# EVAULATION OF THE APPLICABILITY OF VARIOUS POROSIMETRY METHODS IN INVESTIGATION OF THE ULTRASTRUCTURE OF HYDROGELS

[1]Monika TRUDIČOVÁ, [1]Jan ZAHRÁDKA, [1]Jiří SMILEK, [2]Kamila HRUBANOVÁ, [2]Kateřina MRÁZOVÁ, [1]Petr SEDLÁČEK, [1]Miloslav PEKAŘ

*[1]Brno University of Technology, Faculty of Chemistry, Brno, Czech Republic, EU, xctrudicova@fch.vut.cz*

*[2]Institute of Scientific Instruments of the Czech Academy of Sciences, Brno, Czech Republic, EU.*

https://doi.org/10.37904/nanocon.2022.4619

**Abstract**

Properties of hydrogels that define their application potential (e.g., stiffness, mechanical strength, transport properties) are primarily based on the morphology of the internal structure. Accurate analysis of the ultrastructure of hydrogels is therefore important for understanding the parameters affecting the functionality of hydrogels. Accordingly, this work was focused on the optimization and testing of methods suitable for the analysis of the nanostructure of hydrogel materials with a focus on determining the pore size. Conventional porosimetry methods (DSC thermoporometry), direct visualization methods (SEM, cryo-SEM, and AFM) as well as rheology, turbidimetry, and DLS microrheology were chosen as methods for structural analysis. Hydrogels based on polyvinyl alcohol (PVA) were chosen as representative hydrogel systems for testing these methods. The results obtained by the individual methods were compared.

**Keywords:** Hydrogel, polyvinyl alcohol, structural analysis

## 1. INTRODUCTION

Hydrogels are natural or synthetic polymeric materials with a three-dimensional internal structure that evinces versatile chemical, physical, and biological properties. Their structure is formed by physically or chemically connected polymers creating a spatial network. The properties of these materials are unimaginably diverse and applicable in many practical directions. Their great advantage is the ability to absorb a large amount of water into their internal structure for a long period. In this way, they resemble human tissues to a large extent. Obtaining detailed information on the morphology of the internal structure of gels, focusing on the pores, and understanding how they can be modified depending on the desired properties is crucial for preparing these materials for specific applications.

The main goal was to find a method or a set of methods that would be able to precisely define the internal and surface structure with a focus on porosity in materials with a high-water content i.e., hydrogels. Determining the internal structure of hydrogels is crucial to understanding the relationship between the structure and properties of these systems. A significant problem with most of the used methods consists in their sample dehydration prior to the analysis. When converting hydrogels into xerogel form, the microstructure inevitably collapses. Based on the experience with these materials, it could be said that PVA hydrogels show very good mechanical properties from the point of their viscoelastic properties, and therefore should resist growing ice crystals at higher molecular weights and should even limit their growth during the lyophilization process. This assumption is partially true; however, it is necessary to consider that even a small change in experimental conditions can affect the process of ice crystal growth to such an extent that it can disrupt the resulting structure of the hydrogel.

## 2. MATERIALS AND METHODS

In this work, PVA polymers with different molecular weights but the similar degree of hydrolysis were selected to restrict the variation of the structure and material properties among the tested gel solely to the PVA chain length. All polymers used in this work were purchased from Sigma-Aldrich (polyvinyl alcohol (PVA) CAS: 9002-89-5). Concentration series of stock PVA solutions were prepared. The exact amount of PVA polymer was transferred together with the required amount of deionized water into a reagent bottle. First, these mixtures were mixed for 30 minutes at laboratory temperature, and then this suspension was transferred to a water bath in which it was mixed at 95 °C for 3 hours. Subsequently, the polymer dispersion prepared in this way was poured into prepared molds, in which it was subjected to a cross-linking process under different conditions. All samples of PVA hydrogels were prepared by the freeze-thaw method at two freezing speeds. **Table 1** refers to the parameters of the various freezing conditions.

