-hydroxyethyl methacrylate) and polyvinylpyrrolidone to remove adhesive coatings from canvas painting surfaces [16]. The goal was to benefit from the mechanical strength and hydrophilic properties of both polymers in the final hydrogel system [16].A semi-IPN refers to a polymer network where multiple polymers are physically intertwined but not chemically bonded, while a fully IPN is a polymer network where the polymers are chemically crosslinked to form a single interconnected structure. Fully IPNs exhibit improved mechanical properties, while semi-IPNs offer advantages such as flexibility and toughness [17]. Gel systems containing organic solvents, which belong to the family of semi or fully interpenetrating networks (IPNs) of physically or chemically cross-linked and linear polymers, have been extensively studied in various fields [9,18,19]. The properties of these gels, such as porosity, swelling, and viscosity, can be tailored by selecting the appropriate polymer and synthetic routes within the framework of material science [19,20]. Both semi-IPN and fully IPN hydrogels have been reported to exhibit increased mechanical strength and lower degradation rates, as supported by literature [21]. Polysaccharides are commonly chosen as linear polymers in semi-IPNs due to their biocompatibility, non-toxicity, abundance, low production cost, and ease of chemical modification [17]. In a specific study, a semi-IPN structure was investigated by combining a cationic poly(acrylamide-co-(3-methacryloxypropyl) trimethyl ammonium chloride) (poly(AAm-co-MAPTAC)) network with a chitosan polymer, and the swelling behavior, morphological properties, and mechanical stability were analyzed [22]. To the best of our knowledge, polysaccharides have not been employed in an IPN-based hydrogel system for the conservation of cultural heritage purposes thus far [14].

Levan, a fructose homopolysaccharide and fructan, consists of β-D-fructofuranose residues linked by β-(2–6) glycosidic bonds [23]. This natural polysaccharide possesses unique properties, including strong adhesivity, self-assembly into spherical colloids in water, very low intrinsic viscosity, high biocompatibility, and health benefits. Microbial levan can be produced extracellularly and in high yields from sucrose through the hydrolysis and transfructosylation actions of the levansucrase enzyme [24,25]. In recent years, levan has found applications in drug-carrier systems, adhesive multilayers, free-standing and thin films, as well as stimuli-responsive hydrogels, emerging as an active area of research [26,27]. The results of a study indicate that levan demonstrated high adhesive strength and resistance to solvents like jet fuel [28]. This solvent resistance is significant as it ensures the adhesive's stability and integrity even in challenging environments where exposure to harsh chemicals or solvents is possible. This characteristic makes levan a suitable choice for conservation applications that may involve solvent-based treatments or environments. Despite being affected by water, the adhesive could be remoistened and reused for bonding surfaces [28]. This means that once the adhesive has dried and formed a bond, it can be reactivated by moisture and used again to bond surfaces together. This reusability aspect can be advantageous in conservation scenarios where adjustments or repositioning of objects or materials are necessary. Shear strength on anodized aluminum averaged 819 psi, and tensile strength varied depending on the substrate and production method, ranging from 500 to 1500 psi. Modified water-resistant forms of the adhesive maintained good adhesive strength. When compared to other natural adhesives, the microbial levan adhesive showed higher tensile strength [28]. This strong bonding capability makes it effective in adhering surfaces together, which is crucial for conservation purposes where durable and long-lasting adhesion is desired. In a recent study conducted by our group, we examined the chemical changes in paper resulting from the application of various formulations of sizing, ink, and two adhesives: Levan by *Halomonas smyrnensis, Halomonas* Levan (HL), and starch. The results showed that HL effectively preserved the amorphous regions of fibers in samples treated with egg white-alum sizing and exhibited a more alkaline nature compared to starch after aging. This investigation marks the first known application of levan in the field of conservation [29].

Research aim

For a deeper investigation of the potential use of levan polysaccharide in the conservation field, our study aimed to harness its hydrogel-forming ability and developed Levan-polyacrylamide IPN based hydrogels. Previous research has demonstrated the crosslinking of surfactant monomers with acrylamide to produce hydrogel precursors that address common problems encountered when using cleaning solutions on canvas paintings [30]. These efforts have been undertaken to improve swelling capacity and facilitate easy handling during restoration and conservation practices. Additionally, we were interested in exploring the potential effects of structural differences associated with different sources of levan. Consequently, we utilized both linear microbial long chain *Halomonas* levan and branched enzymatically produced long chain *Halomonas*

levan in our study to produce hydrogels, and their mechanical and physical properties were comparatively evaluated.

To assess the hydrogel properties and its potential use in paper conservation, we conducted swelling kinetics, bonding behavior, morphological analysis, and rheological characterization. The results highlighted the modified viscoelastic behavior of the hydrogel. The addition of levan to the polymer network increased the swelling capacity and overall surface area of the hydrogels which is in accordance with the nature of IPN hydrogels as IPN hydrogels often exhibit unique swelling properties compared to single-network hydrogels.

Furthermore, we investigated the practical application of the selected hydrogel on a 19th-century book of Namık Kemal, using Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), X-ray diffraction (XRD), and colorimetric analysis methods. These analyses aimed to evaluate the impact of the hydrogel treatment on the artifact and further support the potential use of levan-based hydrogels in conservation practices.

Material and methods

Microbial and enzymatic levan production

Microbial *Halomonas* levan (HL) was produced under controlled bioreactor conditions using halophilic *Halomonas smyrnensis* bacterial cultures as described before [33]. Stationary phase cultures grown in a sucrose-based medium at 37 °C were centrifuged, and the levan polymer in the supernatant was recovered by precipitation with ice-cold ethanol and then purified by dialysis against distilled water. For enzymatic *Halomonas* levan production, the levansucrase enzyme from *H. smyrnensis,* which was recombinantly expressed in *Escherichia coli*, was added to a sucrose-containing buffered saline substrate solution. After overnight incubation at 15 °C, the enzymatically produced levan (EL) polymer was recovered from the reaction mixture by ethanol precipitation and dialyzed [34]. Levan preparations were then subjected to weak anion exchange chromatography column to further remove charged impurities, ensuring the purity of the levan samples for subsequent experiments and analyses.

