



Rheology-guided experimental design of dynamically crosslinked keratin/ ι -carrageenan hydrogels as potential drug delivery systems

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ABSTRACT

This study establishes a deterministic framework for designing 3D-printable, dynamically crosslinked hydrogel inks for controlled drug delivery via Direct Ink Writing (DIW). By blending cysteine-S-sulfonated keratin with ι -carrageenan through Ca^{2+} ionotropic gelation, shear-responsive networks were created. A Design of Experiments (DoE) approach generated a predictive rheology-to-printability map correlating composition with storage modulus (G'), flow stress (τ_f), and printability index (P_r).

Advanced Large Amplitude Oscillatory Shear (LAOS) profiling revealed a transition from ductile, entanglement-driven networks in keratin-rich blends to brittle yielding via cooperative ionic junction rupture in crosslinked systems. Sequence of Physical Processes (SPP) analysis further identified an intracycle recovery loss factor ($\tan\delta_{\text{rec}}$) that dictates post-deposition shape retention. Structural analyses verified that Ca^{2+} drives network compaction and crystalline β -sheet reorganization.

Crucially, dexamethasone (Dex) loading acts as a dual-functional modulator via supramolecular hydrogen bonding: behaving as an interfacial sliding agent that reduces interchain friction within densely crosslinked matrices, while acting as a secondary physical crosslinker in weakly crosslinked networks, which amplifies elastic energy storage and induces viscous Lissajous-Bowditch loop expansions. Finally, mathematical modeling confirmed that ionic crosslinking shifts drug transport from anomalous diffusion to a relaxation-controlled Super Case II regime governed by the relaxation rate (k_r).

1. Introduction

The introduction of 3D printing into tissue engineering has marked a true revolution in the creation of customized and advanced drug delivery systems, offering innovative and highly precise solutions for controlled and targeted therapeutic release. Among the most promising materials for these applications are hydrogels made from innovative blends of polysaccharides and proteins. These multicomponent networks emerge as frontrunners for next-generation drug delivery due to their exceptional biocompatibility, high water-retention capacity, and tunable structural integrity, all of which are critical to safeguard and modulate the kinetics of therapeutic payloads (Iranmanesh et al., 2021; H. Li et al., 2024; Nashchekina, et al., 2024; Roy Chowdhury et al., 2025). While various formulations considered in this field involve traditional combinations like of sodium alginate and gelatin (Huang

et al., 2024; Roy Chowdhury et al., 2025), chitosan and collagen (Suo et al., 2021), or pectin and albumin (Agarwal et al., 2021), the synergistic potential of keratin and ι -carrageenan remains untapped in the design of next-generation bio-inks.

Keratin is the most abundant protein in nature featuring a unique molecular architecture rich in reactive chemical sites, capable of engaging in precise macromolecular and drug-matrix interactions (Rajabi et al., 2020). High molecular weight α -Keratin from mammalian sources possess a packed secondary structure stabilized by a dense network of inter- and intramolecular disulfide bonds, rendering it inherently water-insoluble and challenging for hydrogel processing (Ranjit et al., 2022). To overcome this limitation, keratin can be functionalized via a controlled sulphitolysis reaction using sodium sulphite salts, yielding water-soluble cysteine-S-sulfonated keratin. This modified form unlocks new possibilities for formulating protein-based

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aqueous inks (Maurizii et al., 2024). Complementary, carrageenans are a group of polysaccharides extracted from different species of red algae, known for their physicochemical and stabilizing attributes (Pourshahrestani et al., 2020). Depending on the degree of sulfation, carrageenan is classified into the variants κ (kappa), ι (iota) and λ (lambda). In particular, the ι -Carrageenan, consists of repeating galactose units with two sulfate groups per disaccharide and is widely used in food and biomedical applications due to its gelling, stabilizing and hydrating properties (Wang et al., 2021).

From a processing perspective, the presence of strongly interacting functional groups along both polymer chains can be exploited to construct dynamically crosslinked hydrogels (DCHs) through divalent ionotropic gelation with calcium ions (Ca^{2+}). DCHs offer several advantages in Direct Ink Writing (DIW) 3D printing, as the transient nature of ionic junctions allows them to dissociate and reform under external stimuli. This reversibility allows the material to flow smoothly during extrusion while maintaining its structural integrity after deposition, resulting in more precise and stable prints (Eckman et al., 2025; Morgan et al., 2022). Nevertheless, engineering these yield-stress inks requires an extraordinarily precise optimization of their rheological landscapes. In drug delivery systems, where strict control over the final geometry directly dictates the dosage and release surface area, establishing predictive relationships between fundamental ink rheology and macroscopic printability is crucial (García-Tuñón et al., 2023; Rau et al., 2023).

Following the roadmap introduced by García-Tuñón et al. (García-Tuñón et al., 2023), which relies on mapping a *printability window* based on the linear viscoelastic storage modulus (G') and the large-deformation flow stress (σ_f), it is hypothesized that a Design of Experiments (DoE) approach can build a deterministic, predictive map. Rather than relying on simple trial-and-error, the DoE model can correlate initial ink composition directly to these fundamental rheological markers (G' and σ_f), which are subsequently linked to the printability index (P_r) to accurately forecast structural fidelity.

Furthermore, a comprehensive optimization requires moving beyond cycle-averaged parameters to capture the complex, transient physical conditions experienced by the inks during extrusion. In this regard, steady-shear flow modeling (such as the Herschel–Bulkley framework) combined with Large Amplitude Oscillatory Shear (LAOS) coupled to the Sequence of Physical Processes (SPP) analysis can provide an unmatched, processing-relevant representation of the ink state. This advanced characterization allows to evaluate the delicate boundary between heterogeneous yielding/flow discontinuities and optimal viscoplastic flow, identifying specific intracycle parameters, such as the recovery loss factor ($\tan\delta_{rec}$), that govern post-deposition shape retention.

In this work, the established predictive framework was used to screen and select optimal ink formulations, which were subsequently loaded with dexamethasone (Dex) as a model hydrophobic drug. To unravel the underlying molecular mechanisms driving both the macroscopic printability and the therapeutic performance, the hydrogels were systematically investigated using a combination of scanning electron microscopy (SEM), macro-rheological modeling, and dual Fourier-transform infrared spectroscopy (FTIR) / X-ray diffraction (XRD) analyses. Specifically, individual binary systems (polymer/cation and polymer/drug) were analyzed to circumvent band overlapping, elucidating how Ca^{2+} coordination induces local network compaction and crystalline β -sheet structural reorganization, and how Dex modulates the network through neutral hydrogen bonding pathways, acting either as a physical interlocking agent or an interfacial plasticizer. Finally, the processed hydrogels were subjected to dissolution testing, and their elution profiles were mathematically fitted via Korsmeyer-Peppas and Peppas-Sahlin models to demonstrate how the ionic crosslinking density and macromolecular relaxation kinetics (k_2) govern the transition from anomalous diffusion to a purely relaxation-controlled Super Case II

transport regime.

Altogether, this study pioneers the synergistic engineering of cysteine-S-sulfonated keratin and ι -carrageenan blends for DIW 3D printing, establishing a scalable, deterministic approach that translates fundamental rheological, morphological, and spectroscopic insights into optimized, highly functionalized drug delivery systems.

2. Materials and methods

2.1. Materials

The ι -carrageenan (Viscarin PC 209, molecular weight between 300 and 1000 kDa, ester sulfate content between 28% and 35% by weight, hereinafter referred to as ι -Car) was purchased by Biochim S.r.l (Milano, Italia); while the cysteine-S-sulphonated keratin at high molecular weight (HMW-Ker, molecular weight between 45 and 65 kDa, ~ 4 mol % of cysteine-S-sulphonated amino acid, hereinafter referred to as Ker) was purchased by Kerline S.r.l. (Bologna, Italia). Dexamethasone (water solubility ~ 0.089 mg/mL at room temperature and hereinafter referred to as Dex) was purchased from TCI (Tokyo, Japan). The supplier of all the other reagents was Sigma-Aldrich (Milano, Italia). All solvents used were of analytical grade.

2.2. Design of experiments for hydrogels formulation

A Box Behnken DoE (BBD) was carried out considering the Ker/ ι -Car blending ratio, the amount of crosslinker (% vs total polymer concentration) and the temperature as input factors, while total polymer concentration was maintained constant at 8% w/V. The low (−1), medium (0), and high (1) levels selected for each input factor are listed in **Table S1**. The fixed total polymer concentration along with the high and low factor levels, were selected based on preliminary trials and in order to avoid both incomplete gelation (which resulted in a predominantly liquid-like behaviour) or hydrogels too solid-like and unable to flow. The elastic modulus in the linear viscoelastic region ($G'_{@LVR}$) and the flow stress (σ_f) intended as the crossover between G' and G'' were considered as output factors. This dynamic oscillatory flow stress serves as a key indicator of the hydrogels' solid-to-liquid transition. The BBD was implemented with three centre points, resulting in 15 experimental conditions, each performed in triplicate (**Table 1**). The response surfaces were elaborated using the Design Expert 13 Software (StatEase®). The standard analysis of variance (ANOVA) was performed to select the significant factors in a multi-factor model, considering a statistical significance level of 5%. The model validation correlating the input factors with the results (output data) was based on *p-value* and regression coefficients R^2 , adjusted R^2 ($\text{adj-}R^2$), and predicted R^2 ($\text{pred-}R^2$). The regression model was considered significant if *p-value* < 0.05, and the best-fitting model for each response was selected based on the highest $\text{adj-}R^2$ and a difference between $\text{pred-}R^2$ and $\text{adj-}R^2$ of less than 0.2. For each output, the coded equation (based on coded factor levels) was used to identify the factors and the interactions that mainly influence the results. Instead, the actual equation (based on actual factor levels) was employed to generate response surface plots illustrating the relationships between process factors and responses. In addition to the 15 experimental conditions of the DoE, two other conditions were considered (as shown in **Table 1**) to validate the developed response surfaces.

2.3. Hydrogel preparation

For the preparation of the inks, stock solutions of Ker (10% w/V) and ι -Car (10% w/V) were prepared by dissolving a proper amount of the biopolymers in distilled water under magnetic stirring (400 rpm). Keratin was dissolved at room temperature, whereas carrageenan was dissolved at 80 °C. After both biopolymers were fully solubilized, a volume of keratin solution corresponding to the desired Ker/ ι -Car blending ratio

Table 1

Experimental runs performed according to the Box-Behnken Design (BBD), runs used to validate the response surfaces, and runs for testing the drug loaded hydrogels. The column Std indicates the standard order of the runs.

Runs according to the Box-Behnken design				
Run	Std	Ker/i-Car	Crosslinker (%)	Temperature °C
1	3	10/90	6	35
2	13	50/50	3	35
3	11	50/50	0	45
4	8	90/10	3	45
5	4	90/10	6	35
6	9	50/50	0	25
7	2	90/10	0	35
8	12	50/50	6	45
9	1	10/90	0	35
10	14	50/50	3	35
11	15	50/50	3	35
12	7	10/90	3	45
13	6	90/10	3	25
14	10	50/50	6	25
15	5	10/90	3	25

Runs used to validate the response surfaces				
Run	Std	Ker/i-Car	Crosslinker (%)	Temperature °C
16	F1	60/40	3	25
17	F2	50/50	6	35

Runs used to test the drug loaded inks				
Std	Drug (% w/w)	Ker/i-Car	Crosslinker (%)	Temperature °C
Std2D	0.1	90/10	0	35
Std12D	0.1	50/50	6	45
Std11D	0.1	50/50	0	45
Std8D	0.1	90/10	3	45

was added to the carrageenan solution, and the mixture was maintained under magnetic stirring (600 rpm) at 80 °C. For hydrogels containing the crosslinker, 2 mL of a 0.1 M or 0.2 M CaCl₂ solution was added which corresponds to a CaCl₂ content of 3 or 6 wt% relative to the total mass of polymers in the system, respectively. The crosslinker solution was added dropwise under continuous stirring (600 rpm) to ensure homogeneous crosslinking.

The inks loaded with Dex were prepared by using selected runs of the BBD (Table 1) such as Std11, Std8, Std2, and Std12. Practically, Dex was incorporated into the formulation using an aqueous drug solution of 0.08 mg/mL to achieve a final drug loading of 0.1% w/w relative to the total polymer mass., corresponding to the concentration commonly used in topical preparations for the treatment of wounds. The aqueous Dex solutions were maintained under stirring (400 rpm) at room temperature for 2 h before being used for inks preparation. Once printed, the samples were frozen at −80 °C and subsequently lyophilized using the Lio 5P lyophilizer (Cinquepascal S.r.l, Italy). These loaded hydrogels were named as reported in Table 1.

2.4. Rheological analysis

LAOS rheology was performed with a strain amplitude test in a transient data acquisition mode, using a MCR 302e (Anton Paar, Austria) rheometer. The measurements were carried out with a plate-plate geometry (PP, 25 mm Ø) using a 1 mm gap and at a controlled temperature specific for each ink (according to the DoE Table 1). The dynamic strain amplitude was varied in the 0.01% -1010% range, maintaining a frequency of 1 Hz. These measurements were carried out

in triplicate for each hydrogel, and the mean response was used. The first harmonic storage (G') and loss (G'') moduli were derived from the raw waveform. Other considered rheological parameters were the storage modulus ($G'_{@LVR}$) and the strain (γ_{LVR}) within the linear viscosity region, the flow stress (σ_f) as well as the critical strain (γ_c) at the crossover ($G' = G''$).

Moreover, the contributions of the higher third harmonic were analyzed in the raw waveforms at different strains to construct the Lissajous-Bowditch plots and to determine the strain-stiffening index (S) and the shear-thickening index (T) (Insua et al., 2025). The Rheo-Compass™ (Anton Paar, Austria) and Origin Pro 2022 (OriginLab Corporation, Northampton, MA, USA) were used for the analysis. The transient waveforms were further analyzed within the Sequence of Physical Processes (SPP) framework. Specifically, the time-resolved storage and loss moduli, (G'_t , G''_t) were calculated and represented as Cole–Cole trajectories in order to obtain a more phenomenological description of the yielding process and to visualize the evolution of elastic- and viscous-dominated states during the oscillation cycle. This SPP analysis was carried out using the software kindly provided by Prof. S. A. Rogers (University of Illinois). Steady-shear flow curves were recorded for the selected formulations, using the same plate–plate geometry and gap. The shear rate varied from 0.01 s^{−1} to 100 s^{−1} according to a continuous logarithmic ramp protocol, and the resulting shear stress and apparent viscosity were recorded.

2.5. Printing test

The hydrogels were 3D printed using a BioX printer (Cellink, Sweden) equipped with a pneumatic print head and loaded with a 3 mL syringe fitted with a 22G plastic nozzle (0.4 mm diameter). The printed scaffold designs were developed using Tinkercad® Computer-Aided Design (CAD) software (Autodesk Inc., USA). For the printability, a square design (23 mm length x 23 mm height x 2.8 mm thickness) was used with an infill of 10%, a print speed of 5 mm/s, and a printhead temperature between 25 and 45 °C, according to the BBD. To print the Dex-loaded hydrogels, the infill was changed at 50% and the printhead temperature was set to 35 or 45 °C according to the BBD. In all tests, the print bed temperature was maintained at 25 °C, and the pressure was adjusted according to the formulation (from 5 kPa to 550 kPa) in order to have an almost continuous ejection. The printing conditions used for each ink are summarized in Table S2. To define the geometric accuracy of the printed object, the Crosshatch method was then adopted to calculate the printability index Pr (Gillispie et al., 2023). Pr is a parameter used to evaluate the resolution of the printed object, especially in the context of bioprinting. It is used to understand how closely the actual shape of a printed object matches the shape predicted in the model in the x-y plane by measuring the circularity of the pores and their deviation from the ideal square shape. Pr is defined by the following equation (Eq. 1):

$$Pr = \frac{\pi}{4} \times \frac{1}{C} = \frac{L_p^2}{16A_p} \quad (1)$$

where C is the circularity of the pore, L_p is the perimeter of the pore, and A_p is the area of the pore (Jongprasitkul et al., 2022). The perimeter and area of the pores were determined by analysing images of the printed objects using ImageJ software. Because pore shapes can vary slightly within the printed structure, 8 pores were measured for each type of printed ink, and the average values were then calculated.

2.6. Characterizations

The scanning electron micrographs were obtained with a microscope SEM JEOL IT200LA (JEOL, Japan). Imaging was performed in low-vacuum mode under a specimen chamber pressure of 10 Pa, with an acceleration voltage of 20.0 kV and a standard probe current (Std-PC).

Magnifications ranged from 30× to 2000×, while the working distance was maintained at 10.0 mm to ensure optimal resolution and depth of field. The Fourier-transform infrared spectra (FTIR) were acquired using a PerkinElmer Spectrum Two spectrometer (MA, USA) equipped with an Attenuated Total Reflectance (ATR) accessory. Data were collected using a spectral range 650–4000 cm^{-1} , a resolution of 4 cm^{-1} and 64 scans. Following acquisition, the resulting spectra were normalized within the 800–4000 cm^{-1} range to ensure consistency for comparison. Powder x-ray diffraction (XRD) measurements were carried on a Miniflex benchtop diffractometer (Rigaku, Tokyo, Japan), using Cu-K α irradiation ($\lambda = 1.5418 \text{ \AA}$) with a D7teX Ultra 2 detector. All the measurements were conducted in the 2θ range of 3–50° with a step size of 0.01° and a scan speed of 5°/min.

