Hydrogels: experimental characterization and mathematical modelling of their mechanical and diffusive behaviour

Journal:	*Chemical Society Reviews*
Manuscript ID	CS-REV-08-2017-000638.R2
Article Type:	Review Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Caccavo, Diego; University of Salerno, Industrial Engineering Cascone, Sara; University of Salerno, Lamberti, Gaetano; Università degli Studi di Salerno, Dipartimento di Ingegneria Industriale Barba, Anna; University of Salerno, Department of Pharmacy

***Chemical Society Reviews* – Referee Guidelines**

Chemical Society Reviews publishes succinct and reader-friendly articles on topics at the forefront of the chemical sciences. **Our aim is to only publish reviews of the very highest quality and international impact.**

Questions to consider when reviewing this article:

- Is the subject of broad general interest?
- Is it timely? Does it genuinely add to/complement the existing literature?
- Does it give an authoritative and state-of-the-art account?
- Are the authors qualified to write this review?
- Would it be more suitable for a specialised journal?

2015 Impact Factor: 34.090

Chemical Society Reviews publishes two article types:

Tutorial Reviews

Tutorial Reviews are concise, authoritative overviews of important contemporary topics in the chemical sciences. They should appeal to advanced undergraduates, the general research chemist who is new to the field, as well as the expert. They provide a solid introduction to the development of a subject, the latest breakthrough results and their implications for the wider scientific community.

Tutorial Reviews must be:

- **Accessible**: to advanced undergraduate students and beyond. Tutorial reviews are often used in advanced undergraduate and Masters studies.
- **Authoritative**: An essential introduction to the field will lay the foundation of knowledge in the area with approximately 25 seminal citations**,** serving as a springboard to further reading, followed by the most important recent advances.
- **Exciting**: the reviews should treat subjects where there has been significant recent advance and promote a rigorous view in a succinct form.
- **Short:** Maximum 12,000 word equivalents; 12 journal pages; 35 pages of A4 or American Quarto (double-spaced text and artwork).
- **Lightly referenced:** Maximum 50 citations; authors should credit the major contributors – old and new – only. The 'and references therein' tag is recommended to direct the reader to further reading. Multiple referencing (the use of a, b, *etc.*) is strongly discouraged.
- **Jargon free:** Specialist terms and symbols should be defined and fundamental ideas simply explained.

Key Learning Points: Tutorial Reviews should now include up to five key learning points that a reader should expect to gain from reading the review.

Review Articles

Review Articles provide an authoritative and in-depth understanding of important topics in the chemical sciences. They give a very high quality state-of-the-art account and a balanced assessment of the current primary literature.

Not suitable?

If you do not recommend publication of this article in *Chemical Society Reviews*, please indicate whether it might be suitable for a more specialised RSC journal (see http://pubs.rsc.org/en/journals).

With best wishes,

May Copsey

Dr May Copsey, MRSC
Editor, *Chemical Society Reviews*

We appreciate your time in serving as a reviewer. To acknowledge this, the RSC offers a **25% discount** on its books.

Gaetano Lamberti
Dipartimento di Ingegneria Industriale
Università di Salerno
Via Ponte don Melillo – 84084 Fisciano (SA) - Italy
Phone +39 089964077 – Fax +39 089964057
E-mail: glamberti@unisa.it

Dear David,

I got your e-mail dated 8th February 2017, with reference to the manuscript entitled "**Hydrogels: experimental characterization and mathematical modelling of their mechanical and diffusive behaviour**" (CS-REV-08-2017-000638.R1), submitted for publication on *Chemical Society Reviews*.

The only point remaining to be modified is related to some missing references, and it has been solved. We thank you and the referees for the work done in revising the manuscript.

Look forward to hear from you

Best Regards,

Fisciano (SA), 9th February 2018

Gaetano Lamberti

Gaetano Lamberti

Comments to the Author. I think the authors have done a fine job in addressing the reviewers' comments, and that the manuscript is nearly ready for final acceptance. There is just one aspect that needs attention: It does not appear that the references recommended by referee 2 were added in section two. In the authors' cover letter it is mentioned that few new references to 2D materials are added in section 2. In the revised text for section 2 the references numbered 50 - 54 are cited in the context of 2D materials, though close inspection of refs. 50 and 51 indicates the new references are different that the ones recommended by reviewer 2. The references numbered 50 - 54 that are currently in the revision do not appear to refer to 2D materials.

Answer. The reference manager software did not interact well with the word-processor revision mode, therefore the references were added to the database but they were not listed in the list of references. Now this problem has been solved.

Chemical Society Reviews

REVIEW

Hydrogels: experimental characterization and mathematical modelling of their mechanical and diffusive behaviour

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

D. Caccavo[a], S. Cascone[a], G. Lamberti [a, †] and A. A. Barba[b]

Hydrogels are materials widely used in countless applications, particularly in biomedical, pharmaceutical, and nutraceutical fields; because of their biocompatibility and their mechanical and transport properties. Several approaches are known to evaluate their properties; but only a few approaches are under development to describe mathematically their behaviour, in terms of how the materials answer to mechanical stimuli and how incorporated active substances are released. In this review, the main properties of hydrogels are summarized and the structure-properties relationships were investigated (i.e. how the macromolecular structure influences the properties of macroscopic samples made of hydrogels). A selection criterion is proposed based on the comparison of three characteristic times: relaxation time, diffusion time, and process time. Then, the most common experimental methods to investigate the hydrogel properties were summarized, along with the state-of-the-art of the mathematical modelling, with reference to mechanical and transport properties of hydrogels, with particular attention to the viscoelastic and to the poroelastic behaviours. Last but not least, some cases histories which can be classified as viscoelastic, poroelastic, or poroviscoelastic behaviours were presented.

1. Hydrogels: generalities, properties and selection

Hydrogels are polymeric networks made of hydrophilic macromolecules, able to absorb large amounts of water, increasing the samples volume, this phenomenon being known as “swelling”[1]. The networks can be made of homopolymers or copolymers, and the structure of the network is due to the presence of crosslinks, of chemical or physical nature. The stability of these crosslinks influences the hydrogel’s behaviour: for example, if these bonds can withstand water exposure for long times, the hydrogel will swell, otherwise, the degradation of the crosslinks will cause the hydrogel erosion.

The applications of hydrogels are numerous, in biomedical, pharmaceutical, material, food sciences and so on. Their properties, mainly relate to the ability of transport solvents (water)/active molecules (e.g. drugs or nutrients), and to the mechanical resistance, dictate the use. Therefore, a deep knowledge of the hydrogel’s properties (and of the structure-properties relationships) is highly desired in order to select the suitable material (and shape, and size) to be used in a given application. A summary of the most relevant parameters, along with their meanings, is given in Table 1.

A full material characterization is useful in order to gain the desired knowledge; furthermore, a detailed mathematical model can forecast the behaviour of materials, saving time and cost required from trial-and-error processes[2], currently followed in the development of novel systems.

The high level of interest in these topics is evident by a query to Web of Science Core Collection database. The papers published with the word ‘hydrogel’ as a ‘topic’ are 40’341 (26’373 of which published in the time span ‘2010-today’). If the query is carried out searching within the ‘topic’ for both the words ‘hydrogel’ and ‘characterization’ the results are 4’024 (2’662 in ‘2010-today’). Therefore, roughly the 10% of the papers regarding hydrogels are also focused on characterization, and about 65% of papers were published in the last 8 years. To have an idea of the relevance of mathematical modelling the query has been repeated using the words ‘hydrogel’ and ‘mathematical’ (since the word ‘model’ is used also with different meanings), and the results are 334 (200 in ‘2010-today’). Less than 1% of papers are then focused on mathematical modelling in the ‘hydrogel’ field of study. For all these data the number of papers and the number of citations show increasing trends, testifying the growing interest on the topic (all the queries have been made in August 2017).

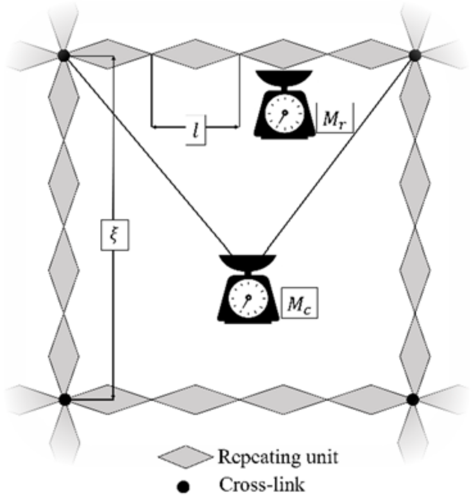
1.1 Hydrogel classification(s) and properties

Hydrogels can be classified according to several criteria[1, 3-5] based on nature of macromolecular structure, on final form of the system, on nature of crosslinks. This last is the most widespread one, dividing the physical (or reversible) hydrogels in simple entanglement systems, ion-mediated or ionotropic networks, and thermally induced networks; and the chemical (or permanent) hydrogels in cross-linked polymers, copolymers obtained by monomer + cross-linker, conversion of hydrophobic polymers into hydrophilic polymers, interpenetrating networks (IPN). Due to the relevance of the crosslinks nature, this will be treated in the next section.

---

[a] *Dept. Industrial Engineering, University of Salerno, Via Giovanni Paolo II, 132, Fisciano (SA)*
[b] *Dept. Pharmacy, University of Salerno, Via Giovanni Paolo II, 132, Fisciano (SA)*
† glamberti@unisa.it, +39089964077

Table 1. Parameters useful to characterize the structure and the properties of hydrogels.

Symbol	Description	Meaning	
Structural parameters: chain-related			
l	Length of the bond along the polymer backbone	It depends on the nature of the polymer chain and on the composition of the repeating unit	
M_r	Molecular weight of the repeating unit	It depends on the type of polymer	
Structural parameters: crosslinks-related			
M_c	Molecular weight between two crosslinks	It is a measure of the degree of reticulation of the hydrogel	
ξ	Mesh size	It is the distance between crosslinking points into the network, thus it can be seen as a measure of the space available between the macromolecular chains. The mesh size can be influenced by the cross-linking density, but also by the external environment including the temperature and solvent chemistry (pH and ionic strength)[6]	
ϕ	Hydration	It is a measure of the amount of water retained in the hydrogel	
Properties parameters			
E	Elastic modulus (Young's modulus)	It is a measure of the stiffness of the hydrogel. It defines the relationship between the applied stress and the consequent strain on the sample	
τ_R	Relaxation time	It defines the time required by a hydrogel to dissipate energy by viscous phenomena. It is an intrinsic property of the network	
$\mathfrak{D}$	Water diffusivity	It is a measure of the ability of water to move into the polymeric matrix	
Process parameters			
τ_D	Diffusion time	It is a measure of the time required by the solvent to travel a characteristic length. It depends on a intrinsic property ($\mathfrak{D}$) and on the geometrical characteristics of the sample	
τ_P	Process time	It is the characteristic time of the process at which the hydrogel is subjected. It depends on the application of the specific process	

Diego Caccavo is a postdoc fellow at University of Salerno. He received his PhD in Industrial Engineering at University of Salerno in the 2017, which focused on the analysis and modelling of the behaviour of hydrogels. In the 2013 he received his MSc in Chemical Engineering (Summa Cum Laude, awarded by AIDIC-GRICU and Rotary International D2100) at the University of Salerno with a thesis partially developed at the Chalmers University of Technology (Sweden). His main research activities concern the analysis and modelling of hydrogel-based systems behaviour for biomedical and agro-food applications, the modelling of drug release and wet granulation processes.

Sara Cascone is a research fellow at University of Salerno. She received her MSc in Chemical Engineering (Summa Cum Laude with Special Mention) and her BSc in Chemical Engineering (Summa Cum Laude, awarded by AIDIC-GRICU) in 2009 and 2007, respectively. She obtained her Ph.D. in "Science and Technology for Chemical, Pharmaceutical, and Food Industry" from University of Salerno in 2013. Dr. Cascone has published more than 30 research papers in peer-reviewed journals. Her research interests include: the development of pharmacokinetic models, the study of the phenomena involved in the drug release, and the characterization of hydrogels' behavior.

1.1.1 Crosslinks and chemical structure

Type and density of bonding between the polymeric chains can greatly influence the property of the resulting hydrogel. Generally, the higher the cross-linking density, the stiffer the hydrogel.

The type of cross-links within a certain polymeric network can be of different nature, i.e. in the most complex case covalent bonded polymeric chains can further bind to each other through noncovalent interactions (hydrogen bonding, dipole-dipole etc.) forming a so called supramolecular structure[7].