NMR analysis was conducted to provide insights into the structural variations between enzymatic and microbial levan and results revealed that they exhibit differences in their branching characteristics where enzymatically produced levan was found to have a branching degree of 10% while microbially produced one is primarily linear with minimal branching observed [31]. On the other hand, based on the analysis of the IR spectra in a previous study [32], it was concluded that both HL ad EL showed similar peaks, suggesting that the chemical structure of EL and HL do not differ significantly. To investigate the impact of these structural disparities, we prepared and utilized both enzymatically and microbially produced levans in our experimental study.

Synthesis of interpenetrated network based hydrogel

In order to optimize the interpenetrated networking conditions, different concentrations of levan were combined with acrylamide and methylene-bis-acrylamide (MBA) in distilled water [35]. As a blank, a polyacrylamide (PA) gel was produced using 0.05 mole of acrylamide (AAm) in a 50 ml buffer solution. Ammonium persulfate (APS) (5 × 10–4 mole) was used as an initiator, and MBA served as the crosslinker [35]. The resulting solution was placed in a water bath at 50 °C. The hydrogels were prepared by combining AAm and levan solutions (50 ml) using the same protocol mentioned above. Levan concentrations of 1%, 3%, and 5% (w/v) were used, and 1,4-Butanediol diglycidyl ether (BDDE) was added as a crosslinker for levan [36]. The hydrogels were then vacuum dried and weighed. The IPN-based Levan-g-PA hydrogels with enzymatically produced levan at concentrations of 1%, 3%, and 5% (w/v) were coded as E1, E3, and E5, respectively. Similarly, the hydrogels with microbially produced levan at concentrations of 1%, 3%, and 5% (w/v) were coded as M1, M3, and M5, respectively [36].

Characterization of IPNs

Chemical composition of the hydrogels as well as the evidence on the network and crosslinking bond formation reaction were determined by FTIR analysis. The hydrogels were analyzed using attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) with the model 4600 from Jasco, Japan. IR spectra were recorded in the range of 400–4000 cm^{-1}. The scanning electron microscopy (SEM) was performed to analyze surface morphology of hydrogels (SEM, EVO MA-10, ZEISS Inc., USA).

Swelling kinetics

The water holding capacity of the hydrogels was determined by first pre-weighing the vacuum-dried hydrogels. Subsequently, the hydrogels were immersed in three different buffer solutions: PBS buffer (pH 7.4), acetate buffer (pH 5.0), and carbonate buffer (pH 9.0) for a duration of 72 h. All experiments were conducted at room temperature. The hydrogels were periodically weighed during immersion until equilibrium weight was reached. To ensure statistical reliability, this procedure was repeated three times for each pH value, and the standard deviation was calculated. The swelling ratio (%SR) and swelling kinetics were then calculated using the following equations:

$$\% SR = \frac{W_s - W_d}{W_d} x\ 100 \quad (1)$$

W_s: weight of swollen gel
W_d: weight of dry gel
Fick's law was used to explain swelling fraction (Eq. (2)) [35]:

$$F = kt^n \quad (2)$$

F: the swelling fraction
k: constant
n: diffusion exponential of the solvent

Specific surface area

The specific surface area of the synthesized hydrogels was determined using the Brunauer-Emmett-Teller (BET) analysis method. Prior to the analysis, the hydrogels were first freeze-dried after they reached equilibrium swelling capacity. Samples were placed in BET analysis apparatus (AUTOSORB-6B) equipped with a gas sorption system. Nitrogen gas was used as the adsorbate, and the outgas temperature was set to 50 °C. The adsorption-desorption isotherms were obtained by incrementally increasing and decreasing the nitrogen pressure at various equilibrium points. The data obtained from the isotherms were used to calculate the specific surface area using the BET equation. Three replicates were performed for each sample, and the average specific surface area and standard deviation were reported.

Rheological behavior

The frequency sweep test is performed in rheological measurements of soft materials. Physical strength and cohesiveness can be analyzed by these dynamic tests. Storage modulus, loss modulus, complex viscosity, loss factors are parameters that this analysis can provide. Additionally, amplitude sweep test is a specific

type of oscillatory analysis in which the strain or stress amplitude is varied while keeping the frequency constant. It is used to determine the linear viscoelastic region (LVER) and characterize the material's response to different strain or stress levels. To determine the viscoelastic behavior of IPN based levan-polyacrylamide hydrogels (EL-PA), frequency sweep and amplitude sweep tests were performed at a fixed temperature of 25 °C by Anton Paar RheoCompass™ Rheometer using plate-plate geometry. Also, steady state analysis was performed. During the frequency sweep test, a constant strain of 0.1% was maintained. The effect of oscillatory frequency range of 0.01–50.0 Hz on loss and storage moduli was measured.

The artifact and characterization of its media

The book titled "Osmanlı Tarihi" (Ottoman History) was originally published in 1910 by Mahmud Bey Press in Istanbul and authored by the renowned Turkish writer Namık Kemal [37]. For the purpose of conservation, two bindings of the book were selected for investigation. In the second binding, an ordinary pressure sensitive tape was applied to the backing, while an old strip of paper was used as backing in the first binding. However, over time, these repair materials have lost their binding properties in certain areas, leading to color changes and damage on the paper surface. To address this ongoing deterioration, it was decided to remove the old repair. However, the conventional method of using water or solvents to soften the adhesive could potentially cause staining on the book. To mitigate this risk, solvents were applied using the EL-PA hydrogel. The use of this IPN hydrogel offers a safer and more controlled approach for adhesive removal and book conservation.

Prior to the application of the EL-PA hydrogel for adhesive removal, a characterization process was conducted on the book. The focus of the characterization was primarily on the binding paper, which included paper tape and modern pressure sensitive tape. Analytical studies were carried out on a selected 1 cm^2 area of the binding paper and the detached adhesive tapes to assess the effectiveness of the hydrogel application and evaluate the book's conservation status. A key parameter to consider in preserving the visual originality of the artifact is chromatic change. Colorimetric measurements were performed using the Datacolour Spectroflash SF 600 instrument, and the CIE L*ab** coordinates were recorded. The L* coordinate represents the level of brightness, the a* coordinate corresponds to the red–green color axis, and the b* coordinate indicates the yellow–blue color axis. These measurements provided valuable insights into the chromatic properties of the book and aided in assessing the impact of the adhesive and the subsequent hydrogel after treatment.

