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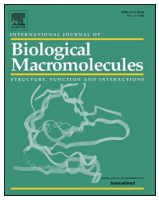

Plasticized agarose films: A physicochemical, mechanical and thermal study

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ABSTRACT

Agarose uniquely forms moldable biodegradable films, making it a promising renewable material with exceptional biocompatibility, thermo-reversibility, and flexibility. However, agarose films lose most of their flexibility at low moisture content. One way to assuage this issue is to incorporate plasticizing agents. In this study, four plasticizers (i.e., sucrose, urea, glucose, and glycerol) were chosen and combined in various concentrations and combinations to produce an agarose-based composite. The study examined how four different plasticizers affect agarose's intermolecular interactions, impacting its mechanical, morphological, thermal, and physicochemical properties. Techniques like Fourier Transform Infrared Spectroscopy (FTIR), Thermogravimetric Analysis (TGA), X-ray Scattering, electric actuation, and tensile testing were used to analyze the effects of plasticizers on agarose-based films. The findings reveal that the mechanical and thermal properties of agarose films are influenced to varying degrees by the four plasticizers studied. Plasticizers with high hydroxyl content and smaller molecular size demonstrated the most significant improvements in film flexibility and stretchability. These variations in performance can be attributed to differences in intermolecular interactions, driven by changes in hydrogen bonding groups, as observed through FTIR and X-ray Scattering analyses. A deeper understanding of how hydrogen bonds affect the agarose-plasticizer matrix could pave the way for precisely tailoring the properties of agarose films.

1. Introduction

Developing renewable, durable, bioderived materials is pertinent in solving humanity's growing environmental issues and rapidly advancing production needs in the modern construction and manufacturing industries. Research into biopolymers, such as polysaccharides and proteins, has grown in popularity due to their ideal biocompatible, biodegradable, and renewable properties [1]. Agarose, in particular, is a biopolymer that is finding a wide range of applications, including uses in drug delivery, cell encapsulation, immunological analysis, protein extraction, electrophoresis, wound healing, bone regeneration, gold separation, biosensors, cartilage formation, angiogenesis, and tissue engineering [2,3]. Recently, agarose has even been investigated as a component for sustainable concrete and a replacement for petroleum-based binders in construction [4,5].

Agarose is synthesized by first extracting agar from certain species of marine red algae [6]. Next, agaropectin is removed from the agar through acetylation or other chemical methods, leaving behind only agarose, the gelling component of agar. Agarose's molecular structure is composed of alternating 3,6-anhydro-α-L-galactose and β-D-galactose

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units oriented in a linear polysaccharide [3]. This molecular composition provides agarose with a nonionic character but also a high number of hydroxyl groups, enabling it to form several inter and intramolecular hydrogen bonds and even form thermo-reversible gels. The gelling process requires heating agarose in water to 80–90 °C and then cooling to room temperature. During cooling, agarose will self-organize via hydrogen bond interaction to go from a random coil configuration into a helical structure [7]. Agarose's gelation process occurs in three steps: induction, gelation, and pseudoequilibrium, resulting in a helical-shaped structure [2]. Xiong et al. [8] summarized agarose's gelation mechanism by proposing a nucleation and growth mechanism as the initiating step whose kinetics are defined by the formation of nuclei in the polymer-rich region [8]. However, some of the intricacies of agarose's structure during the gelation process are still being debated, and the question of whether it forms a single helix or double helix conformation is still unanswered [9]. Early modeling theorized that agarose had a particular double helix structure with an extended chain configuration [10]. However, later studies found that single-chain states and shorter chains with wider diameters seemed to form after gelation [11,12]. To resolve these conflicting findings, future studies could determine the actual structure of agarose gel by examining the energetics of chain conformations and investigating how agarose chains interact with guest molecules.

Once successfully gelled, agarose can be dried to form freestanding films. These films are strong, transparent, and heat-sealable. However, once dried to a low moisture content, agarose films become very stiff, brittle, and inflexible, thus limiting their prevalent usage [13]. To ameliorate these weaknesses, a plasticizer can be incorporated to substantially improve film flexibility and durability. Plasticizers decrease a film's inherent brittleness by reducing intermolecular forces and increasing polymer chain mobility [14]. Specifically, plasticizers work to disrupt and expand hydrogen bonds by diffusing between polymer chains and supplementing the hydrogen bond network. This expansion of the hydrogen bond network thus prevents the primary polymer from being entrapped by its network of more restrictive polymer-polymer hydrogen bonds. Furthermore, by diffusing a plasticizer between polymer chains, the spacing between polymer chains is increased, the polymer-polymer interactions are decreased, and the relative movements between polymers are made easier [15]. Recently, plasticized agarose films have shown the potential to be utilized as an eco-friendly alternative material in everyday objects, including display devices, windows, biodegradable packaging, and more [16]. Different plasticizers affect agarose films uniquely due to variations in factors like molecular weight, hydroxyl content, and functional groups, which influence their interactions with the polymer [17]. For instance, a plasticizer with a lower molecular weight can penetrate a polymer network more easily, resulting in increased flexibility [14].

This study hypothesizes that an agarose composite film's mechanical and physicochemical properties depend on the molecular interaction between its polymer chains and plasticizer molecules. These properties can thus be tuned by incorporating plasticizers with various molecular weights and hydrogen bonding groups. The primary objective of this study was to investigate the influence of distinct plasticizer properties on modifying the structural and functional characteristics of agarose films. By incorporating different plasticizers into agarose with distinct molecular properties, agarose's molecular interactions, especially its hydrogen bond network, will experience changes, manifesting as changes to agarose's mechanical, morphological, and physicochemical properties. Four plasticizers—sucrose, glucose, urea, and glycerol—were systematically selected and incorporated either individually or in combination to evaluate their effects on the material's properties. These four plasticizers were selected due to their cost effectiveness, availability, biocompatibility, and comparable characteristics. Sucrose and urea were chosen due to their distinct hydrogen bonding groups involving hydroxyl vs amine groups and variations in their molecular sizes. Additionally, D-glucose, commonly referred to as dextrose, was selected based on its structural similarity to sucrose while having a smaller molecular size. On the other hand, glycerol, one of the most ubiquitous plasticizers available, was selected for its comparatively small molecular size, being comparable to urea, but differs with enhanced hydrogen bonding capabilities, stemming from the presence of hydroxyl groups. Once the various agarose composite films were generated, the films were characterized using Fourier transform infrared spectroscopy (FTIR), thermal gravimetric analysis (TGA), X-ray Scattering, electric actuation, and tensile strength to study the different physicochemical, thermal, mechanical, and morphological changes elicited by the various plasticizers.