Mathematical deconvolution of the structural profiles was performed to quantify individual domain fractions. For the FTIR Amide I band, the 1450–1800 cm^{-1} region was analyzed after baseline correction using the Peak Analyzer tool in OriginPro 2022 (OriginLab Corp., Northampton, MA, USA). The number and initial positions of the underlying secondary structure components were established by second-order derivative analysis. For the XRD profiles, the broad amorphous halo and overlapping sharp crystalline reflections were resolved across the 3°–50° 2θ range using Fityk software (version 1.3.1) after baseline correction.

In both methodologies, individual sub-peaks were resolved by fitting wide and narrow Voigt functions. Peak parameters (center position, FWHM, and height) were iteratively optimized via the Levenberg-Marquardt algorithm until statistical convergence ($R^2 > 0.99$) was achieved. The relative area percentage ($A_{\%}$) of each structural phase was calculated as $A_{\%} = (A_i / \sum A_{\text{total}}) \times 100$, where A_i is the individual integrated peak area.

2.7. Swelling test

The water absorption and swelling behaviour of the crosslinked matrices (6%Ca²⁺) were evaluated in PBS (pH 7.4) at controlled temperature (37 °C) using a gravimetric approach. Dry samples were accurately weighed and placed on filter paper previously soaked in the medium. The filter paper was positioned on top of a sponge completely saturated with the medium and placed in a Petri dish (Ø 22 cm) filled with the medium until soaking 2/3 of the sponge to maintain saturation conditions of the sponge with PBS for the whole duration of the swelling test. At predetermined time intervals (t), the filter paper with the swollen sample was weighed and then replaced on top of the sponge. The procedure was repeated until equilibrium swelling was obtained. All measurements were performed in triplicate ($n = 3$).

To elucidate the swelling mechanism and water transport kinetics, the experimental data were fitted to Schott's second-order kinetic model and the Korsmeyer–Peppas model.

2.8. Drug release test

For the release test, about 80 mg of each lyophilized Dex hydrogel were inserted into cellulose dialysis membranes (molecular cut-off of 12–14 kDa) and immersed in 100 mL of PBS (pH 7.4), which acted as the release medium under strict sink conditions, defined as keeping the final drug concentration (C) below 10% of its saturation solubility (C_s). The test was conducted at 37 °C under stirring (200 rpm) and in triplicate. Drug release was evaluated at the following time points: 5, 10, 15, 30, 45, 60 min, 2, 4, and 24 h. To quantify the concentration of drug released over time, the collected samples (1 mL) were analyzed by UV-HPLC Agilent 1260 Infinity II (Agilent, USA) using an Agilent InfinityLab Poroshell HPH-C18 column, 3.0 × 100 mm, 2.7 μm (USA). The mobile phases used were 0.5% v/v trifluoroacetic acid (TFA) in water and acetonitrile in a 60:40 ratio. The flow rate was set to 1 mL/min, while the injection volume was 20 μL . Analyte detection was performed by a diode array detector at a wavelength of 242 nm. A calibration curve (R^2

= 0.9999) was previously constructed to quantify the released drug.

3. Results and discussion

3.1. The rheology-to-printability map

For a formulation to be considered printable, it must accurately reproduce the shape of the CAD-designed object and maintain its structure throughout and after the printing process. In the case of hydrogels, printability is evaluated based on established criteria in the literature. An ink is deemed printable with the DIW technology if the filament produced during extrusion is continuous, smooth, and homogeneous and the extrusion is steady and uninterrupted to ensure consistent printing quality without nozzle clogging. Moreover, once deposited on the print bed, the filament must retain its structure to support the weight of subsequent layers. Effective layering is crucial for the printed object to maintain its intended shape, without collapsing or deforming (Insua et al., 2025). According to the printability criteria outlined earlier, a non-printable hydrogel will display characteristics such as a rough surface and excessively hard texture. These properties make extrusion difficult, as they create high resistance to the hydrogel passing through the narrow nozzle, increasing the risk of clogging and interrupting the printing process. Additionally, non-continuous extrusion disrupts the uniform deposition of material, which compromises both the precision and final shape of the printed object, leading to deformations or incomplete prints. Hydrogels that are too liquid also struggle with printability, as they form drops instead of continuous filaments. This behaviour interferes with proper layering and prevents the formation of a stable, well-defined structure (Amorim et al., 2021). The printability index Pr was here used to compare the printability of the prepared inks. The evaluation was based on the following criteria: a) $Pr = 1$ indicates perfectly square pores, signifying excellent print quality; b) $Pr > 1$ represents deformed pores with sharp edges and narrowing, caused by the irregularity of the filament shape; and c) $Pr < 1$ indicates rounded pores, typically resulting from a more liquid filament consistency (Gillispie et al., 2023). Therefore, the closer the Pr value is to 1, the better the ink's ability to produce a printed object with high geometric accuracy.

In Fig. 1a, the Pr values obtained for the inks of the first 15 runs were here used to build the Ashby-type printability maps for the Ker/iCar hydrogels as proposed by Garcia-Tunon et al (García-Tuñón et al., 2023). This is a chart based on the coordinates $G'_{@LVR}$ and σ_f serving as a useful tool to guide formulation design for DIW 3D printing. The map establishes critical structural boundaries as the ratio $G'_{@LVR}/\sigma_f$ equal to 20 and the vertical lines of the σ_f limits (20 Pa < σ_f < 320 Pa) for printable formulations based on Ker/iCar hydrogels. Four different regions associated with different levels of printability can be highlighted in the map. The clogged/interrupted extrusion region (Gray) is characterized by excessively high rheological values ($G'_{@LVR} > 1.2 \times 10^4$ Pa and $\sigma_f > 320$ Pa) resulting in an extrusion pressure exceeding the operating limits of the printer; this causes discontinuous flow or nozzle blockage (e.g., Std 3, which demands an exceptionally high extrusion pressure of 550 kPa), making the formulations unprintable. In the over-gelled/poor printability region (Purple), a decrease in yield stress below 320 Pa enables extrusion even at high elasticity ($G'_{@LVR} > 10^4$ Pa, e.g., Std 7, requiring 63–70 kPa). However, within the range of 1.4×10^3 Pa < $G'_{@LVR}$ < 2.8×10^4 Pa where the ratio $G'_{@LVR}/\sigma_f < 20$, highly elastic behaviour dominates. This mismatch yields a highly distorted, rough filament with poor surface finish, leading to high printability values ($Pr > 1.1$, e.g., Std 1, 5 which require elevated pressures of 72–74 kPa and 140–145 kPa, respectively, to force the overly stiff network through the nozzle). The optimal printability region (Green) is bounded by 2×10^2 Pa < $G'_{@LVR}$ < 1.4×10^3 Pa and 20 Pa < σ_f < 320 Pa, while strictly maintaining the ratio

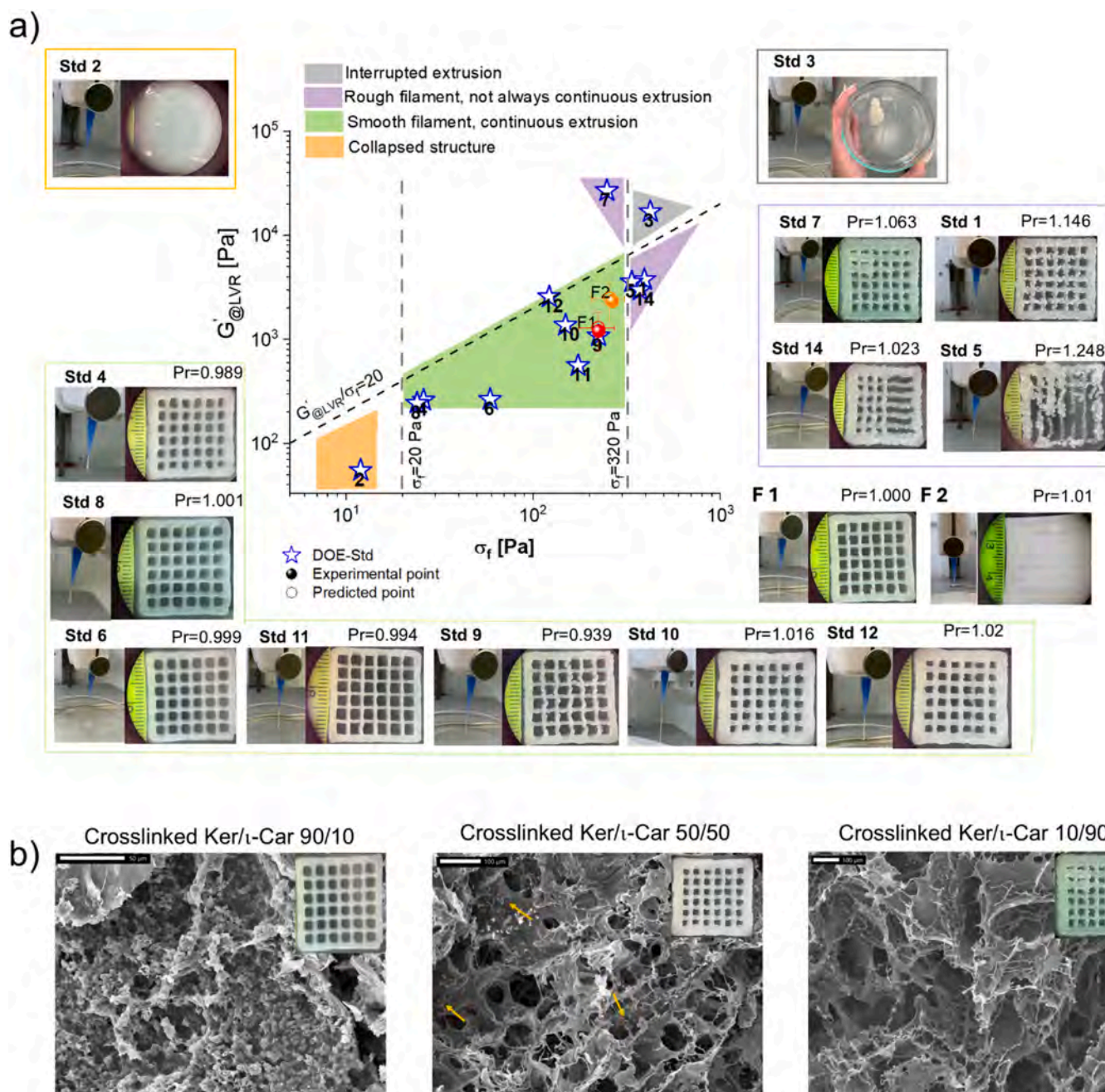


Fig. 1. a) Printability map based on an Ashby-type chart correlating the storage modulus in the linear viscoelastic region ($G'_{@LVR}$) and the flow yield stress (σ_f) with the printability ratio Pr (Eq. 1). Critical boundaries are marked by the dashed line $G'_{@LVR}/\sigma_f = 20$ and specific yield stress limits ($20 \text{ Pa} < \sigma_f < 320 \text{ Pa}$), which delineate the transition between successful extrusion and printing (green region) and bad extrusion (violet region), interrupted extrusion (gray region) or collapsed structure (orange region).

b) SEM micrographs of the crosslinked Ker/ ι -Car formulations at different blending ratio.

$G'_{@LVR}/\sigma_f \leq 20$. Formulations falling into this zone (e.g., Std 4, 6, 8, 9, 10, 11, 12, and validation runs F1, F2) exhibit highly manageable and steady operating pressures ranging from a minimal 4 kPa (Std 4, 8) up to a moderate 60 kPa (Std 9). These balanced viscoelastic profiles permit smooth, continuous filament extrusion, highly defined, multi-layered square grids, and highly reproducible printability parameters ($Pr \approx 1$). Finally, in the under-gelled/collapsed structure region (Orange), the ink possesses insufficient mechanical properties ($G'_{@LVR} < 10^2 \text{ Pa}$, $\sigma_f < 20 \text{ Pa}$, and $G'_{@LVR}/\sigma_f < 20$). A smooth filament can still be extruded, but it lacks the structural integrity to support consecutive layers (e.g., Std 2, at just 2 kPa). Consequently, the extruded filaments

rapidly flow and merge post-deposition, causing the printed structure to collapse and making the quantitative evaluation of Pr unfeasible.

To complement the oscillatory flow curves used to construct the printability map, the steady-shear flow curves of selected uncrosslinked and Ca^{2+} -crosslinked formulations are reported in Fig. S1. The formulations showing good printability exhibited a clear viscoplastic response, well described by the Herschel–Bulkley model, with a yield stress, $\sigma_{y,HB}$, followed by pronounced shear-thinning behaviour. The fitted flow-behaviour index, n , ranged from 0.20 to 0.50, confirming the pseudo-plastic character of these inks. This rheological response is favorable for DIW, since the yield stress limits uncontrolled flow and filament

spreading at rest, whereas shear thinning reduces the apparent viscosity under the high-shear conditions developed within the nozzle, thereby facilitating continuous extrusion. However, some formulations located in the high- G'_{LVR} , high- σ_f region of the map exhibited flow discontinuities around the yielding transition. These features are consistent with heterogeneous yielding and flow localization, possibly associated with shear banding, indicating that excessively strong and elastic networks may fail to develop a stable and homogeneous flow through the nozzle.

The internal microstructures of the lyophilized ker/i-Car hydrogel blends were evaluated using SEM to investigate the influence of the polymer blending ratio on network morphology (Fig. 1b). The keratin-rich hydrogel (ker/i-Car 90/10) displays a highly irregular and dense surface dominated by globular aggregation behaviour (Li et al., 2022). In the balanced blend (ker/i-Car 50/50), a transitional, hybrid morphology is observed. The structure opens into an interconnected network, within which distinctive globular keratin aggregates remain entrapped and dispersed along the main polymeric pathways (highlighted by the orange arrows). This configuration represents an intermediate stage where the linear polysaccharide chains begin to template and organize the self-assembling protein domains. Conversely, the i-Car-rich hydrogel (Ker/i-Car 10/90) exhibits a morphology characterized by thin, smooth, sheet-like walls and highly defined, interconnected macropores.

3.2. Response surfaces for $G'_{@LVR}$ and σ_f

To better navigate the developed rheology-to-printability map, response surfaces correlating the $G'_{@LVR}$ and the σ_f with the input factors of Table S1 were determined. The mathematical models (Table 2) that best correlate the factors with the outcomes were selected based on the p -values from the ANOVA test and the fit statistics as described in the experimental section (Table S3). Specifically, both the coded and actual equations for each result are provided in Table 2. The coded equations help identify the factors and their interactions that have the greatest impact on the results. The actual equations, which underlie the response surfaces (Fig. 2), can be used for predictive purposes (Matos et al., 2025). As observed, the $\log_{10}G'_{@LVR}$ is significantly influenced (p -values < 0.05) by the Ker/i-Car blending ratio in a linear manner, by the blending ratio/temperature interaction and by the crosslinker in a quadratic manner. In general, the $G'_{@LVR}$ increases as the Ker/i-Car blending ratio decreases and the crosslinker amount increases. These effects are significant only at blending ratios below 30/70 and they are more pronounced at higher temperatures (Fig. 2a).

The $\sqrt{\sigma_{flow}}$ is significantly influenced in a linear quadratic manner by all the factors, even though interactions among the factors do not appear to have an effect. In general, the σ_f decreases with increasing the Ker/i-Car blending ratio and for blending ratio it reaches maximum values when the crosslinker is about 3%. This trend is more pronounced at a temperature of 35 °C (Fig. 2b).

Table 2
Coded and actual equations of the refined mathematical models.

Response	Refined Mathematical Model
G'_{LVR}	<u>Coded Equation:</u>
	$\log G'_{LVR} = 3.35 - 0.8516A + 0.2611B + 0.1033C - 0.2279AC + 0.1396BC - 0.1303A^2 - 0.20120B^2$
	<u>Actual Equation:</u>
σ_{flow}	$\log G'_{LVR} = 2.82923 - 0.006801A + 0.088156B + 0.024856C - 0.000570AC + 0.004654BC - 0.000081A^2 - 0.022350B^2$
	<u>Coded Equation:</u>
	$\sqrt{\sigma_{flow}} = 19.09 - 6.67A - 0.3160B - 1.04C - 4.02A^2 - 2.83B^2 - 3.41C^2$
	<u>Actual Equation:</u>
	$\sqrt{\sigma_{flow}} = -19.48 - 0.085A + 1.78B + 2.28C - 0.0025A^2 - 0.3148B^2 - 0.03407C^2$

To validate the developed response surfaces, they were employed predictively to identify the conditions necessary to obtain formulations that occupy the desired regions on the printability map. To achieve this, the $G'_{@LVR}$ and σ_{flow} for the runs 16 and 17 (listed in Table 1) were calculated using the actual equations provided in Table 2. The predicted values were then compared to the experimentally determined ones.

