Palkovits, D., Domingo-Roca, R., Lam, K.-H., & Windmill, J. F. C. (2025). Hydro-locked hydrogel-based retinal phantom development for ultrasound imaging applications. In 2025 IEEE International Ultrasonics Symposium (IUS) (pp. 1-4). (2025 IEEE International Ultrasonics Symposium (IUS)). IEEE. https://doi.org/10.1109/ius62464.2025.11201550

Hydro-locked hydrogel-based retinal phantom development for ultrasound imaging applications[1]

Dorottya Palkovits
Department of Electronic & Electrical Engineering
FUSE CDT
Glasgow, UK
dorottya.palkovits@strath.ac.uk

Roger Domingo-Roca
Department of Electronic & Electrical Engineerin)
University of Strathclyde
Glasgow, UK
roca@strath.ac.uk

Kwok-Ho Lam
Centre for Medical and Industrial Ultrasonics
University of Glasgow
Glasgow, UK
kwokho.lam@glasgow.ac.uk

James F.C. Windmill
Department of Electronic & Electrical Engineering
University of Strathclyde
Glasgow, UK
james.windmill@strath.ac.uk

***Abstract*— Hydrogel-based tissue phantoms are widely used in ultrasound imaging research due to their ability to replicate the acoustic and mechanical properties of biological tissues. However, conventional hydrogels suffer from rapid dehydration and poor long-term stability, limiting their effectiveness in reliable or repeatable imaging studies—particularly for delicate structures such as the retina. To address this limitation, we present a scalable method for extending hydration stability in hydrogel-based retinal phantoms through the development of a hydro-locking polymer network. Two sulphuric acid (H_2SO_4) treatment approaches were investigated: (1) pre-polymerization incorporation into the hydrogel precursor, and (2) post-polymerization immersion. Acid concentrations ranging from 10% to 40% (w/w) were evaluated, with thermal processing at 65 °C applied to stabilize chemical bonding. Supplementary storage measures, including refrigeration and cling film wrapping, were implemented to further preserve phantom integrity. Results demonstrate that H_2SO_4 promotes ionic and hydrogen bonding between water and methacrylated monomers, significantly reducing water loss. The most effective treatment extends hydration stability from minutes to several weeks without compromising acoustic performance. These findings support the development of durable, reusable hydrogel-based retinal phantoms suitable for ophthalmic ultrasound system calibration and testing, with ongoing imaging validation studies in progress.**

Keywords— ***dehydration, hydrogel, imaging, phantom, retina, sulphuric acid, ultrasound***

I. INTRODUCTION

1.1 Phantoms

Eyeball phantoms are synthetic models fabricated from tissue-mimicking materials that replicate the acoustic characteristics of ocular structures, including the retina, lens, cornea, and vitreous and aqueous humour. Commonly used materials—such as silicone-based compounds, agarose, gelatine, polyacrylamide, alginate, polyurethane, and epoxy—are carefully calibrated to match the speed of sound and attenuation coefficients of biological tissues [1].

A range of fabrication techniques, including 3D printing, bioprinting, and precision moulding, has been developed to capture the complex anatomical features of the eye. These phantoms have diverse applications in ultrasound imaging research and clinical practice, including diagnostic imaging, therapeutic ultrasound development, and professional training, offering a safe and cost-effective platform prior to pre-clinical and clinical studies. Hydrogel phantoms are widely used in these applications and are also employed as scaffolds in tissue engineering and as models of the extracellular matrix in biological studies [2].

Despite significant progress, challenges remain in achieving high anatomical fidelity, long-term material stability, and reproducibility [3,4]. In particular, dehydration and microbial contamination limit the durability of gel- and agar-based models. A further challenge for ultrasound ocular phantoms lies in maintaining these acoustic properties over extended periods. Hydrogels, while ideal for mimicking soft tissue acoustics, are prone to dehydration and microbial degradation, compromising their long-term stability and usability. Addressing these limitations, this study explores the integration of a sulphuric acid-based hydro-locking strategy into hydrogel-based eyeball phantoms to enhance water retention, preserve acoustic performance, and extend shelf life, thereby enabling reliable use in ophthalmic ultrasound calibration, training, and therapeutic development.

1.2 Hydrogels

Hydrogels are increasingly utilised in the development of ophthalmic phantoms for ultrasound applications due to their ability to replicate the acoustic properties of biological tissues by allowing fine-tuning of their mechanical and acoustic properties. Their high water content and biocompatibility make them particularly effective for simulating ocular structures such as the retina, cornea, and vitreous humour.