The physical state of the paper can be explained by the crystallinity index. The increase in crystallinity of polymers means the polymer is rich in ordered regions thus, becomes more fragile when subjected to mechanical deformation in elastic mode. In other words, it has less amorphous regions which have more freedom for absorbing energy. All measurements were carried out by Bruker D2 Phaser CuKα radiation which was generated at 30.0 kV and 10.0 mA. The CuKα radiation has a wavelength of 0.154184 nm. Scans were obtained from 5 to 30° 2θ in 0.01° steps for 19.2 s per step. The peaks of diffractograms were analyzed using Python version 3.8.8, and the crystalline index was calculated using the Segal Method (3):

$$X_c = \frac{I_{002} - I_{am}}{I_{002}} \quad (3)$$

where I_{002} stands for the maximum intensity of lattice diffraction and I_{am} is the intensity of the lattice diffraction in arbitrary units.In addition to the crystallinity index, ATR-FTIR spectra of the adhesive residues of the paper tape were recorded in the range of 400–4000 cm^{-1}. Also, the surface morphology of paper specimens was investigated via SEM analysis to track the effectiveness of the adhesive removal process and overall effect of aged adhesive on paper.

Results and discussion

Synthesis and characterization of hydrogels

The hydrogels were synthesized, and their proposed formation mechanism can be explained as follows: First, the HL macromolecule was utilized in the formation of the initial network, where HL chains were cross-linked using BDDE. Subsequently, free AAm monomers underwent polymerization to create the second network. The hydrogel networking process is believed to involve three types of coordination: (i) interaction between BDDE ions and Levan, (ii) covalent interaction between methylene-bis-acrylamide (MBA) and AAm monomers, and (iii) noncovalent interactions between polyacrylamide chains and levan [38].

Structural characterization of the hydrogels

Building upon previous investigations [32], the IR spectra revealed distinct bands that shed light on the structural characteristics of HL and EL. Notably, the IR spectrum of enzymatic levan (EL) closely resembled that of native HL, suggesting similar structural features between these two variants. The infrared (IR) spectra of enzymatic levan (EL), and the enzymatic levan-polyacrylamide (EL-PA) mixture were analyzed to investigate potential differences (Fig. 1). Specifically, prominent bands at 3200 cm^{-1}, 2900 cm^{-1}, and 2950 cm^{-1} were attributed to the vibrations of the hydroxyl group (–OH), the –CH2OH group, and the C–H stretching of fructofuranose residues, respectively. Furthermore, the region spanning 1430–1200 cm^{-1} exhibited bands associated with C–H vibrations and aromatic skeletal vibrations. Consistent with the earlier findings, characteristic peaks were observed at 1120–1020 cm^{-1} and 950 cm^{-1}, indicating the presence of glycosidic linkages and C–O–C vibrations within fructofuranose rings.

The FTIR spectrum of enzymatic levan-polyacrylamide hydrogel (EL-PA) and polyacrylamide gel (PA) shows typical characteristics of amide structure. As seen in Fig. 1a, the peak at 3300 cm^{-1} and 1646 cm^{-1} belongs to the N–H group and C=O group, respectively. The aliphatic C–H stretching vibration band is seen at 2927 cm^{-1} which is a result of crosslinking bridges. Deformation vibration bands belonging to C–N double bond and C–H stretching can be seen between 1400 and 1000 cm^{-1} wavenumbers, respectively. In Fig. 1b, strong band at 1012 cm^{-1} with shoulder at 1116 cm^{-1}(C–O–C bonds of fructofuranose chain. Presence of a broad band at 3331 cm^{-1} in IPN hydrogels could be assigned to O–H and N–H stretching in the hydrogel matrix. Characteristic bands of the saccharide structure are seen at 927, 870 and 808 cm^{-1} [35,39].

Swelling kinetics

The swelling behavior of vacuum-dried hydrogels was investigated in various buffer solutions to evaluate their response under different pH conditions. The hydrogels were immersed in PBS buffer (pH 7.4), acetate buffer (pH 5.0), and carbonate buffer (pH 9.0) for a duration of 72 h, and the equilibrium swelling ratios were determined and presented in Table 1. Additionally, the time-dependent swelling behavior of the interpenetrating network (IPN) hydrogels was provided in the supplementary data (S1). Notably, no significant differences in the swelling behavior were observed between the IPN hydrogels produced using enzymatic and microbial levan. However, the incorporation of 1% levan resulted in an approximate threefold increase in the swelling ratio at both pH