**Table 1** Conditions of the freeze/thaw method during the preparation procedure of hydrogels

| | **Temperature (°C)** | **Freezing time (min)** | **Melting time (min)** | **The average number of freeze/thaw cycles** |
|---|---|---|---|---|
| Laboratory freezer | −20 to −15 | ~135 | ~15 to 30 | 5 |
| Salt ice bath | −2 to 0 | ~315 | | 9 |

A wide portfolio of methods was selected to determine the internal porous structure, which should cover the most frequently used methods that are used for these purposes.

## 3. RESULTS AND DISCUSSION

### 3.1. DSC thermoporometry

The literature [1,2] provides a high-quality mathematical apparatus for many porosimetric methods. Among them, determination of the pore size via monitoring a decrease in the melting temperature of intraporous ice by DSC (differential scanning calorimetry) represents a simple and widely available experimental option [1]. For any DCS experiment, there is a certain correlation between the heating rate of the experiment and the resolution of the signal, which provides information on the pore size. During the evaluation, it is necessary to determine the decrease in the melting temperature that corresponds to the intrapore water. In the case of microporous solids, the signal is often represented by the separated melting peak that occurs at the subzero temperature. The measured PVA hydrogels (using TA Instruments, Q 2000) do not have even a hint of a separate peak that would define the porous phase and therefore it is not possible to characterize the pore size in the quantitative manner. Despite this, it is possible to extract somewhat interesting qualitative information from the measured data. With the additional TGA measurement (using TA Instruments, TGA 5500), it is possible to determine the amount of freezable bound water. Based on the detected amount of water and the values measured by DSC, it is possible to evaluate that the onset temperature of the hydrogels shifts to lower temperatures as the molecular weight of the polymer increases. It indicates that the greater amount of freezing water, the more the normal and stable form of water is disturbed. Therefore, the higher the freezing water content, the larger the pores in the hydrogel.

### 3.2. Direct visualization methods

In the evaluation of methods focused on direct visualization of the gel ultrastructure, atomic force microscopy (AFM; Bruker Corporation, NanoWizard 4 XP BioScience), scanning electron microscopy (SEM; Carl Zeiss AG, EVO LS 10), and cryogenic scanning electron microscopy (CryoSEM; Thermo Fisher Scientific, Magellan 400) were used.

AFM is fundamentally different from other types of microscopes since the resulting image is not created by focusing light or electrons on the surface of the sample. Using AFM, the sample is spatially mapped based on the atomic forces between the sample and a sharp probe consisting of a tip (probe), which is located at the end of the beam (cantilever). Therefore, the samples could be measured in their native swollen state and the structure of the sample could not be destroyed by artifacts created during sample preparation for measurement (e.g., freezing). A big advantage of AFM images (**Figure 2A**) compared to SEM images is their homogenous illumination and contrast. The images taken of PVA hydrogels did not have as well discernable polymer network structure as we have previously found for macroporous gels, e.g., agarose (data not shown). Most likely this was caused by the fact that we are two to three orders of magnitude lower than the agarose hydrogels. The resulting pore sizes confirm the trend (**Figure 1**) of decreasing pore size with increasing PVA concentration, as described in [3]. Even these values for the lower molecular weight sample match the values reported in this paper.