2. Materials and methods

2.1. Materials

Invitrogen UltraPure Agarose (Invitrogen: 16500100) was acquired from Fisher Scientific. Dextrose (SKU D9434-250G), sucrose (SKU S9378-500G), urea (SKU U5128-100G), and glycerol (SKU G9012-500ML) were purchased from Sigma-Aldrich. All chemicals were used as received.

2.2. Fabrication method

To fabricate agarose composite films, a solution containing water, agarose powder polymer, and plasticizer polymer was mixed together. This solution was 94 % water by weight and 6 % polymers by weight. Of the total polymer weight used, 67 % by weight of the polymer was agarose. The remaining 33 % were plasticizers added in the relevant ratio. The resulting solution was then heated to 85 °C for 1 h at a slow stirring speed and cast into a 90 × 15 mm glass petri dishes. Samples were then treated following Lim and Gong's [18] procedure for stretchable films, where the Petri dishes were covered and placed in a 60 °C oven for 12 h. Afterwards, the petri dishes were uncovered and placed in a 50 % humidity chamber for 3 h [18]. When necessary, samples were dried overnight in a vacuum oven set to 30 in Hg and 50 °C before running characterization tests.

2.3. Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared (FTIR) spectroscopy was analyzed using a Bruker's ALPHA-Platinum Attenuated Total Reflectance Fourier Transforms Infrared (ATR-FTIR) Spectrometer with a platinum-diamond sample module. For each film, a spectrum range from 4000 cm^{-1} to 400 cm^{-1} was performed per scan at a resolution of 4 cm^{-1}. 128 background scans were completed prior to 32 sample scans conducted at 6 different sample locations. The average of the 6 sample locations was taken, and a min-max normalization was applied to all spectra. IR analysis was performed using Opus 7.2 software.

2.4. Thermal gravimetric analysis (TGA)

Thermalgravimetric analysis (TGA) was performed using the TA Instruments Discovery system on a ~3 mg sample. All tests were conducted under a 25 mL/min nitrogen gas purge at 30 °C. The TGA procedure entailed a one-minute isothermal period, followed by a ramping temperature with a thermal rate of 10 °C/min up to 600 °C. Step transition analysis and derivative plots were used to determine the temperature of the onset of decomposition (T_{onset}) and the weight-loss percentage of the sample.

2.5. X-ray scattering

The morphological studies were conducted using a Dual Environmental X-ray Scattering System (XRS) under vacuum. The Xeuss 2.0 by XENOCS has a Cu X-ray source, computer-controlled focusing and

transmission incident sample geometries, a 1 M pixel Pilatus detector (2D), and a smaller detector for simultaneous small-angle scattering (SAXS) and wide-angle scattering (WAXS) acquisition. A full-flux collimation was used with a slit size of 1.2 mm × 1.2 mm. Each sample run was executed for a total of 600 s. The reported intensity is not absolute and thus is in arbitrary units (a.u.). All samples were taped to a sample holder and placed under a vacuum for data acquisition. The X-ray scattering profiles were evaluated using Foxtrot 3.4.9; the isotropic 2D scattering patterns were azimuthally integrated to yield intensity versus scattering vector (q).

2.6. Electrical actuation

Prior to gelling, an agarose solution was poured into a glass petri dish that included a mold constructed from two glass slides and a spacer that was placed between the slides. Once the hydrogel had formed and cooled, the mold was removed from the petri dish, and any excess agarose material was cut away from the edges of the mold. From the mold, a uniform agarose rectangular strip was retrieved. The dimensions of the agarose strips measured, on average, 24 mm in length, 12 mm in width, and 0.6 mm thick. These measurements were confirmed and recorded for each strip prior to testing using electronic calipers. The strips were immersed in water for 10 min, then gently blotted with tissue paper to remove excess water. They were then placed, at one end, between a pair of copper electrodes mounted to the tips of customized tweezers. The electrodes, with a contact area measuring 9 mm long by 6 mm wide, delivered a bias voltage from a DC power supply (Keithley model 2231A-30-3) set to 15.0 V. The nominal distance from the electrode's end to the agarose strip's end was about 12 mm. A CMOS camera (Sentech model STC-MBS241U3V) equipped with a Computar macro zoom 0.3×–1×, 1:4.5 lens was utilized to monitor the deflection of the agarose strip. The deflection was gauged by placing a small metal ruler near the strip's end, and an image was captured after 3 min of application of the bias voltage. Final average displacement values were calculated after three trials.

2.7. Tensile strength

All the tensile tests to measure Young's modulus and the tensile strength were conducted with a Shimadzu mechanical Tester -a tabletop model (Autograph AGS-X –Shimadzu Scientific Instruments, Inc. 7102, Riverwood Drive, Columbia) (gauge length: 20 mm; cross-head speed: 10 mm/min) equipped with a 5000 N capacity load cell at 25 ± 0.5 °C and 60 ± 5 % relative humidity. For testing, samples were fabricated in I-shaped/Dog bone Shape and stretched till breakage.

3. Results and discussions

All agarose films containing plasticizers had the same 2:1 ratio of agarose to total plasticizer content. Moving forward, agarose films will be referred to by the percentage of plasticizers used, as listed under Film Reference in Table 1. Fig. 1 displays an image of the nine studied agarose composite films. All films have a similar colorless, translucent appearance except 33 % urea, which appears translucent initially but will start forming white crystals across its surface after about one to two weeks.