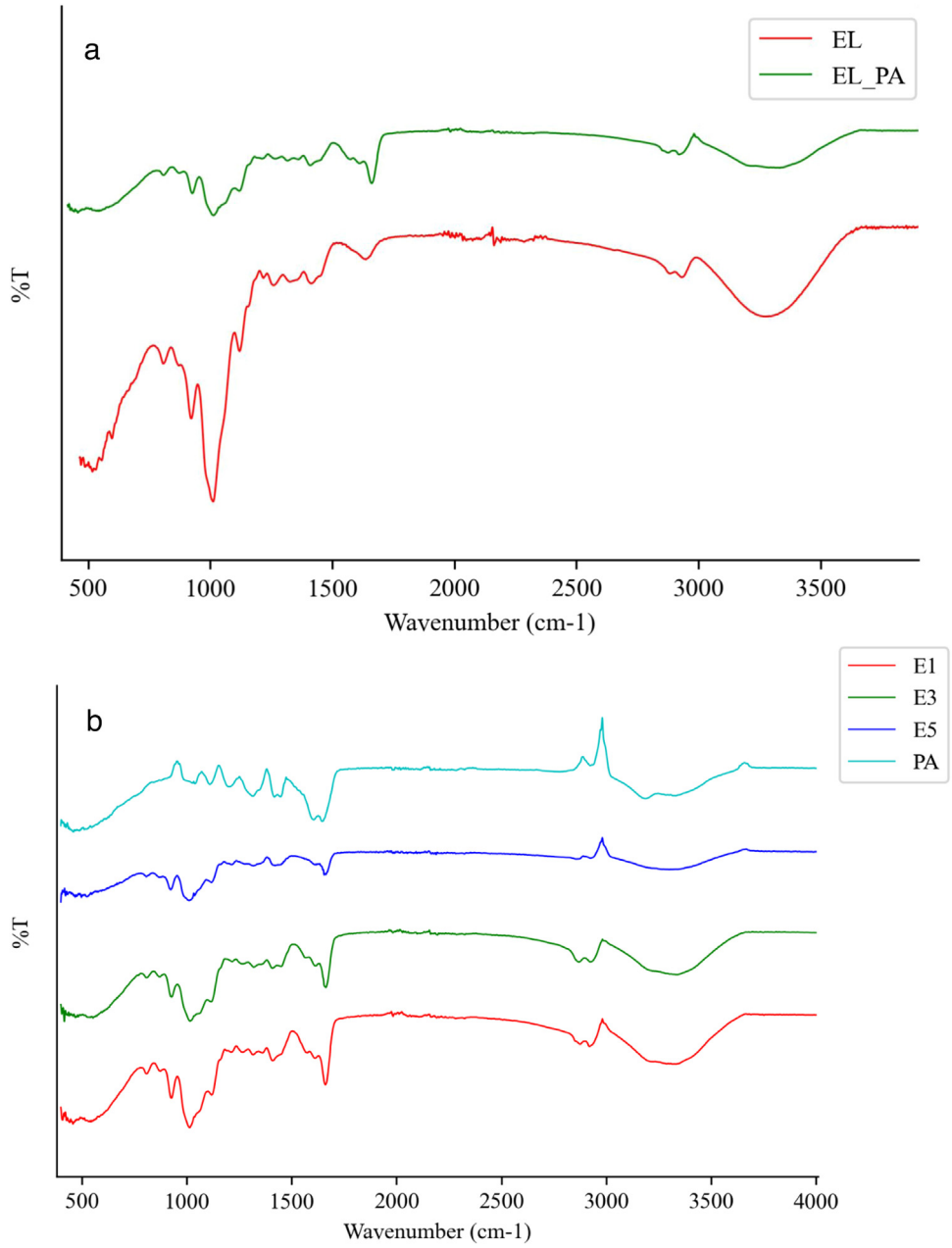

Fig. 1. (a) FTIR spectra of enzymatic levan (EL) and IPN based enzymatic levan-polyacrylamide hydrogel (EL-PA) (b) FTIR spectra of EL-PA hydrogels at varying levan compositions 1.0% (w/v) enzymatic levan (E1) 3.0% (w/v) enzymatic levan (E3), 5.0% (w/v) enzymatic levan (E5) and blank formulation polyacrylamide (PA).

Table 1
Equilibrium swelling ratios (%) of PA, enzymatic (E) and microbial levan (M) containing hydrogels at varying concentrations and pH values.

Samples	ph5.0	pH 7.2	pH 9.0
E1	1253.18	2296.18	2966.11
E3	1034.66	1733.78	2105.38
E5	896.80	1366.63	1622.26
M1	1271.41	2174.25	2896.72
M3	1076.34	1909.55	2137.20
M5	909.45	1479.10	1800.04
PA	1190.97	985.45	1533.66

7.4 and pH 9.0. This enhancement in swelling can be attributed to the increased hydrophilicity and water retention capacity of the hydrogel matrix due to the presence of levan. Interestingly, as the concentration of levan increased beyond 1%, the swelling ratio gradually decreased. This phenomenon can be ascribed to the higher crosslinking density introduced by the greater concentration of levan, limiting the water uptake capability of the hydrogels. Overall, the IPN hydrogels exhibited higher swelling ratios in alkaline media, demonstrating their sensitivity to pH variations. In comparison to the blank samples composed of polyacrylamide (PA) alone, the IPN hydrogels displayed significantly higher water uptake capacities. However, it is noteworthy that the swelling ratios at pH 5.0 were comparable between the IPN hydrogels and the blank samples. This observation can be attributed to the protonation of ionizable alcohol groups present in the IPN hydrogels at acidic pH, leading to reduced availability of functional groups for hydrogen bonding. Consequently, this reduced interaction between the hydrogel matrix and water molecules accounts for the lower water uptake capacity of the IPN hydrogels under these conditions.

The diffusion mechanism of water within the hydrogels was investigated using Fick's law, which is considered accurate for the initial 60% of the diffusion process [40]. Consequently, the swelling data obtained during the first 3 h were utilized for model calculations. According to Fick's law, three distinct types of swelling mechanisms exist: (I) When the rate of diffusion is slower than the relaxation of the polymer chains ($n = 0.5$), (II) Case II transport, where diffusion is solely controlled by solvent concentration ($n > 0.5$), and (III) anomalous transport, where both mechanisms are comparable ($0.5 < n < 1.0$) [19,39]. To investigate the diffusion mechanism of enzymatic and microbial IPN hydrogels at different pH levels, a plot of the natural logarithm of the swelling ratio (ln F) against the natural logarithm of time (ln t) was constructed. Detailed values of the diffusion exponentials (n), rate constants (k), and the coefficient of determination (R2) can be found in the supplementary data (S2). Notably, all diffusion exponentials corresponding to the swelling of IPN-based hydrogel samples exhibited non-Fickian water transport behavior ($0.5 < n < 1.0$). This distinctive behavior can be attributed to the repulsion forces between the ionizable groups of levan within the hydrogel matrix. The relaxation of the polymer network during swelling further suggests that the overall diffusion mechanism is predominantly controlled by relaxation processes. This analysis provides valuable insights into the water diffusion behavior of the IPN-based hydrogels and contributes to our understanding of the intricate swelling mechanisms involved. The non-Fickian transport behavior observed underscores the complex nature of the hydrogel matrix and highlights the role of repulsion forces in modulating water uptake and release.