As can be seen in Fig. 1, for both formulations, the predicted points (empty circles) are close to the experimental ones (full circles), demonstrating the goodness of the developed response surfaces. Moreover, both formulations fell in the green region and, as expected, they showed a good printability characterized by continuous extrusion, smooth filament and good layering, thereby demonstrating a good functionality of the $G'_{@LVR}$ vs σ_f printability map.

3.3. Effect of the blending ratio and the crosslinker on the rheological behaviour of the hydrogels

i-Carrageenan, along with the keratin, features negatively charged functional groups (e.g. sulfate groups and/or carboxylate groups) that can interact with positively charged ions, like calcium, promoting ionic crosslinking. This crosslinking can occur either *within* the polysaccharide and keratin chains and/or *between* the keratin and i-Carrageenan chains (Fig. 3a). However, this is a transient crosslinking that can be broken under deformation, such as that occurring during extrusion in the DIW printing process (Kopnar et al., 2025).

To gain deeper insight into the behaviour of the transient ionic crosslinking under applied strain, the LAOS data were analyzed as described in detail below. Specifically, for the sake of brevity, the study focused on the impact of the blending ratio and the amount of crosslinking agent on both the linear and nonlinear responses of the Ker/i-Car hydrogels.

3.3.1. Response to large strain

In Fig. 4 a-c, the first harmonic derived dynamic moduli (G' and G'') of both uncrosslinked and crosslinked hydrogels with CaCl_2 6%, at different blending ratios and all at a temperature of 35 °C, are reported as a function of the applied strain amplitude γ_0 . The yielding transition is delimited by the γ_{LVR} and γ_c ; while the flow transition occurs at $\gamma > \gamma_c$.

At low strain level (up to 1.5%) G' remains rather constant for all the uncrosslinked samples, signifying the gels are within the LVR region. In this region, G' was higher than G'' indicating that all the hydrogels exhibit a viscoelastic solid-like behaviour. The uncrosslinked hydrogels at higher keratin content show a lower G' , a more extended LVR region and a higher γ_c (Fig. 4 a-c). This data suggests that as keratin becomes the dominant phase, the blend shifts from behaving like a rigid polysaccharide gel to behave like a viscoelastic protein network. In this protein-rich network, the chains can unfold and rearrange when stress is applied, allowing the material to deform more before it fails. As a result, although the stiffness may decrease, the material becomes more capable of withstanding large deformations before flow.

It is also worth noting that the uncrosslinked Ker/i-Car 10/90 hydrogel shows an overshoot in G'' before the crossover between the moduli (Fig. 4a). This Type III behaviour is typical of a rigid, junction-zone-dominated gel: as strain increases, the carrageenan network undergoes collective junction-zone rupture, leading to a peak in energy dissipation (G'' overshoot) just before losing elasticity, followed by a rapid collapse of elasticity, characteristic of brittle and abrupt yielding (Moraes & Hilliou, 2024). In the uncrosslinked Ker/i-Car 50/50 hydrogel, the G'' overshoot falls at the crossover point (Fig. 4b). This indicates a balanced, co-continuous network where maximum viscous dissipation occurs at the point where elastic and viscous responses are equal. This means that the structural breakdown and loss of elasticity happen simultaneously. As a result, yielding is progressive, with gradual damage accumulation and network fluidization, characteristic of ductile failure (Moraes & Hilliou, 2024). Finally, the uncrosslinked Ker/i-Car 90/10

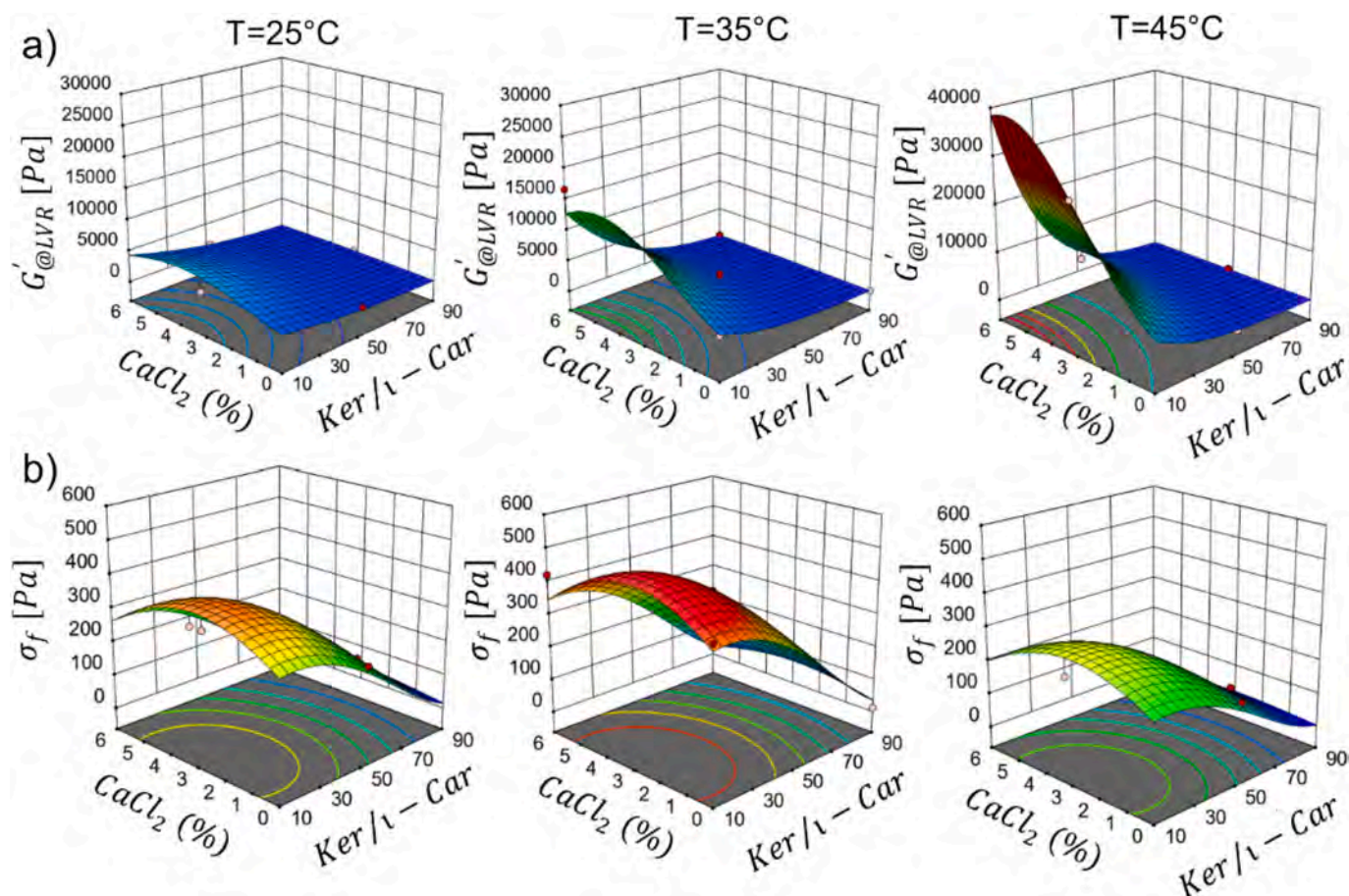


Fig. 2. Response surfaces illustrating the influence of calcium chloride concentration (%) and the keratin/ ι -carrageenan ratio (Ker/ ι - Car) on $G'_{@LVR}$ and σ_f .

hydrogel does not show a G' overshoot (Fig. 4c) indicating a Type I behaviour, according to which the system behaves as an entanglement-dominated viscoelastic network, where deformation occurs via chain alignment, unfolding, slippage, and gradual disentanglement. Therefore, the structure reorganizes continuously rather than failing through abrupt bond breakage (Hyun et al., 2011). As also emerged from the response surfaces analysis, the presence of the crosslinker increases the G' of all the hydrogels. A G' overshoot before its rapid decrease can be observed in the crosslinked hydrogels, indicating a strain-stiffening effect preceding structural failure. As strain increases, the network initially resists deformation, the polymer chains between crosslinks become stretched and aligned. This stretching temporarily increases the elastic response before breaking, reflecting a stretch-dominated, tightly crosslinked network that ultimately fails once its structural limit is exceeded (Hyun et al., 2011). Moreover, when the crosslinker is added, the LVR becomes smaller in all the blends, meaning that the structure departs from linear behaviour at lower strain. The deformation is transferred directly to crosslinks points rather than dissipated by chain mobility and the network becomes less able to rearrange under small deformations (Fig. 3b). Therefore, the crosslinking makes the systems more rigid and less strain-tolerant in the small-deformation regime.

In phase-dominated hydrogels (Ker/ ι -Car 10/90 and 90/10 blends) the crosslinker reinforces the already continuous network since more strain is required to reach the crossover point. This means that the dominant network becomes more resistant to macroscopic yielding. While the crosslinked carrageenan-rich hydrogel becomes more resistant to junction rupture, the crosslinked keratin-rich hydrogel gains stronger inter-chain connectivity. The result is that the failure in the crosslinked hydrogel is delayed to higher strain with respect to the related uncrosslinked one (Fig. 4a and c). In the balanced Ker/ ι -Car 50/

50 blend, crosslinking could introduce structural constraints and heterogeneity, since stress concentration points may form at phase interfaces. Therefore, instead of reinforcing a single continuous network, crosslinking may disrupts cooperative deformation, reduces network flexibility, and promotes earlier mechanical decoupling, causing the system to yield at lower strain (Fig. 4b).

3.3.2. Evaluation of non-linear rheological responses using elastic and viscous Lissajous-Bowditch (L-B) plots

When a large-amplitude sinusoidal strain is applied, the resulting stress response is no longer purely sinusoidal and instead contains significant higher-order harmonics. To better understand the molecular mechanisms involved in the yielding behaviour of the selected hydrogels, the 3rd harmonic analysis of the waveform deriving from the materials' response during the in dynamic deformation has been included.

In Fig. 4 d-i, the L-B plots from the elastic perspective (stress vs strain) are shown. The normalized curves, in which σ/σ_0 is reported in function of γ/γ_0 are useful to follow the evolution of the mechanical response across the strain amplitude. In the elastic L-B plots, a perfect elliptical curve indicates a linear viscoelastic behaviour of the material, while distorted ellipses indicate a nonlinear viscoelastic regime. At lower strain ($\gamma_0 < 1\%$), all the hydrogels show an intracycle curve as a narrow ellipse, which becomes wider with increasing the amplitude strain suggesting a progressive change from elastic to more viscous properties. When the blend hydrogels experienced an increase of the amplitude strain, the curves transitioned from an elliptical shape to a slightly distorted ellipse, indicating that non-linear effects emerge (Rogers et al., 2011). At higher strain the curve assumes a near parallelogram shape (curves flattening at the top), where a constant value of

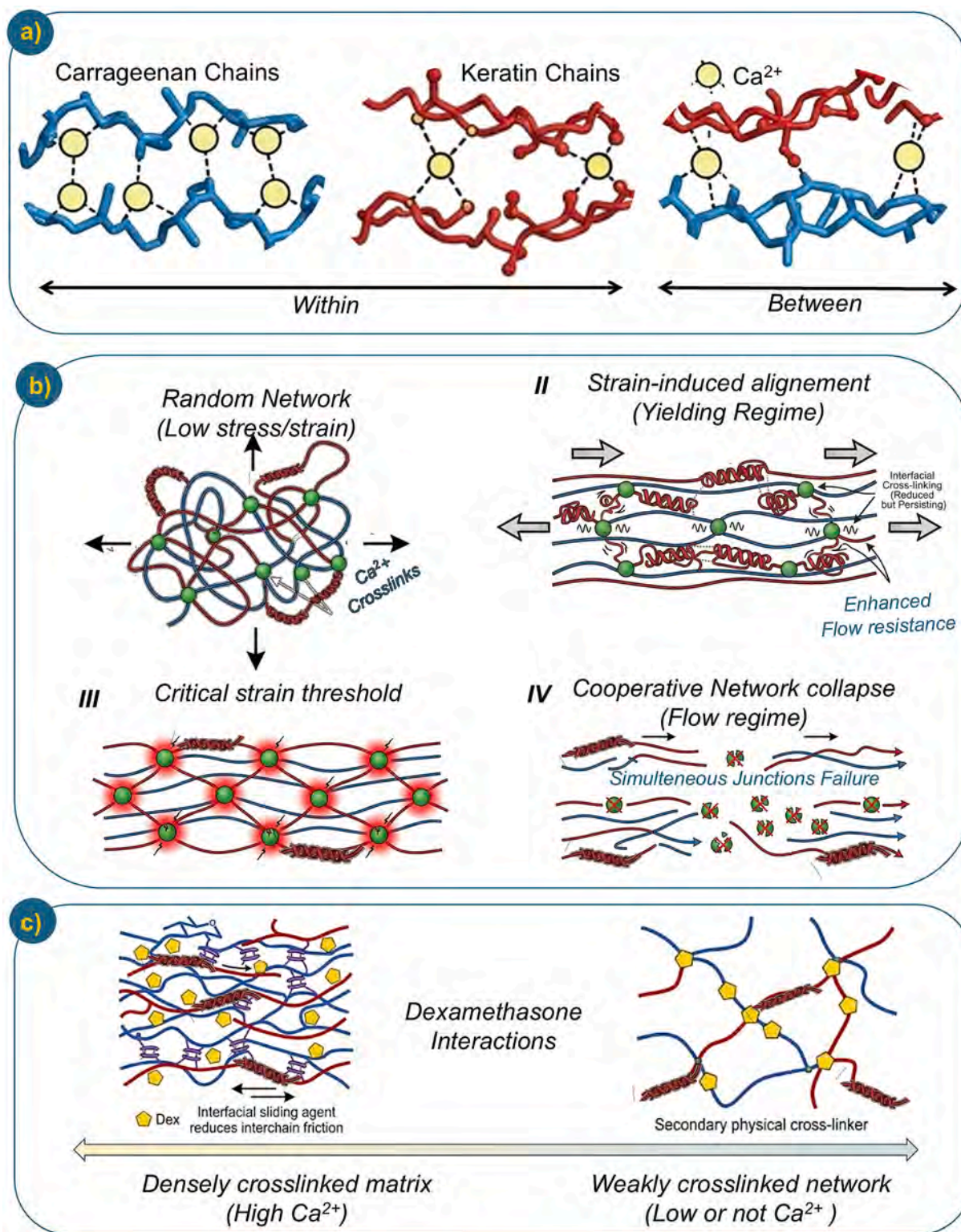


Fig. 3. Comprehensive schematic representation of crosslinking mechanisms, large-strain network evolution, and matrix-dexamethasone interactions. a) Cross-linking Mechanisms (Within / Between): Molecular illustration of the Ca^{2+} -mediated coordination networks. Intrachain/interchain stabilization (Within) occurs via electrostatic bridges targeting negatively charged groups of κ -carrageenan chains (blue) and of the keratin backbone (red). b) Network Evolution Under Large Strain (LAOS): Four-stage progression of the hybrid gel under large-amplitude oscillatory shear: (I) Isotropy under quiescent/low-strain conditions (Random Network); (II) Microstructural rearrangement and Strain-Induced Alignment within the yielding regime, marked by Transient Elastic Energy Storage and localized stress accumulation; (III) Maximum network elongation upon reaching the Critical strain threshold; and (IV) Macroscopic network rupture within the flow regime governed by a Simultaneous Junction Failure. c) Dexamethasone Interactions: Dex acts as an interfacial sliding agent that reduces interchain friction within densely cross-linked matrices, while acting as a secondary physical cross-linker in weakly cross-linked networks.