3.1. Molecular interaction

Fourier transform infrared (FTIR) spectroscopy was conducted to verify the successful blending of agarose and plasticizer. The molecular structure of agarose and the four plasticizers are shown in Fig. 2a. Fig. 2b illustrates a model of agarose's crosslinked gel structure. The IR spectra in Fig. 2c-e were normalized so that the peak positions would remain constant for comparison. Agarose, sucrose, glycerol, and dextrose all share similar molecular compositions, being comprised of purely oxygen, hydrogen, and carbon. Thus, their FTIR spectra in Fig. 2c share similar FTIR profiles with several of the same key functional groups. Specifically, the broad bands with strong intensity at the 3500–3100 cm^{-1} region can be attributed to hydroxyl groups' O—H stretching. Rajesh and Popat [19] reported a similar O—H peak in their agarose samples at 3415 cm^{-1} [19]. Furthermore, the medium intensity bands at 2950 to 2850 cm^{-1} can be attributed to the alkane groups' C—H stretching, while the small peak at 1640 cm^{-1} should be attributed to bound water molecules. The C—H stretching peak can be seen at 1430 $cm^{-1,}$ while the ether groups' C-O-C stretching can be seen at 1200 cm^{-1}. Lastly, the intense peak at 1050 cm^{-1} is ascribed to the alcohol groups' C—O stretching. These peaks are present in all agarose, sucrose, glycerol, and dextrose-containing materials [3].

When urea was incorporated as a plasticizer, as seen in Fig. 2d and e, urea's unique amine and carbonyl functional groups were integrated into agarose's FTIR profile. Specifically, N—H stretching and deformation appear at 3450 cm^{-1} and 1580 cm^{-1}, respectively, while C—N stretching appears at 1370 cm^{-1}. Lastly, the 1680 cm^{-1} peak is attributed to C═O stretching [16]. Thus, these new peaks' appearance confirms urea's successful incorporation into the materials. Furthermore, as seen in Fig. 2d and e, utilizing different plasticizers other than urea caused urea's C—N stretch to disappear, as expected. Based on these results, all samples with their various plasticizers and concentrations were fabricated successfully.

Table 1
List of agarose composite film names, percentage of agarose and plasticizers used in each film, and the reference name used.

Film name	% polymer composition	Film reference
Agarose with urea film	67 % agarose, 33 % urea	33 % urea
Agarose with sucrose film	67 % agarose, 33 % sucrose	33 % sucrose
Agarose with glycerol film	67 % agarose, 33 % glycerol	33 % glycerol
Agarose with dextrose film	67 % agarose, 33 % dextrose	33 % dextrose
Agarose, sucrose, urea film	67 % agarose, 25 % sucrose, 8 % urea	25 % sucrose, 8 % urea
Agarose, sucrose, glycerol film	67 % agarose, 25 % sucrose, 8 % glycerol	25 % sucrose, 8 % glycerol
Agarose, dextrose, urea film	67 % agarose, 25 % dextrose, 8 % urea	25 % dextrose, 8 % urea
Agarose, dextrose, glycerol film	67 % agarose, 25 % dextrose, 8 % glycerol	25 % dextrose, 8 % glycerol

3.2. Morphology

Small-angle X-ray scattering (SAXS) and wide-angle X-ray scattering (WAXS) were performed to examine morphological changes between the different agarose films. An example of a SAXS 2D diffraction pattern of 100 % agarose can be seen in Fig. 2f, which shows that agarose is x-ray scattering is isotropic with only two distinct regions. From the q vector graphs in Fig. 2g and h, agarose has two diffuse broad peaks at the scattering vector, q = 9.5 and 14.0 nm^{-1}, which are attributed to agarose's semi-crystalline matrix [20]. Yuan et al. [21] saw a similar set of peaks in their X-ray scattering analysis, which they attributed to the helix formation of agarose molecular chains where the helical structure of agarose increases the order of agarose [21]. Conversely, adding plasticizers often lowers a material's degree of crystallinity [22]. By comparing the different peak locations in Fig. 2g and h, we see there are slight shifts in the position and relative intensity of the first agarose peak around q = 9.5 nm^{-1}. This is likely due to changes in the intermolecular forces between differing agarose films. By incorporating plasticizers that alter agarose's hydrogen bonding network, agarose's wider gel structure experiences slight changes, resulting in the peak shifts that are recorded. Yuan et al. [21] saw a similar phenomenon with the addition of konjac glucomannan (KGM), where KGM's interaction with agarose decreased the aggregation of the helical structure of agarose molecular chains,