Morphological characterization

Fig. 2 presents the scanning electron microscopy (SEM) micrographs of the blank (PA) hydrogel and IPN hydrogels, offering insights into their surface morphology. As shown in Fig. 2a, the blank sample (PA) exhibits a surface devoid of pores compared to the hydrogels incorporating levan. Notably, Figs. 3b to 3d display the IPN hydrogels with 1% levan (E1) and 3% levan (E3), showcasing a porous structure. This pronounced change in surface morphology confirms the successful formation of the IPN and highlights the influence of levan in creating a porous hydrogel architecture. The SEM images also provide support for the observed swelling behavior of the hydrogel samples. The inclusion of more than 1% crosslinked levan into the hydrogel network leads to a reduction in swelling. Specifically, the E1 hydrogel exhibits greater porosity and pore size, whereas an increase in levan concentration results in a decrease in porosity density. This finding aligns with the swelling analysis results, which explain the higher swelling ratios observed for the E1 hydrogel sample. As the levan concentration increases, the water uptake capacity of the hydrogels decreases. The initial augmentation in porosity and water absorption upon incorporating 1% levan can be attributed to the repulsive forces between the hydrophilic groups of levan, facilitating the creation of larger pore sizes. However, increasing the crosslinking density by raising the levan concentration in the polymer network reduces the pore sizes of the hydrogel samples, as the levan molecules occupy space within the pores despite possessing numerous ionic and hydrophilic groups. Utilizing scanning electron microscopy (SEM) images, we were able to ascertain the pore diameters of various hydrogel formulations. The SEM micrographs provided high-resolution insights into the porous structure of the hydrogels (Fig. 2e). For the E1 hydrogel, the average pore diameter was deduced to be approximately 67.8 µm, encompassing a range from about 63.3 µm to 82.7 µm. The hydrogel E3 showcased a mean pore size of around 46.8 µm, with diameters spanning between 40.8 µm and 68.5 µm. In the case of E5 hydrogel, the SEM images reflected a more constrained average pore diameter of 28.1 µm, varying from 22.6 µm to 41.0 µm. On the other hand, the PA hydrogel exhibited the most compact pore structure with an average diameter of about 14.1 µm and pores ranging from 8.7 µm to 20.0 µm. All measurements were carried out using ImageJ software, version 1.53.

Table 2
BET results of PA and EL-PA hydrogels.

Samples	P/Po	BET surface area (m^2g^{-1})
E1	33.61	0.31161
E3	29.67	0.31164
E5	27.70	0.31169
PA	20.70	0.31177

Specific surface area

The specific surface area analysis was performed using the Brunauer-Emmett-Teller (BET) method, which provides valuable information about the specific surface area and adsorption capacity of materials. Comparing the results of the BET analysis, the E1 mixture exhibited the highest BET specific surface area of 0.31161 m^2/g at a P/Po value of 33.61. where the PA gel exhibited a relatively lower specific surface area of 0.31177 m^2/g at a slightly higher P/Po value of 20.70 (Table 2). Type I isotherm was observed, which implies monolayer adsorption and typical for most microporous materials. These findings indicate that the EL-PA mixtures possess larger specific surface areas compared to the PA gel. Incorporating levan increased the specific surface area of the solid matrix. This enhancement in specific surface area pertains to the solid phase, and not the hydrogel matrix.

Rheological analysis

Amplitude sweep test

In the results of the amplitude sweep test, it was observed that EL-PA hydrogels exhibited a narrower Linear Viscoelastic Region (LVER), compared to pure PA hydrogels, (Fig. 3a-b) . A smaller LVER region indicates that the linear viscoelastic behavior of the material is confined to a narrower range of frequencies or strains. This implies that the material deviates from linearity and begins to display non-linear viscoelastic behavior. These findings highlight the distinct viscoelastic characteristics and behavior of EL-PA hydrogels compared to pure PA hydrogels.

Frequency sweep test

The frequency sweep analysis revealed a pronounced crossover point in the viscoelastic response of the PA gel, characterized by a shift in the behavior from predominantly elastic to predominantly viscous as the frequency increased (Fig.3c-f). This observation suggests the presence of structural reorganization or relaxation processes within the gel network. Although in 3c shows a single crossover point, it might be associated with the sudden increase in the loss modulus at the end of the experiment and can be attributed to the rheometer. Despite the efforts made to capture a crossover point in the frequency sweep with an additional experiment, it was not feasible due to the gel decomposition. The crossover point provides valuable insights into the viscoelastic nature of the gel. It indicates the point where the gel transitions from a predominantly elastic behavior to a predominantly viscous behavior. At the crossover point, the relaxation time of the gel becomes comparable to or exceeds the characteristic time scale of the experiment or observation. The absence of this characteristic behavior in the Levan-Polyacrylamide mixtures indicates a different mechanism at play, possibly due to altered network structure or interactions introduced by the levan component.