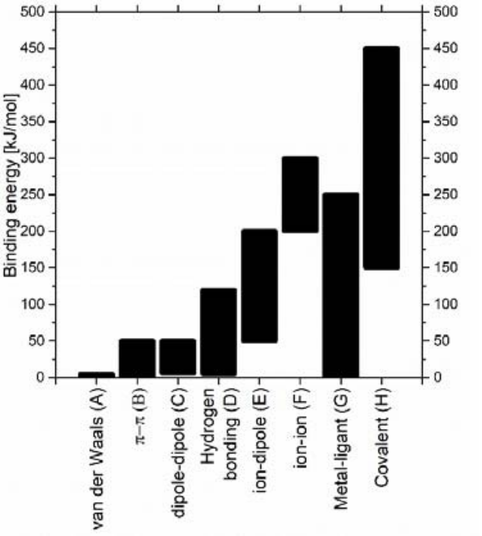


Fig. 1 Binding energy depending on the type of bond. Each type of bond is denoted by a capital letter, A to H. Figure redrawn from[8].

Depending on the strength of the bonding, the behaviour of the hydrogel can be greatly different. A covalent bonded structure tends to manifest and elastic behaviour, due to persistence of the cross-links that tightly holds the polymeric chains and confers a rubber-like behaviour to the hydrogel. Hydrogels formed by secondary bonds (i.e. hydrogen bonds (Agarose) or hydrophobic interaction (Hydroxy-Propyl-Methyl-Cellulose, HPMC) tends to manifest a viscoelastic (or at limit a viscoplastic) behaviour, due to the mechanism of breaking and reformation of the cross- links, which allows the reorganization of the polymeric structure. The previous reasoning can be extended to the other types of cross-links (both primary and secondary) (Fig. 1, in which each kind of bond is denoted by a letter), considering that the higher the binding energy the lower the possibility to reorganize the polymeric network and therefore the higher the elastic contribution over the viscoelastic one.

1.1.2 Elastic modulus

The elasticity of the hydrogel, or its stiffness, can be expressed with several mechanical parameters. The most immediate are the Young's modulus ($E, [Pa]$), which is a measure of the tensile elasticity (ratio of the structure tensile stress to the extensional strain[9]) and the shear modulus ($G, [Pa]$), also known as modulus of rigidity, that is the ratio of shear stress to the shear strain[9]. These moduli are related with functionality of increasing complexity depending on the complexity (anisotropy) of the material. Having in mind of reasoning about the order of magnitude of the stiffness of the hydrogels, the assumption of perfectly incompressible isotropic material leads to the well-known relation $E = 3G$ [10]. The shear modulus of the dry hydrogel (G_{dry}) can be related, with the aid of the rubber elasticity theory (assuming a Gaussian chains distribution and using the affine network theory)[11, 12], to the density of cross-links in the structure. It can be demonstrated [13] that the shear modulus of the swollen hydrogel can be scaled with the polymer volume fraction, ϕ_2, most commonly (in a θ solvent) to the power of 1/3 [6, 14]:

$$G = G_{dry}\phi_2^{\frac{1}{3}} = \nu RT\phi_2^{\frac{1}{3}} = \frac{\rho_2}{M_c}RT\phi_2^{\frac{1}{3}} \qquad (1)$$

Where ν is the cross-links density $[mol/m^3]$, R is the gas constant $[8.314\ J/(mol \cdot K)]$ and T the temperature $[K]$. The

Prof. Gaetano Lamberti, Ph.D., is a chemical engineer, and he teaches Transport Phenomena at Department of Industrial Engineering, University of Salerno, Italy. His research interests are focused on the applications of transport phenomena in pharmaceutical/biomedical sciences, as well as in food science, and on polymer transformation processes, with special emphasis on flow induced crystallisation. The results of his research and an overview on his research group are summarized on the web site gruppotpp.unisa.it. Gaetano Lamberti is the single author or a co-author of more than 110 papers published on international journals on these subjects.

Prof. Anna Angela Barba, Ph.D., is a chemical engineer. She teaches Industrial Pharmaceutical Plants at Department of Pharmacy, University of Salerno, Italy. Her research activities are focused on the development of non-conventional techniques, involving microwave energy and ultrasonic energy, in the production of active molecules delivery systems and in the agro-food treatments. The results of her scientific activities are reported in numerous articles published in international journals (over 80), in national technical journals, in communications at international and national conferences (over 100), in books/monographs (over 20). An overview on her research group are available on the web site gruppotpp.unisa.it cross-links density can be related to the dry mass polymer concentration ρ_2 $[g/m^3]$ ($\sim 1200\ kg/m^3$) and to the number average molecular weight of the chain between two cross-links M_c $[g/mol]$.

In Fig. 2 the Young's modulus versus M_c is reported for several hydrogels. The data, in terms of E or G (calculated in some cases from the storage and loss moduli, see "relaxation time" section), were derived from literature works[15-34], along with the polymer volume fraction, and M_c was obtained from Eq. 1. Different approaches could be used to calculate M_c, for example from the Flory-Huggins free volume theory and its simplifications (i.e. Eq. 4) but this would require the knowledge of further parameters (such as the Flory-Huggins solvent-polymer interaction parameter). Such kind of approaches, for ideal polymeric network (affine network) in a θ solvent would lead to the same results reported in Fig. 2, whereas nonidealities (of the network or in the interaction polymer-solvent) would produce a deviation from the trend reported in Fig. 2. However this approach goes beyond the aim of this section, which is to display a simple but yet general relation between the elastic constant and the degree of reticulation.

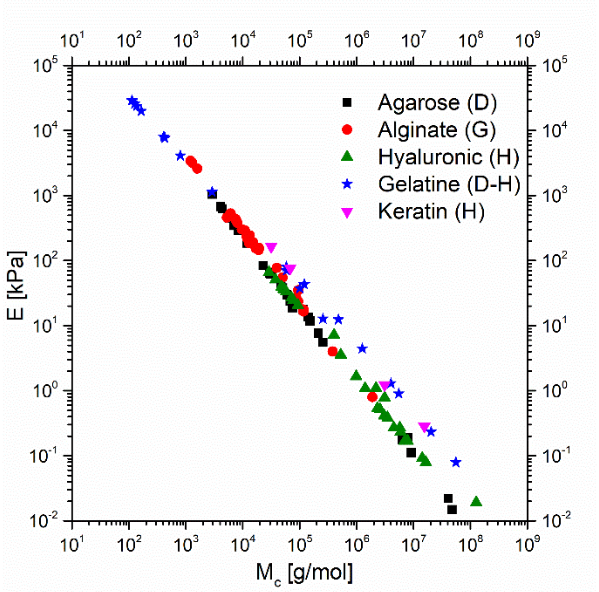


Fig. 2 Young's modulus function of the molecular weight between two cross-links for several hydrogels. The value of the elastic moduli have been obtained from: Agarose[17, 18, 23, 27, 31, 32, 34], Alginate[22, 24, 25, 30, 32], Hyaluronic[16, 20, 26, 33], Gelatine[15, 19, 28, 29], Keratin[21]. In the legend, the capital letters between brackets indicate the prevalent bond type (Fig. 1) that was used to form the hydrogels.

As it can be seen, the higher M_c the lower the elastic modulus, E. This is due to the lower density of cross-links or, in other terms, due to the longer distance between the cross-links, which produces softer hydrogels. It results from this consideration that properly tuning the molecular weight between the cross-links it is possible to obtain hydrogels of the same type with elastic properties that differ in order of magnitude. From Eq. 1 it is evident that to increase the stiffness, the polymer concentration can be increased or M_c decreased, which translates in the requirement of higher quantity of cross-linkers.

1.1.3 Relaxation time

The polymeric network that forms the skeleton of the hydrogel rarely behaves as a purely elastic solid. Due to conformational changes of the structure (i.e. breaking and reformation of cross-links) the energy can be dissipated generating viscoelastic behaviours. The viscoelastic nature of the hydrogels, most of the time, is studied with the oscillatory rheometry, obtaining the mechanical moduli G' and G'' in the frequency domain, which are the storage and the loss moduli, respectively. The first is a measure of the energy "stored" by the sample, therefore is directly related to the elasticity. The latter is a measure of the energy "loss" by the sample through viscous dissipations, therefore it is related to the viscosity of the sample [35]. It is of a great practical use to convert dynamic mechanical data, which are easy to measure, from frequency domain to time domain, obtaining the relaxation modulus $G(t)$ [35]:

$$G(t) = G'(\omega) - 0.4G''(0.40\omega) + 0.014G''(10\omega) \qquad (2)$$

where $t = 1/\omega$. The relaxation modulus $G(t)$ can be described with several rheological models that combining springs and dashpots elements aim to describe the material viscoelasticity. For linear viscoelasticity (where the elastic moduli do not depend on deformation) the most general viscoelastic model is the so called "generalized Maxwell" model, which combines a spring in parallel with "n" Maxwell elements [36, 37]. Each Maxwell element (composed by a series of a spring and a dashpot) is characterized by an elastic modulus and a relaxation time. The ensemble of the relaxation times of the "n" Maxwell elements defines the relaxation spectra of the material. In this review, for sake of simplicity, the relaxation modulus obtained by using Eq. 2 from the storage and loss moduli, derived from literature works, has been fitted with the Standard Linear Solid (SLS) rheological model, which corresponds to a generalized Maxwell with only a Maxwell element in parallel with a spring. The experimental exponential decay of $G(t)$ has been fitted with the exponential decay predicted by the SLS model:

$$G(t)_{SLS} = G_1 + G_2 \exp\left(-\frac{t}{\tau_R}\right) \qquad (3)$$

Where G_1 (also noted as G_∞ or G_R) correspond to the elastic modulus of the relaxed (R) hydrogel and it is representative of the stable cross-links which confer a pure elastic behaviour to the system. G_2 and τ_R (or in general G_j and τ_{R_j} with $j = 2 \dots n$ Maxwell-elements) are representative of the viscoelasticity of the system and they are related to the amount of transient cross-links that with their breakage and reformation produce the viscous dissipations. The sum of G_1 and G_2 (in general $G_1 + \sum_{j=2}^{n} G_i$) is representative of the mechanical short time response of the unrelaxed (U) hydrogel (also called G_0 or G_U). Taking experimental $G(t)$ data from several literature works[16-18, 24, 28, 30, 33, 34], the relaxation time τ_R [s] was obtained by the fitting of the SLS model to the experimental $G(t)$ for different hydrogels. Then, in Fig. 3, the relaxation time, τ_R $[s]$, is reported against the number average molecular weight of the chain between two cross-links, M_c $[g/mol]$.

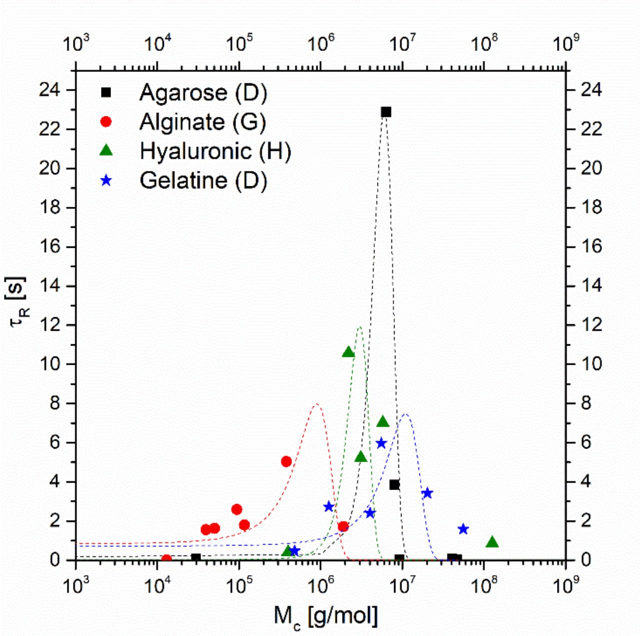


Fig. 3 Viscoelastic relaxation time function of the molecular weight between two cross-links for several hydrogels. The dashed lines (Gaussian curves) are intended to be a qualitative aid in recognizing the trend of the experimental data (just a "guide for the eye"). The rheological data are from: Agarose[17, 18, 34], Alginate[24, 30], Hyaluronic[16, 33], Gelatine[28]. In the legend, the capital letters between brackets indicate the prevalent bond type (Fig. 1) that was used to form the hydrogels.

The values for M_c have been obtained using Eq. 1 and G_1 as representative shear elastic modulus. It can be seen that for very stiff systems, which are characterized by high values of G_1 and therefore low values of M_c, the relaxation time tends to zero, being the hydrogel elasticity predominant over the viscous dissipations, in other terms the system is purely elastic. For low values of G_1 and therefore high values of M_c, the system present very few cross-links and tend to be more viscous than elastic, therefore, like a liquid, the relaxation time tend to zero due to the instantaneous possibility to rearrange the structure. For intermediate range of M_c the viscoelastic behavior is enhanced and the relaxation time present a maximum.