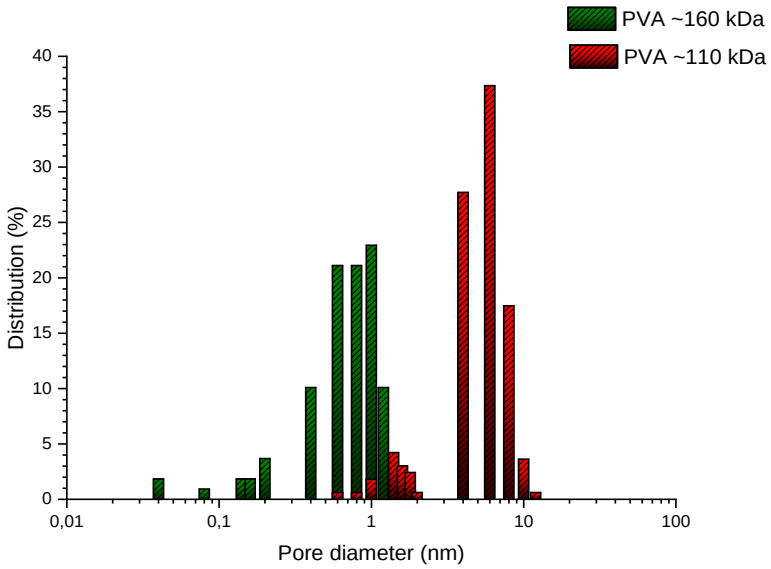


**Figure 1** Histogram of the distribution of pore sizes obtained from AFM measurements and image analysis for PVA hydrogels (results of image analysis provided by Particle analysis tool in ImageJ as described previously [4])

Compared to AFM, samples for SEM measurements were converted into xerogel form. This modification of the sample is likely to create artifacts that can disrupt the internal structure of the samples. No measurement using this method produced adequate results and these results showed (**Figure 2B**) a distorted structure. A possible solution to this problem could be measurement under cryogenic conditions using the CryoSEM technique.

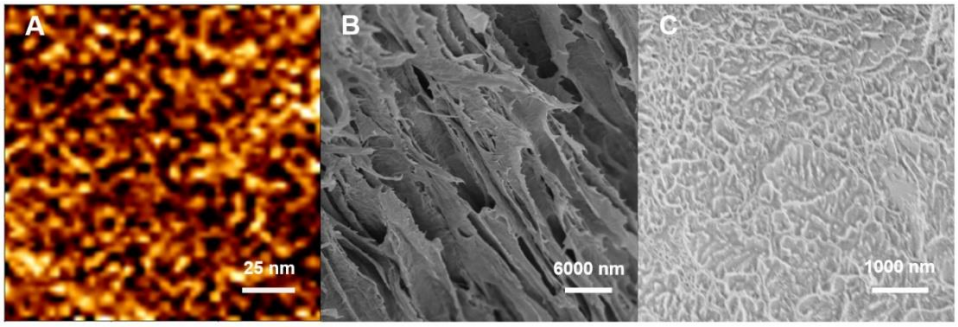


**Figure 2** PVA sample (110 kDa and 10 wt.% of polymer) visualized by AFM (A), SEM (B), and CryoSEM (C)

The results obtained with CryoSEM show similar nanostructures (**Figure 2C**) as with AFM, except that the AFM images are displayed at a scale almost 10 times larger. CryoSEM shows a lower contrast, which could increase with further modification of the samples, however, these results cannot yet be used for image analysis. Compared to classic SEM a swollen structure can be analyzed, this structure could be considered as real, however, it is necessary to take into account all modifications that could alter the sample structure during sample preparation for analysis.

### 3.3. Rheology

The mechanical properties depending on the internal structure of the fully swollen hydrogels were characterized using a rotational rheometer (TA Instruments, Discovery Hybrid Rheometer (HR-2)). Since hydrogels are viscoelastic materials (i.e., they exhibit both viscous and elastic behavior), rheology (the measure of flow and deformation behavior of liquids and solids) is an appropriate method for characterizing hydrogel mechanical properties. The sample is placed between the plates with a defined gap, and the upper plate performs an oscillating movement. All hydrogels showed a common plateau in the frequency sweep, i.e., the elastic moduli were frequency independent in the range from about 0.1 to 10 rad/s. Therefore, these hydrogels exhibit a soft rubbery behavior. Polymers with lower concentrations of PVA and molecular weights show lower values of elastic moduli, and their plateau has a relatively narrow range. In contrast, hydrogels with higher mass concentrations and molecular weights have the opposite trend. The change in toughness or stiffness of these hydrogel networks can therefore be correlated with their composition and internal structure.