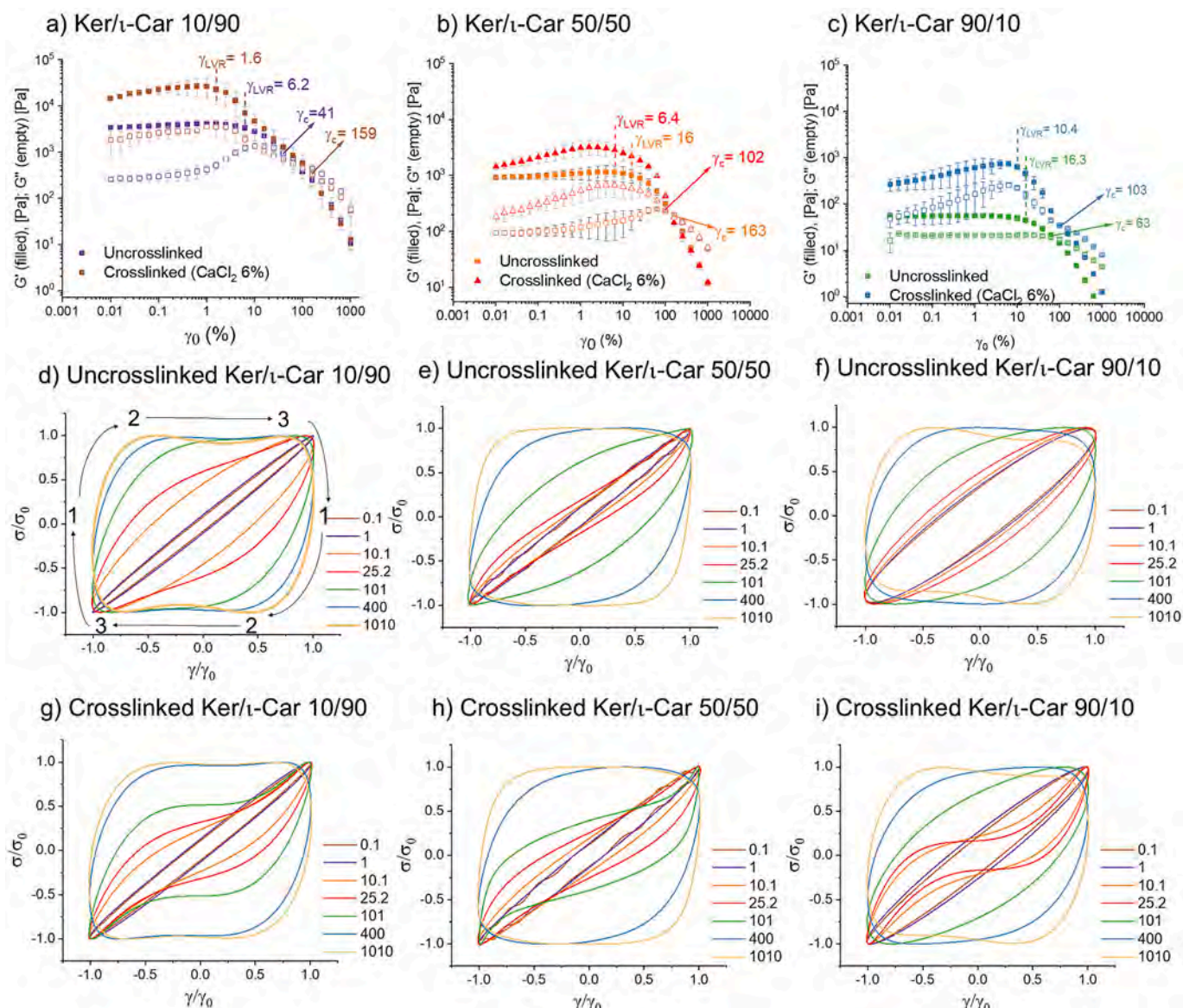


Fig. 4. Rheological Properties and Nonlinear Deformation of Ker/ι-Car Hydrogels. a–c) Amplitude Sweeps: Storage modulus (G') and loss modulus (G'') as a function of strain amplitude (γ_0) for three Ker/ι-Car ratios: 10/90 (a), 50/50 (b), and 90/10 (c). d–i) Elastic Lissajous-Bowditch Plots: Normalized stress (σ/σ_0) vs. strain (γ/γ_0) loops for uncrosslinked samples (d–f) and crosslinked hydrogels (g–i) at various strain amplitudes (0.1% to 1010%).

stress for many values of strain was observed. Examining in detail this LB curve at higher amplitude strain and beginning at zero shear stress (Fig. 4d), the hydrogel first exhibited elastic strain as the sample is sheared, highlighted by the curve segment 1→2. In this stage, the polymer chains align in the direction of shear. When the strain is increased further, the maximum stress is reached (2) and at this point the breakdown of the polymers' network induces plastic flow (2→3). At this point, the shear stress relaxes (3) as the direction of strain is reversed and the polymers' network reorganizes (3→1).

The uncrosslinked Ker/ι-Car 10/90 hydrogel becomes nonlinear very early ($\gamma_0 \geq 10\%$), confirming that the extended carrageenan domains in this blend form junction zones which confer a significant physical rigidity to the network, which tends to break or reorganize at relatively low deformation. In this hydrogel, the nonlinearity starts at $\gamma_0 \geq 25\%$, probably because keratin modifies the carrageenan network which becomes more tolerant to deformation before rearranging (Fig. 4d). The uncrosslinked Ker/ι-Car 90/10 hydrogel is the highest strain-tolerant since distorted ellipses occur only at $\gamma_0 \geq 100\%$. Keratin-rich blends are more deformable and flexible, exhibiting the behaviour of a softer

and more ductile material (Fig. 4f). The crosslinked hydrogels show more pronounced distortions than the uncrosslinked ones with sharp stress upturns at maximum strain, in the $10\% < \gamma_0 < 100\%$ amplitude range (Fig. 4g–i). The sharp upturn within some cycles indicates a sudden increase in the material's resistance to further deformation and can be interpreted as a pre-yield intracycle strain-stiffening response of the ionic network. In line with recent LAOS/SPP analyses of crosslinked biopolymer gels, this behaviour is consistent with increasing instantaneous nonlinear elasticity (Eckman et al., 2025). As the strain amplitude increases, local configurational changes become increasingly restricted, and the applied load is transferred more directly to polymer strands constrained between ionic junctions. Their stretching and alignment enhance the instantaneous resistance of the network to deformation, leading to the observed rise in stress. Once a critical strain is reached, however, the network can no longer redistribute the applied stress efficiently, and yielding follows through junction rupture and/or structural reorganization.

This suggests that the crosslinking prevents the chains from sliding past one another easily, leading to more strain accumulation in the

structure before deformation (Fig. 3b). The upturns are more pronounced in the Ker/*l*-Car 10/90 and 90/10 blends (Fig. 4g and i) than in the Ker/*l*-Car 50/50 one (Fig. 4h). Probably, when one component dominates, there is a dominant continuous charged phase which allows more effective crosslinking. The upturn effect is weaker in the crosslinked Ker/*l*-Car 50/50 blends (Fig. 4h), since the system may be more heterogeneous and phases may be partially segregated. Therefore, the crosslinking points may form locally but not create a fully cooperative network.

This could lead to a more distributed stress accommodation. In the crosslinked Ker/*l*-Car 90/10 blend (Fig. 4i), the upturns occur at lower strains (10–25%). This suggests that crosslinked keratin domains are stiffer at small deformation, and they may begin to reorganize, slip, or partially break earlier. While normalized curves facilitate the comparison across the samples, plotting overlapped L-B curves without normalization enables for a better understanding of the yielding and flow transitions, thereby allowing the identification of differences between the selected hydrogels (Dahl et al., 2024). As shown in Fig. 5,

while the strain increases, the area enclosed by the curves expands, indicating a rise in dissipated energy. This is due to the growing influence of the viscous response and the onset of non-linear effects.

As observed, all the crosslinked blend hydrogels show a stress overshoot as strain amplitude increased, since some of the intracycle curves reached a higher stress value than the plateau stress observed in the flow regime. In all the crosslinked hydrogels, the maximum stress is of about 1.5 times higher than the stress at flow.

Particularly, these blend hydrogels exhibited a sudden transition from the yielding region to the flow region, occurring between two successive strain amplitudes (Fig. 5 b,e and h). The hydrogels' network first resists deformation strongly and stores elastic energy (lower entrapped area). Suddenly, it breaks and/or reorganizes thereby flowing at a lower steady stress. Accordingly, the stress overshoot observed across successive strain amplitudes can be regarded as the intercycle counterpart of the intracycle upturn observed in the elastic L-B plots, both reflecting transient elastic energy storage prior to cooperative yielding. As reported by Eckman et al. (Eckman et al., 2025), this

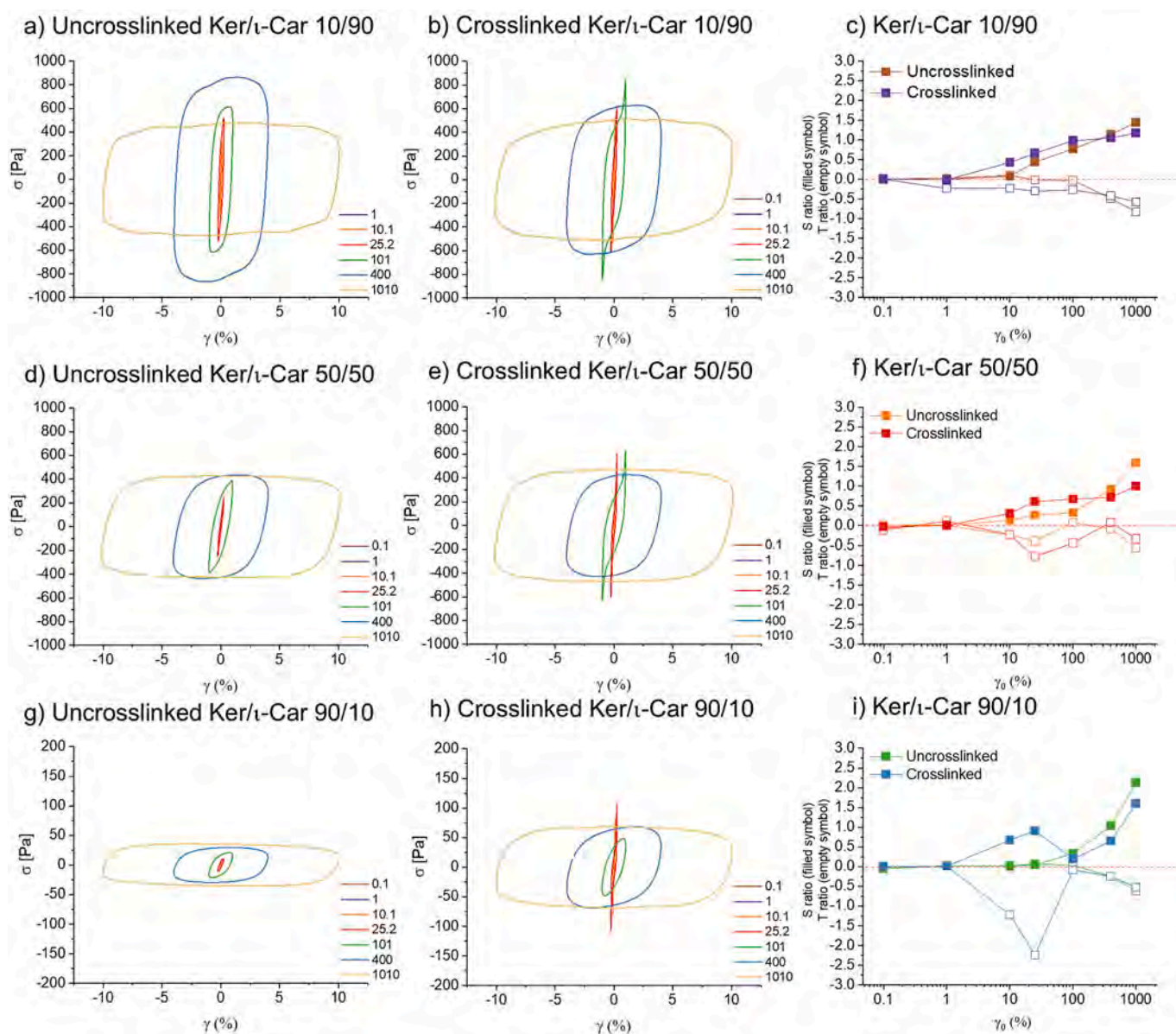


Fig. 5. Nonlinear Viscoelastic Analysis of Ker/*l*-Car Hydrogels: Elastic Lissajous plots representing the total stress (σ) as a function of instantaneous strain (γ) across various strain amplitudes (0.1% to 1010%) for uncrosslinked hydrogels (a,d,g) and for crosslinked hydrogels (b,e,h). S and T ratios for crosslinked and uncrosslinked hydrogels for three distinct Ker/*l*-Car blends: 10/90 (c), 50/50 (f) and 90/10 (i).

behaviour can be explained by the progressive orientation of polymer chains as strain increases: the chains align with the direction of the applied shear stress, and the cross-linked regions provide greater resistance to flow, storing elastic energy (reduced enclosed area). At a critical strain, the crosslinking points (junctions) undergo rupture almost simultaneously dissipating energy (wider enclosed area). The sudden transition between the yielding regime to flow regime occurring at two successive strain amplitudes indicates that the yielding process is not gradual, but it is cooperative. Instead of progressive bond breaking, a collective rupture of the ionic junctions occurs at a critical strain threshold and the network collapses. This behaviour is typical of physically crosslinked ionic hydrogels (Eckman et al., 2025) with cooperative junction rupture. Carrageenan-rich blends (with their higher sulfate density) create a more efficient ionic network for energy storage, resulting in a higher stress overshoot. Keratin-rich blends have less efficient crosslinking (due to weaker network formation or different chain dynamics), which results in a lower stress overshoot.

In addition to the elastic Lissajous plots, the viscous L-B plots (shown in Fig. S2) give information about the energy dissipation and flow heterogeneity during LAOS. These shear stress-strain rate loops exhibit a gradual transformation from an elliptical shape to a nearly S-shaped curve, indicating a nonlinear viscoelastic response with pronounced shear thinning at higher strains. As can be seen, the uncrosslinked samples show secondary loops at 1010% strain, suggesting structurally unstable flow associated with heterogeneous deformation, temporary shear banding or local yielding, or the coexistence of elastic recoil and viscous flow within the same oscillation cycle. These secondary loops are less evident in the blends containing the same amount of keratin and carrageenan. This could mean that neither polymer forms a fully continuous dominant network and the blend results more interpenetrated or phase mixed.

3.3.3. *S and T factors*

To quantify the evolution of the elastic Lissajous curves, the dimensionless strain-stiffening (*S*) and shear thickening (*T*) indexes of nonlinearity were calculated (Fig. 5 c, f and i). All blend hydrogels exhibited strain stiffening ($S > 0$) and shear thinning ($T < 0$) behaviour at higher strain, which was in line with the L-B curves. In the case of uncrosslinked hydrogels, both strain stiffening and shear thinning persisted throughout the entire strain range. In agreement with the first harmonic G' behaviour, for the carrageenan rich blend, elastic nonlinearities started at a lower strain and were more pronounced compared to the other blend hydrogels, as evidenced by higher *S* values. Additionally, for all the blends, within the 1–100% strain range, the increase in *S* was more rapid in the crosslinked hydrogels compared to the uncrosslinked ones. However, at higher strain ($\gamma > 100\%$), this increase became less pronounced.

For the Ker/ι-Car 90/10 blend, *S* reached a maximum before decreasing and then increasing again. The strain stiffening-softening-stiffening behaviour corresponds to a shear thinning-thickening response (Fig. 5c). These transitions are indicative of the alignment of polymer chains in the direction of shear, which is more pronounced in the crosslinked blends due to the enhanced energy storage capability of the network before yielding. The network deforms elastically until a certain strain amplitude, causing the dissociation of crosslinks—likely through a combination of bond breakage and stress redistribution—leading to strain-softening behaviour (Insua et al., 2025). Notably, the strain at which *S* peaks or plateaus is lower in the crosslinked Ker/ι-Car 90/10 hydrogel, confirming that this blend yields more easily than the others.

3.3.4. *Cole–Cole analysis within the SPP framework*

To gain a more phenomenological view of the yielding transition, the time-resolved storage and loss moduli were represented as Cole–Cole trajectories (G'_t vs G''_t) within the Sequence of Physical Processes (SPP) framework. In the sense originally proposed by Rogers (Rogers et al.,

2011), the nonlinear LAOS response can be interpreted as a sequence of transient elastic- and viscous-dominated states whose relative contributions evolve continuously throughout the oscillation cycle, rather than as a single cycle-averaged response. Thus, in the present work, the Cole–Cole representation is used as a geometrical complement to the Lissajous and *S/T* analyses, aimed at visualizing how the balance between elastic energy storage and viscous dissipation evolves during yielding. Fig. 6 shows that, at low strain amplitudes, all formulations display relatively compact trajectories located in the linear viscoelastic response with $G'_t > G''_t$, consistent with a predominantly elastic behaviour. As the strain amplitude increases, the trajectories progressively expand and distort into deltoid-like shapes, indicating the onset of intracycle nonlinearity. The uncrosslinked hydrogels show a more gradual evolution of the Cole–Cole paths, with the keratin-rich Ker/ι-Car 90/10 blend maintaining the most compact trajectories over the widest strain range, in agreement with its higher strain tolerance. By contrast, the Ca^{2+} -crosslinked hydrogels exhibit much larger deltoids, indicating a broader separation between elastic-dominant and viscous-dominant states within each cycle. This behaviour is consistent with a network that initially stores elastic energy efficiently but then redistributes stress more abruptly as the ionic junctions approach their limit of stability.

The weaker symmetry and more distributed trajectories of the crosslinked 50/50 blend further suggest a more heterogeneous response, whereas the marked distortion already observed at intermediate strain amplitudes for the crosslinked 90/10 formulation is consistent with an earlier onset of intracycle rearrangement. Additionally, for the crosslinked formulations, part of the trajectory extends into the $G''_t < 0$ region, interpreted as a signature of viscous backflow (Park & Rogers, 2020).