Hydrogel synthesis includes polymeric precursors, which need to undergo crosslinking by either a chemical, thermal, or photo-initiated method. Commonly used polymers include polyethylene glycol diacrylate (PEGDA), behenyl methacrylate

---

[1] This work was supported by the Engineering and Physical Sciences Research Council [grant number EP/S023879/1, EPSRC Centre for Doctoral Training in Future Ultrasonic Engineering]. For the purpose of open access, the author has applied a Creative Commons Attribution (CC BY) license to any Author Accepted Manuscript version arising.

(BEMA), gelatin, methacryloyl (GelMA), polyacrylamide, and agarose [5]. The preparation process involves dissolving these polymers in aqueous solutions, adding crosslinking agents or photo-initiators, and then curing the solution under controlled conditions to achieve a solid hydrogel with specific structural properties [6].

Tuning the acoustic properties of hydrogels is critical for their application in ultrasound imaging. Parameters such as polymer concentration, crosslink density, and the inclusion of additives or scatterers are adjusted to replicate the acoustic properties of ocular tissues [6]. As an example, the hydrogel's density and stiffness can be induced by increasing the polymer concentration, which can directly influence its acoustic behaviour; or using additives (such as microspheres or nanoparticles) to improve the hydrogel's echogenicity, making it more reflective [8].

Regardless of their versatility, they can face similar challenges, such as dehydration, material degradation, or long-term stability, which are based on the materials applied to create the hydrogel [7]. Their adaptability compensates for their disadvantages, making them essential in the development of ultrasound phantoms, and thereby contributing to the improvement of ophthalmic diagnostic techniques in the long term.

The concept of "hydro-locking" using sulfuric acid for hydrogels, particularly alginate-polyacrylamide double networks, was introduced by Zhang *et al*. [9]. This approach immobilizes water molecules within the polymer network, thereby protecting the primary chemical structure from collapsing under wide temperature ranges. As a result, the hydrogels retain their stretchability and functional integrity across extreme circumstances. The method is hypothesized to be broadly applicable to other hydrogel systems, offering potential for the preservation of diverse hydrogel materials.

II. METHODS

The development of the retina phantom involved designing the phantom based on the specific acoustic properties of the retina and the hydrogel materials, including speed of sound, attenuation, acoustic impedance, thickness, and density. The acoustic parameters of various ocular components were systematically compared with those of potential candidate materials. The material closest to the eyeball characteristics was the 20% bisphenol-A ethoxylate diacrylate (BEMA) hydrogel. With either direct incorporation, or post-polymerization immersion, different concentrations of 1M solution of sulphuric acid (10%, 20%, 30%, 40%) were introduced, followed by thermal processing at 65 °C. Ethyl phenyl(2,4,6-trimethylbenzoyl) phosphinate (TPO-L) was used as a photo-initiator, and tartrazine (TTZ) as a photo-blocker. These materials were photo-crosslinked using an ASIGA MAX X27 (385 nm) 3D printer, with varying exposure times systematically applied. For each exposure time, measurements were conducted in triplicate across all material concentrations. Thickness measurements were obtained using a calliper and subsequently analysed in MATLAB to calculate the precise energy required for gelation.

Acoustic testing of the materials was conducted in a water tank using ultrasound transducers with center frequencies of 5 and 10 MHz to measure their speed of sound, attenuation, and impedance. Once the phantom achieved the desired acoustic accuracy, simulation results were validated using COMSOL to compare the real-life eye values with experimentally obtained data. To facilitate this comparison, 2D computer models were developed for COMSOL and set up using the nonlinear pressure acoustics module, simulating a realistic eyeball, and then the phantom eyeball. Ultrasound simulations were performed on this model at 10MHz, thereby establishing a benchmark for analysing ultrasound propagation through the eye.

III. RESULTS AND DISCUSSION

A. BEMA phantoms

The baseline phantom models were fabricated using 20% bisphenol-A ethoxylate diacrylate (BEMA) hydrogels with photoinitiator (TPO-L) and photoblocker (TTZ). Two sulphuric acid treatment pathways were evaluated: (1) immersion of fully polymerized phantoms in solutions of varying acid concentrations, and (2) direct incorporation of the acid into the pre-polymer mixture prior to printing. Material formulations and component ratios were systematically tested and adjusted to achieve acoustic properties closely matching those of the human eye. The acoustic properties of the BEMA material closely matched those of the retina, with measured sound speeds of 1527 m/s and 1528 m/s, acoustic impedances of 1.63 MRayl and 1.59 MRayl, and densities of 1070 kg/m³ and 1036 kg/m³, respectively.