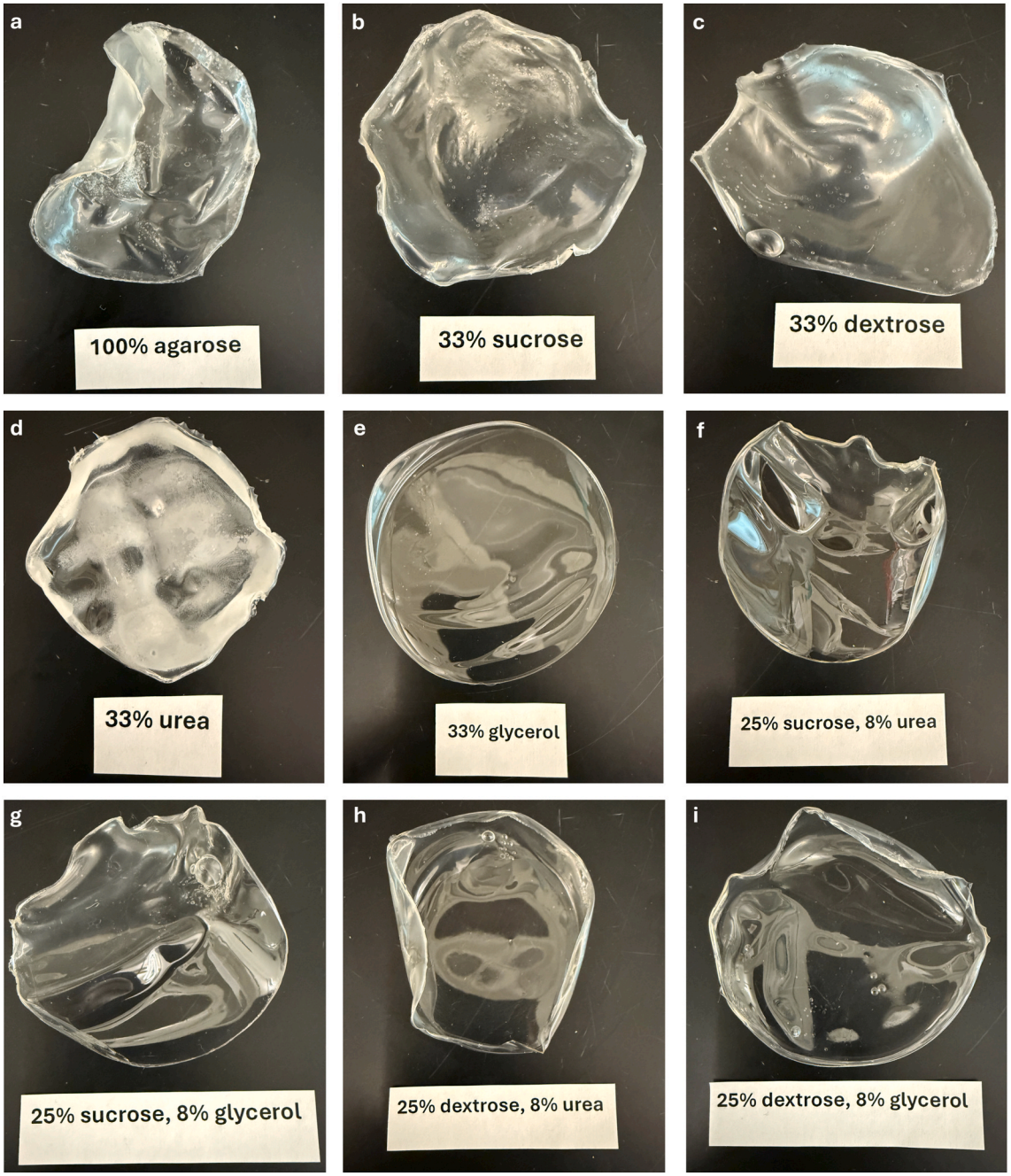

Fig. 1. Images of the nine unique agarose composite films, each prepared from a 20 mL solution and dried in room conditions. The films presented are as follows: a) 100 % agarose, b) 33 % sucrose, c) 33 % dextrose, d) 33 % urea, e) 33 % glycerol, f) 25 % sucrose, 8 % urea, g) 25 % sucrose, 8 % glycerol, h) 25 % dextrose, 8 % urea, and i) 25 % dextrose, 8 % glycerol.

resulting in a decrease in the intensity of the first peak [21]. Overall, adding the selected plasticizers to agarose can slightly affect agarose's gel structure and X-ray profile, specifically on the first peak, but does not cause any major shifts or new peaks indicative of a widely reordered morphology.

3.3. Thermal decomposition

Thermogravimetric analysis (TGA) was conducted to detect changes in the thermal properties of the various materials and gain insights into how agarose's intermolecular interactions change with the incorporation of different plasticizers. Fig. 3a shows an example TGA sample set up with an agarose film sample loaded into an aluminum pan prior to being enclosed in the TGA's furnace. In Fig. 3c-e, sample weight loss over a constant increase in temperature ranging from 35 to 600 °C is shown. Table 2 lists sample T_{onset} values for Fig. 3c-e. The initial weight lost in a sample from around 50 to 100 °C can be attributed to water evaporating out of the sample. In contrast, any sample mass lost between 170 and 600 °C was likely due to the thermal degradation of the sample. Fig. 3c shows the thermal profiles of the individual film components. Overall, pure agarose powder was found to have a higher thermal stability, having the highest T_{onset} value of 265 °C, than any other component. The second closest component material was sucrose, with a T_{onset} 45 °C below agarose's. Thus, we could infer that combining a plasticizer with agarose will, in all cases, decrease agarose's thermal stability. This decrease in thermal stability is due to the weakening of