Accordingly, the stress upturn observed in the elastic Lissajous–Bowditch curves is associated with the pre-yield build-up of elastic stress, whereas negative G''_t reflects the onset of flow reversal within the viscous contribution and therefore marks a later stage of the yielding process, in which the network can no longer sustain the imposed deformation in a purely recoverable manner. In this sense, the Cole–Cole plots suggest that pre-yield strain stiffening and viscous backflow are sequentially connected features of the same yielding dynamics: first, the network transiently stiffens and accumulates elastic stress; then, as structural constraints are exceeded, viscous backflow and cooperative rearrangement emerge. This behaviour is also reported by Fontaine-Seiler et al. for composite biopolymer network composed of co-polymerized fibrin and gelatin networks (Fontaine-Seiler & G. J. D. E. D. G. D. L. B., 2025).

In this context, LAOS coupled with SPP analysis provides a processing-relevant rheological representation of the repeated flow and rest conditions experienced by hydrogel inks during DIW. Within each oscillation cycle, the material evolves from a highly structured state at low strain rate, through initial yielding and high-strain-rate flow, and subsequently undergoes structural recovery and relaxation as the strain rate decreases. These transient states can be related, respectively, to ink storage in the reservoir, flow initiation through the contraction region, viscoplastic flow during nozzle passage, and post-extrusion recovery after deposition. Therefore, the transient elastic and viscous moduli obtained from SPP analysis complement the conventional cycle-averaged moduli, providing information on the rheological transitions that govern extrusion and post-deposition shape retention (Insua et al., 2025). Therefore, the Cole–Cole trajectories were further analyzed to obtain a quantitative information of the structure recovered within the LAOS cycle. During the recovery trajectory, as the strain rate decreases after the flow-dominated part of the cycle, the hydrogel network reorganizes and the transient elastic modulus increases. Accordingly, $G'_{t,\max}$ was taken as a measure of the maximum elastic response recovered within the cycle [(Insua et al., 2025)]. The transient viscous modulus evaluated at the same point, $G''_{t|G'_{t,\max}}$, was used to define, in the present work, an intracycle recovery loss factor: $\tan\delta_{\text{rec}} = \frac{G''_{t|G'_{t,\max}}}{G'_{t,\max}}$. This

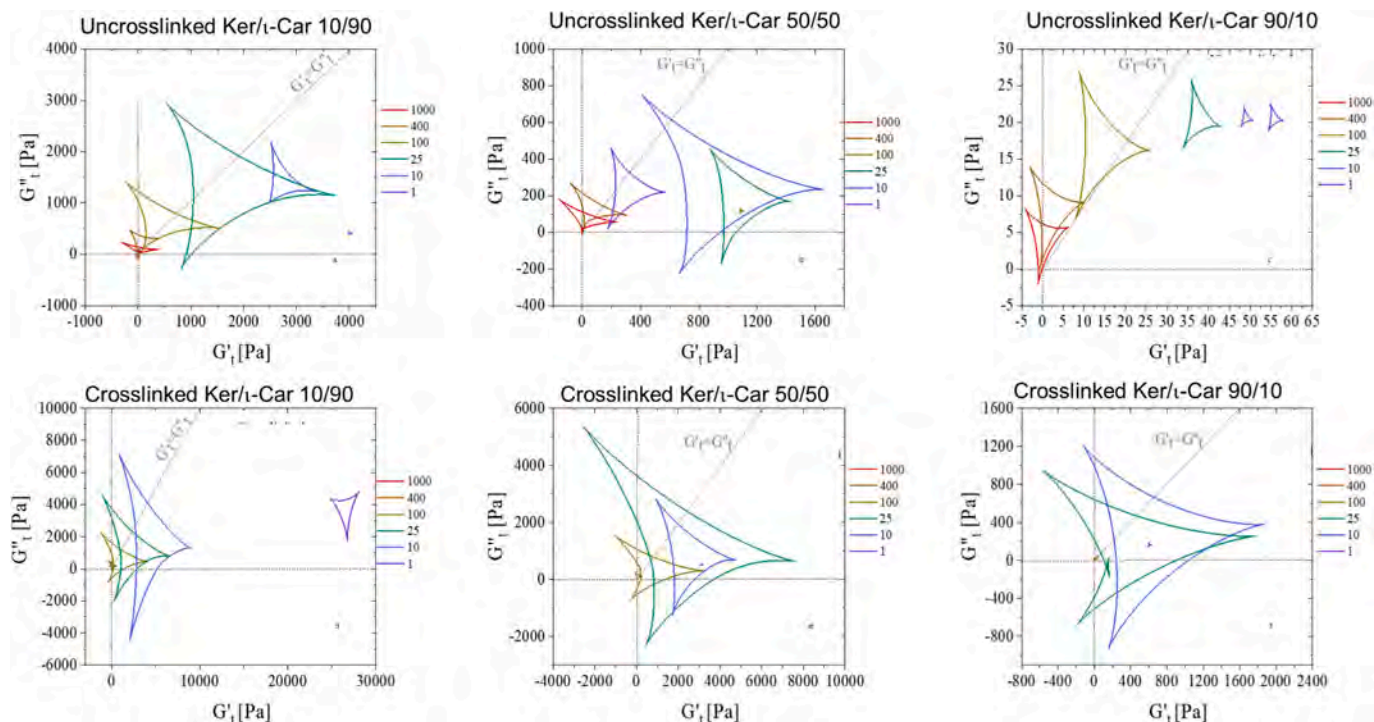


Fig. 6. Time-dependent Cole–Cole plots (G'' vs G') of uncrosslinked (a–c) and Ca^{2+} -crosslinked Ker/I-Car hydrogels (d–f) at Ker/I-Car ratios of 10/90, 50/50 and 90/10, obtained at increasing strain amplitudes. The line $G' = G''$ marks the transition between elastic- and viscous-dominated transient states. The progressive expansion and distortion of the trajectories with increasing strain amplitude reflect the development of intracycle nonlinear viscoelasticity.

parameter describes the balance between the viscous and elastic contributions at the point of maximum recovered elasticity. Lower $\tan\delta_{\text{rec}}$ values therefore indicate a more elastic recovered state, whereas higher values indicate that viscous dissipation remains more relevant after network reorganization. It should be noted that $\tan\delta_{\text{rec}}$ characterises the elastic–viscous balance of intracycle recovery at the timescale imposed by LAOS. The values obtained for the selected formulations are reported in Fig. S3. Focusing on the recovery parameter evaluated in the strain-amplitude range $\gamma_0 = 100$ –400%, in which the Cole–Cole trajectories encompassed both viscous-dominated ($G'' > G'$) and elastic-dominated ($G' > G''$) states within a single oscillation cycle, we observed the following: Std8 exhibited the lowest $\tan\delta_{\text{rec}}$ values (0.40–0.60) and a printability index closest to the ideal value ($Pr = 1.001$). Std11 also showed a predominantly elastic recovered state, with $\tan\delta_{\text{rec}} = 0.5$ –0.6 and $Pr = 0.994$, whereas values of $\tan\delta_{\text{rec}} = 0.70$ and 0.85 were obtained for Std12 ($Pr = 1.020$) and Std4 ($Pr = 0.989$), respectively. Thus, all formulations producing well-defined printed structures exhibited the lowest $\tan\delta_{\text{rec}}$ values, indicating that the structure recovered within the cycle remained predominantly elastic. By contrast, Std2, which underwent excessive flow and could not be printed, showed a pronounced increase in $\tan\delta_{\text{rec}}$ over the same strain range. Although the relationship was not strictly monotonic across all printable formulations, the overall trend indicates that P_r values closer to unity were associated with a more elastic recovered state. These results support the relevance of recovering an elastic-dominated structure after yielding for limiting filament spreading and preserving shape after deposition. In this respect, $\tan\delta_{\text{rec}}$ complements G'_{LVR} and σ_f by describing the elastic–viscous balance re-established after yielding. Taken together, the first-harmonic moduli, the Lissajous–Bowditch plots, the S and T indices, and the SPP analysis provide complementary information on the rheological states relevant to DIW. In this framework, G'_{LVR} reflects the solid-like strength of the undisturbed ink, σ_f identifies the stress required to reach macroscopic flow, and $\tan\delta_{\text{rec}}$ describes the elastic–viscous balance re-established after yielding. The uncrosslinked hydrogels

generally undergo a more gradual structural rearrangement; however, when the network lacks sufficient strength, as observed for Std2, the ink undergoes excessive flow and cannot retain the deposited geometry. By contrast, Ca^{2+} crosslinking increases the elastic strength of the hydrogels and leads to a more pronounced nonlinear yielding response, while the dynamic nature of the ionic junctions still permits partial network reorganization. Thus, Ca^{2+} -mediated interactions act as effective constraints at small deformation but remain dynamic under the larger deformations associated with extrusion. Nevertheless, the effect of increasing network elasticity is not necessarily beneficial beyond a certain level. An insufficiently elastic network may show poor structural recovery and excessive filament spreading after deposition, whereas an excessively strong network may resist yielding, resulting in difficult or discontinuous extrusion. Optimal printability therefore requires a compromise between sufficient elasticity for post-deposition shape retention, adequate flowability through the nozzle, and the ability to re-establish an elastic-dominated structure after yielding.

3.4. Effect of the drug on the rheological behaviour of the hydrogels

Initially only std12, std11 and std8 blends were considered for loading with dexamethasone, since they fell into the printability green zone (Table 1). As observed in Fig. 7, the addition of the dexamethasone caused a significant increase of the G'_{LVR} . Moreover, with the exception of Std12, the addition of Dex increased also the σ_f . Consequently, it was decided to also consider the Std2 hydrogel, which is in the orange zone of the printability map. Although adding the drug to the Std2 hydrogel increased both G'_{LVR} and σ_f , the loaded hydrogel (Std2D) still did not exhibit rheological properties sufficient to fall within the printable zone. Indeed, as illustrated in the Fig. 7, the freeze-dried Std2D sample exhibited an irregular morphology, attributable to the collapse of the printed architecture. On the other hand, upon incorporation of the drug, the Std11 hydrogel transitioned into the gray zone and became non-extrudable, mirroring the extrusion behaviour of Std3 (Fig. 7). This finding further substantiates the practical relevance of the developed

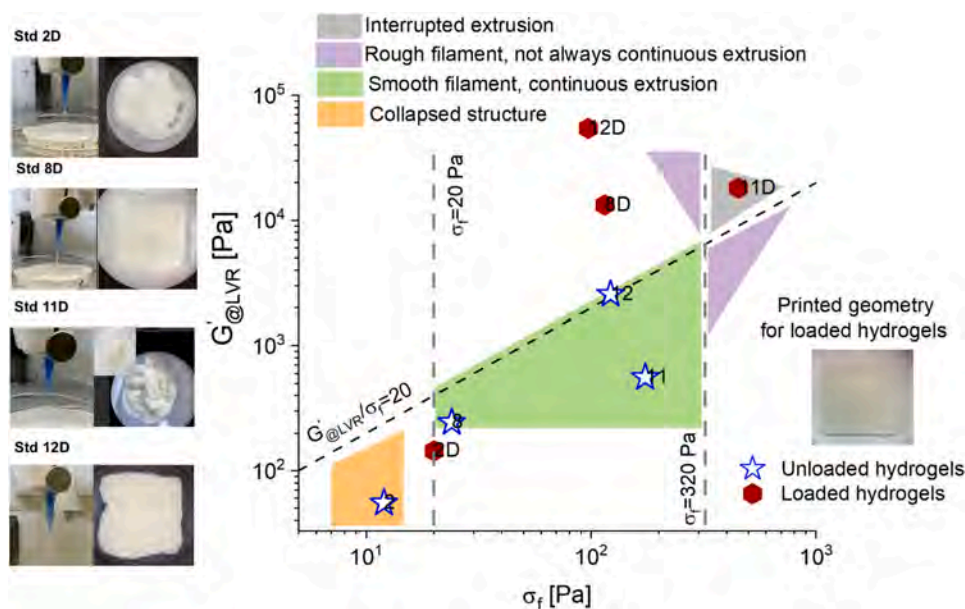


Fig. 7. Ashby-type chart correlating the storage modulus in the linear viscoelastic region ($G'_{@LVR}$) and the flow yield stress (σ_f): comparison between unloaded and Dex loaded hydrogels.

printability map.

In Fig. 8, the first harmonic derived moduli (G' and G''), as well as the elastic L-B plots in the yielding and flow regions of the drug loaded hydrogels were compared with the related unloaded ones. As observed, the incorporation of the drug reduces the LVR across all hydrogels. However, this effect is only marginal in the Std12D hydrogel (Fig. 8g), whereas it is markedly more pronounced in the Std11D hydrogel (Fig. 8l). Moreover, it is worth noting that the two formulations falling in the not printable region such as Std3 (crosslinked Ker/i-Car 10/90) and Std8D have in common an extended yielding region characterized by a γ_c is 100 times higher than γ_{LVR} (Figs. 7a and 8l).

Examining the elastic L-B curves, it can be observed that in the yielding region (Fig. 8 e,h,m), the area enclosed by the curves for the drug-loaded hydrogels is larger than that of the unloaded hydrogels, indicating an increased energy dissipation.

Moreover, except for the Std2D blend, all other blends exhibited a sharp upturn at the maximum strain within each cycle of the yielding region, suggesting that the drug enhances resistance to flow during amplitude cycles. In addition, Std8D and Std11D showed an abrupt transition between cycles, whereas Std12D displayed a more ductile intercycle transition. In Fig. S4 the viscous L-B plots of the yielding region are shown (Fig. S4 a,c,e,g). The drug-loaded hydrogel exhibited a much larger loop area and significantly higher peak stress compared to the drug-free control (green). This indicates that the drug molecules do not merely act as fillers but actively reinforce the network, increasing the material's ability to store elastic energy during flow. The “bow-tie” expansion in the curves of drug loaded hydrogels signifies a transition to a highly non-linear regime. The high stress at low shear rates, followed by the narrowing of the loops at higher rates, confirms a robust shear-thinning behaviour.

In the flow region (Fig. 8 f,l,n), except for the Std8D, all the other drug loaded hydrogels showed elastic LB curves characterized by a higher stress and higher enclosed area, compared to the related unloaded ones. Moreover, it is possible to observe stress overshoots in the cycles at higher γ_0 , slightly accentuated in Std2 and even more accentuated in Std12 and Std11. Instead, in the Std8 blends, for $\gamma_0 > 400\%$, no differences were observed in the elastic L-B cycles between the drug loaded and unloaded hydrogels.

The viscous L-B plots (Fig. S4 b, d, f, h) demonstrates that drug loading significantly increases the elastic energy storage during flow

compared to the unloaded hydrogel (larger loops area), suggesting a more resilient dynamic network that promotes superior shape-fidelity under shear rate.