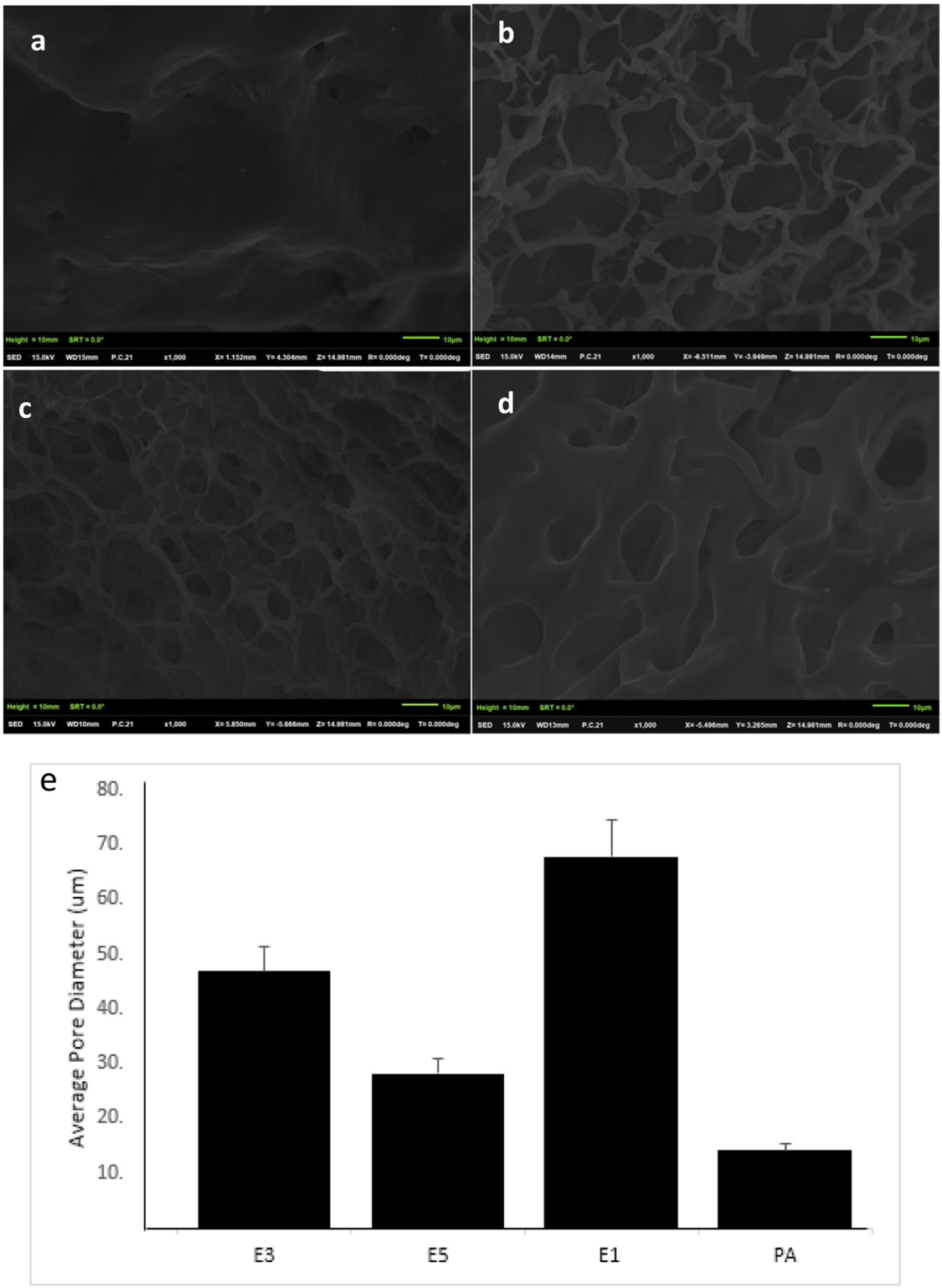

Fig. 2. SEM images of (a) blank (PA) (b) Enzymatic 1% levan -PA (E1) (c) enzymatic 3% Levan-PA (E3) (d) enzymatic 5% Levan-PA (E5) (e) Average pore diameter of hydrogels.

Oscillatory-analysis

Lastly, a comprehensive oscillatory analysis was conducted to elucidate the flow behavior and rheological properties of the hydrogel after levan addition into the gel matrix. Complex viscosity was observed to decrease with increase in frequency (Fig. 3g). At low frequency, viscosity was observed to increase with increase in levan content.

Characterization of paper media

In addition to the characterization of hydrogels, the color and crystallinity index of the media on the paper surface were also investigated. Color measurements were conducted to determine the L*, a*, and b* values of paper samples with and without adhesive coatings. The paper samples without old adhesive coatings exhib-

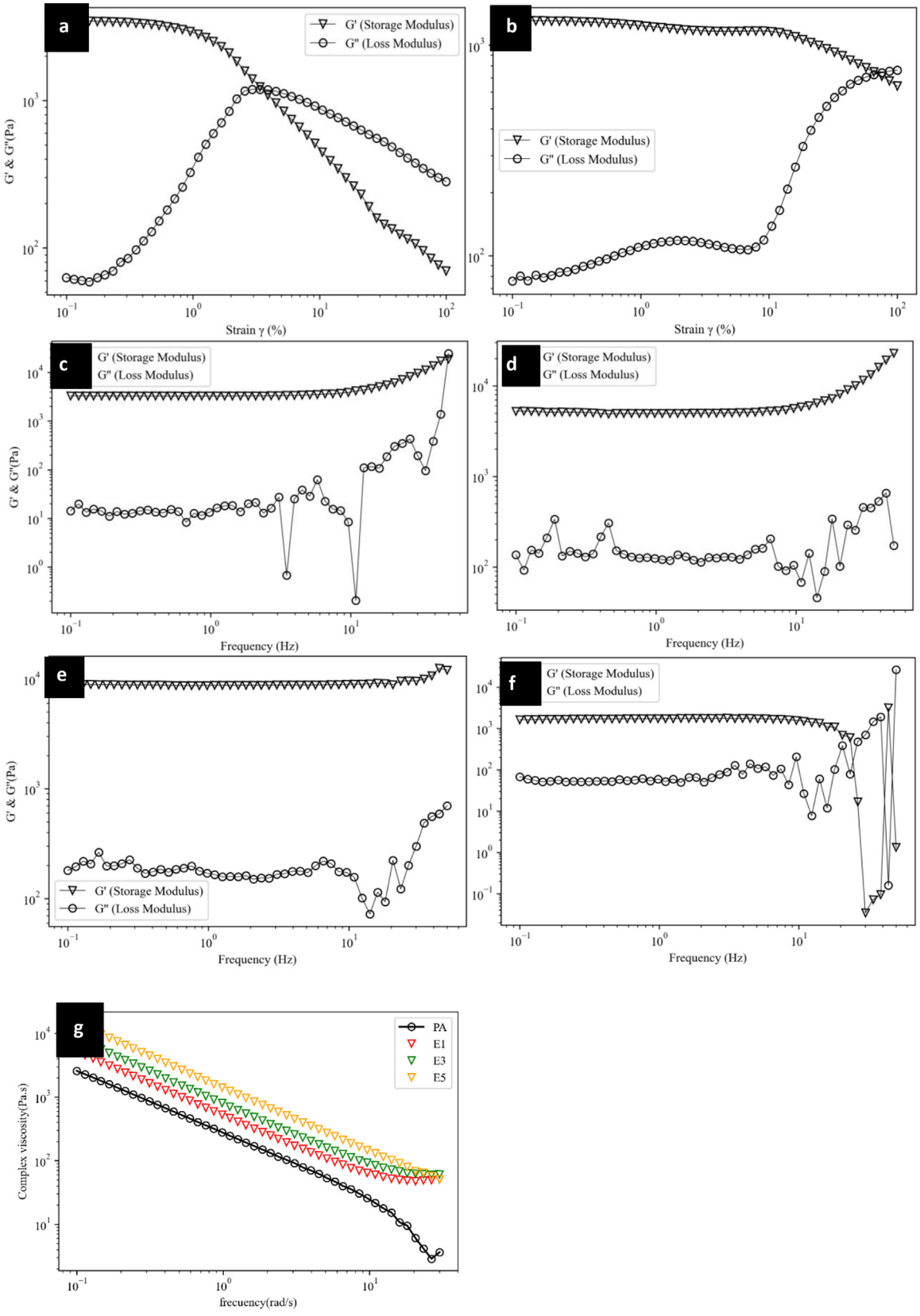

Fig. 3. (a) Amplitude sweep test of EL-PA hydrogels (b) PA hydrogel (c) Frequency sweep test of PA (d) E1 (e)E3 (f) E5 (g)Oscillatory analysis- complex viscosity vs frequency plot for EL-PA and PA hydrogels.