1.1.4 Water diffusivity

Hydrogels are characterized by the presence of huge amounts of water (or water-based solutions) within the polymeric network, which can enter or leave the system, giving rise to volumetric deformations. The water transport in the gel phase is complex and mainly driven by diffusion process, despite most of the time it does not follow a pure Fickian behavior[1]. Two limiting cases can be identified to describe the water diffusion in hydrogels, the matrices being in rubber or in glass state. When hydrogels are in the rubber conditions (i.e. when the water concentration is high enough to cause system to be above the glass-rubber transition), the water transport is similar to the diffusion in viscous media, and the Fick's law can be used to describe molecular transport, using diffusion coefficients expressed as function of hydrogel's water content. When hydrogels are in the glass state, the diffusion process can be compared to diffusion in solid elastic media, which can be described by the Fick's law with a constant diffusivity coefficient. However, another intermediate diffusion behavior can occur, the so called "anomalous transport" [38], which is representative of viscoelastic diffusion (diffusion in relaxing media). In this case the solvent transport within the hydrogel matrix is affected by a series of complex phenomena, involving even the system mechanics.

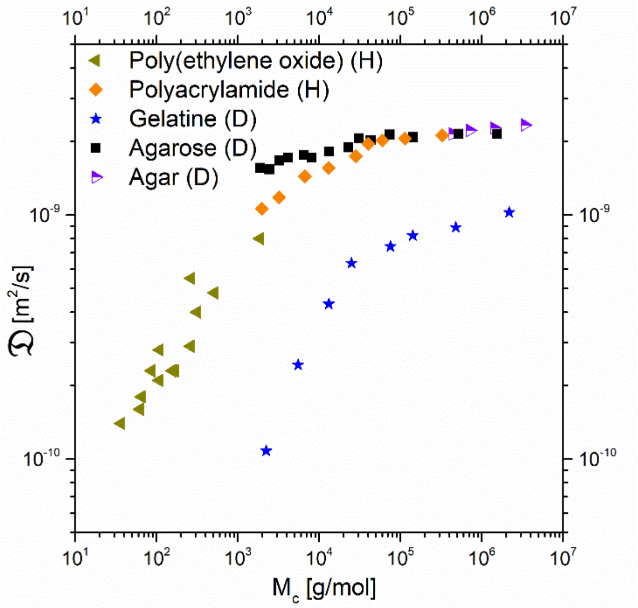


Fig. 4. Water diffusivity in the hydrogels as a function of the molecular weight between two cross-links for several hydrogels. The experimental data are from Poly(ethylene oxide)[39], Polyacrylamide[40], Gelatin[41], Agarose[42], and Agar[43]. In the legend, the capital letters between brackets indicate the prevalent bond type (Fig. 1) that was used to form the hydrogels.

Solvent transport within hydrogels mainly occurs between the spaces delineated by the polymer chains. Consequently, any factor that modifies these spaces affects the water movement and its diffusion coefficient across the polymer chains. These factors include structural parameters, such as the network mesh size (accounting for length and molecular weight between two crosslinks, which determine the chain mobility), physical parameters, such as the size of the solute (compared with the size of the openings between polymer chains), and chemical parameters, such as the presence of charged groups in the polymer network, which can bind solute molecules. In general, the water diffusivity through a cross-linked hydrogel decreases with increasing the cross-linking density and increases with the volume fraction of water within the gel. The dependence of the water diffusivity on these parameters can be explained and quantified by several theories (i.e. free volume, hydrodynamic, obstruction theories) or a combination of them[1, 44].

In Fig. 4 the diffusion coefficient of water in several hydrogels[39-43], $\mathfrak{D}\,[m^2/s]$, is reported as function of the number average molecular weight of the chain between two cross-links M_c $[g/mol]$. The diffusivity values are taken from literature, whereas the M_c values were derived from a simplified expression of that proposed by Peppas and Merrill[45]:

$$M_c = -\frac{\frac{\Omega_1}{\bar{v}}\cdot\left({\phi_{2s}}^{\frac{1}{3}} - \frac{\phi_{2s}}{2}\right)}{\ln(1-\phi_{2s}) + \phi_{2s} + \chi_{12}\,{\phi_{2s}}^{2}} \qquad (4)$$

Where: Ω_1 is the molar volume of water (18 cm^3/mol); $\bar{v}$ is the specific volume of the bulk polymer in the amorphous state; ϕ_{2s}

is the final swollen equilibrium polymer volume fraction; and χ_{12} is the Flory-Huggins solvent-polymer interaction parameter.

Looking at Fig. 4 it can be seen that the diffusivity of water within a hydrogel increases as the molecular weight between crosslinks increases. This is due to a longer distance between crosslinks, which allows an easier diffusion of water between the polymer chains: the network's mesh size is larger. Depending on the hydrogel nature, which involves its structure, bonds, and crosslinking, the diffusivity value can vary order of magnitude changing M_c value. It can be concluded that, if M_c is conveniently set, the transport properties of the final hydrogel can be tuned for specific applications.

1.2 Hydrogel selection criterion

The mechanical response of the hydrogel can be coupled with solvent movement accounting for a complex phenomenon known as poroviscoelasticity. With this term, it is intended that a viscoelastic behaviour is associated with a poroelastic one. The viscoelasticity is generated by conformational changes of the structure, i.e. cross-links reformation or "short-range" motion of the water molecules, with the change of the hydrogel's shape at constant volume. The poroelasticity, instead, is produced by "long-range" motion of the water molecules, where the number of solvent molecules locally are not conserved, which results in the swelling or shrinking of the hydrogel[46].

1.2.1 Coupling diffusion, viscous relaxation and process times

The hydrogels behaviour at last can be described by the resultant of the combination of the diffusion time (τ_D), viscous relaxation time (τ_R) and process time (τ_P).

The diffusion time can be calculated as the ratio of the characteristic length of diffusion L_{CH} to the second power over the diffusivity of the solvent $\mathfrak{D}$:

$$\tau_D = L_{CH}^2/\mathfrak{D} \qquad (5)$$

The viscous relaxation time τ_R is the material characteristic introduced in previous section 1.1.3.

The process time τ_P is the characteristic time of the process, in which the sample made of hydrogel is intended to be used.

Comparing these times it is possible to identify the behaviour of hydrogels.

1.2.1.a Limiting conditions

Looking at the limiting conditions, first four cases can be individuated as summarized in Table 2.

Table 2. Strong differences between characteristic times

Case	Conditions	The hydrogel behaves …
1	$\begin{cases}\tau_P \ll \tau_R \\ \tau_P \ll \tau_D\end{cases}$	… as an elastic solid with unrelaxed moduli with negligible migration of solvent.
2	$\begin{cases}\tau_P \ll \tau_R \\ \tau_P \gg \tau_D\end{cases}$	… as an elastic solid with unrelaxed moduli with pronounced migration of solvent.
3	$\begin{cases}\tau_P \gg \tau_R \\ \tau_P \ll \tau_D\end{cases}$	… as an elastic solid with relaxed moduli with negligible migration of solvent.
4	$\begin{cases}\tau_P \gg \tau_R \\ \tau_P \gg \tau_D\end{cases}$	… as an elastic solid with relaxed moduli and pronounced migration of solvent.

Cases 1-4 are all characterized by an elastic behaviour, because the relaxation is slower (cases 1 and 2) or faster (cases 3 and 4) than the process. Meanwhile the diffusion can be slower than the process, causing a minimum solvent transport (cases 1 and 3), or faster than the process, causing a large solvent transport (cases 2 and 4).

When the process time is comparable with the diffusion or with viscous relaxation time, another four cases can be individuated as in Table 3.

Table 3. Process time is comparable with diffusion time or with viscous relaxation time.

Case	Conditions	The hydrogel can manifest …
5	$\begin{cases}\tau_P \gg \tau_R \\ \tau_P \sim \tau_D\end{cases}$	… poroelastic relaxation with relaxed moduli.
6	$\begin{cases}\tau_P \sim \tau_R \\ \tau_P \ll \tau_D\end{cases}$	… viscoelastic relaxation with negligible migration of solvent.
7	$\begin{cases}\tau_P \ll \tau_R \\ \tau_P \sim \tau_D\end{cases}$	… poroelastic relaxation with unrelaxed moduli.
8	$\begin{cases}\tau_P \sim \tau_R \\ \tau_P \gg \tau_D\end{cases}$	… viscoelastic relaxation with relevant migration of solvent.

In cases 5 and 7 the process time is comparable with diffusion time, then the solvent transport takes place during the process. The macromolecules relaxation, on the other side, is faster than the process (case 5) or slower than the process (case 7), therefore the viscoelastic behaviour is not shown by these systems. Cases 5 and 7, then, are classified as poroelastic behaviours.

Since the process time is comparable with the relaxation time, cases 6 and 8 are characterized by a viscoelastic behaviour (due the relaxation of macromolecules, which takes place in the same timescale of the process). In case 6 the diffusion is slower than the process, therefore there is a limited solvent transport; in case 8 the diffusion is faster than the process, therefore there is a large solvent transport.

1.2.1.b Dynamic conditions

In dynamic conditions the process time represents the extreme of the time interval $t = [0, \tau_P]$. Considering τ_P greater than the viscous relaxation time and the diffusion time three cases can be individuated, and they are listed and commented in Table 4. All these cases can be represented on a plane τ_D/τ_R versus t/τ_R, represented in Fig. 5, where the dashed lines $t = \tau_D$ and $t = \tau_R$ define four regions, representative of the cases 1-4, instead the four segments generated by these dashed lines represent the cases 5-8. Continuous lines represent the dynamic cases 9-10.

Table 4. Dynamic conditions, with (final) processing time larger than diffusion time and than viscous relaxation time.

Case	Condition	The hydrogel experiences …
9	$\{\tau_R \ll \tau_D$	… first the viscoelastic relaxation and then the poroelastic relaxation with relaxed moduli.
10	$\{\tau_R \gg \tau_D$	… first the poroelastic relaxation with unrelaxed moduli and then the viscoelastic relaxation.
11	$\{\tau_R \sim \tau_D$	… the superposition of the viscoelastic and poroelastic relaxation.

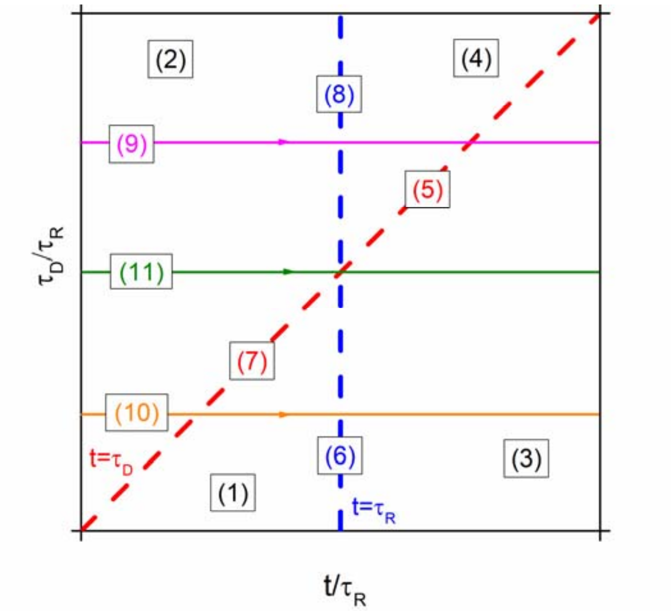


Fig. 5 Behaviour of the hydrogel depending on the characteristic times: diffusion time τ_D, viscous relaxation time τ_R and process time τ_P (limiting conditions) or t (dynamic). The ordinate is also known as the inverse of the diffusional Deborah number $N_{De.D}^{-1}$ [1]. The vertical (blue) dashed line represents $t = \tau_R$, the oblique (red) dashed line represents $t = \tau_D$. The number 1-8 individuate the limiting cases described in Table 2 and in Table 3. The number 9-11 individuate the dynamic behaviour describe in Table 4. Figure reinterpreted from [47].

The Fig. 5, qualitative in this case, can be built for each specific hydrogel once the relaxation time, the diffusion time and the process time are known. The interesting fact is that the dynamic response can be changed from case 9 to case 11 by changing the diffusion time τ_D, which depends on the characteristic length of diffusion. Therefore properly choosing the hydrogel (with its intrinsic characteristics τ_R and $\mathfrak{D}$) and tuning the characteristic length of diffusion L_{CH}, it is possible to modify the hydrogel behavior in the process time of interest. A quantitative example is given in the simulation study reported in section 4.3. The map in Fig. 5 can be used as a selection criterion for hydrogels in the following way. Let assume that, for example, the system is expected to have a poroelastic behaviour, meaning that the solvent has to be expelled or absorbed because of an external mechanical stimulus. In this case, the process time has to be, in order of magnitude, equal or greater than the diffusion time (cases 5, 7 or 2, 8, 4 of previous classification). This means that, if the hydrogel's diffusivity is not tuneable (e.g., acting on M_c as in Fig. 4), then the sample has to be built with a given size, in order to realize the characteristic length of diffusion, L_{CH}, to get the diffusion time (by Eq. 5) matching the process time. Similarly, if the scope is to minimize the solvent migration, the cases 1, 3, or 6 are of interest. Therefore, the processing time has to be kept lower than the diffusion time (acting on $\mathfrak{D}$ or on L_{CH}).