Dexamethasone can interact with the keratin-carrageenan matrix primarily through non-covalent interactions, including hydrogen bonding between its hydroxyl and carbonyl groups and the polar functional groups of both polymers, alongside hydrophobic interactions with its steroid group and lipophilic regions of keratin. Nevertheless, in an aqueous environment, the hydrophobic interactions are likely hindered, as the protein's lipophilic domains spontaneously self-assemble into shielded internal cores to minimize water exposure. In the Std12 formulation (6% Ca^{2+}), the high concentration of divalent cations promotes a densely crosslinked, compact iota-carrageenan network and a reduced availability of polar groups for binding the drug via hydrogen bonds. This results in the significantly more ductile intercycle transition observed in the drug-loaded Std12 sample compared to the other drug loaded formulations as Std8D and Std11D (Fig. 8 d, g and l). Conversely, the Std8 formulation is characterized by a network with a lower amount of crosslinker (3% Ca^{2+}) and a higher availability of polar groups accessible for drug binding. Under these conditions, the drug reduces the chain sliding during deformation, leading to a stark increase in shear resistance (higher stress values) that surpasses that of the drug-free related formulation (Fig. 8e). However, as the stress amplitude increases into the large deformation zone, these stabilizing hydrogen-bonded networks progressively weaken and rupture under the escalating shear. This mechanical breakdown eventually triggers a structural collapse, leading to the observed convergence and reduced rheological differences between the drug-loaded and drug-free hydrogels in the continuous flow regime (Fig. 8f). On the contrary, the Std11D hydrogel features the highest carrageenan content formulated without ionic crosslinkers, thereby maximizing the number of free functional sites available for drug-polymer hydrogen bonding. In the absence of divalent cations, dexamethasone effectively takes over as a secondary physical crosslinker, establishing an extensive network of interchain hydrogen bonds. The pronounced stress overshoot observed in the viscous Lissajous-Bowditch (L-B) curves within the flow regime provides clear evidence of significant structural rearrangements during the strain cycle. This specific non-linear fingerprint is directly associated with the dynamic, shear-induced rupture and subsequent re-formation of these drug-matrix hydrogen bonds, which temporarily resist deformation

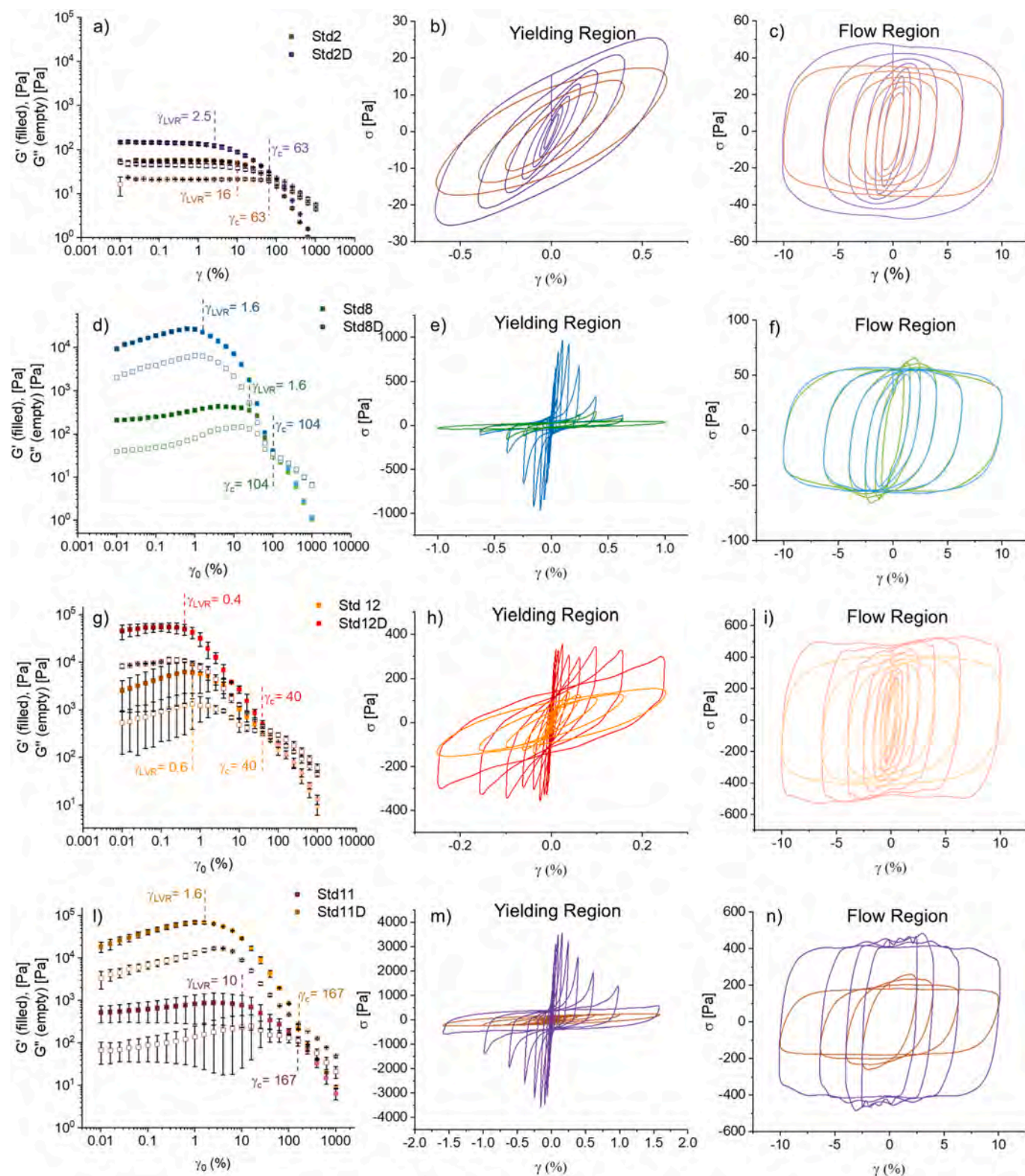


Fig. 8. Nonlinear viscoelastic analysis of selected unloaded and dexamethasone loaded hydrogels: Storage modulus (G') and loss modulus (G'') as a function of strain amplitude γ_0 (a,d,g,j); Elastic L-B curves of the yielding region (b,e,h,n) and of the flow region (c,f,i,n).

before yielding under escalating strain (Fig. 8 m and n).

The Cole-Cole/SPP analysis of the unloaded and drug-loaded formulations (Fig. S5) supports the trends already inferred from the amplitude sweeps and Lissajous–Bowditch plots. In general, dexamethasone enlarged deltoid-like trajectories, indicating a greater transient build-up of elastic stress before yielding.

This effect was strongest in Std11D, which also showed a clearer extension into the $G'' < 0$ region, consistent with viscous backflow and a more abrupt cooperative rearrangement of the network. Std8D exhibited more moderate changes, as a result of reduced drug/polymer matrix interactions, whereas Std12D showed an intermediate and more balanced response. In contrast, the enlargement observed for Std2D

remained insufficient to confer the transient network strength required for printable behaviour.

3.5. FTIR study of polymer Interactions with the crosslinker and dexamethasone

To investigate the molecular interactions between keratin and ι -carrageenan chains, as well as the effect of their ionic interactions with Ca^{2+} and Dex Fourier-transform infrared (FTIR) spectroscopy was performed (Fig. 9). Fig. 9a shows the FTIR spectra of the pure Ker and ι -Car,

as well as their uncrosslinked and crosslinked blends. As observed, carrageenan-rich blends exhibit strong absorption bands at approximately 3400 cm^{-1} and 1650 cm^{-1} associated with O—H stretching and bending vibrations, respectively. The bands at 1215 , 1025 cm^{-1} , $^{-1}$ are attributed to sulphate ester groups ($\text{S}=\text{O}=\text{S}$) and glycosidic bands of the saccharide moiety, respectively. Finally, the adsorptions at 930 cm^{-1} , 845 cm^{-1} and 805 cm^{-1} are associated to the C—O—C vibration of 3,6-anhydro-D-galactose, C—O—S axial stretching at C-4 of galactose and C—O—S stretching at C-2 of 3,6-anhydro-D-galactose, respectively (Pereira et al., 2013).

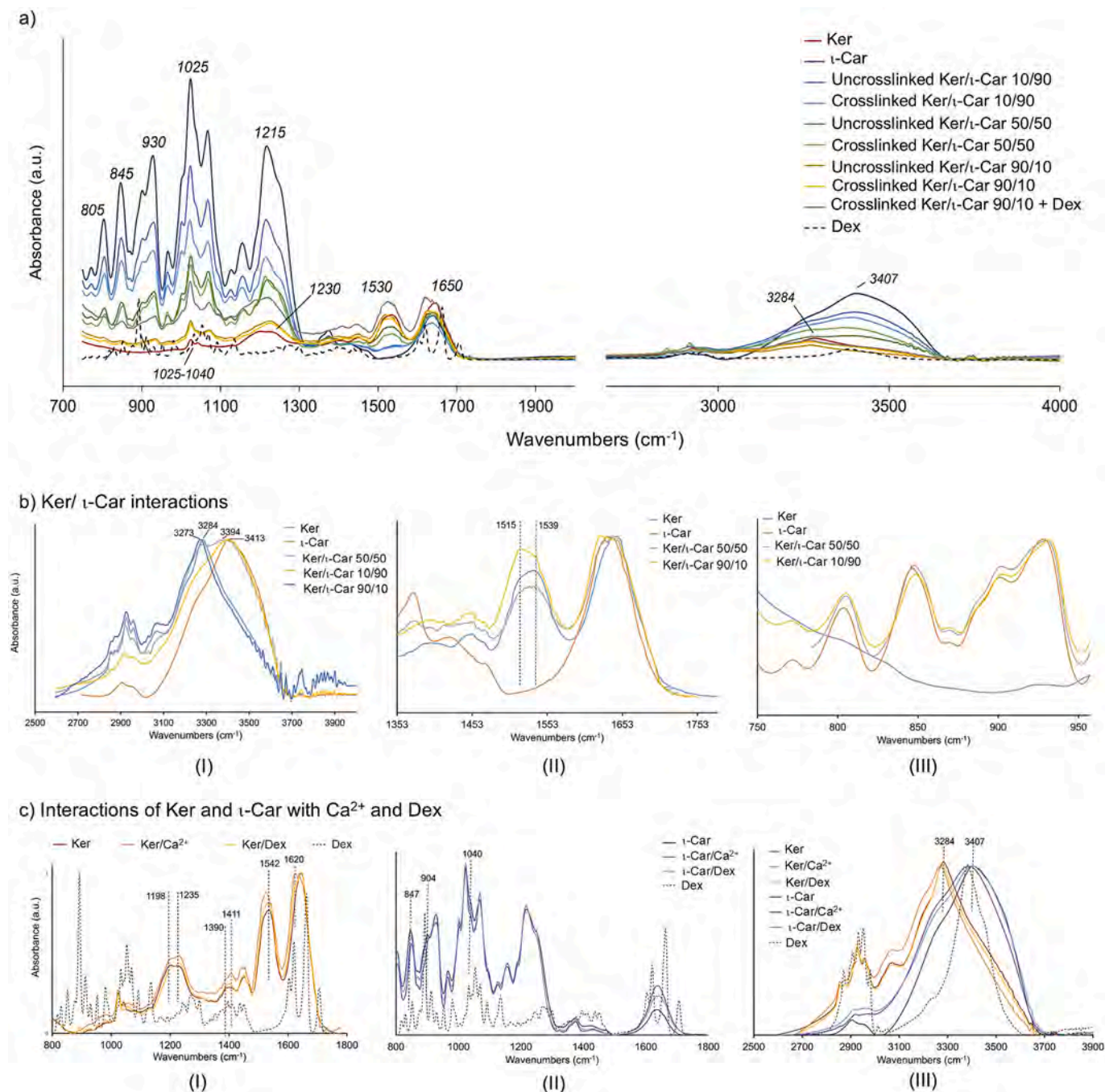


Fig. 9. ATR-FTIR spectra. a) spectra of uncrosslinked and crosslinked Ker/ ι -Car hydrogels at different blending ratio and crosslinked Ker Ker/ ι -Car hydrogel 90/10 containing dexamethasone. b) uncrosslinked Ker/ ι -Car hydrogels at different blending ratio normalized in the spectral ranges of (I) $2500\text{--}3000\text{ cm}^{-1}$; (II) $1350\text{--}1753\text{ cm}^{-1}$ and (III) $750\text{--}950\text{ cm}^{-1}$. c) spectra of (I) pure keratin, keratin/calcium ions (Ker/ Ca^{2+}) and keratin/dexamethasone (Ker/Dex) binary systems, normalized in the $800\text{--}1800\text{ cm}^{-1}$ range; (II) ι -carrageenan/calcium ions (ι -Car/ Ca^{2+}) and ι -carrageenan/dexamethasone (ι -Car/Dex) binary systems, normalized in the $800\text{--}1800\text{ cm}^{-1}$ range and (III) pure keratin, pure ι -carrageenan and all the binary systems normalized in the $2500\text{--}4000\text{ cm}^{-1}$ range.

In contrast, keratin-rich blends displayed the characteristic absorption bands of keratin, including Amide A centered at 3284 cm^{-1} , due to N—H stretching vibrations, and Amide I (1650 cm^{-1}) and Amide II (1514 cm^{-1}), corresponding to C=O and C—N stretching vibrations of the peptide bonds, respectively. Additional, less intense keratin-related bands including Amide III (1230 cm^{-1}) and absorptions at 1190 cm^{-1} and 1025 cm^{-1} associated to the S=O asymmetric and symmetric stretching vibration, respectively, of the sulphonate groups were observed (Aluigi et al., 2007).

The FTIR spectra of the uncrosslinked blends exhibited characteristic absorptions reflecting changes in the hydrogen-bonding environment of the polymer matrix. Specifically, in the high-wavenumber region ($3000\text{--}3500\text{ cm}^{-1}$, Fig. 9b-I), the hydroxyl (O—H) peak of carrageenan at 3413 cm^{-1} undergoes a red shift as the keratin (Ker) content increases. Concurrently, the amine/amide (N—H) peak of keratin exhibits a red shift from 3284 cm^{-1} to 3273 cm^{-1} with increasing the carrageenan content. These shifts indicate an intensification of intermolecular hydrogen bonding between the hydroxyl groups of carrageenan and the amide groups of keratins. This hypothesis is further supported by structural alterations in the keratin amide II band (Fig. 9b-II), a region free of overlapping carrageenan absorptions that is highly sensitive to out-of-phase C—N stretching and N—H bending vibrations. Notably, an increase in carrageenan content induces a significant intensity increase of the component at 1515 cm^{-1} relative to the peak at 1539 cm^{-1} , confirming that the N—H chemical environment is altered due to direct binding with the carrageenan matrix. Finally, looking to the $950\text{--}750\text{ cm}^{-1}$ region, which contains characteristic absorption bands α -carrageenan, the peak corresponding to the C—O—S axial sulfate stretch remains essentially unchanged at 845 cm^{-1} in the presence of keratin. This stability indicates that the primary sulfate ester backbone of α -carrageenan is preserved within the polymer blend matrix without major structural disruption. (Fig. 9b-III).

To investigate the specific interactions of Ca^{2+} and dexamethasone within the keratin and carrageenan domains, FTIR analysis will be conducted on the individual binary systems keratin/ Ca^{2+} , keratin/Dex, carrageenan/ Ca^{2+} , and carrageenan/Dex. This approach effectively circumvents the issue of overlapping bands in the complex blend spectra (Fig. 9c).

For the Ker/ Ca^{2+} binary system, the addition of Ca^{2+} ions induces a net red shift in the Amide A (Fig. 9c-I), Amide I and Amide II bands (Fig. 9c-II). This shift reflects alterations in the protein's secondary structure and hydrogen-bonding network, likely driven by electrostatic interactions between Ca^{2+} and acidic side chains, which induce local conformational rearrangements in the keratin matrix.

Specifically, the downshift of the Amide I band (primarily C=O stretching) and Amide A band (N—H stretching) indicates that these groups act as electron donors to the metal cation, weakening the original covalent bonds and altering the local hydrogen-bonding network. Furthermore, a relative reduction of the peak at 1198 cm^{-1} compared to the peak at 1235 cm^{-1} is observed, alongside a distinct intensity increase of the 1411 cm^{-1} peak relative to the 1390 cm^{-1} peak (Fig. 9c-I). These specific alterations point to conformational adjustments in the side-chain groups (such as carboxylate or C—O environments) involved in chelating the calcium ions. This behaviour aligns with previous FTIR characterizations of calcium-bound albumin (Patel et al., 2022) and crosslinked caseinate networks (Zhao et al., 2025), where divalent cation coordination to protein side chains drives local structural compaction and matrix stabilization. This chelation process is driven by a mechanism of competitive ion exchange and deprotonation, macroscopically evidenced by a distinct drop in the solution pH from 5.5 to 4.6. As the incoming divalent Ca^{2+} cations actively coordinate with the protein matrix, they displace bound protons from the partially protonated carboxylic acid groups on the acidic amino acid side chains, converting them into fully coordinated carboxylate groups and shifting the local spectral balance.

Curve fitting and deconvolution of the Amide I band ($1600\text{--}1700\text{ cm}^{-1}$) were conducted to monitor *relative structural transitions* in the keratin/ α -carrageenan backbone across different ratios (Fig. S6-a). It is worth noting that peak deconvolution of ATR-FTIR spectra provides semi-quantitative estimation of relative secondary structure trends rather than absolute conformational quantification. As shown in Fig. S6-a, Amide I band deconvolution revealed a relative decrease in the proportion of α -helical structures upon calcium addition. This trend suggests a local destabilization and loss of native intramolecular hydrogen bonding within the helical domains (Atri et al., 2015).

This structural disruption is also corroborated by the complementary XRD data, where the characteristic α -helix reflection not only decreases in relative area but also shifts toward a lower scattering angle, moving from 10.0° to 9.7° in 2θ (Fig. S6-b). This downshift directly indicates an expansion of the interplanar d -spacing between the coiled-coil helical chains, probably associated to the Ca^{2+} intercalation which partially alters the native helical framework. Conversely, the random coil and β -sheet structures undergo a red shift from 1640 cm^{-1} to 1635 cm^{-1} and from 1624 cm^{-1} to 1618 cm^{-1} , respectively. These downshifts are an indicator of matrix compaction, where the electrostatic crosslinking shorten and strengthen the interchain hydrogen bonds (Byler & Susi, 1986). This compaction mechanism is validated by the complementary XRD data (Fig. S6-b), where the primary β -sheet crystalline reflection undergoes a significant shift to a higher scattering angle (from 21.80° to 22.1°), directly confirming a reduction in interplanar d -spacing (Murthy, 2016). Furthermore, while FTIR suggests the absolute number of β -sheet carbonyl bonds remains stable, XRD registers a notable increase in the relative β -sheet peak area at the expense of the amorphous halo. Taken together, these dual-technique observations reveal that Ca^{2+} incorporation does not induce the neoformation of β -sheets; rather, it drives the spatial reorganization and tight packing of pre-existing, structurally semi-disordered β -strands into highly ordered, long-range crystalline β -sheet lattices (Murthy, 2016).