B. Adding the Sulphuric Acid

The introduction of sulphuric acid induces protonation and facilitates the formation of both ionic and hydrogen bonds between water molecules, the acid, and the methacrylated monomers (Fig. 1).

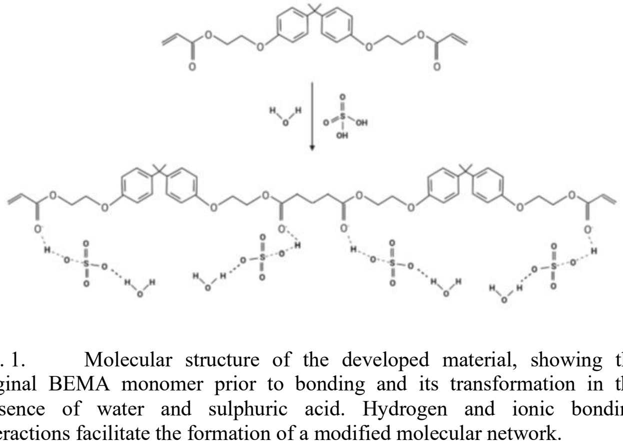


Fig. 1. Molecular structure of the developed material, showing the original BEMA monomer prior to bonding and its transformation in the presence of water and sulphuric acid. Hydrogen and ionic bonding interactions facilitate the formation of a modified molecular network.

This molecular interaction effectively immobilizes free water molecules, thereby markedly reducing dehydration. Hydrogen bonding occurs when a hydrogen atom covalently bonded to a highly electronegative atom—such as nitrogen, oxygen, sulphur, or phosphorus—acquires a partial positive charge, creating an electrostatic attraction to another electronegative atom. In this system, hydrogen bonds form between the nitrogen-bound hydrogen atoms in BEMA and the sulphur atoms of the sulphuric acid, stabilizing the hydrogel network through combined electrostatic and hydrogen-bonding effects.

Both treatment methods improved water retention, with the most effective approach extending hydration stability from a few minutes to multiple weeks.

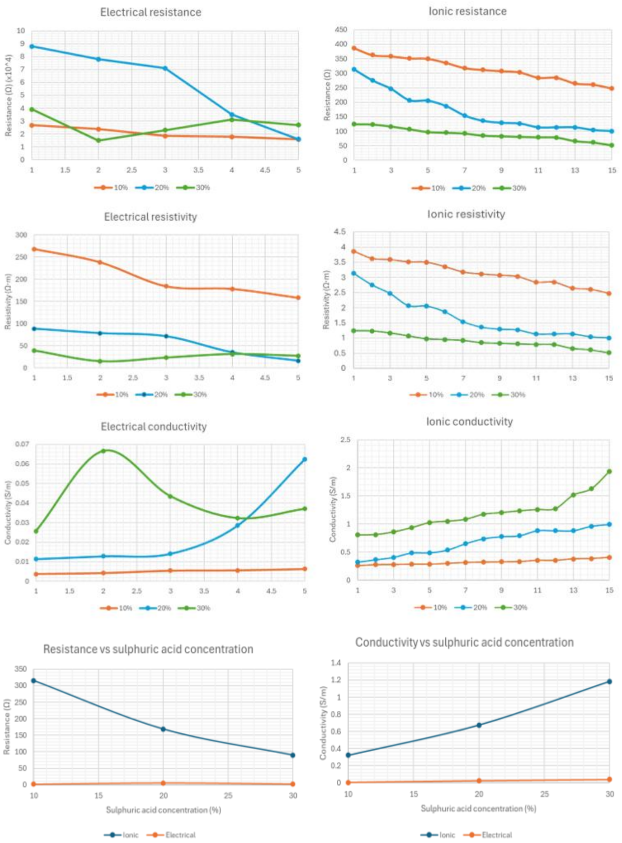
C. *Resistance and conductivity*

Given the chemical composition and structural characteristics of these materials, it is hypothesised that, while sulphuric acid molecules form hydrogen bonds with the BEMA monomers and ionic bonds with water molecules, the number of available binding sites is finite. Consequently, a fraction of water molecules remains unbound, and some sulphite ions (HSO_4^- or SO_4^{2-}) may be protonated but not fully bonded. This results in the presence of free protons (H^+) and ions within the hydrogel matrix, adding potential ionic conductivity to the material.