The mesh size of the hydrogels was calculated based on the rheological data using the rubber elasticity theory [2]. In hydrogels, the crosslinking density increases with increasing polymer concentration. This leads to the increase in the elastic modulus and at the same time to the decrease in the molecular weight of the chain between the two nodes. Hydrogel networks with a higher concentration and molecular weight of the polymer show a smaller average mesh size. In a wide range of concentrations, the trend of decreasing porosity with increasing concentration and molecular weight of PVA was well reproducible, as you can see in **Figure 3**.

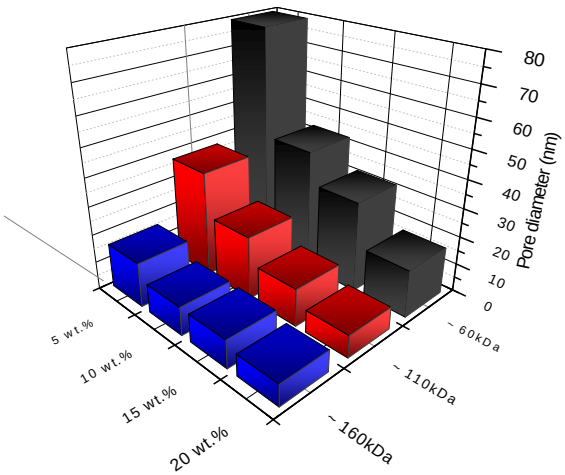


**Figure3** Dependence of the input parameters of the PVA polymer on the resulting pore sizes; hydrogels prepared in a laboratory freezer

### 3.4. DLS microrheology

The DLS (dynamic light scattering) measurement is based on observing the movement of tracer particles with defined particle size in the sample via monitoring the intensity of the light scattered by these particles. The selection of suitable particles for microrheological measurements can critically affect the measurement results. Based on theoretical knowledge [3] of the framework pore size in the selected PVA hydrogels, polystyrene (PS) particles with an average size of 50 nm, which are larger than the predicted meshes of the polymer network, were selected. If the selected particles were smaller, their free passage through the mesh would occur and the method would not provide an accurate rheological response. Furthermore, if the particles were much larger, there would be complex interactions of the particles with the entire network. Micrometer-sized particles also begin to exhibit non-Brownian behavior with thermal fluctuations reduced below realistically measurable levels. The measured data (using Malvern Panalytical, ZetaSizer Nano ZS) of the MSD curves were transformed into the form of viscoelastic moduli using complex mathematical apparatus. The subsequent

evaluation was based only on the middle part of the average elastic moduli, the so-called plateau. To evaluate this part of the modules, we decided to use the mathematical apparatus of inverse derivation. The results of these measurements provide data that are almost correlated with the values discussed in the literature [3].

### 3.5. Turbidimetry

Polymer networks creating pores in hydrogels are responsible for the turbidity of final hydrogels. Measuring the loss of intensity of transmitted light caused by the light scattering due to this turbidity can offer information about the internal structure. The turbidimetric measurement (performed using UV-VIS spectrophotometer: Hitachi High-Technologies Corporation, U-3900H) of PVA hydrogels was carried out based on an article [5] in which the author dealt with agarose hydrogels. To evaluate the measured data, the part of the spectrum that corresponds to the shape of the scattering spectrum without radiation interference was selected. The part of the spectrum in which the interference occurred was fundamental to the final calculation and the limitation of the correlation curve was based on the wave exponents associated with this part. Therefore, it was not possible to calculate the exact pore sizes. Nonetheless, it was possible to determine the predicted trends regarding the porosity of these hydrogel materials. According to the directions of the individual curves, a decreasing trend can be confirmed depending on the increasing molecular weight, e.i., the trend that was observed for all previous methods. For ordinary comparison of set of samples, this method is suitable and simple to perform.

## 4. CONCLUSION

This work focused on the optimization and testing of methods suitable for the analysis of the microstructure of hydrogel materials with a particular focus on the determination of pore size. Each method tested has specific advantages and disadvantages (summarized in **Table 2**).