In contrast, the keratin/Dex binary system displays a distinct interaction profile. A slight redshift is observed across the Amide A, I, and II bands (Fig. 9c-I and II) upon addition of the drug, confirming localized hydrogen bonding directly along the protein backbone. Furthermore, a distinct alteration in the $1198/1235\text{ cm}^{-1}$ peak ratio is observed, while no detectable changes or shifts are registered for the 1025 cm^{-1} band (Fig. 9c-II). This suggests that the drug does not engage in direct coordination or hydrogen bonding with the bulk sulphonate groups; rather, the observed perturbation of this intensity ratio is most likely driven by a supramolecular rearrangement of the keratin matrix induced by the drug. Conversely, the absence of major spectral variations in the aliphatic C—H region confirms that the drug does not deeply penetrate or chemically disrupt the primary internal hydrophobic cores of the protein folds (Fig. 9c-I). It is worth noting that despite the slightly alkaline pH of the added drug formulation (pH 8.2), the overall pH of the binary system remains entirely unaltered. This behaviour indicates that the drug associates primarily via neutral hydrogen bonding rather than electrostatic ion-exchange mechanisms that would alter proton equilibria. These spectroscopic findings contextualize the rheological models: they suggest that while dexamethasone possesses a chemical affinity for accessible polar regions along the protein backbone (driving the bridging effect seen in Std8), it leaves the tightly packed hydrophobic folds largely unperturbed. Consequently, in formulations like Std12, where dense ionic crosslinking of carrageenan sterically excludes the drug from these polar backbone sites, Dex is forced to localize at the macro-domain interfaces, reducing interchain friction without altering native hydrophobic assemblies.

Deconvolution of the Amide I region upon Dex incorporation reveals a significant increase in the random coil band area at 1641 cm^{-1} . At the same time, XRD shows that the relative contribution of the broad “amorphous halo” is substantially reduced. This means the drug acts like a wedge, locally disrupting some parts of the matrix, while forcing other

disordered segments to pack together more tightly.

Furthermore, FTIR Amide I deconvolution revealed a relative reduction in both the β -turn and total β -sheet band areas, accompanied by a redshift from 1668 cm^{-1} to 1665 cm^{-1} and from 1624 cm^{-1} to 1618 cm^{-1} , respectively.

The sharp red shift of the β -sheet and β -turn signal implies that the drug favors a tighter, more strongly hydrogen-bonded compaction.

Interestingly, while FTIR tracks a net decrease in the relative contribution of β -sheet vibrational conformations, XRD registers an increase in the relative β -sheet crystalline reflection. This operational distinction highlights the different structural scales probed by the two techniques: FTIR reflects local, short-range chemical bond environments, whereas XRD reflects longer-range spatial periodicity.

A plausible explanation for this behaviour is that Dex incorporation induces local steric perturbations that disrupt loosely organized or peripheral β -sheet segments (reflected in the increased random coil content in FTIR). Concurrently, the localized spatial constraints introduced by the drug matrix may facilitate the closer alignment of the remaining, stable β -strands into better-ordered domains that scatter X-rays more constructively. Thus, the observed reduction in the XRD amorphous halo likely reflects the spatial reorganization and packing of pre-existing segments rather than the net de novo formation of chemical β -sheet bonds.

Both techniques confirm that the α -helix content remains virtually unchanged. The drug does not destroy or unfold these helices. Instead, Dex sterically shields the helices from water, which causes a blue shift in the FTIR signal (1652 cm^{-1} to 1658 cm^{-1}) due to reduced hydration (Fig. S6).

Regarding the Ca^{2+} /i-Car binary system, the introduction of Ca^{2+} induces a clear red shift of the broad hydroxyl band at 3407 cm^{-1} (Fig. 9c-I), indicating that the metal coordination disrupts the existing intra- and intermolecular hydrogen-bonding networks of the polysaccharide, establishing a new, more constrained coordinated matrix. In the skeletal region (Fig. 9c-III), a slight decrease in the intensity of the shoulder at 1040 cm^{-1} relative to the peak 1020 cm^{-1} is observed, alongside a similar reduction of the 904 cm^{-1} shoulder compared to the 930 cm^{-1} peak. These relative intensity alterations suggest that Ca^{2+} binding affects the local conformation and orientation of the carrageenan polymeric chain. Meanwhile, the characteristic C–O–S sulfate peak at 847 cm^{-1} remains positionally unaltered, confirming that the primary sulfate groups maintain their structural identity during Ca^{2+} crosslinking.

Moreover, a pH increases from 4.6 to 6.3 is registered upon addition of Ca^{2+} into carrageenan solutions. This shifts toward neutrality implies that the macrostructural condensation and the electrostatic pairing between Ca^{2+} and the sulphate groups screens the anionic backbone which alters the local pKa or availability of acidic protons. Interestingly, the spectral transitions observed for Ca^{2+} /i-Car systems, are mirrored in the i-Car/Dex binary system (Fig. 9c-III), even if less pronounced. This suggests that Dex interacts via non-ionic hydrogen bonding pathways rather than rigid electrostatic bridges. This interaction profile is further supported by a rise in the solution pH from 4.6 to 7.8 upon adding the dexamethasone formulation. This shift indicates that as the drug locks across the polymer chains, it restricts the availability of the carrageenan matrix's acidic protons. Because the carrageenan matrix loses its ability to resist pH changes, the alkaline components of the incoming drug formulation easily neutralize the bulk phase.

Notably, these spectroscopic findings provide a direct molecular explanation for the macroscopic behaviour observed in the rheological characterization of the uncrosslinked Std11D formulation. Because this system lacks divalent calcium ions, the free sulfate and hydroxyl sites remain entirely unblocked. This allows dexamethasone to efficiently anchor itself across the carrageenan chains, acting as a secondary physical crosslinker through these very same hydrogen-bonding networks and sulfate interactions. This extensive, highly dynamic drug-

polymer interlocking network is what ultimately gives rise to the pronounced stress overshoots and non-linear energy dissipation fingerprints recorded during large-amplitude shear flow.

3.6. Swelling and rehydration kinetics

The water absorption capacity and swelling kinetics of the cross-linked matrices (6% wt, relative to the total mass of polymers) were investigated to elucidate the intrinsic hydration capacity and network mesh relaxation of the biopolymer backbone across different Ker/i-Car ratios. Table 3 summarizes the kinetic parameters obtained by fitting the experimental data to the Schott's second order (Schott, 1992) and Korsmeyer–Peppas kinetic models (S_∞ , k_s , r_0 , and n). The equilibrium swelling capacity (S_∞) exhibited a strong dependence on the formulation composition, significantly increasing with higher carrageenan content. Std3 (Ker/i-Car 10/90) achieved the highest swelling capacity ($S_\infty = 26.11$), which was more than double that of Std10 (Ker/i-Car 50/50) and Std4 (Ker/i-Car 90/10). This pronounced water-holding capacity is attributed to the polyanionic nature of i-carrageenan, whose abundant negatively charged sulfate groups ($-\text{SO}_3^-$) induce electrostatic repulsion, opening the network mesh and facilitating solvent penetration. Conversely, higher proportions of keratin (Std4) yielded a denser, more hydrophobic polymeric framework, that sterically constrained hydrogel expansion.

Interestingly, evaluating the second order swelling constant (k_s) and the initial swelling rate ($r_0 = k_s \cdot S_\infty^2$) revealed a non-linear trend indicative of synergistic polymer interactions. While Std3 demonstrated rapid hydration kinetics ($r_0 = 24.57\text{ min}^{-1}$) driven by strong hydrophilic affinity, Std10 exhibited a substantial decline in both k_s (0.02 min^{-1}) and r_0 (3.08 min^{-1}). This kinetic retardation suggests the formation of a tightly interpenetrated complex network between domains of keratin and the moieties of carrageenan at equal ratios, resulting in enhanced network density and increased tortuosity for water uptake.

The diffusion exponent (n) obtained from the drug-free swelling profiles reflects the intrinsic water penetration mechanism into the blank matrix: Std10 $n = 0.52$ exhibited anomalous (non-Fickian) transport governed simultaneously by diffusion and viscoelastic chain relaxation, whereas Std3 ($n = 0.32$) and Std4 ($n = 0.10$) displayed pseudo-Fickian behaviour. While these measurements provide baseline insight into the structural hydration dynamics of the blank hydrogel network, the presence of loaded dexamethasone, which acts as a structural modulator, further alters the matrix relaxation and drug release kinetics discussed in Section 3.7.

3.7. Drug release

In Fig. 10, the drug release profiles of the selected formulations have been reported. As observed, all the formulations release the 100% of the drug in the first 1400 min. However, the rate of drug release in the first 20 min is different in different formulations (Fig. 10, detail in the box). Practically, a lag time of 5 min is observed for all formulations except for Std2D.

As observed in the XRD spectra of drug loaded Ker and i-Car (Fig. S6 and S7), a highly attenuated, narrow peak remained visible at 15.1° , indicating the presence of a minor, residual crystalline fraction of Dex, co-existing alongside the dominant amorphous phase.

To elucidate the physical transport mechanisms governing dexamethasone release, the release profiles of formulations Std2D, Std11D, Std8D, and Std12D were fitted using both the Korsmeyer–Peppas and Peppas–Sahlin kinetic models. The Korsmeyer–Peppas model is described by the eq. 2:

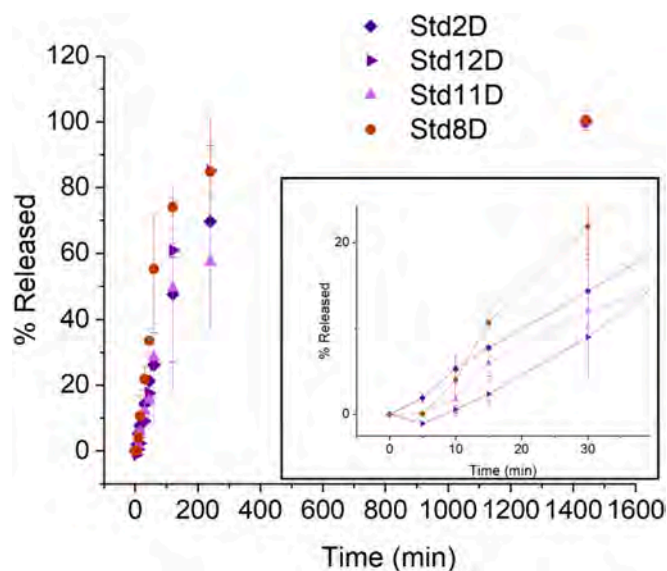
$$\frac{M_t}{M_\infty} = k_{KPT} t^n \quad (2)$$

where M_t denotes the mass of drug released over time t , M_∞ is the mass

Table 3

Kinetic parameters of the swelling profiles evaluated using the Schott and Korsmeyer-Peppas mathematical models.

Sample	SCHOTT				KORSMEYER-PEPPAS	
	S_{∞}	K_s (min ⁻¹)	r_0 (min ⁻¹)	R^2	n	R^2
Std3	26.11 ± 1.21	0.17 ± 0.02	24.57 ± 1.96	0.999	0.32 ± 0.07	0.990
Std10	12.85 ± 0.97	0.02 ± 0.01	3.08 ± 0.74	0.995	0.52 ± 0.05	0.930
Std4	10.87 ± 1.09	0.10 ± 0.03	11.38 ± 1.13	0.999	0.10 ± 0.02	0.890

**Fig. 10.** Drug release profiles of the dexamethasone loaded hydrogels. Detail of the profile in the first 30 min of the release.

of drug released when t approaches infinity (its upper limit should be the total mass of drug carried by the delivery system), and k_{KP} is the kinetic constant. The exponent of release n provides information on the possible mechanism(s) of drug release at play. To determine the exponent n , it is recommended to use the portion of release curve until the point where $M_t/M_{\infty} < 60\%$. For planar films, $n = 0.5$ indicates Fickian diffusion (Case I), $0.5 < n < 1.0$ corresponds to non-Fickian (anomalous) transport governed by coupled diffusion and polymer relaxation, and $n = 1.0$ indicates Case II (zero-order) transport driven predominantly by polymer chain relaxation and swelling.

To further decouple the relative contributions of diffusion and polymer relaxation, the release profiles were fitted with the Peppas-Sahlin model (Eq.3)

$$\frac{M_t}{M_{\infty}} = k_1 t^m + k_2 t^{2m} \quad (3)$$

where k_1 represent the kinetic constant related to the diffusional Fickian

contribution and k_2 , the kinetic constant related to the Case II relaxational contribution (Peppas & Sahlin, 1989). To exclude mathematical overparameterization the Fickian exponent m was fixed to its theoretical value for film-type geometrical shape ($m = 0.5$) and boundary constraints for the kinetic constants ($k_1 > 0$; $k_2 > 0$) were applied.

The resulting fitting parameters (n , K_{KP} , K_1 , K_2 , and R^2) are summarized in Table 4. The Korsmeyer-Peppas release exponent (n) varied significantly across hydrogel compositions. Formulations Std2D ($n = 0.86 \pm 0.02$) and Std11D ($n = 0.7 \pm 0.1$) displayed exponents falling within the classic range for cylindrical geometries ($0.45 < n < 0.89$), indicating anomalous (non-Fickian) transport. In these systems, drug release is driven by the coupled contribution of Fickian solvent penetration and macromolecular chain relaxation. This dual mechanism is strongly supported by the Peppas-Sahlin fitting parameters, where the Fickian diffusion rate constants ($k_1 = 0.8 \pm 0.2$ for Std2D and 1.9 ± 0.8 for Std11D) predominated over their respective relaxation rate constants ($k_2 = 0.33 \pm 0.02$ and 0.14 ± 0.07). Nevertheless, the relaxation contribution (k_2) is substantially higher in Std2D than in Std11D, which aligns with their distinct rheological fingerprints (Fig. 8). Std11D forms a remarkably dense and rigid network (Fig. 8I), strongly constraining physical chain relaxation during hydration. Conversely, Std2D exhibits a far more compliant network (Fig. 8a), offering lower resistance to chain relaxation upon contact with water.

Conversely, the crosslinked matrices Std8D ($n = 1.4 \pm 0.1$) and Std12D ($n = 1.32 \pm 0.05$) yielded diffusion exponents exceeding unity ($n > 1.0$). Exponents above the upper theoretical bound of the Korsmeyer-Peppas model ($n = 0.89$) indicate that drug movement is no longer governed by standard Fickian diffusion dynamics. When these profiles were evaluated using the Peppas-Sahlin model with a fixed Fickian exponent ($m = 0.5$), the diffusion contribution collapsed to zero, while, the relaxation term ($k_2 t^{1.0}$) completely dominated the release kinetics ($k_2 = 1.0 \pm 0.3$ and 0.7 ± 0.2 , respectively).

These combined model outputs demonstrate clear physical convergence: Fickian diffusion plays a negligible role in Std8D and Std12D. Instead, drug release in these formulations is governed almost exclusively by rapid polymer network relaxation, matrix disentanglement, and progressive hydrogel erosion (Mady & Donia, 2015). The shift from anomalous transport in uncrosslinked formulations (Std2D, Std11D) to relaxation-dominated Case II transport in ionic crosslinked matrices (Std8D, Std12D) stems from structural reorganization induced by Ca^{2+} ions. In uncrosslinked matrices, a loose network permits rapid water penetration and immediate Fickian diffusion. In crosslinked

Table 4

Kinetic parameters of the release profiles evaluated by using the Korsmeyer-Peppas and Peppas-Sahlin mathematical models.

Sample	KORSMEYER-PEPPAS			PEPPAS-SHLIN			Inferred Mechanism*
	k_{KP} (min ⁻ⁿ)	n	R^2	k_1 (min ^{-0.5})	k_2 (min ⁻¹)	R^2	
Std2D	0.76 ± 0.06	0.86 ± 0.02	0.998	0.8 ± 0.2	0.33 ± 0.02	0.997	Anomalous (Non-Fickian)
Std12D	0.11 ± 0.02	1.32 ± 0.05	0.996	0	0.7 ± 0.2	0.94	Anomalous (Non-Fickian)
Std11D	1.3 ± 0.7	0.7 ± 0.1	0.916	1.9 ± 0.8	0.14 ± 0.07	0.901	Relaxation/Erosion-dominated
Std8D	0.20 ± 0.08	1.4 ± 0.1	0.992	0	1.0 ± 0.3	0.91	Relaxation/Erosion-dominated

* Evaluated for cylindrical geometry. For $n \leq 0.45$, mechanism is Fickian diffusion; $0.45 < n < 0.89$ indicates anomalous (non-Fickian) transport; $n = 0.89$ corresponds to Case II transport. Values of $n > 1.0$ exceed the classical upper validity boundary of the Korsmeyer-Peppas model ($n = 0.89$). When evaluated alongside Peppas-Sahlin parameters ($k_1 \approx 0$ and $k_2 > 0$, fixed $m = 0.5$), this behaviour indicates release driven predominantly by macromolecular polymer chain relaxation, network disentanglement, and hydrogel matrix erosion rather than standard Fickian diffusion.

formulations, dense gel-vitreous domains act as physical barriers that suppress free diffusion ($k_1 = 0$), making release dependent on front-like hydration and mechanical chain relaxation. Furthermore, k_2 was higher for Std8D than for Std12D. Std12D possesses a rigid, tightly crosslinked network characterized by a narrow linear viscoelastic region and a low critical yield strain (γ_c , Fig. 8), limiting macromolecular mobility and slowing water penetration. Conversely, Std8D displays a lower storage modulus and higher critical yield strain, yielding a more compliant matrix that undergoes rapid hydration and relaxation, yielding a higher relaxation rate constant (k_2).