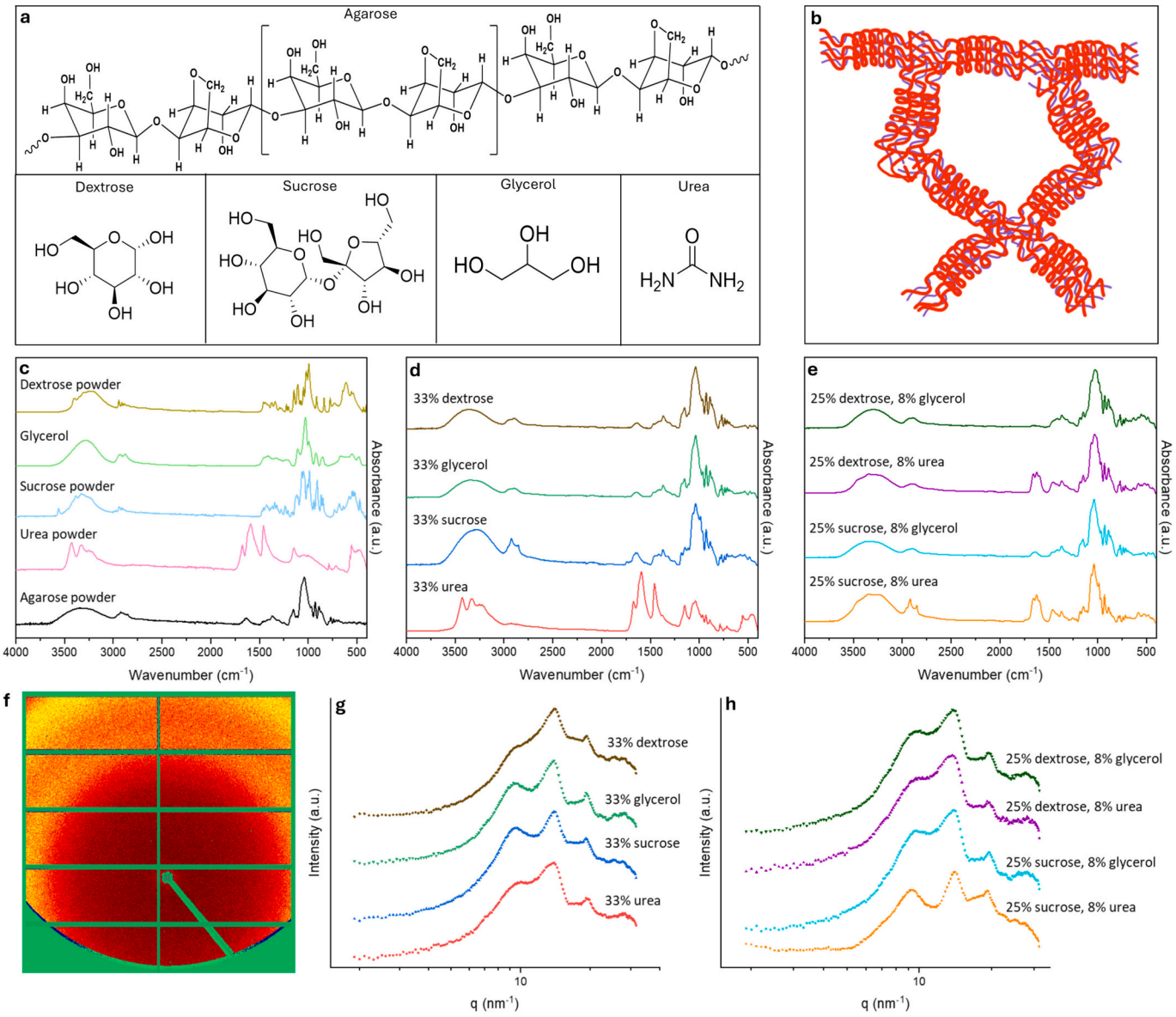

Fig. 2. Molecular structure and organization of agarose and the four plasticizers. a) Chemical structure of agarose, dextrose, sucrose, glycerol, and urea. b) Model of agarose's organized gel network. FTIR plots of c) pure components, d) agarose films with a single plasticizer, and e) agarose films with two plasticizers. f) 100 % agarose's 2D small-angle diffraction pattern with the converted X-ray plots of g) agarose films with a single plasticizer and h) agarose films with two plasticizers. All tests were performed on dried agarose films.

agarose's intermolecular forces, specifically its hydrogen bonds. Plasticizers can form hydrogen bonds with agarose polymers, supplementing agarose's polymer-polymer hydrogen bonds. This supplementation allows for an increase in agarose chain separation and a decrease in film thermal stability. Furthermore, when comparing TGA profiles of the pure powders in Fig. 3c, urea powder was the least thermally stable material, as seen in its low T_{onset} temperature. Urea would thus be expected to form the least thermally stable agarose films when utilized as a plasticizer. Examining Fig. 3d, this reduction in thermal stability is exactly what we see with the 33 % urea plasticized agarose film having a T_{onset} reduced to 190 °C, the lowest of the plasticized films. This low thermal stability is likely due to urea's amine groups which form weaker hydrogen bonds with agarose, leading to films that degrade more easily. Kasapis and Al-Marhoobi [23] noted a similar deleterious effect from urea in their agarose samples [23]. Of the remaining films, films plasticized with i) 25 % dextrose and 8 % glycerol, ii) 25 % dextrose and 8 % urea, iii) 25 % sucrose and 8 % glycerol, and iv) 33 % glycerol, as depicted in Fig. 3d and e, all had thermal stabilities equal to or marginally weaker than agarose powder. This would indicate that these combinations of plasticizers did not compromise or reduce the intermolecular forces that agarose is comprised of. The only other exception is the 33 % dextrose plasticized film in Fig. 3d. This film had a T_{onset} of 218 °C, making it thermally weaker than films plasticized with 33 % glycerol or 33 % sucrose. From a molecular standpoint, when comparing dextrose to sucrose, a single dextrose molecule has a lower hydroxyl count and thus forms fewer hydrogen bonds. This could explain why the films plasticized with dextrose had a lower thermal stability than films plasticized with sucrose. In contrast, glycerol also has fewer hydrogen bond-forming hydroxyl groups but is a much smaller molecule. Thus, plasticizing with glycerol should create less polymer chain separation, thus maintaining a higher thermal stability.

Lastly, Table 3 presents the percentage of a sample's mass lost due to water evaporating out of the sample during a TGA run. It is hypothesized that samples with high ambient water retention capabilities exhibited a more significant loss in water mass during TGA analysis and, thus, had more robust or complex hydrogen bond networks to retain water