2. Experimental methods for hydrogel preparation and characterization

2.1 Methods of preparation

As presented above, hydrogels are hydrophilic polymeric networks cross-linked in some fashion to produce elastic structure. In theory, any technique, which is suitable to create cross-linked polymers, can be used to produce a hydrogel[5]. The cross-linking of the polymer chains can be realized in a number of different ways: i) using a chemical reaction, ii) using ionizing radiation to generate main-chain free radicals, which can recombine as cross-link junctions, iii) using physical interactions. From a preparative point of view, cross-linked hydrogels can be obtained by graft polymerization, cross-linking polymerization, and radiation cross-linking[5].

The main drawbacks of the single-network hydrogels are the weak mechanical properties and the slow response at swelling[48]. In order to improve these features, multicomponent networks as interpenetrating polymer networks (IPNs) have been designed. IPNs result from the combination of cross-linked polymers, at least one of them being synthesized within the immediate presence of the other. The preparation of these systems can take place according two main methods: i) the precursors of both networks are mixed and the two networks are synthesized at the same time by independent, noninterfering routs, obtaining simultaneous IPN, or ii) a single-network hydrogel swells into a solution containing the mixture of monomer, initiator, activator, and cross-linker, obtaining sequential IPN[48]. Thus, the macromolecular structure of hydrogels depends on the constituents used during the preparation: linear co-polymers, block co-polymers, graft copolymers, hydrophilic networks stabilized by hydrophobic domains, H-bonded complexes and IPNs or physical blends can be obtained[49].

During the last years, attention has been paid on the use of inorganic 2D mineral lattices as fillers[50]. They are incorporated into polymers to improve the bulk properties such as shrinkage on molding, stiffness, heat resistance, or electrical conductivity[51]. There are several procedures to incorporate inorganic layered 2D materials into a polymer matrix: (i) to insert molecular layers of polymer chains between the sheets of the inorganic host, obtaining intercalated hybrids[52], (ii) to disperse homogeneously into the polymer matrix exfoliated layers of the inorganic host, obtaining delaminated hybrids, or (iii) to produce systems between these two extremes[53].

2.2 Methods of characterization

2.2.1 Structural parameters

The composition of the nano- and microstructure of polymer networks is of crucial relevance to determine the macroscopic characteristics of the hydrogel. In the following section the methods commonly used to evaluate the structural parameters of hydrogels' network are described.

2.2.1.a Hydration

The hydration degree of a hydrogel is the measurement of the solvent amount retained into the polymer network. The phenomenon of water uptake (or release) through a hydrogel network is complex and it can be described mainly by diffusion mechanism. There are several theories connecting the water diffusion process to other structural parameters[1]. The simplest and most used experimental method to determine the hydration degree of a hydrogel is the gravimetric technique. It is based on the weighting of the samples in dry and hydrated state to calculate the water absorbed. Strongly connected to the hydration, the polymer volume fraction in swollen state, which is given by the ratio between the dry polymer volume and the swollen gel volume, is an important parameter. It can be seen also as the reciprocal of the volumetric swelling ratio.

2.2.1.b Molecular weight between two crosslinks

The molecular weight between two crosslinks is a key parameter to identify the crosslinking degree of hydrogels. This parameter has to be evaluated on swollen hydrogels in the equilibrium state. The most diffused expression to evaluate M_c is that derived by Flory and Rehner[54]:

$$\frac{1}{M_c} = \frac{1}{M_n} - \frac{\bar{\upsilon} \cdot \left[\ln(1-\phi_{2s}) + \phi_{2s} + \chi_{12}\, {\phi_{2s}}^2\right]}{\Omega_1 \cdot \left({\phi_{2s}}^{\frac{1}{3}} - \frac{\phi_{2s}}{2}\right)} \qquad (6)$$

Where: M_n is the number-average molecular weight of the polymer, $\Omega_1$is the molar volume of water (18 cm^3/mol); $\bar{\upsilon}$ is the specific volume of the bulk polymer in the amorphous state; ϕ_{2s} is the final swollen equilibrium polymer volume fraction; and χ_{12} is the Flory-Huggins solvent-polymer interaction parameter. Equation 4 is a simplified version of this expression. Later, equation 6 has been modified to take into account the hydration level of the polymer after the crosslinking and before the swelling[55]. Thus, known the polymer characteristics (molecular weight, specific volume, Flory-Huggins solvent-polymer interaction parameter), M_c can be derived from hydration experiments (both in the equilibrium state and after the crosslinking).

As mentioned in section 1.1.2, the molecular weight between two crosslinks can be connected, by means of the rubber elasticity theory, to the shear modulus (equation 1). It results that an evaluation of M_c can be deduced from the rheological analyses, measuring the shear modulus of the hydrogel.

2.2.1.c Mesh size

Mesh size, usually identified with ξ, is defined as the distance between crosslinking points into the network. It is a measure of the space available between the macromolecular chains. The mesh size is strictly connected with the molecular weight between two crosslinks and it can be evaluated as[1]:

$$\xi = l \cdot {\phi_{2,s}}^{-\frac{1}{3}} \cdot \left(C_n \cdot \frac{2 \cdot M_c}{M_r}\right)^{\frac{1}{2}} \qquad (7)$$

Where: l is the length of the bond along the polymer backbone, C_n is the Flory characteristic ratio[56] (or rigidity factor), M_r is the molecular weight of the repeating unit. The mesh size can be influenced by the cross-linking density, but also by the external environment including the temperature and solvent chemistry (pH and ionic strength)**[6]**, thus there is room during the swelling to customize the mesh size of the final hydrogel. Once again, from an experimental point of view, only hydration tests are needed to evaluate ξ, if the polymer characteristics are known.

2.2.2 Properties parameters

In the following section, the main experimental techniques used to test the properties of hydrogels are described. Concerning the mechanical properties of hydrogels, they can be measured using two main classes of methods: i) tensile analyses, useful to determine the rubber elastic behaviour, and ii) dynamic-mechanical analyses, useful to describe the viscoelastic behaviour. Coupling these techniques with the evaluation of solvent transport, the poroviscoelastic behaviour of a hydrogel can be characterized.

2.2.2.a Elastic modulus

The simplest method to determine the elastic modulus of a swollen hydrogel is to perform a tensile test on an equilibrated hydrogel[57, 58]. From the peak of the stress-strain curve, failure point can be determined; from the average slope of the curve (in linear, rubberlike zone) the elastic modulus can be evaluated[59].

Young's modulus can be calculated starting from force-deformation curves, which can be obtained using instruments such as texture analysers. These devices are equipped with a probe, which can be of different shapes (i.e. spherical, conical, or cylindrical), that applies a certain deformation. In cases of indentation tests, the probe penetrates into the sample, in case of compression tests, the probe compresses the sample's surface. In both cases, the instrument measures, with the aid of a loading cell, the force opposed by the sample during the penetration, obtaining the force-deformation behavior. For hydrogels, the Young's modulus can be related with the force applied and the total displacement of the indenter[23], according to theoretical models[25]. I.e., using a spherical indenter, and applying small deformations, which means that the indentation depth, δ, is much smaller than the sample thickness, the Hertz equation can be used to evaluate the Young modulus, the Taylor and Kragh equation to evaluate the shear modulus[60]:

$$F = \frac{4 \cdot E}{3 \cdot (1-\nu^2)} \cdot R^{\frac{1}{2}} \cdot \delta^{\frac{3}{2}} \qquad (8)$$

$$G = \frac{0.36 \cdot F}{R^{\frac{1}{2}}} \cdot \left(\frac{h-\delta}{\delta}\right)^{\frac{3}{2}} \qquad (9)$$

Where E, G, and ν are the Young's modulus, the shear modulus, and Poisson's ratio, respectively. R is the radius of the spherical indenter, F is the measured force, h is the sample thickness. A major limit of these equations is that the radius of the indenter is kept constant, however, during the mechanical test, the contact radius between the probe and the sample changes, due to the penetration inside the hydrogel. Then, these equation have been later adjusted to take into account the deviations from Hertz mechanics since the substrate stiffness influences the geometric relationship between contact radius and both indentation depth and stress field[61].

The Young's modulus can be calculated starting from the shear modulus, as mentioned above (section 1.1.3). One of the methods used to evaluate the shear modulus is to perform Dynamic Mechanical Analysis (DMA), which can be performed using a rheometer. Usually, the DMA is performed in frequency sweep mode, in which the instrument moves from the start frequency to the stop frequency at a specified sweep rate at a fixed strain, in parallel-plate geometry, even if the cone-plate geometry can be adopted[62]. By these tests both the storage and the loss moduli (and then the complex modulus) can be obtained as a function of frequency[17], as well as the dynamic viscosity[63].

2.2.2.b Relaxation time

The relaxation time can be evaluated studying the rheology and mechanical behavior of materials, i.e. the stress-relaxation technique[64, 65] can be used. Stress relaxation experiments provide firstly the application of a step strain deformation to the sample and then the monitoring of the stress decay over time as the specimen is held in the same constrained state.

However, the most diffused technique to evaluate the relaxation time of a hydrogel is the oscillatory rheometry, which allows to measure the storage and loss moduli. The exponential decay of the shear modulus during the relaxation phase can be modelled with several theoretical models, among them, the most general viscoelastic model is the generalized Maxwell model (see section 1.1.3). In general, each Maxwell element is described by an elastic modulus and a relaxation time, thus, once the rheological model has been chosen, the rheological parameters, and among them the relaxation time, derive from the fitting of experimental data, i.e. using a non linear regression analysisMoreover, the viscoelastic properties of the hydrogel can be found by measuring the creep behaviour of the sample[32]. This method consists in the measure of the sample deformation during the time when a constant stress is applied. Describing the experimental behavior using the standard linear model or Zener model, which consists of two springs and one dashpot to describe the material's elasticity and viscosity, respectively, the relaxation time for constant stress can be evaluated.

2.2.2.c Water diffusivity

Concerning the water transport within hydrogels, it can be attributed mainly to the diffusion process, thus it can be quantified, experimentally, measuring the water diffusivity. A simple method to evaluate the water diffusivity is based on the measure of water permeability across a hydrogel membrane. In general, the experimental devices are composed of two different chambers, usually separated by porous plates, within which a hydrogel film is mounted. One of the two chambers (the upper) is then filled with distilled water and air pressure is applied in the other chamber, measuring the flow rate[66]. The driving force for the water transport is the difference between the saturation pressure of the water and the partial pressure of water in air (accounting for the relative humidity of air). Starting from the experimental flux values, the water permeability across the hydrogel and, after some considerations on the equilibrium chemical potential in the membrane (described in [67]) the water diffusivity can be evaluated.

Diffusion is the most crucial form of molecules transport. A convenient and non-invasive technique to measure translational motion is the pulsed-field gradient nuclear magnetic resonance (PFG-NMR), whose basic principles are reported in [68]. Using this method the water self-diffusion coefficients (the starting point in the evaluation of diffusional processes in polymeric systems), both at the equilibrium water content and as a function of reduced water content, can be measured[69]. By the Fourier transform version of the pulsed field gradient spin-echo NMR technique, the diffusional behaviour of molecules in complex and multicomponent polymer matrices can be characterized[70].

3. Case histories

Hydrogels, in principle, could always be able to manifest the poroviscoelasticity but, with the right shrewdness, the focus can be given to the viscoelasticity or to the poroelasticity. Depending on the final application, it could be preferable to have a viscoelastic behaviour rather than a poroelastic or vice versa to have a poroelastic rather than viscoelastic one. Moreover, some applications could benefit from both the behaviours and therefore prefer a poroviscoelastic response.

This reasoning holds also for applications regarding the same sector. I.e. controlled drug release systems based on hydrogels predominantly exploit the poroelasticity of the system, using solvents as carrier to dissolve and transport active substances [71], however also the poroviscoelasticity could be of interest to tune the release ("anomalous transport") [72, 73].

Tissue engineering applications are often direct to the poroelastic behaviour as well, using hydrogels as scaffold for cell growth and the solvent as carrier to transport nutrients [74, 75]. However, it could also aim to exploit the viscoelasticity to simulate the short time mechanical behaviour of tissues [76, 77], as well as to the poroviscoelasticity to grow cells in systems that mimic the real tissues and that can promote the right cells differentiation[78-80].

In the following several case histories involving hydrogels and their exploitation as poroviscoelastic materials: first some literature work that focused on the viscoelasticity will be reported; then some works focused on the poroelasticity will be described; finally works that considered the poroviscoelastic behaviour will be treated.