4. Conclusion

In this work a design of experiments approach was successfully used for mapping the printability of Ker/iCar hydrogels using an Ashby-type chart ($G'_{@LVR}$ vs σ_{flow}). By integrating response surfaces with this rheological map, this study established a predictive framework that accurately identifies the optimal blending ratios and crosslinker concentrations required to achieve high-fidelity 3D printing of Ker/i-Car hydrogels via Direct Ink Writing (DIW).

The Ashby-type printability map successfully established critical boundaries ($G'_{@LVR}$ and σ_f) for Ker/i-Car hydrogels, demonstrating that $2 \times 10^2 \text{ Pa} < G'_{@LVR} < 1.4 \times 10^3 \text{ Pa}$ and $20 \text{ Pa} < \sigma_f < 320 \text{ Pa}$, while strictly maintaining the ratio $G'_{@LVR}/\sigma_f \leq 20$ are essential conditions to avoid nozzle clogging or structural collapse during direct ink writing. Predictive response surfaces effectively mapped the complex dependencies of hydrogel elasticity and yield stress on input factors. The close agreement between the predicted and experimental validation runs confirms the robustness of this optimization strategy for engineering reliable DIW 3D printing inks.

Time-resolved Cole–Cole trajectories and Lissajous–Bowditch curves successfully mapped the transient structural transitions—spanning pre-yield strain stiffening, cooperative junction rupture, and viscous backflow—that mirror the real-world extrusion and deposition phases of DIW. Specifically, the characterization of the first derived moduli reveals that uncrosslinked blends transition from a rigid, junction-zone-dominated carrageenan network to a more strain-tolerant entanglement-driven system as the keratin content increases. Conversely, the addition of a calcium crosslinker shifts the yielding mechanism toward a brittle, cooperative rupture of ionic junctions, which eliminates flow heterogeneities (secondary loops) but results in a sudden, collective structural collapse at critical strain thresholds. The strain-stiffening (S) and shear-thinning (T) indices confirm that while ionic crosslinking accelerates the onset of nonlinear elasticity, the complex ‘stiffening-softening-stiffening’ profile observed in keratin-rich blends highlights a highly tunable network where chain alignment and cooperative junction dissociation dictate the material's failure threshold. The validation of the intracycle recovery loss factor ($\tan\delta_{rec}$) provides a robust, processing-relevant metric to screen hydrogel formulations for rapid elasticity recovery and geometric fidelity. Drug loading narrowed the linear viscoelastic region and significantly enhanced transient elastic energy storage before yielding, causing key formulations to shift into a different position in the printability map. Concurrently, multi-technique spectroscopic and structural analyses successfully elucidated the molecular mechanisms driving hydrogel assembly and drug integration. FTIR and XRD characterizations confirmed that while divalent calcium (Ca^{2+}) ions establish robust ionic crosslinking through direct coordination with the keratin backbone and carrageenan sulfate groups, inducing matrix compaction and highly ordered β -sheet crystalline registries. The dexamethasone interacts primarily via non-ionic, supramolecular hydrogen bonding. Crucially, these molecular insights provide a definitive rationale for the system's macroscopic rheological performance. In the absence of ionic crosslinkers, Dex effectively acts as a dynamic physical crosslinker by anchoring across open polymer sites, resulting in pronounced non-linear viscoelastic responses. Conversely, in densely

crosslinked networks, steric exclusion forces the drug to localize at macro-domain interfaces. Finally, mathematical modeling of the drug release kinetics confirmed a profound transition from diffusion-controlled anomalous transport in loose, uncrosslinked formulations to a purely relaxation-driven Super Case II transport mechanism in Ca^{2+} -crosslinked matrices. This kinetic shift was beautifully validated by experimental swelling rates and viscoelastic signatures, showing that while dense ionic crosslinking suppresses Fickian diffusion to zero ($k_1 = 0$), the absolute rate of relaxation-driven release (k_2) can be finely regulated by adjusting the biopolymer ratio to modulate network rigidity and hydration dynamics.

CRedit authorship contribution statement

Costanza Fratini: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Mattia Tiboni:** Writing – review & editing, Visualization, Validation, Investigation. **Itxaso Calafel:** Writing – review & editing, Validation, Investigation. **Mercedes Fernández:** Writing – original draft, Validation, Methodology, Investigation, Data curation, Conceptualization. **Robert Aguirresarobe:** Writing – review & editing, Validation, Methodology, Data curation, Conceptualization. **Annalisa Aluigi:** Writing – original draft, Validation, Supervision, Investigation, Data curation, Conceptualization. **Luca Casettari:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.carbpol.2026.125842>.

Data availability

Data will be made available on request.

References

- Agarwal, T., Costantini, M., & Maiti, T. K. (2021). Extrusion 3D printing with Pectin-based ink formulations: Recent trends in tissue engineering and food manufacturing. *Biomedical Engineering Advances*, 2, Article 100018. <https://doi.org/10.1016/j.bea.2021.100018>
- Aluigi, A., Zoccola, M., Vineis, C., Tonin, C., Ferrero, F., & Canetti, M. (2007). Study on the structure and properties of wool keratin regenerated from formic acid. *International Journal of Biological Macromolecules*, 41(3), 266–273. <https://doi.org/10.1016/j.ijbiomac.2007.03.002>
- Amorim, P. A., d'Ávila, M. A., Anand, R., Moldenaers, P., Van Puyvelde, P., & Bloemen, V. (2021). Insights on shear rheology of inks for extrusion-based 3D bioprinting. *Bioprinting*, 22, Article e00129. <https://doi.org/10.1016/j.bprint.2021.e00129>
- Atri, H., Bidram, E., & Dunstan, D. (2015). Reconstituted Keratin Biomaterial with Enhanced Ductility. *Materials*, 8(11), 7472–7485. <https://doi.org/10.3390/ma8115392>
- Byler, D. M., & Susi, H. (1986). Examination of the secondary structure of proteins by deconvolved FTIR spectra. *Biopolymers*, 25(3), 469–487. <https://doi.org/10.1002/bip.360250307>
- Dahl, J. F., Gregersen, S. B., Andersen, U., Schulz, H.-J., & Corredig, M. (2024). Small and large deformation rheology on pizza cheese as an example of application to study

- anisotropic properties of food soft materials. *Food Hydrocolloids*, 148, Article 109456. <https://doi.org/10.1016/j.foodhyd.2023.109456>
- Eckman, N., Grosskopf, A. K., Jiang, G., Kamani, K., Huang, M. S., Schmittlein, B., ... Appel, E. A. (2025). Crosslink strength governs yielding behavior in dynamically crosslinked hydrogels. *Biomaterials Science*, 13(6), 1501–1511. <https://doi.org/10.1039/D4BM01323A>
- Fontaine-Seiler, W. A., & G. J. D. E. D. G. D. L. B. (2025). *Exploring the Nonlinear Rheology of Composite Hydrogels: A New Paradigm for LAOS Analysis*. *Soft Condensed Matter*.
- García-Tuñón, E., Agrawal, R., Ling, B., & Dennis, D. J. C. (2023). Fourier-transform rheology and printability maps of complex fluids for three-dimensional printing. *Physics of Fluids*, 35(1). <https://doi.org/10.1063/5.0128658>
- Gillispie, G. J., Copus, J., Uzun-Per, M., Yoo, J. J., Atala, A., Niazi, M. K. K., & Lee, S. J. (2023). The correlation between rheological properties and extrusion-based printability in bioink artifact quantification. *Materials & Design*, 233, Article 112237. <https://doi.org/10.1016/j.matdes.2023.112237>
- Huang, T., Sun, Z., Heath, D. E., O'Brien-Simpson, N., & O'Connor, A. J. (2024). 3D printed and smart alginate wound dressings with pH-responsive drug and nanoparticle release. *Chemical Engineering Journal*, 492, Article 152117. <https://doi.org/10.1016/j.cej.2024.152117>
- Hyun, K., Wilhelm, M., Klein, C. O., Cho, K. S., Nam, J. G., Ahn, K. H., ... McKinley, G. H. (2011). A review of nonlinear oscillatory shear tests: Analysis and application of large amplitude oscillatory shear (LAOS). *Progress in Polymer Science*, 36(12), 1697–1753. <https://doi.org/10.1016/j.progpolymsci.2011.02.002>
- Insua, I., Etzold, O., Calafel, I., Aguirresarobe, R., Calderón, M., & Fernández, M. (2025). Rheological Insight into the 3D Printability of Carboxymethyl Cellulose-Based Hydrogels. *Gels*, 11(4), 259. <https://doi.org/10.3390/gels11040259>
- Iranmanesh, P., Gowdini, M., Khademi, A., Dehghani, M., Latifi, M., Alsaadi, N., Hemati, M., Mohammadi, R., Saber-Samandari, S., Toghraye, D., & Khan, A. (2021). Bioprinting of three-dimensional scaffold based on alginate-gelatin as soft and hard tissue regeneration. *Journal of Materials Research and Technology*, 14, 2853–2864. <https://doi.org/10.1016/j.jmrt.2021.08.069>
- Jongprasitkul, H., Turunen, S., Parihar, V. S., & Kellomäki, M. (2022). Two-step crosslinking to enhance the printability of methacrylated gellan gum biomaterial ink for extrusion-based 3D bioprinting. *Bioprinting*, 25, Article e00185. <https://doi.org/10.1016/j.bprint.2021.e00185>
- Kopnar, V., O'Connell, A., Shirshova, N., & Aufderhorst-Roberts, A. (2025). Mechanistic Origins of Yielding in Hybrid Double-Network Hydrogels. *Macromolecules*, 58(16), 8610–8621. <https://doi.org/10.1021/acs.macromol.4c02431>
- Li, B., Sun, Y., Yao, J., Wu, H., Shen, Y., Zhi, C., & Li, J. (2022). An environment-friendly chemical modification method for thiol groups on polypeptide macromolecules to improve the performance of regenerated keratin materials. *Materials & Design*, 217, Article 110611. <https://doi.org/10.1016/j.matdes.2022.110611>
- Li, H., Wang, Y., Guo, L., Huang, L., Li, X., & Gao, W. (2024). Polysaccharide hydrogels for diabetic wounds: Reasonable customization and regulatory mechanisms. *Chemical Engineering Journal*, 497, Article 154143. <https://doi.org/10.1016/j.cej.2024.154143>
- Mady, O. Y., & Donia, A. A. (2015). A new mathematic method for calculation of peppas-sahlin model constants and interpret the results in relation to zero order, higuhi, korsmeyer-peppas models and microcapsule structure image. *World Journal of Pharmaceutical Research*, 4(12), 2199–2246.
- Matos, A. F., Pricci, A., Benedetti, S., Tiboni, M., Aluigi, A., & Casertari, L. (2025). Optimized microfluidic blow spinning process for single-step manufacturing of crosslinked chitosan nanofilaments using a quality-by-design approach. *International Journal of Biological Macromolecules*, 321, Article 146279. <https://doi.org/10.1016/j.ijbiomac.2025.146279>
- Maurizii, G., Valentini, L., Sotgiu, G., Zamboni, R., Tonetti, C., Vineis, C., Canonico, B., Montanari, M., Tiboni, M., Casertari, L., & Aluigi, A. (2024). The dark side of the wool? From wool wastes to keratin nanofilaments through the solution blow spinning process. *International Journal of Biological Macromolecules*, 275, Article 133722. <https://doi.org/10.1016/j.ijbiomac.2024.133722>
- Moraes, I. C. F., & Hilliou, L. (2024). Viscoelastic Reversibility of Carrageenan Hydrogels under Large Amplitude Oscillatory Shear: Hybrid Carrageenans versus Blends. *Gels*, 10(8), 524. <https://doi.org/10.3390/gels10080524>
- Morgan, F. L. C., Fernández-Pérez, J., Moroni, L., & Baker, M. B. (2022). Tuning Hydrogels by Mixing Dynamic Cross-Linkers: Enabling Cell-Instructive Hydrogels and Advanced Bioinks. *Advanced Healthcare Materials*, 11(1). <https://doi.org/10.1002/adhm.202101576>
- Murthy, N. S. (2016). X-ray Diffraction from Polymers. In *Polymer Morphology* (pp. 14–36). Wiley. <https://doi.org/10.1002/9781118892756.ch2>
- Nashchekina, Y., Militina, A., Elokhoyskiy, V., & Ivan'kova, E., Nashchekin, A., Kamalov, A., & Yudin, V. (2024). Precisely Printable Silk Fibroin/Carboxymethyl Cellulose/Alginate Bioink for 3D Printing. *Polymers*, 16(8), 1027. <https://doi.org/10.3390/polym16081027>
- Park, J. D., & Rogers, S. A. (2020). Rheological manifestation of microstructural change of colloidal gel under oscillatory shear flow. *Physics of Fluids*, 32(6). <https://doi.org/10.1063/5.0006792>
- Patel, D., Haag, S. L., Patel, J. S., Ytreberg, F. M., & Bernards, M. T. (2022). Paired Simulations and Experimental Investigations into the Calcium-Dependent Conformation of Albumin. *Journal of Chemical Information and Modeling*, 62(5), 1282–1293. <https://doi.org/10.1021/acs.jcim.1c01104>
- Peppas, N. A., & Sahlin, J. J. (1989). A simple equation for the description of solute release. III. Coupling of diffusion and relaxation. *International Journal of Pharmaceutics*, 57(2), 169–172. [https://doi.org/10.1016/0378-5173\(89\)90306-2](https://doi.org/10.1016/0378-5173(89)90306-2)
- Pereira, L., Gheda, S. F., & Ribeiro-Claro, P. J. A. (2013). Analysis by Vibrational Spectroscopy of Seaweed Polysaccharides with Potential Use in Food, Pharmaceutical, and Cosmetic Industries. *International Journal of Carbohydrate Chemistry*, 2013, 1–7. <https://doi.org/10.1155/2013/537202>
- Pourshahrestani, S., Zeimaran, E., Kadri, N. A., Mutlu, N., & Boccaccini, A. R. (2020). Polymeric Hydrogel Systems as Emerging Biomaterial Platforms to Enable Hemostasis and Wound Healing. *Advanced Healthcare Materials*, 9(20). <https://doi.org/10.1002/adhm.202000905>
- Rajabi, M., Ali, A., McConnell, M., & Cabral, J. (2020). Keratinous materials: Structures and functions in biomedical applications. *Materials Science and Engineering: C*, 110, Article 110612. <https://doi.org/10.1016/j.msec.2019.110612>
- Ranjit, E., Hamlet, S., George, R., Sharma, A., & Love, R. M. (2022). Biofunctional approaches of wool-based keratin for tissue engineering. *Journal of Science: Advanced Materials and Devices*, 7(1), Article 100398. <https://doi.org/10.1016/j.jsamd.2021.10.001>
- Rau, D. A., Bortner, M. J., & Williams, C. B. (2023). A rheology roadmap for evaluating the printability of material extrusion inks. *Additive Manufacturing*, 75, Article 103745. <https://doi.org/10.1016/j.addma.2023.103745>
- Rogers, S. A., Erwin, B. M., Vlassopoulos, D., & Cloitre, M. (2011). A sequence of physical processes determined and quantified in LAOS: Application to a yield stress fluid. *Journal of Rheology*, 55(2), 435–458. <https://doi.org/10.1122/1.3544591>
- Roy Chowdhury, S., Dey, K., & Basu, B. (2025). 3D Extrusion-Printed Alginate–Gelatin Hydrogel Modified with Nanoscale Hydroxyapatite: A Comprehensive Understanding of Process Science and Evaluation of the Antimicrobial Property. *ACS Omega*. <https://doi.org/10.1021/acsomega.4c10743>
- Schott, H. (1992). Kinetics of swelling of polymers and their gels. *Journal of Pharmaceutical Sciences*. <https://doi.org/10.1002/jps.2600810516>
- Suo, H., Zhang, J., Xu, M., & Wang, L. (2021). Low-temperature 3D printing of collagen and chitosan composite for tissue engineering. *Materials Science and Engineering: C*, 123, Article 111963. <https://doi.org/10.1016/j.msec.2021.111963>
- Wang, Y.-Q., Han, Y.-T., Yan, J.-N., Du, Y.-N., Jiang, X.-Y., & Wu, H.-T. (2021). Gel properties and network structure of the hydrogel constructed by iota-carrageenan and Ala-Lys dipeptide. *International Journal of Biological Macromolecules*, 182, 244–251. <https://doi.org/10.1016/j.ijbiomac.2021.04.001>
- Zhao, Y., Truong, T., & Chandrapala, J. (2025). Milk Powder Formulations with Varying Casein to Whey Ratios and Calcium Addition: Physico-Chemical and Structural Properties and the Effect of Low-Frequency Ultrasound. *Foods*, 14(4), 685. <https://doi.org/10.3390/foods14040685>