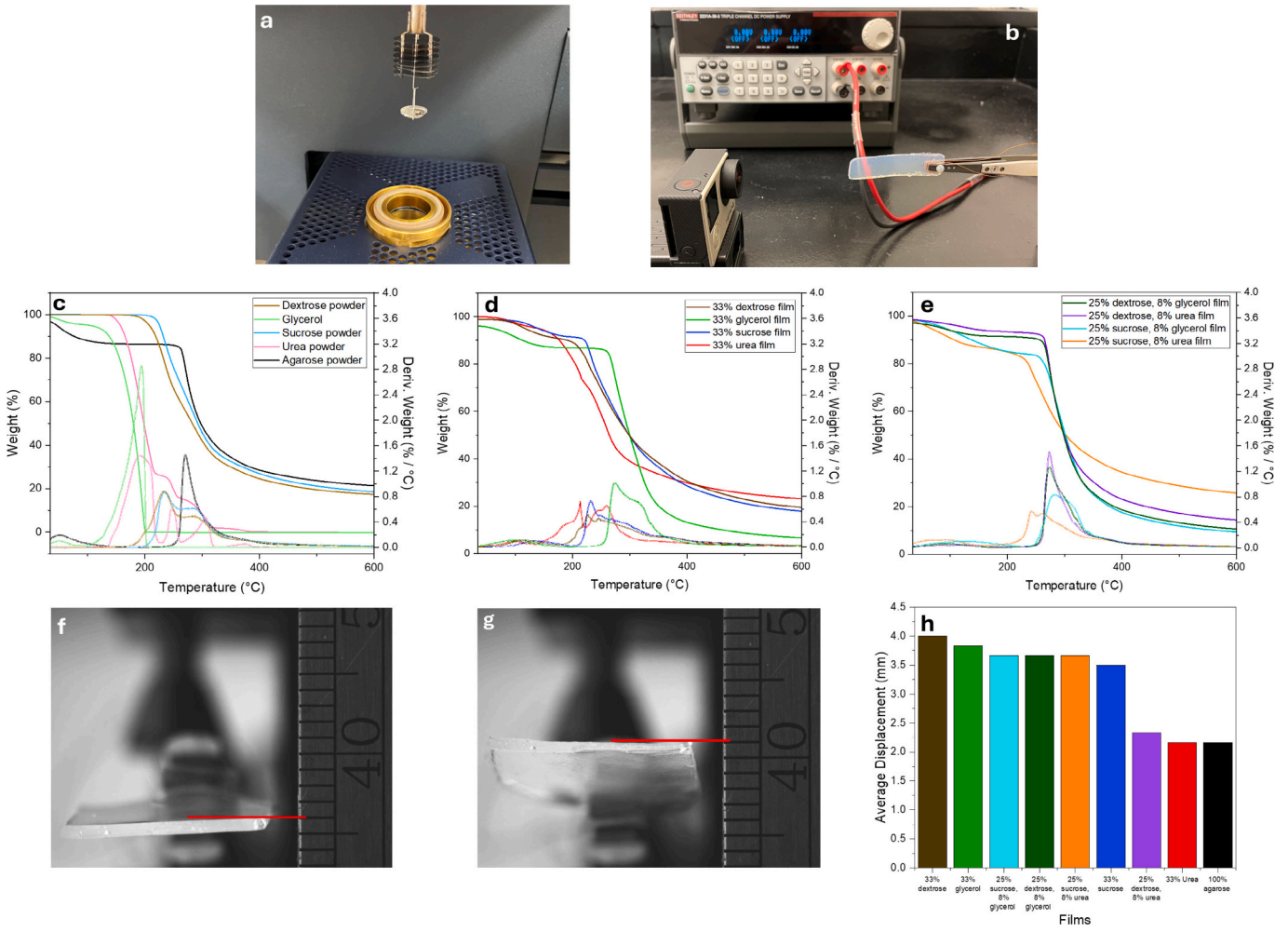

Fig. 3. Analyses of the thermal and electrical properties of agarose films. a) TGA set up with a ~3 mg dry agarose sample loaded in an aluminum pan and lowered into the TGA's furnace. b) Electrical actuation set up with a water saturated agarose film clamped between two electrodes soldered onto the tips of a tweezer. Once power was supplied to the tweezers, the total displacement of the agarose film was measured using a millimeter rule and camera recording software. TGA profiles with percentage weight loss over increasing temperature (solid lines in upper half) and derivative weight over increasing temperature (empty lines at lower half) for c) pure powders, d) agarose films with a single plasticizer, and e) agarose films with two plasticizers. Still images from the recording of an actuating sample f) before and g) during induced bending. h) Average displacement of each agarose film type.

Table 2
List of T_{onset} values for TGA graphs.

Agarose film material	T_{onset} (°C)
Agarose powder	265
Urea powder	164
Sucrose powder	220
Glycerol	140
Dextrose powder	215
33 % urea	190
33 % sucrose	225
33 % glycerol	262
33 % dextrose	218
25 % sucrose, 8 % urea	240
25 % sucrose, 8 % glycerol	263
25 % dextrose, 8 % urea	265
25 % dextrose, 8 % glycerol	263

molecules. After comparing water mass losses, it can be observed that the top two water-retaining films were ones that contained some combination of sucrose along with a second plasticizer. It is likely that sucrose's high hydroxyl count benefited water retention by providing more hydrogen bonding sight for water to interact with. Additionally, looking at the derivative of 33 % sucrose in Fig. 3d, even though the 33 % sucrose film had a lower water content compared to its double plasticized counterparts, we also see that 33 % sucrose's water loss peak was shifted noticeably upfield. Most films had their water loss peak occur around the 50 °C to 150 °C range while 33 % sucrose instead ranged from 100 °C to almost 200 °C. This shows an increased water retention in the 33 % sucrose film, likely due to its high hydroxyl count providing more hydrogen bond interaction opportunities for water. Conversely, in Table 3, it then follows that the 33 % urea film had the lowest water retention and thus the least amount of sample mass lost due to water due

Table 3
List of films and the percentage of mass lost due to water evaporation during TGA analysis.

Agarose film	Film % mass loss from water
33 % urea	4.71
33 % sucrose	6.68
33 % glycerol	8.35
33 % dextrose	7.33
25 % sucrose, 8 % urea	11.43
25 % sucrose, 8 % glycerol	12.88
25 % dextrose, 8 % urea	5.31
25 % dextrose, 8 % glycerol	5.44

to urea's weaker hydrogen-boding amine groups.

Overall, TGA analysis shows that by incorporating plasticizers into agarose, changes in agarose's intermolecular interactions can occur. Plasticizers work to supplement the agarose-agarose hydrogen bond networks, leading to a weakening in said bonds. This then causes a weakening of agarose's thermal stability and shifts in water retention.

3.4. Electrical actuation

The electro-induced bending responses of the agarose composite films were examined under the application of a 15 V DC bias, as depicted in Fig. 3b. Fig. 3f and g displays still images from a recording used to measure actuation displacement. Final averaged deflections are shown in Fig. 3h. There are three mechanisms for agarose's bending response under an electric field. First, the dielectrophoresis force created from the non-symmetric electric dipoles within the agarose structure creates the initial electro-induced bending. Secondly, the hydrogen and hydroxide ions of water operate as the mobile ions that generate the ionic polarizations within the agarose films, resulting in the induced bending. Thirdly, the hydroxyl groups attached to an agarose chain can withdraw the electrons from the agarose carbon backbone, resulting in a net negative charge that will cause agarose to bend towards the positive charged electrode under an applied electric field [24].

Given these three mechanisms, plasticizers can impact agarose electro-induced bending by decreasing general film stiffness, allowing the same electric force to push a film further, and by adding further hydroxyl groups to increase the net negative charge. Note that the second mechanism of water ion movement could also play an important role in explaining the different bending displacements seen between the agarose composite films, especially in light of the TGA data from Table 3 showing the different water content of the films. However, unlike in the TGA procedure, the agarose films for electrical actuation were all saturated in water moments before testing and thus all films had a similar abundance of water ions.