3.1 Focus on viscoelastic behaviour

Tuning the viscoelasticity of hydrogels is of great relevance for tissue engineering applications, especially in their use as synthetic extra cellular matrices for three-dimensional culture. In this particular application, the attention has been payed for years only to the elasticity of the system, leading to the production of several chemically cross-linked hydrogel scaffolds[74, 75]. Despite the scaffold rigidity (elasticity) has been proved to modify the cell spreading behaviour, motility, proliferation and stem cells differentiation[79], recently it has been proved that the stress-relaxation behaviour (viscoelasticity), which can mime the native tissue property, plays the major role[78-81]. Viscoelasticity, as it has been treated so far, is an intrinsic property of the polymeric networks that through breaking and reformation of cross-links can dissipate energy and relax the structure. Therefore, the modulation of this characteristic requires the structural modification of the polymeric network. Cameron et al. [79] produced polyacrylamide (PAM) hydrogels varying the ratio monomer (acrylamide)/ cross-linker (bis-acrylamide) and the total polymer concentration. With these variations Cameron et al. managed to keep constant the storage modulus (G') while obtaining different loss moduli (G''), therefore changing the viscoelastic properties only. McKinnon et al.[80] worked with polyethylene glycol (PEG) hydrogels crosslinked with reversible covalent bonds (hydrazone bonds). The advantages of this type of hydrogel was the capability to display a viscoelastic behaviour while maintaining a stable material property over time. Bartnikowski et al. [82] tailored the viscoelasticity of their hydrogels, blends of gelatin methacrylamide (GelMA) and gellan gum, varying the amount of chemical (GelMA) and physical (gellan gum) cross-links in the system. Chaudhuri et al.[78] used ionical cross-linked alginate and varied the alginate molecular weight and the amount of cross-linker (Ca^{2+}) to obtain the same initial elastic modulus (G_u) but different relaxation times. All these studies, while demonstrating the importance of the viscoelasticity on the cells culture, demonstrated also the possibility to modulate the viscoelastic response of hydrogels, varying their relaxation time (τ_R) of order of magnitudes (from fraction of seconds to one hour). It has also to be said that in all these analysed works, the poroelasticity is not considered and the hydrogel is seen as a simple viscoelastic material (case 6 in Table 3 and Fig. 5). This can be done as far as the diffusion time is higher than the process and relaxation time $\tau_D \gg \tau_P \sim \tau_R$. Considering that cells responds to force oscillation over a timescale of 1 s, exert traction forces on a timescale of minutes and undergo cell spreading on a time scale of minutes to hours[78], as far as the characteristic length of diffusion (L_{CH}) is macroscopic (i.e. the entire scaffold) this imbalance is satisfied. However, if the L_{CH} is of the same order of the cell dimension, as it should be to study the local stress evolution, than $\tau_D \sim \tau_R \sim \tau_P$ (case 11 in Table 4 and Fig. 5) and the poroviscoelasticity has to be considered.

Hydrogel viscoelasticity is also useful to mimic the behaviour of natural tissues like brain, liver, adipose tissue, muscles etc. In this case, the characteristic length L_{CH} is macroscopic (order of centimetres) and this holds the case 6 conditions (in Table 3 and Fig. 5), which is characteristic of viscoelastic relaxation with negligible migration of solvent. I.e. Fitzgerald et al. [77] produced and tested alginate-PAM IPN hydrogels to mimic the muscle tissue behaviour. In the timescale of the mechanical analysis, $\tau_P \sim 1000\,s$, the macroscopic hydrogels, $L_{CH} \sim 1\,cm$, displayed an adjustable viscoelastic stress-relaxation behaviour through the modification of the amount of PAM cross-linker.

3.2 Focus on poroelastic behaviour

Hong et al.[83] extended the poroelastic theory to model the behaviour of hydrogels and, since then, several modelling [84-89] and experimental works[90-93] have been published to confirm its applicability and validity. I.e. Cai et al. [90] studied the porelastic behaviour of covalently cross-linked alginate hydrogels under compression. Ding et al. [91] considered the poroelastic behaviour triggered by temperature variations. The temperature sensitivity of the hydrogel was modelled using a Flory-Huggins interaction parameter dependent on the temperature ($\chi_{12}(T)$) in a simplified version of the model proposed in section 4.1 and validating the model with experimental results obtained from poly(n-isopropylacrylamide, PNIPA) hydrogels. Nowadays the theory is well established and most of the hydrogel applications can be traced back to a poroelastic behaviour.

Most of the literature works dealing with hydrogel-based drug delivery systems treat hydrogels as poroelastic materials[94]. The solvent penetrates inside the structure, produces the swelling of the system with the stretching of the polymeric networks (glass-rubber transition) and the dissolution of the active substance that diffuses toward the external dissolution medium. The network viscoelasticity is often disregarded stating that $\tau_P \gg \tau_R$ [95]. Under these phenomenological hypotheses, the system behaves according to the case 4 or case 5 conditions (in Table 3, Table 4 and Fig. 5). In literature, this behaviour sometimes is defined through the diffusional Deborah number[1], $N_{De.D} = \tau_R/\tau_D$, which being lower than one is characteristic of a diffusion process through a viscous mixture (the swollen rubbery hydrogel) and can be described by conventional Fick's law, using coefficients of diffusion which are a strong function of water/polymer concentrations [96]. Despite this approach can be found in the majority of the literature works, and it is always true for hydrogels with strong cross-link bonds with limited viscoelasticity, it is not always the right one. Physical cross-linked hydrogels can manifest great viscoelastic relaxation at the glass-rubber transition front, which produces a relaxed structure that can absorb even more water (stiffer gels equilibrate with a given external water solution absorbing less water than softer gels [46]). In particular conditions, when $\tau_R \sim \tau_D$ (L_{CH} being proportional to the thickness of the glass-rubber transition region and not to the macroscopic system), the so called anomalous (or Case II) transport can be obtained, in which the viscoelastic relaxation is concomitant with the water penetration and a sharp front separating the glassy dry core and the rubbery fully swollen layer is present [72, 73, 97]. Of course, this case cannot be described with a poroelastic vision but a poroviscoelastic behaviour (case 11 in Table 4 and Fig. 5) has to be considered.

Hydrogels poroelasticity and their volumetric changes can be used to produce actuators and to perform mechanical work on the micro- and macroscale (microfluidic applications and/or micro-machines). Ionov [98] reviewed several papers that used hydrogels as actuators thanks to their ability to bend and/or twist in response to external stimuli. This bending/twisting behaviour can be "easily" obtained in two ways: applying inhomogeneous stimuli to a homogeneous hydrogel (i.e. gradient of temperature or concentration etc.) or obtaining inhomogeneous hydrogels (i.e. variation of the cross-linking density, layers of hydrogels with different swelling properties etc.). A particular class of hydrogel-based actuators is represented by the stimuli-responsive (micro)valves, which can be a very smart alternative to conventional valves, especially for microfluidic application where the scaling down of conventional valves is complex and often unfavourable. Liu et al. [99] produced acrylic acid (AA)/2-hydroxyethyl methacrylate (HEMA) pH-responsive hydrogels chemically crosslinked by ethylene glycol dimethacrylate in a photopolymerization process. The system was proved to respond to external pH variations by swelling or shrinking and therefore regulating the flow rate. To minimize the time of response of the valve (maximize the kinetic of swelling and shrinking) the use of multiple micro-valves per channel was suggested. In this manner the characteristic length of diffusion (L_{CH}) was decreased, decreasing the diffusion time (τ_D) and therefore the time required to open/close the channel. Mazaheri et al. [100] performed a modelling study for temperature sensitive PNIPA hydrogels, using a simplified version of the model proposed in section 4.1 and $\chi_{12}(T)$, and compared the simulation with experimental results.

3.3 Focus on poroviscoelastic behaviour

Poroelasticity and viscoelasticity, separated, can only describe part of the hydrogels behaviour. As it has been shown in the previous paragraphs, several applications that currently consider separated these phenomena should instead combine them and aim to describe the poroviscoelasticity of the system. Zhao et al. [65] analysed the stress-relaxation behaviour of alginate hydrogels, ionically and chemically cross-linked, subjected to compression tests. Despite the poroelasticity (covalent alginate gel) and the viscoelasticity (ionic alginate gel) were still separated, the discussion was in full poroviscoelastic style. The size independent relaxation of the ionic alginate (viscoelastic) and the gel size dependent relaxation of the covalent alginate (poroelastic) were demonstrated. Hu & Suo [47] laid the basis for modelling the poroviscoelasticity and Wang et al. [101], in 2014, for the first time, combined modelling and experiments to clearly show the poroviscoelastic behaviour and how to decouple poroelasticity and viscoelasticity. In their experiments on polyacrylamide-alginate IPN hydrogels the case 9 conditions (in Table 4 and Fig. 5) were verified and an experimental graph with the same meaning of the following Fig. 6b was obtained. Recently, the Mercadé-Prieto's research group investigated the poroviscoelastic behaviour of whey protein hydrogels[102, 103]. The indentation tests performed and the consecutive modelling, with the approach showed in section 4.2, proved that the major role in whey protein relaxation is given by poroelasticity and a smaller contribution is given by the viscoelasticity in the first seconds. Caccavo et al. [104], investigated experimentally and modelling, the poroviscoelastic behaviour of agarose hydrogels subjected to stress-relaxation tests. However, in the timescale analysed only the viscoelastic relaxation was recorded, suggesting that a case 9 behaviour was analysed but, with the process time $\tau_P \ll \tau_D$ so that the poroelasticity did not have time to manifest.

4. Modelling of hydrogels' behaviour

The complex hydrogel behaviour can be analysed with the aid of mathematical models, which should be able to describe and predict the main phenomena. Several approaches have been proposed in literature, and categorized in two classes[94]: the multiphasic models and the monophasic models. The first class considers the hydrogels as made of multiple phases (liquid (water), solid (polymer network), etc.) that interact giving rise to the overall hydrogel behaviour. The second class include all the models that consider hydrogel as a continuum, in which multiple components coexist (water, polymer, etc.) and their interaction give rise to the overall hydrogel behaviour. The main difference is that with the first class each phase has its own properties (i.e. viscosity and density for the water phase, elastic/viscoelastic parameters for the solid phase, etc.) which should be representative of the pure phase, plus other properties which are related to the interaction between the phases (i.e. friction factor between the phases, etc.). The second class instead looks at the hydrogels as a single-phase matter and therefore the properties are not of the single phase but of the combination of all the species.

As a result, the multiphasic models require more numerical effort (more equations to be coupled and solved) and much more experimental work to measure the physical parameters required (some of them being scarcely accessible to measurement). On the contrary, the monophasic models require less numerical effort and it is based on physically based parameters, which are measurable in principle, with the only drawback of more complex constitutive equations.

All the following paragraphs deal with the more recent and comprehensive monophasic approach. Firstly, the most general multicomponent monophasic poroviscoelastic model[105] will be shown and secondly some ready-to-use equations will be given. Despite the development of the model given in section 4.1 is quite elegant and robust, and its resolution gives useful information to describe the hydrogel dynamic behaviour (swelling, poroviscoelasticity etc.), the difficulties related to its resolution through a numerical based method (i.e. FEM) can discourage most of the researchers not used to this level of mathematical modelling. Therefore, a different approach is given in section 4.2, which can allow the use of mathematical models even to non specialist, of course with some simplifying assumptions, among them the presence of small deformations.

4.1 The multicomponent poroviscoelastic model for finite deformations

The mass and momentum balances, written in the lagrangian frame, completely define the hydrogel behaviour in terms of species concentration ($i = 1 \ldots N, i = 1\ water, i = 2\ polymer, i = 3 \ldots N\ other\ species$) and stress/deformation:

$$\begin{cases} \dfrac{\partial \mathrm{c_i}}{\partial \mathrm{t}} = -\overline{\nabla} \cdot \overline{\mathrm{h}}_{\mathrm{i}} \quad \forall i \neq 2 \\ \overline{\nabla} \cdot \overline{\overline{\mathrm{P}}} = 0 \end{cases} \tag{10, 11}$$

Where $\mathrm{c_i}$ is the molar concentration of i-th species, $\overline{\mathrm{h}}_{\mathrm{i}}$ is its molar flux, $\overline{\overline{\mathrm{P}}}$ is the first Piola-Kirchoff stress tensor.

The hydrogel is subjected to the volumetric constraint, which relates the deformation to the amount diffusing species:

$$\mathrm{J} = 1 + \sum_{\substack{i=1 \\ i\neq 2}}^{N} \Omega_i (c_i - c_{i0}) \tag{12}$$

Where J is the Jacobian of the deformation gradient, which represents the volumetric changes, Ω_i is the molar volume of the i-th species and c_{i0} is the initial concentration of the i-th species.