**Table 2** Comparison of discussed methods

| | **Advantages** | **Disadvantages** | **Economic demands of operation** |
|---|---|---|---|
| DSC thermoporometry | small amount of material; both dried and hydrated samples can be measured | poor results for aqueous solvents, better results with organic solvents | $$$ |
| SEM | topography measurement | impossible to measure hydrated samples; deformation of the sample during preparation | $$$$ |
| CryoSEM | measurement of the samples in the hydrated state | possible sample deformation during cryofixation or sublimation | $$$$$ |
| AFM | universal method; allows obtaining qualitative and quantitative information about physical parameters, 3D | low scan speed; both the sample and the cantilever can be damaged during the measurement; prolonged optimization of new materials | $$ |
| Rheology | quick and easy method; samples are measured in the hydrated state | it is not always possible to prepare a sample of the same size | $$ |
| DLS microrheology | small amount of material; fast method with higher measurement ranges | complex sample preparation; selection of a suitable probe for measurement | $ |
| Turbidimetry | the simplest of the commented techniques | complex sample preparation; there is no mathematical apparatus connecting the issues for a wider range of materials | $ |

The AFM method shows admirable and, in our opinion, the most reasonable results, which, however, are redeemed by relatively complex measurements and optimization for each measured sample. Therefore, it would be advisable to combine this technique with the simpler technique for a faster determination of e.g., rheology, microrheology, turbidimetry, or DSC. The optimization of the combination of these two chosen

methods should be carried out on a wide range of different materials and should try to find a connection between the results, thanks to which it should be possible to subsequently use a simpler and faster variant in the routine porosity-mapping assays. However, the limitations of individual methods and the dynamic nature of hydrogel polymer networks must still be considered.

## REFERENCES

[1] LANDRY, Michael R. Thermoporometry by differential scanning calorimetry: experimental considerations and applications. *Thermochimica Acta.* 2005, vol. 433, pp. 27-50. Available from: https://doi.org/10.1016/j.tca.2005.02.015.

[2] PESCOSOLIDO, Laura, FERUGLIO, Luigi, FARRA, Rossella, FIORENTINO, Simona, COLOMBO, Italo, COVIELLO, Tommasina, MATRICARDI, Pietro, HENNINK, Wim E., VERMONDEN, Tina, GRASSI, Mario. Mesh size distribution determination of interpenetrating polymer network hydrogels. *Soft Matter.* 2012, vol. 8, pp. 7708-7715. Available from: https://doi.org/10.1039/C2SM25677K.

[3] HICKEY, Alla S., PEPPAS, Nikolaos A. Mesh size and diffusive characteristics of semicrystalline poly(vinyl alcohol) membranes prepared by freezing/thawing techniques. *Journal of Membrane Science.* 1995, vol. 107, pp. 229-237. Available from: https://doi.org/10.1016/0376-7388(95)00119-0.

[4] TRUDICOVA, Monika, SMILEK, Jiri, KALINA, Michal, SMILKOVA, Marcela, ADAMKOVA, Katerina, HRUBANOVA, Kamila, KRZYZANEK, Vladislav, SEDLACEK, Petr. Multiscale Experimental Evaluation of Agarose-Based Semi-Interpenetrating Polymer Network Hydrogels as Materials with Tunable Rheological and Transport Performance. *Polymers.* 2020, vol. 12, pp. 1-25. Available from: https://doi.org/10.3390/polym12112561.

[5] AYMARD, Pierre, MARTIN, David R., PLUCKNETT, Kevin P., FOSTER, Tim J., CLARK, Allan H., NORTON, Ian T. Influence of Thermal History on the Structural and Mechanical Properties of Agarose Gels. *Biopolymers.* 2001, vol. 59, pp. 131-144. Available from: https://doi.org/10.1002/1097-0282(200109)59:3<131::AID-BIP1013>3.0.CO;2-8.