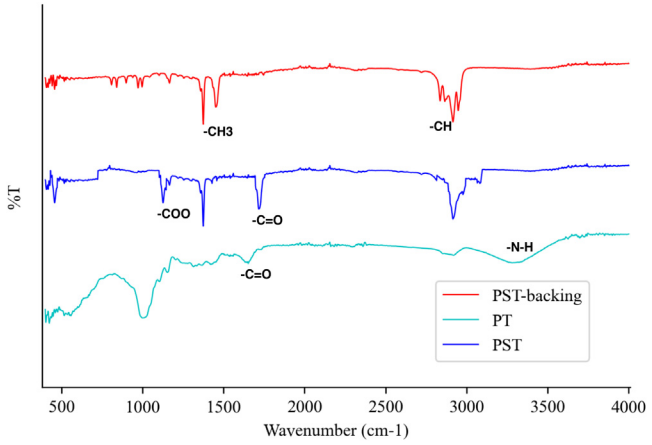

Fig. 4. FTIR spectrum of adhesive residues of paper tape (PT), backing of the pressure sensitive tape (PST-backing) and its adhesive (PST).

ited a lightness value (L*) of 82.30, while those with adhesive coatings showed a lower lightness value of 59.62. The adhesive coating caused a noticeable color change, as indicated by the increased values of a* (from 2.68 to 9.04) and b* (from 19.01 to 26.40) for the paper samples. These color coordinates demonstrated the influence of adhesive residues on the natural aging of the paper [29]. The crystallinity index (CI) was also determined to assess the impact of adhesive residues on paper specimens. The paper specimen without adhesive exhibited a CI of 0.305, whereas the paper specimen with adhesive residue had a higher CI of 0.538. The diffractogram related to the latter can be found in the supplementary data (S4). The increased crystallinity suggests that the application of adhesive made the paper fibers more rigid, resulting in brittleness. Therefore, the removal of adhesive residues from the paper surface becomes an important step in the conservation process.

Structural characterization of paper specimens

Fig. 4 presents the ATR-FTIR spectra of the adhesive residues from the paper tape used (PT), as well as the backing of the pressure-sensitive tape used (PST). The spectra of the adhesive residues from the paper tape exhibits characteristic absorption bands associated with animal glue. These include the presence of C–O at 1600 cm^{-1} and stretching of the νN–H group at 3300 cm^{-1}, confirming the presence of amide groups. Additionally, bands observed at 2950, 2917, 2871, and 2835 cm^{-1} correspond to CH3 stretching and aliphatic C–H stretching vibrations. The spectrum of the pressure-sensitive tape backing reveals the infrared pattern of acrylic adhesive tapes. The spectra displays characteristic absorption bands of propylene, including C–H stretching of CH2 and CH3 groups in the aliphatic chain at 2950, 2982, and 2865 cm^{-1}, as well as bending vibrations of CH2 and CH3 at 1455 and 1375 cm^{-1}. For the adhesive material of PST, the peak at 1700 cm^{-1} indicates the presence of the ester functional group (C=O) O of polyacrylate. Also peaks at 1138 cm^{-1} may correspond to the C–O stretching of the ester functional groups (-COO-) present in the polymer structure.

Morphological characterization of paper surface

The surface morphology of paper specimens was investigated via SEM analysis to track the effectiveness of the adhesive removal process and overall effect of aged adhesive on paper. As it is shown in Fig. 5a, the aged adhesive residues caused cracked and layered formation on the surface. Also, Fig. 5b shows that hydrogel application was successful in the removal of adhesive tape.

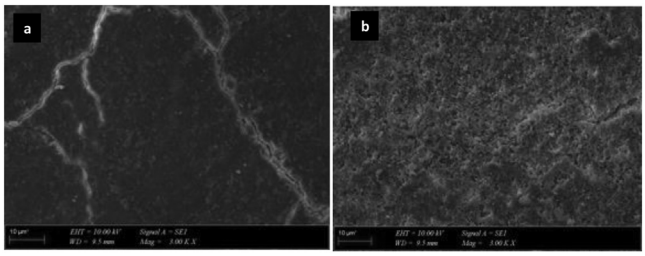

Fig. 5. SEM micrographs of paper surface (a) with aged adhesive layer (b) after removal (PST-backing) and its adhesive (PST).

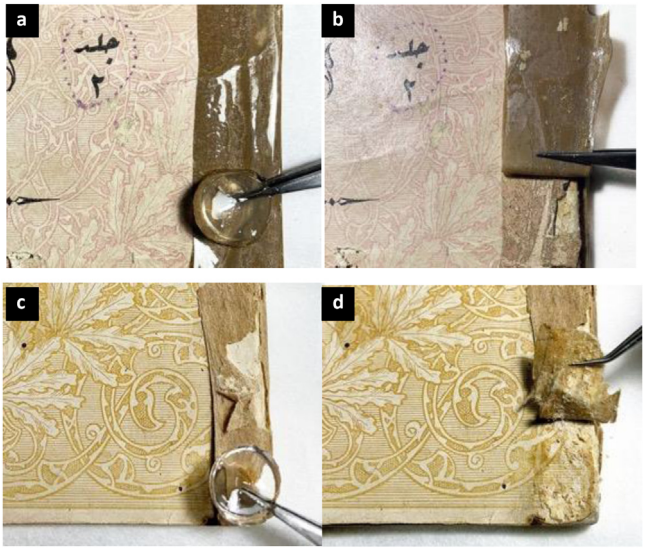

Fig. 6. Two bindings of "Ottoman History" and IPN based EL-PA hydrogel application. (a) Hydrogel application on the pressure sensitive tape of the second binding (b) Second binding paper surface after removal of the backing (c) hydrogel application on paper tape of the first binding (d) Paper surface after removal of the paper tape.