Overall, all films plasticized with hydroxyl-containing plasticizers (dextrose, glycerol, and sucrose) experienced over a 60 % increase in bending displacement vs. pure agarose. These hydroxyl-containing plasticized films all performed similarly, with the 33 % dextrose film performing the best, indicating it likely reduces agarose's rigidity the most. 33 % urea, on the other hand, performed the worst, leading to no increase in deflection. This is likely due to urea having no hydroxyl groups, it forms weaker hydrogen bonds, and urea is a comparatively smaller molecule than most of the other plasticizers. Thus, urea likely does not reduce agarose's rigidity nor enhance its net negative charge

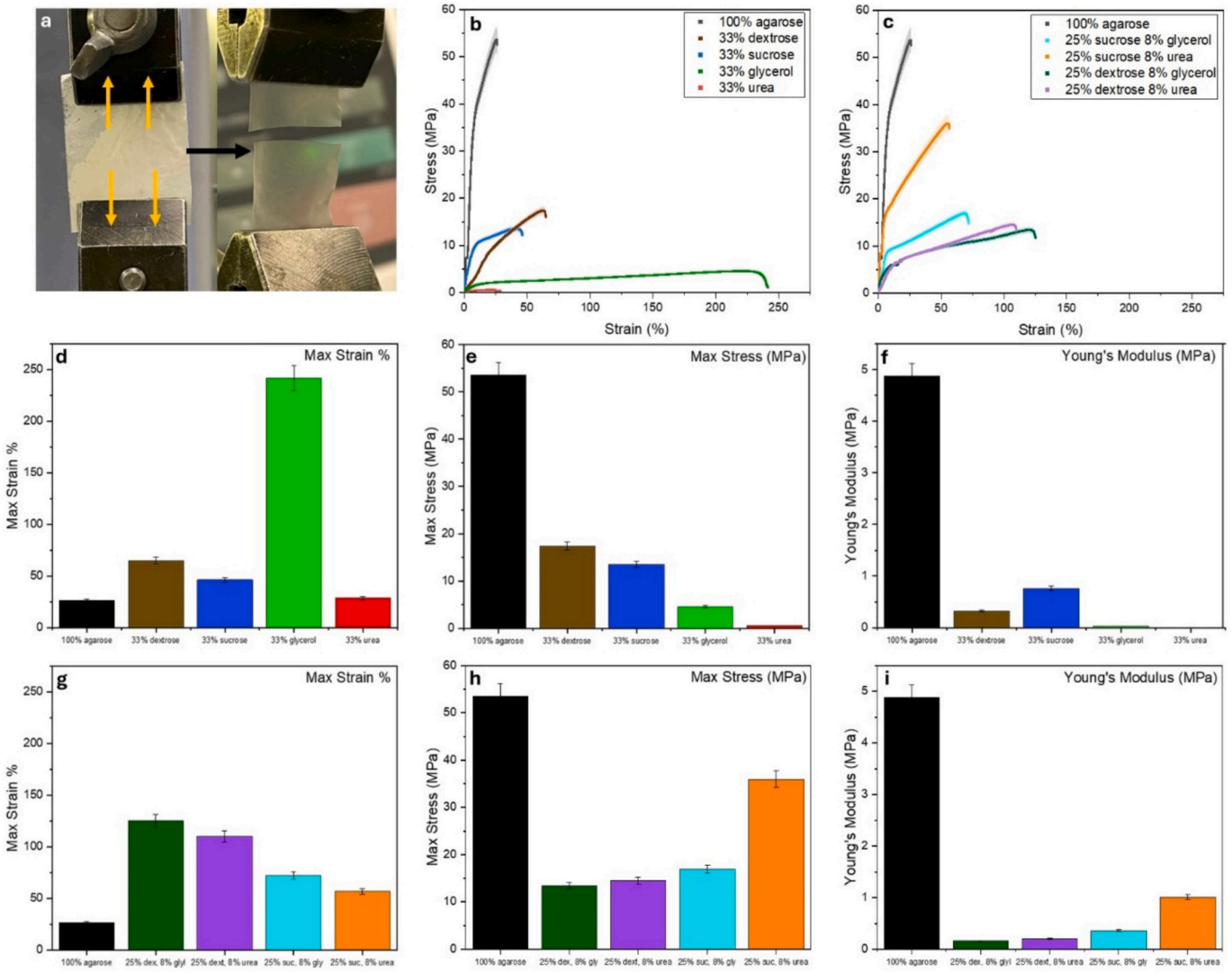

Fig. 4. Analyses of the mechanical properties of agarose. a) Image of the before and after of an agarose film undergoing tensile testing. Tensile strength profiles of agarose films with b) a single plasticizer and c) agarose films with two plasticizers. Bar graphs of max strain, max stress, and Young's modulus of d-f) agarose films with a single plasticizer, and g-i) agarose films with two plasticizers, respectively. All values have an error margin within ±5 %.

when under an electric field.

3.5. Mechanical properties

The mechanical properties of the films were examined via tensile testing until breakage, as depicted in Fig. 4a. Looking at the tensile data in Fig. 4, the 100 % agarose sample reportedly had the highest stress resistance, maxing out at 53.60 MPa, but also the lowest strain elongation, reaching only 26.25 %. When a plasticizer was added to agarose, the resulting film had improved strain elongation but reduced stress resistance compared to pure agarose, as seen from the max strain and max stress data reported. This effect of plasticizers on materials' strain and stress properties has been reported by Galdeano et al. in similar studies [25]. However, as illustrated in Fig. 4b and c, each plasticizer had a unique influence on agarose's mechanical properties.

In the tensile graph seen in Fig. 4b of single plasticized agarose films, the plasticizers from weakest stress resistance to strongest are urea, glycerol, sucrose, and dextrose. Urea likely exhibited weaker tensile properties due to its weaker hydrogen bonding amine groups. This was similarly seen in the thermal decomposition analysis and electrical actuation. Weak hydrogen bonds cause a film to break under less stress but also affect strain properties. Film elongation, or strain, improves with plasticization because plasticizers reduce the number of polymer-polymer intermolecular bonds and substitute those bonds with plasticizer-polymer hydrogen bonds. This disruption and reconstruction of polymer chain interactions results in reduced rigidity and promotes film flexibility by allowing greater chain mobility [15]. Given urea's weaker hydrogen bonding amine groups, urea is unable to considerably disrupt and substitute agarose's hydrogen bond network and thus does not promote increased chain mobility. Comparatively, films with hydroxyl-containing plasticizers, such as sucrose, performed better than urea, likely in part due to the stronger hydrogen bonds hydroxyl groups form. Maurer et al. [26] similarly saw an increase in film elasticity when plasticized with sucrose [26]. However, according to Fig. 4b, films plasticized with sucrose still exhibited weaker mechanical performance than films plasticized with dextrose. Both sucrose and dextrose contain hydroxyl groups so the difference in mechanical performance must be due to differences in the plasticizing molecule's size and its total hydroxyl content. In fact, sucrose is the only plasticizer used whose monomer has a larger molecular weight than that of an agarose monomer. Sucrose is thus more likely to experience steric hindrance, which would, in turn, lead to reduced plasticizing and hydrogen bonding. Dextrose, on the other hand, has a smaller molecular weight and thus does not experience as much steric hindrance.