The constitutive equations can be derived from non-equilibrium thermodynamics using the "free energy imbalance"[106]:

$$\frac{\partial \mathrm{A_R}}{\partial \mathrm{t}} - \overline{\overline{\mathrm{P}}} : \dot{\overline{\overline{\mathrm{F}}}} + \sum_{\substack{i=1 \\ i\neq 2}}^{N} \left(-\mu_{\mathrm{i}} \dot{c}_{\mathrm{i}} + \overline{\mathrm{h}}_{\mathrm{i}} \cdot \overline{\nabla} \mu_{\mathrm{i}} \right) \leq 0 \tag{13}$$

Where A_R is the relaxed version of the Helmholtz free energy of the hydrogel, $\overline{\overline{\mathrm{F}}}$ is the deformation gradient and μ_i is the chemical potential of the i-th species. The relaxed version of the free energy is used to enforce the volumetric constraint and to obtain constitutive equations that satisfy it. This can be done by adding the volumetric constraint, through a Lagrange multiplier (p), to the Helmholtz free energy of the system, obtaining a relaxed version:

$$\mathrm{A_R} = \mathrm{A} - \mathrm{p}\left(\mathrm{J} - 1 - \sum_{\substack{i=1\\ i\neq 2}}^{N} \Omega_i (c_i - c_{i0})\right) \qquad (14)$$

Where p is the Lagrange multiplier that has the meaning of physical pressure.

The Helmholtz free energy of a (neutral) hydrogel is the sum of the elastic (generally called elastic, but could also be viscoelastic) free energy owed by the polymeric network and of the mixing contribution generated by the interactions of the species:

$$\mathrm{A} = \mathrm{A^{el}} + \mathrm{A^{mix}} \qquad (15)$$

For ionic hydrogels, other contributions have to be taken into account[86, 107-113]. Normally the elastic contribution is derived from rubber-elasticity theory, using Gaussian[105, 114-118] or non-Gaussian[12, 84, 118-121] expressions, whereas the mixing term can be derived from the Flory-Huggins mixing model[11, 112] considering the entropic and the enthalpic terms.

In Caccavo et al.[105] using a standar linear solid model (SLS) to describe the polymer network viscoelasticity with the aid of the affine network theory[115, 116] and assuming that only the interaction of water and polymer produce an enthalpic effect (while the other species contribute to the free energy only with the entropic effect) the following expression has been derived:

$$\begin{aligned}\mathrm{A}\left(\overline{\overline{\mathrm{F}}}, \mathrm{c_1}, \overline{\overline{\mathrm{F}}}^{\mathrm{visc}}\right) = \frac{1}{\mathrm{J_0}}\Bigg\{ & \frac{\mathrm{G_1}}{2}\left[\overline{\overline{\mathrm{F}}}_0\overline{\overline{\mathrm{F}}} : \overline{\overline{\mathrm{F}}}_0\overline{\overline{\mathrm{F}}} - 3 - 2\ln(\mathrm{J_0 J})\right] + \\ & + \frac{\mathrm{G_2}}{2}\Bigg[\overline{\overline{\mathrm{F}}}_0\overline{\overline{\mathrm{F}}}\,\overline{\overline{\mathrm{F}}}^{\mathrm{visc}^{-1}} : \overline{\overline{\mathrm{F}}}_0\overline{\overline{\mathrm{F}}}\,\overline{\overline{\mathrm{F}}}^{\mathrm{visc}^{-1}} - 3 \\ & \qquad - 2\ln\left(\mathrm{J_0 J}\det(\overline{\overline{\mathrm{F}}}^{\mathrm{visc}})^{-1}\right)\Bigg] + \\ & + \mathrm{RT}\left[\mathrm{J_0 c_1}\ln(\phi_1) + \sum_{\substack{i=1\\ i\neq 2}}^{\mathrm{N}} \mathrm{J_0} c_i \ln(\phi_\mathrm{i}) + \mathrm{J_0 c_1}\chi_{12}\phi_2\right]\Bigg\}\end{aligned} \qquad (16)$$

Where the first term on the RHS represents the elastic contribution to the free energy, characterized by the shear modulus parameter $\mathrm{G_1}$. The second term on the RHS represents the viscoelastic contribution described with a Maxwell element, characterized by the shear modulus parameter $\mathrm{G_2}$ and function of the viscous deformation $\overline{\overline{\mathrm{F}}}^{\mathrm{visc}}$. The third term on the RHS represents the mixing contribution to the free energy. The deformation gradient $\overline{\overline{\mathrm{F}}}_0$ and its Jacobian $\mathrm{J_0}$, were used to perform a change of reference frame to adapt the affine network theory (which is developed for dry substances) to an already homogenous swollen system with a volumetric deformation equal to $\mathrm{J_0}$.

Once the proper Helmholtz free energy expression is obtained, the constitutive equations can be derived differentiating it with respect to time and substituting it in the free energy imbalance (Eq. 13), which has to satisfy the following equations in order to be verified:

$$\begin{cases} \dfrac{\partial \mathrm{A}}{\partial \overline{\overline{\mathrm{F}}}} = \overline{\overline{\mathrm{P}}} + \mathrm{pJ}\overline{\overline{\mathrm{F}}}^{-\mathrm{T}} \\ \dfrac{\partial \mathrm{A}}{\partial \mathrm{c_i}} = \mu_\mathrm{i} - \mathrm{p}\Omega_\mathrm{i} \quad i = 1 \dots N \end{cases} \qquad (17)$$

From which it is possible to derive the relation between stress $(\overline{\overline{\mathrm{P}}})$ and deformation $(\overline{\overline{\mathrm{F}}})$ as well as the relation between the concentration $(\mathrm{c_i})$ and the chemical potential (μ_i). The pressure field (p) influences both the system stress and the chemical potential fields. The free energy imbalance provides also conditions on the admissible kinetic expressions for the molar flux and the viscous relaxation of the structure, which have to respect the following imbalances:

$$\begin{cases} \overline{\mathrm{h}}_\mathrm{i} \cdot \overline{\nabla}\mu_\mathrm{i} \leq 0 \\ \dfrac{\partial \mathrm{A_R}}{\partial \overline{\overline{\mathrm{F}}}^{\mathrm{visc}}} \dfrac{\partial \overline{\overline{\mathrm{F}}}^{\mathrm{visc}}}{\partial \mathrm{t}} \leq 0 \end{cases} \qquad (18)$$

Normally the kinetic law for the molar flux is a Fick type equation:

$$\overline{\mathrm{h}}_\mathrm{i} = -\overline{\overline{\mathrm{D}}}_\mathrm{i} \cdot \overline{\nabla}\mu_\mathrm{i} \qquad (19)$$

Where $\overline{\overline{\mathrm{D}}}_\mathrm{i}$ is a positively defined mobility tensor. The kinetic law for the viscous relaxation can be of the type:

$$\frac{\partial \overline{\overline{\mathrm{F}}}^{\mathrm{visc}}}{\partial \mathrm{t}} = -\frac{1}{\eta}\frac{\partial \mathrm{A_R}}{\partial \overline{\overline{\mathrm{F}}}^{\mathrm{visc}}} \qquad (20)$$

Where η is the viscosity of the dashpot representing the viscous dissipations.

The mass and the momentum conservation equation (Eq. 10 and 11) along with the volumetric constraint (Eq. 12) imposed through the lagrange multiplier p can be solved (with the proper intial and boundary conditions) for the (N-1)+2 fields variables: N-1 concentrations, the deformation $\overline{u}$ and the pressure field p. It has to be noted that the model parameters correspond to physical based and measurable quantities: the mechanical parameters $\mathrm{G_1}$ and $\mathrm{G_2}$ and η (or $\tau_R = \eta/\mathrm{G_2}$) are related to amount and nature of cross-links. The modulus $\mathrm{G_1}$ corresponds to the elastic modulus of the relaxed (R) system, $\mathrm{G_2}$ and τ_R (or in general $\mathrm{G_j}$ and τ_{Rj} with $j = 2 \dots M$ Maxwell-elements) are representative of the viscoelasticity of the system. The mobility tensor $\overline{\overline{\mathrm{D}}}_i$ can be traced back to diffusivity or to permeability and it is a measure of the easiness of the species movement within the structure. The last parameters χ_{12} (in general χ_{ij}) is the Flory-Huggins interaction parameters, representative of the miscibility of the interested species.

4.2 Ready-to-use equations

The development and implementation of a (multicomponent) poroviscoelastic models for finite deformations is not a trivial step and sometimes this can be disheartening. However, since the characterization of the poroviscoelastic properties of a material is of great interest, a different and easier approach has been developed, based on the utilization of master curves.

The force-relaxation behaviour of a hydrogel subjected to a compression or indentation test (stress-relaxation test) can be described considering the superimposition of a poroelastic and a viscoelastic relaxation.

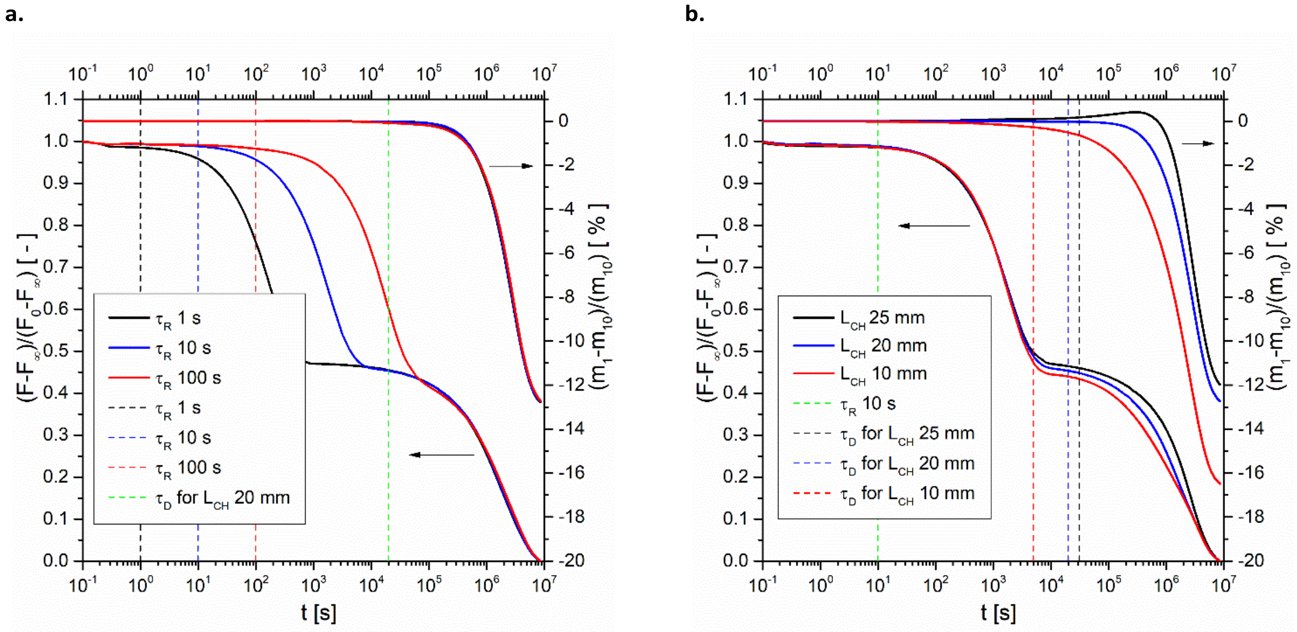


Fig. 6. Stress-relaxation simulations of hydrogel cylinders deformed at 10%. The used model is the one proposed by Caccavo and Lamberti[46] ($G_1 = 100\ kPa, G_2 = 1000\ kPa$, $\mathfrak{D} = 2 \times 10^{-8}\ m^2/s$, τ_R explicitly reported in the graphs). Both graphs are representative of the "case 9" depicted in Fig. 5, where $\tau_R \ll \tau_D$, and the viscoelastic relaxation takes place always before the poroelastic relaxation. The normalized force is on the left axis (F_0 is the peak force and F_∞ is the force at infinite time) and the percentage of water loss (m_{10} is the initial water mass) is on the right axis. In a. the effect of the relaxation time τ_R is shown on a hydrogel with characteristic length of diffusion L_{CH} of 20 mm (which gives a τ_D of 20'000 s). In b. the effect of the diffusion time τ_D is shown by changing the characteristic length of diffusion. A gel with same elastic moduli and solvent diffusivity can behave differently by changing the relaxation time (structural modification) or by changing the geometry (geometrical modification), a. and b. respectively. Similar graphs can also be found in [101].