Application of hydrogels

When considering adhesive tapes in conservation projects, conservators take great care to avoid any rough mechanical actions during their removal. This precaution is necessary because the adhesive can form crosslinking within the fibers of the book, leading to additional damage if the tapes are removed improperly. Therefore, the selection of an appropriate solvent for removal becomes crucial. Several factors such as the porosity of the paper, thickness, and strength of the adhesive, as well as the backing material of the tape, must be taken into account before choosing the suitable solvent. The solvent should possess the ability to efficiently penetrate the backing material and soften the adhesive. The expected mechanism of action for the solvent is twofold: first, it should penetrate the backing effectively, but not excessively, to prevent any bleeding of ink, dye, or other components. Second, it should solubilize the adhesive in a controlled manner to avoid further spreading of the adhesive into the fiber matrix. By following these guidelines, conservators can ensure the safe and effective removal of adhesive tapes during conservation projects without causing any loss of valuable information or compromising the artifact's integrity.

A microemulsion system, named EAPC, was used for the purpose of removing adhesive tapes, following a proposed method [41]. The EAPC system comprises ethyl acetate (EA), propylene carbonate (PC), 1-pentanol (1-PeOH), and sodium dodecyl sulfate (SDS). This system is composed of an organic phase and surfactants, leading to the formation of micro-emulsion droplets on a

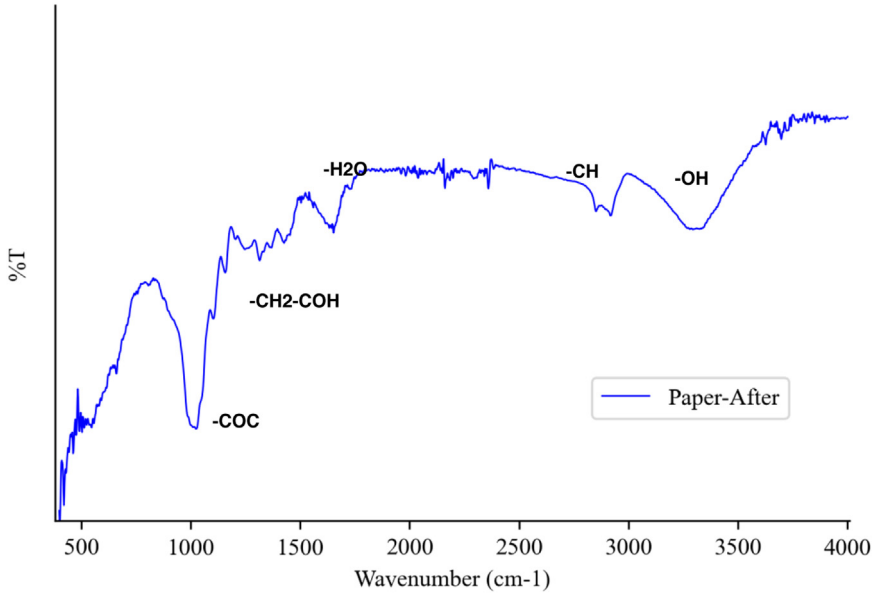

Fig. 7. FTIR spectrum of paper sample after treatment with EL-PA hydrogel.

nanoscale. The hydrogel was immersed in the micro-emulsion for a duration of 12 h. Prior to its application on the book, the effectiveness of the system was tested on mock-up samples. These samples were created by dyeing Whatman Grade 1 filter paper with blue ink and affixing ordinary pressure-sensitive tapes onto them. The performance of neat solvents and the proposed hydrogel system loaded with the microemulsion were compared. For the removal of pressure-sensitive tapes, ethyl alcohol (70%) and ethyl acetate were employed as two neat solvents. Additionally, a Levan-g-PA hydrogel loaded with EAPC was applied to another sample, allowing for 6 min to solubilize the adhesive coating of the pressure-sensitive tape. Visual examination revealed bleeding after the application of neat solvents, while no such results were observed with the IPN hydrogel application (refer to supplementary data S5). Based on preliminary investigations conducted on inked dispersed Whatman samples, it was determined that hydrogels would be utilized on the artifact (see Fig. 6). During application, solvent-loaded hydrogels were placed on the adhesive coatings for 15 min, with an additional 5 min allotted for control. As a result of this process, both bindings were successfully freed from their adhesive backings.

The paper sample treated with EL-PA hydrogel exhibits a FTIR spectrum that is devoid of any residual information. Upon analysis, only the typical bands associated with cellulose are observed, suggesting the successful removal of any additional compounds or contaminants that might have been present on the paper surface (Fig. 7). This indicates the effectiveness of the EL-PA hydrogel treatment in selectively targeting and eliminating undesired substances, while preserving the inherent cellulose structure of the paper.

Conclusion

Throughout the last decade, there has been a mounting request for natural polymers as they can be used in various fields [33]. This research was focused on the use of levan polysaccharide in the field of conservation of cultural heritage by introducing novel hydrogels. Levan-polyacrylamide hydrogels that were produced using both microbial and enzymatic levan polysaccharide, were compared and investigated according to their swelling characteristics, mechanical and morphological properties. There was no significant behavioral difference between hydrogels with microbial and enzymatic levan. The swelling of the hydrogels was increased almost 3-fold with the addition of levan into the polymer network. The highest swelling was achieved with 1% levan addition at pH 9.0. Also, SEM results indicated that hydrogels with 1% addition of levan showed larger surface area which is directly related to swelling content. The results obtained also revealed that EL-PA hydrogels demonstrate higher swelling capacity as the medium gets more alkaline. This tendency can also be useful for different high-value applications such as targeted drug delivery systems or stimuli responsive hydrogels. We demonstrated that the viscoelastic behavior of gels has changed due to the presence of levan. After characterization, solvent loaded hydrogels were applied onto two bindings of a 19th century printed book written by Namık Kemal, *Ottoman History*, with the aim of removing adhesive tapes that would cause further damage in the future. Already existing degradation of paper was shown by analysis of paper specimens of the two bindings. In addition to the findings mentioned above, further investigations are warranted to determine the exact nature of the IPN (Interpenetrating Polymer Network) structure formed by the levan-polyacrylamide (EL-PA) hydrogels. The characterization performed in this study did not conclusively determine whether the hydrogels formed a semi-IPN or a full IPN structure. By conducting these additional experiments, we aim to gain a more thorough understanding of the EL-PA hydrogels' interpenetrating network structure and its implications for their mechanical strength, stability, and long-term performance. This will contribute to the ongoing development and refinement of levan-based hydrogel systems for their successful implementation in the conservation science field.

Availability of data and materials

All the available data have been included in the article.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.culher.2023.10.013.

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