Lastly, for the single plasticized films, examining the effects of glycerol, we see the largest gain among all sample types in strain elongation but also the second lowest stress resistance. Barrangou et al. [27] noted that adding glycerol improved agarose's strain modulus, while Martin and Avérous [28] found glycerol to be the least effective plasticizer out of a list of six commercial plasticizers, agreeing with the below results [27,28]. Glycerol seems to promote high chain mobility and disrupt agarose's hydrogen bond network but, at the same time, does not form strong bonds between agarose. Overall, smaller molecular-sized plasticizers can improve agarose's strain modulus but also greatly reduce the stress modulus. Then, as the plasticizing molecule increases in size, the stress modulus increases while the strain modulus decreases until a certain threshold in size is reached, where steric hindrance likely becomes an issue, as seen in sucrose's effect on mechanical properties.

Looking at Fig. 4c now of double plasticized agarose films, it can be seen that the 25 % dextrose and 8 % glycerol film exhibited stress and strain capabilities that fell somewhere between the capabilities of its individual plasticizing components, 33 % dextrose and 33 % glycerol. However, the 25 % dextrose and 8 % glycerol film was overall closer in performance to the 33 % dextrose film, likely due to the higher concentration of dextrose in the film. The 25 % dextrose and 8 % urea film yielded similar results, though interestingly, it saw a slightly higher max stress, but reduced strain compared to 25 % dextrose with 8 % glycerol. Thus, double-plasticized films where dextrose is the main component seem to result in films whose mechanical properties are roughly averaged between the mechanical properties of the respective single-plasticized films. Adding urea as the minority plasticizer in a dextrose-dominant double-plasticized system yields films with a slightly diminished max stress over the 33 % dextrose films but an improved max strain. Adding glycerol instead of urea as the minority plasticizer leads to an even greater gain in max strain but at the cost of further stress resistance. While the expected trend would be for the minority glycerol film to have greater stress resistance over the minority urea film, given the 33 % glycerol film's greater max stress and glycerol's stronger hydrogen bonding hydroxyl groups, it has been reported that glycerol in smaller quantities (15 % *w*/w or lower) can have an anti-plasticizing behavior due to its strong hygroscopicity and hydrophilic nature [22]. This anti-plasticizing behavior occurs due to the plasticizing molecules attaching to the main polymer and restricting the polymer-polymer interactions that would be necessary for a material to absorb any applied mechanical energy [29]. Thus, adding small quantities of a plasticizer such as glycerol can be beneficial for improving a film's maximum strain but detrimental to other properties. However, this was not always the case if sucrose was included, as the film plasticized with 25 % sucrose and 8 % glycerol improved both max strain and stress. Additionally, when glycerol was replaced for urea in the 25 % sucrose and 8 % urea film, it resulted in a massive gain in max strain while maintaining a larger max stress than both 33 % sucrose and 33 % urea, though not relatively as high stain capabilities as 25 % sucrose, 8 % glycerol. Thus, in the case of a sucrose-dominant double plasticized system, the resulting films will have strain and stress capabilities that surpass either of the two plasticizers used compared to their single plasticized film counterparts. Films plasticized with minority urea will result in significant gains in stress, while minority glycerol films will result in more significant gains in strain. These trends are likely due to the fact that sucrose, a relatively large molecule, now has an additional smaller molecule that can penetrate into the agarose matrix and bolster the system in places that sucrose cannot.

4. Conclusion

In the design of agarose films, the selection of plasticizers plays a critical role in determining the film's morphological, mechanical, and thermal properties. The findings show that the four plasticizers tested influenced these properties to varying degrees due to differences in intermolecular interactions, specifically related to variations in hydrogen bonding groups. FTIR and X-ray Scattering results show that incorporating different plasticizers can alter agarose's wider gel structure. Tensile testing and electric actuation revealed that plasticizers with a high hydroxyl content and small molecular size provided the greatest enhancement in flexibility and stretchability. Among single plasticizers, dextrose offered the highest stress resistance, while glycerol significantly improved strain elongation. When two plasticizers were used together, dextrose exhibited an averaging effect, while sucrose saw enhancements in both strain and stress. Overall, the optimal strategy appears to involve combining a larger plasticizer with high hydroxyl content with a smaller molecule that forms weaker hydrogen bonds for improved mechanical performance. However, this work does not explore the exact mechanism behind the agarose-plasticizer interactions or quantify the changes to agarose's hydrogen bond network. This work was also limited in the range of plasticizer variety and did not explore how altering the ratio of agarose to plasticizers affected the system. In the future, we hope to employ computational modeling to gain a deeper understanding at the molecular level of how agarose and the different plasticizers interact to produce the various results discussed in this paper. This model will also provide an opportunity to explore the unresolved question of agarose's ambiguous structural transitions during gelation. Furthermore, we aim to utilize agarose as the active

component in a bilayer system to develop self-morphing structures.

CRediT authorship contribution statement

Creston A. Singer: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Hajara Abdul-Karim:** Methodology, Conceptualization. **Kyle Printon:** Investigation. **Nagireddy Poluri:** Writing – review & editing, Investigation. **Teng Teng:** Writing – review & editing, Visualization, Investigation. **Mostafa Akbari:** Writing – review & editing, Visualization, Investigation. **Behzad Modanloo:** Writing – review & editing, Visualization. **Laia Mogas-Soldevila:** Writing – review & editing, Methodology, Conceptualization. **Masoud Akbarzadeh:** Supervision, Project administration, Methodology, Conceptualization. **Xiao Hu:** Supervision, Resources, Project administration, Methodology, Conceptualization. **Sean M. O'Malley:** Writing – review & editing, Resources, Methodology. **Hong Fang:** Writing – review & editing, Conceptualization. **David Salas-de la Cruz:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare no conflict of interest.

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Data availability

Data will be made available on request.

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