The poroelastic relaxation can be described as:

$$\frac{F_{PE}(t) - F(\infty)}{F_0 - F(\infty)} = f_{PE}\left(\frac{t}{\tau_D}\right) \tag{21}$$

Where $F_{PE}(t)$ is the time depended force due to poroelastic (PE) relaxation, $F(\infty)$ is the force at infinite time and F_0 is the force at the end of compression step (peak force). This ratio can be described with the master curve $f_{PE}(t/\tau_D)$, which depends mainly on the geometrical characteristics of the investigated system (i.e. indenter geometry). These master curves have been obtained by solving poroelastic models (similar to the one in the previous paragraph) with a FEM approach, and are available in [93], with the general form:

$$f_{PE}\left(\frac{t}{\tau_D}\right) = \sum A_i e^{-B_i\left(\frac{t}{\tau_D}\right)^{C_i}} \tag{22}$$

For poroelastic relaxation, the the total extent of force relaxation for an indentation test can be obtained considering that in the short time limit the hydrogel respond to the compression as an incompressible material (undrained Poisson ratio $\nu_{un} \sim 0.5$), so that $F_0 = \varepsilon_0 G_1/2(1+\nu_{un}) \sim \varepsilon_0 G_1/3$. Where G_1 is the shear elastic modulus. At long time limit, when the solvent is in equilibrium with the external solution, the gel behaves like a compressible elastic solid, and the force in equilibrium is the same as the force on an indenter pressed into a compressible elastic solid $F_{PE}(\infty) = \varepsilon_0 G_1/2(1+\nu_d)$, where ν_d is the drained Poisson ratio. Therefore, the extent of relaxation in poroelastic systems is determined by the Poisson's ratio of the drained hydrogel [93]:

$$\frac{F_{PE}(\infty)}{F_0} = \frac{3}{2(1+\nu_d)} \tag{23}$$

The viscoelastic relaxation can be described with a generalized Maxwell model:

$$F_{VE}(t) = \varepsilon_0 G(t) = \varepsilon_0 G_1 + \sum_{i=2}^{N} \varepsilon_0 G_i e^{-t/\tau_{R_i}} \tag{24}$$

Where ε_0 is the deformation and G_i the elastic moduli in the generalized Maxwell model. Considering an unrelaxed shear modulus $G_U = \sum_{i=1}^{N} G_i$, and knowing that $\varepsilon_0 G_U = F_0$, the previous expression can written with the Prony series:

$$\frac{F_{VE}(t)}{F_0} = 1 - \sum_{i=2}^{N} g_{VE_i}\left(1 - e^{-t/\tau_{R_i}}\right) \tag{25}$$

Where $g_{VE_i} = G_i/G_U\ \forall i \geq 2$.

The viscoelastic and poroelastic relaxation, following [122], can be coupled to describe the poroviscoelastic relaxation:

$$F_{PVE}(t) = \frac{F_{VE}(t) F_{PE}(t)}{F_0} \tag{26}$$

Using this last equation, and with the proper adjustment of the equation coefficients (Eq. 18, 20), it is possible to describe and study the poroviscoelastic relaxation of a hydrogel subjected to stress-relaxation tests [102, 103].

An estimation of the total extent of force relaxation for an indentation test can be obtained considering that in the short time limit the hydrogel respond to the compression as an incompressible material ($\nu_{un} \sim 0.5$), with the unrelaxed modulus G_U, so that $F_0 = \varepsilon_0 G_U/2(1+\nu_{un}) \sim \varepsilon_0 G_U/3$. At long time limit, when the solvent is in equilibrium with the external solution, the gel behaves like a compressible elastic solid with the relaxed modulus G_1, and the force in equilibrium is the same as the force on an indenter pressed into a compressible elastic solid: $F_{PVE}(\infty) = \varepsilon_0 G_1/2(1+\nu_d)$. The poroelastic relaxation in presence of viscoelasticity reads:

$$\frac{F_{PVE}(\infty)}{F_0} = \frac{G_1 3}{G_U\ 2(1+\nu_d)} = \left(1 - \sum_{i=2}^{N} g_{VE\,i}\right)\frac{3}{2(1+\nu_d)} \qquad (27)$$

(The same results could be derived using Eq. 26, 25 and 23 at infinite time). In this case, apart from the drained Poisson ratio, the extent of relaxation depends also on the ratio between relaxed and unrelaxed moduli. The closer this ratio to 0 (poroviscoplasticity) the higher the extend of relaxation, the closer this ration to 1 (poroelasticity) the lower the extent of relaxation.

4.3 A simulation study

In Fig. 6 an example of the case 9 behaviour in a stress-relaxation simulation is reported. The case 9 is the most common behaviour to be expected working with macroscopic systems, which normally ensure $\tau_R \ll \tau_D$. Under these conditions, the hydrogel experiences first a viscoelastic and then a poroelastic relaxation. This behaviour has been described experimentally and modelling also in [101, 102].

In Fig. 6a the effect of the relaxation time is reported: the smaller the τ_R the sooner the appearance of the viscoelastic relaxation. It can be noted that this type of relaxation is not accompanied by water movement (on macroscopic scale), and the hydrogel hydration remains constant. When the process time becomes comparable with the diffusion time, a second step of relaxation, a poroelastic relaxation, takes place. This step is accompanied by volumetric changes of the system due to solvent migration. As τ_R approaches the value of τ_D (i.e. $\tau_R = 100s$ in Fig. 6a, while $\tau_D = 20'000s$) the two steps of relaxation become less pronounced, generating a single step in which the viscoelastic and the poroelastic phenomena are concomitant ("case 11" in Fig. 5). In Fig. 6b the effect of the diffusion time is reported, in these simulations τ_D was changed varying the characteristic length of diffusion (geometrical modification). The higher the τ_D the later the appearance of the poroelastic relaxation and the related water movement.

5. Conclusions

In this review, two main aspects on the use of hydrogels have been analysed.

In particular, the attention was firstly focused on the characterization processes, answering the two questions:

i) To what parameters should the researcher look at?, and

ii) Which are the techniques used to measure them?

The first question was answered in section 1.1, and the second question was answered in section 2.

Once the key parameters were identified, the use of three characteristic times (relaxation time, diffusion time, and processing time) allowed to define and to detail a "selection criterion" for the hydrogel to be used, in terms of which kind of dominant mechanical relaxation and of transport phenomena have to take place during a given application. This selection criterion was presented in section 1.2.

The second relevant aspect of the topic was the mathematical modelling of the hydrogels' behaviour, i.e. the translation of the physical reality into a system of mathematical equations. By solving the equations, the behaviour of the system can be simulated *a priori*, i.e. without the need to carry out any experimental test (or by limited experimental work), and thus saving time and resources.

The review includes some case histories taken from literature presented in section 3 to assess the selection criterion and the modelling approaches.

Finally yet importantly, section 4 is devoted to the review of the state-of-the-art in this field, along with the presentation of most recent modelling approaches, with some simulation results matching the qualitative criterion reported in section 1.2.

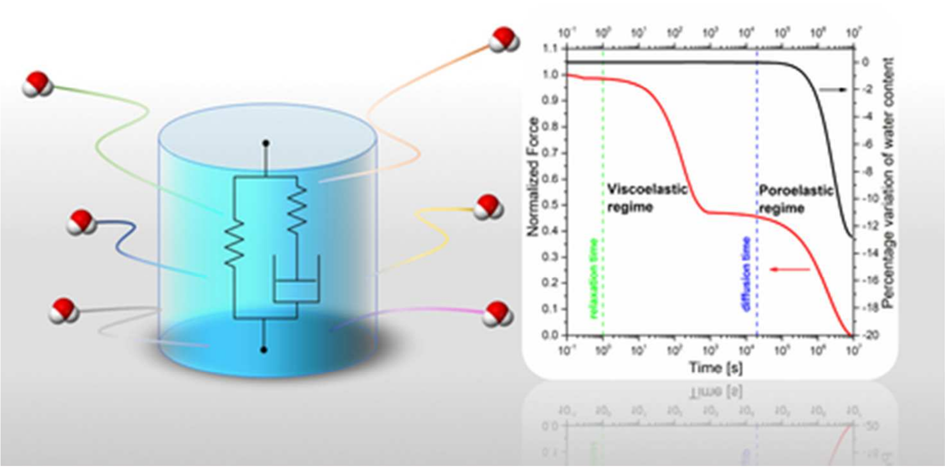
The knowledge of characterization methods, along with the modelling approaches (the use of which is possible following references [46] and [123]) and the proposed selection criterion, will facilitate the informed use of hydrogels for any application.

Notes and references

1. D. Caccavo, S. Cascone, G. Lamberti, A. A. Barba and A. Larsson, in *Smart Drug Delivery System*, ed. A. D. Sezer, InTech Open, 2016, pp. 237-303.
2. G. Lamberti, *International Journal of Pharmaceutics*, 2015, **496**, 157-158.
3. A. S. Hoffman, *Advanced drug delivery reviews*, 2012, **64**, 18-23.
4. F. Ullah, M. B. H. Othman, F. Javed, Z. Ahmad and H. M. Akil, *Materials Science and Engineering: C*, 2015, **57**, 414-433.
5. E. M. Ahmed, *Journal of Advanced Research*, 2015, **6**, 105-121.
6. M. L. Oyen, *International Materials Reviews*, 2014, **59**, 44-59.
7. S. Seiffert, E. Kumacheva, O. Okay, M. Anthamatten, M. Chau, P. Y. Dankers, B. W. Greenland, W. Hayes, P. Li and R. Liu, *Supramolecular Polymer Networks and Gels*, Springer, 2015.
8. S. Seiffert, *Supramolecular Polymer Networks and Gels*, Springer, 2015.
9. A. D. McNaught, A. Wilkinson and A. D. Jenkins, *IUPAC Compendium of Chemical Terminology: The Gold Book*, International Union of Pure and Applied Chemistry, 2006.
10. J. S. Bergstrom, *Mechanics of Solid Polymers: Theory and Computational Modeling*, Elsevier Science, 2015.
11. P. J. Flory, *Principles of Polymer Chemistry*, Cornell University, 1953.
12. F. Horkay and G. B. McKenna, in *Physical Properties of Polymers Handbook (Second Edition)*, ed. J. E. Mark, Springer, 2007.
13. Y. Hu, X. Chen, G. M. Whitesides, J. J. Vlassak and Z. Suo, *Journal of Materials Research*, 2011, **26**, 785-795.
14. S. P. Obukhov, M. Rubinstein and R. H. Colby, *Macromolecules*, 1994, **27**, 3191-3198.
15. A. Bigi, G. Cojazzi, S. Panzavolta, K. Rubini and N. Roveri, *Biomaterials*, 2001, **22**, 763-768.
16. J. F. Kennedy, G. O. Phillips, P. A. Williams and V. C. Hascall, *Hyaluronan: Proceedings of an International Meeting, September 2000, North East Wales Institute, UK*, Elsevier Science, 2002.
17. TA Instruments, *Determining the Viscoelastic Behavior of Agarose Gel Using the ElectroForce 3200 from Bose ElectroForce Systems*, http://www.azom.com/article.aspx?ArticleID=5351, Accessed 25/07/2017.
18. K. J. Le Goff, C. Gaillard, W. Helbert, C. Garnier and T. Aubry, *Carbohydrate Polymers*, 2015, **116**, 117-123.

19. A. Karimi and M. Navidbakhsh, in *Biomedical Engineering / Biomedizinische Technik*, 2014, vol. 59, p. 479.
20. K. Edsman, L. I. Nord, Å. Öhrlund, H. Lärkner and A. H. Kenne, *Dermatologic Surgery*, 2012, **38**, 1170-1179.
21. S. Tomblyn, E. L. Pettit Kneller, S. J. Walker, M. D. Ellenburg, C. J. Kowalczewski, M. Van Dyke, L. Burnett and J. M. Saul, *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, 2016, **104**, 864-879.
22. W.-P. Voo, C.-W. Ooi, A. Islam, B.-T. Tey and E.-S. Chan, *European Polymer Journal*, 2016, **75**, 343-353.
23. M. Ahearne, E. Siamantouras, Y. Yang and K.-K. Liu, *Journal of The Royal Society Interface*, 2009, **6**, 471-478.
24. M. Abrami, I. D'Agostino, G. Milcovich, S. Fiorentino, R. Farra, F. Asaro, R. Lapasin, G. Grassi and M. Grassi, *Soft Matter*, 2014, **10**, 729-737.
25. G. Kaklamani, D. Cheneler, L. M. Grover, M. J. Adams and J. Bowen, *Journal of the Mechanical Behavior of Biomedical Materials*, 2014, **36**, 135-142.
26. O. Jeon, S. J. Song, K.-J. Lee, M. H. Park, S.-H. Lee, S. K. Hahn, S. Kim and B.-S. Kim, *Carbohydrate Polymers*, 2007, **70**, 251-257.
27. Y. Mori, S. Kanazawa, M. Watanabe, H. Suenaga, K. Okubo, S. Nagata, Y. Fujihara, T. Takato and K. Hoshi, *Materials Sciences and Applications*, 2013, **4**, 73.
28. P. M. Gilsenan and S. B. Ross-Murphy, *Journal of Rheology*, 2000, **44**, 871-883.
29. M. Czerner, L. S. Fellay, M. P. Suárez, P. M. Frontini and L. A. Fasce, *Procedia Materials Science*, 2015, **8**, 287-296.
30. P. Duan, N. Kandemir, J. Wang and J. Chen, *MRS Advances*, 2017, **2**, 1309-1314.
31. V. Normand, D. L. Lootens, E. Amici, K. P. Plucknett and P. Aymard, *Biomacromolecules*, 2000, **1**, 730-738.
32. M. Ahearne, Y. Yang, A. J. El Haj, K. Y. Then and K.-K. Liu, *Journal of The Royal Society Interface*, 2005, **2**, 455-463.
33. T. Iannitti, A. Ö. Bingöl, V. Rottigni and B. Palmieri, *International Journal of Pharmaceutics*, 2013, **456**, 583-592.
34. T. Dapčević-Hadnađev, M. Hadnađev and A. Torbica, *Food Processing, Quality and Safety (Serbia)*, 2009.
35. J. D. Ferry, *Viscoelastic properties of polymers*, John Wiley & Sons, 1980.
36. J. N. Reddy and J. N. Reddy, *An Introduction to Continuum Mechanics*, Cambridge University Press, 2013.
37. D. Gutierrez-Lemini, *Engineering Viscoelasticity*, Springer US, 2013.
38. F. Ganji, S. Vasheghani-Farahani and E. Vasheghani-Farahani, *Iran Polym J*, 2010, **19**, 375-398.
39. H. Ju, A. C. Sagle, B. D. Freeman, J. I. Mardel and A. J. Hill, *Journal of Membrane Science*, 2010, **358**, 131-141.
40. T.-P. Hsu, D. S. Ma and C. Cohen, *Polymer*, 1983, **24**, 1273-1278.
41. Y. B. Mel'nichenko, V. Klepko and V. Shilov, *Polymer*, 1993, **34**, 1019-1023.
42. W. Derbyshire and I. Duff, *Faraday discussions of the chemical society*, 1974, **57**, 243-254.
43. F. Nakayama and R. Jackson, *The Journal of Physical Chemistry*, 1963, **67**, 932-933.
44. B. Amsden, *Macromolecules*, 1998, **31**, 8382-8395.
45. N. A. Peppas and E. W. Merrill, *Journal of Applied Polymer Science*, 1977, **21**, 1763-1770.
46. D. Caccavo and G. Lamberti, *Materials Science and Engineering: C*, 2017, **76**, 102-113.
47. Y. Hu and Z. Suo, *Acta Mechanica Solida Sinica*, 2012, **25**, 441-458.
48. E. S. Dragan, *Chemical Engineering Journal*, 2014, **243**, 572-590.
49. V. K. Thakur and M. K. Thakur, *International Journal of Biological Macromolecules*, 2015, **72**, 834-847.
50. M. J. Crane, M. B. Lim, X. Zhou and P. J. Pauzauskie, *Microsystems & Nanoengineering*, 2017, **3**, 17032.
51. M. B. Lim, M. Hu, S. Manandhar, A. Sakshaug, A. Strong, L. Riley and P. J. Pauzauskie, *Carbon*, 2015, **95**, 616-624.
52. M. A. Worsley, P. J. Pauzauskie, T. Y. Olson, J. Biener, J. H. Satcher Jr and T. F. Baumann, *Journal of the American Chemical Society*, 2010, **132**, 14067-14069.
53. G. Kickelbick, *Progress in polymer science*, 2003, **28**, 83-114.
54. P. J. Flory and J. Rehner Jr, *The Journal of Chemical Physics*, 1943, **11**, 512-520.
55. J. C. Bray and E. W. Merrill, *Journal of Applied Polymer Science*, 1973, **17**, 3779-3794.
56. U. Demirci and A. Khademhosseini, *Gels Handbook: Fundamentals, Properties and Applications (In 3 Volumes) Volume 1: Fundamentals of HydrogelsVolume 2: Applications of Hydrogels in Regenerative MedicineVolume 3: Application of Hydrogels in Drug Delivery and Biosensing*, World Scientific, 2016.
57. K. S. Anseth, C. N. Bowman and L. Brannon-Peppas, *Biomaterials*, 1996, **17**, 1647-1657.
58. C. H. Yang, M. X. Wang, H. Haider, J. H. Yang, J.-Y. Sun, Y. M. Chen, J. Zhou and Z. Suo, *ACS applied materials & interfaces*, 2013, **5**, 10418-10422.
59. A. Nakayama, A. Kakugo, J. P. Gong, Y. Osada, M. Takai, T. Erata and S. Kawano, *Advanced Functional Materials*, 2004, **14**, 1124-1128.
60. A. Barnes, P. H. Corkhill and B. J. Tighe, *Polymer*, 1988, **29**, 2191-2202.
61. E. P. Chan, Y. Hu, P. M. Johnson, Z. Suo and C. M. Stafford, *Soft Matter*, 2012, **8**, 1492-1498.
62. V. Michailova, S. Titeva, R. Kotsilkova, E. Krusteva and E. Minkov, *International journal of pharmaceutics*, 2000, **209**, 45-56.
63. V. Michailova, S. Titeva, R. Kotsilkova, E. Krusteva and E. Minkov, *International journal of pharmaceutics*, 2001, **222**, 7-17.
64. M. Watase and K. Nishinari, *Rheologica Acta*, 1980, **19**, 220-225.
65. X. Zhao, N. Huebsch, D. J. Mooney and Z. Suo, *Journal of Applied Physics*, 2010, **107**, 063509.
66. M. F. Refojo, *Journal of Applied Polymer Science*, 1965, **9**, 3417-3426.
67. G. Hoch, A. Chauhan and C. Radke, *Journal of Membrane Science*, 2003, **214**, 199-209.
68. W. S. Price, *Concepts in Magnetic Resonance Part A*, 1997, **9**, 299-336.
69. P. McConville and J. Pope, *Polymer*, 2000, **41**, 9081-9088.
70. P. Gao and P. E. Fagerness, *Pharmaceutical research*, 1995, **12**, 955-964.
71. J. Li and D. J. Mooney, 2016, **1**, 16071.
72. A. Salehi, J. Zhao, T. D. Cabelka and R. G. Larson, *Journal of Controlled Release*, 2016, **224**, 43-58.
73. D. Caccavo, G. Lamberti, A. A. Barba, S. Abrahmsén-Alami, A. Viridén and A. Larsson, *International Journal of Pharmaceutics*, 2017, **528**, 705-713.
74. J. A. Burdick and K. S. Anseth, *Biomaterials*, 2002, **23**, 4315-4323.
75. J. A. Rowley, G. Madlambayan and D. J. Mooney, *Biomaterials*, 1999, **20**, 45-53.

76. S. Kazemirad, H. K. Heris and L. Mongeau, *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, 2016, **104**, 283-290.
77. M. M. Fitzgerald, K. Bootsma, J. A. Berberich and J. L. Sparks, *Biomacromolecules*, 2015, **16**, 1497-1505.
78. O. Chaudhuri, L. Gu, D. Klumpers, M. Darnell, S. A. Bencherif, J. C. Weaver, N. Huebsch, H.-p. Lee, E. Lippens, G. N. Duda and D. J. Mooney, *Nat Mater*, 2016, **15**, 326-334.
79. A. R. Cameron, J. E. Frith and J. J. Cooper-White, *Biomaterials*, 2011, **32**, 5979-5993.
80. D. D. McKinnon, D. W. Domaille, J. N. Cha and K. S. Anseth, *Advanced Materials*, 2014, **26**, 865-872.
81. O. Chaudhuri, L. Gu, M. Darnell, D. Klumpers, S. A. Bencherif, J. C. Weaver, N. Huebsch and D. J. Mooney, 2015, **6**, 6365.
82. M. Bartnikowski, R. Wellard, M. Woodruff and T. Klein, *Polymers*, 2015, **7**, 1539.
83. W. Hong, X. Zhao, J. Zhou and Z. Suo, *Journal of the Mechanics and Physics of Solids*, 2008, **56**, 1779-1793.
84. S. A. Chester and L. Anand, *Journal of the Mechanics and Physics of Solids*, 2010, **58**, 1879-1906.
85. W. Hong, Z. Liu and Z. Suo, *International Journal of Solids and Structures*, 2009, **46**, 3282-3289.
86. W. Hong, X. Zhao and Z. Suo, *Journal of the Mechanics and Physics of Solids*, 2010, **58**, 558-577.
87. M. K. Kang and R. Huang, *Journal of Applied Mechanics*, 2010, **77**, 061004.
88. A. Lucantonio, P. Nardinocchi and L. Teresi, *Journal of the Mechanics and Physics of Solids*, 2013, **61**, 205-218.
89. J. Zhang, X. Zhao, Z. Suo and H. Jiang, *Journal of Applied Physics*, 2009, **105**, -.
90. S. Cai, Y. Hu, X. Zhao and Z. Suo, *Journal of Applied Physics*, 2010, **108**, 113514.
91. Z. Ding, Z. Liu, J. Hu, S. Swaddiwudhipong and Z. Yang, *International Journal of Solids and Structures*, 2013, **50**, 2610-2619.
92. J. Li, Y. Hu, J. J. Vlassak and Z. Suo, *Soft Matter*, 2012, **8**, 8121-8128.
93. Y. Hu, X. Zhao, J. J. Vlassak and Z. Suo, *Applied Physics Letters*, 2010, **96**, 121904.
94. D. Caccavo, S. Cascone, G. Lamberti, A. A. Barba and A. Larsson, *Current drug delivery*, 2017, **14**, 179-189.
95. G. Lamberti, I. Galdi and A. A. Barba, *International Journal of Pharmaceutics*, 2011, **407**, 78-86.
96. L. Masaro and X. X. Zhu, *Progress in Polymer Science*, 1999, **24**, 731-775.
97. J. Wilmers and S. Bargmann, *Heat and Mass Transfer*, 2014, **50**, 1543-1552.
98. L. Ionov, *Materials Today*, 2014, **17**, 494-503.
99. R. H. Liu, Q. Yu and D. J. Beebe, *Journal of microelectromechanical systems*, 2002, **11**, 45-53.
100. H. Mazaheri, M. Baghani, R. Naghdabadi and S. Sohrabpour, *Smart Materials and Structures*, 2015, **24**, 045004.
101. Q.-M. Wang, A. C. Mohan, M. L. Oyen and X.-H. Zhao, *Acta Mechanica Sinica*, 2014, **30**, 20-27.
102. W. Hu, C. Corbera-Sabaté, X. D. Chen and R. Mercadé-Prieto, *Food Hydrocolloids*, 2017, **72**, 237-246.
103. R. Mercadé-Prieto, J. Lopez and X. D. Chen, *Food Hydrocolloids*, 2016, **54**, 221-226.
104. D. Caccavo, S. Cascone, S. Poto, G. Lamberti and A. A. Barba, *Carbohydrate Polymers*, 2017, **167**, 136-144.
105. D. Caccavo, A. Vietri, G. Lamberti, A. A. Barba and A. Larsson, in *Quantitative Systems Pharmacology: Models and Model-Based Systems with Applications*, ed. D. Manca, Elsevier, in press.
106. M. E. Gurtin, E. Fried and L. Anand, *The Mechanics and Thermodynamics of Continua*, Cambridge University Press, New York, 2010.
107. J. Ricka and T. Tanaka, *Macromolecules*, 1984, **17**, 2916-2921.
108. Z. Liu, W. Toh and T. Y. Ng, *International Journal of Applied Mechanics*, 2015, **07**, 1530001.
109. H. Yan, B. Jin, S. Gao and L. Chen, *International Journal of Solids and Structures*, 2014, **51**, 4149-4156.
110. Z. Suo, X. Zhao and W. H. Greene, *Journal of the Mechanics and Physics of Solids*, 2008, **56**, 467-486.
111. P. Chiarelli and D. De Rossi, in *Ionic Interactions in Natural and Synthetic Macromolecules*, John Wiley & Sons, Inc., 2012, pp. 581-620.
112. L. Brannon-Peppas and N. A. Peppas, *Chemical Engineering Science*, 1991, **46**, 715-722.
113. R. Marcombe, S. Cai, W. Hong, X. Zhao, Y. Lapusta and Z. Suo, *Soft Matter*, 2010, **6**, 784.
114. G. A. Holzapfel, *Nonlinear Solid Mechanics a Continuum Approach for Engineering*, John Wiley & Sons Inc, Chichester, 2000.
115. J. E. Mark, *Physical Properties of Polymers Handbook*, Springer New York, 2007.
116. J. E. Mark and B. Erman, *Rubberlike Elasticity: A Molecular Primer*, Cambridge University Press, 2007.
117. M. Rubinstein and R. H. Colby, *Polymer Physics*, OUP Oxford, 2003.
118. L. R. G. Treloar, *The Physics of Rubber Elasticity*, OUP Oxford, 2005.
119. A. S. Argon, *The Physics of Deformation and Fracture of Polymers*, Cambridge University Press, 2013.
120. J. E. Bischoff, E. M. Arruda and K. Grosh, *Rubber chemistry and technology*, 2001, **74**, 541-559.
121. S. A. Chester, *Soft Matter*, 2012, **8**, 8223-8233.
122. D. G. T. Strange, T. L. Fletcher, K. Tonsomboon, H. Brawn, X. Zhao and M. L. Oyen, *Applied Physics Letters*, 2013, **102**, 031913.
123. D. Caccavo, *PoroViscoElastic model to describe hydrogels' behavior – Model implementation in COMSOL*, http://gruppotpp.unisa.it/en/poroviscoelastic-model-describe-hydrogels-behavior-model-implementation-comsol/, Accessed August, 2017.



39x19mm (300 x 300 DPI)