To verify this hypothesis, ionic and electrical characterisation tests were performed, including measurements of conductivity, resistivity, and resistance (Fig. 2). These parameters were calculated according to the relationship defined in (Eq.1.), where resistance (*R*) and conductivity (*σ*) can be calculated from the material's resistivity (*ρ*), sample length (*l*), and cross-sectional area (*A*):

$$\sigma = \frac{1}{\rho} = \frac{l(m)}{R(\Omega)A(m^2)} \tag{1}$$

This approach allows conductivity and resistance to be determined directly from measured resistivity, providing quantitative insight into the ionic transport behavior of the modified hydrogel network.


Electrical resistance

Ionic resistance

Electrical resistivity

Ionic resistivity

Electrical conductivity

Ionic conductivity

Resistance vs sulphuric acid concentration

Conductivity vs sulphuric acid concentration

Fig. 2. The ionic and electrical resistance and conductivity of the materials based on their sulphuric acid concentration, with confirming the theory that there are free ions in the materials.

The results support the theory that these materials are ionically conductive but electronically insulating, with ion conductivity increasing as the acid concentration rises, while the resistance decreases, remaining below 6 S/m. Investigating the point of saturation would be of interest to fully characterize the conductivity behavior and determine the concentration at which ion transport reaches a plateau.

D. *Comparison of hydro-locked phantoms*

Water retention capacity was assessed by measuring sample dimensions before and after a three-week dehydration period to evaluate hydro-locking performance. Measurements were performed using calipers, with three replicates per sample, and averaged for analysis.

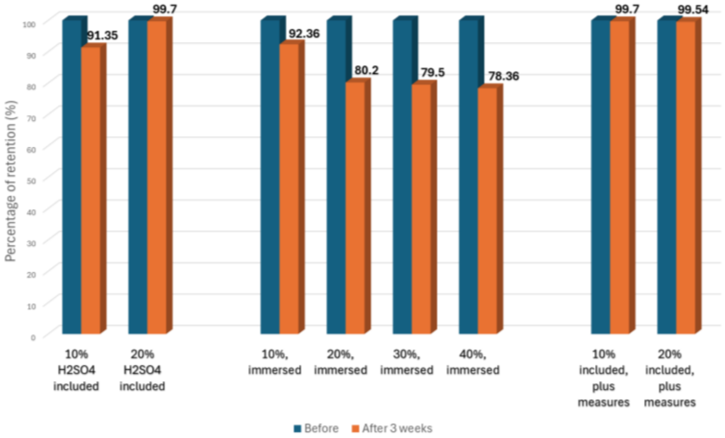


Fig. 3. Graph illustrating the percentage of water retention of the hydrogels after three weeks, relative to their original size, calculated based on the size reduction before and after the dehydration period.

This study includes materials treated via immersion in sulfuric acid (10%, 20%, 30%, and 40%) as well as direct incorporation (10% and 20%), without additional measures being introduced (Fig. 3). Directly incorporated samples (10% and 20%) were further subjected to extra precautions, including cling film wrapping and refrigerated storage.

The results indicate that water retention decreased with increasing sulfuric acid concentration during immersion, whereas direct incorporation showed minimal variation. When additional precautions were applied, both concentrations maintained over 99% of their original size after three weeks without water exposure. The highest performance was observed for BEMA materials directly incorporated with a 10% sulfuric acid solution, extending their effective water retention from a few minutes to three weeks.

IV. SUMMARY AND FUTURE WORK

Hydrogel-based tissue phantoms are widely used in ultrasound imaging research due to their ability to replicate the acoustic and mechanical characteristics of biological tissues; however, conventional formulations exhibit rapid dehydration, limiting their long-term applicability. This study presents a method for enhancing hydration stability in hydrogel-based retinal phantoms through the integration of a hydro-locking polymer network. Bisphenol-A ethoxylate diacrylate hydrogels were modified using two sulphuric acid (H_2SO_4) treatment strategies—pre-polymerization incorporation and post-polymerization immersion—at concentrations of 10–40% (w/w), followed by thermal processing at 65 °C. The acid promoted ionic and hydrogen bonding between water molecules and methacrylated monomers, effectively reducing free water loss while preserving acoustic performance.

Acoustic characterisation at 5 MHz and 10 MHz confirmed the values of speed of sound, attenuation, and impedance. Electrical measurements indicated ionic conductivity proportional to acid concentration, consistent with the presence of protons and ions within the hydrogel matrix. Hydration stability testing demonstrated that direct incorporation of 10% and 20% H_2SO_4, combined with refrigeration and cling film wrapping, retained over 99% of the phantom's original size after three weeks, extending usability from minutes to weeks. The proposed approach enables the fabrication of durable, reusable retinal phantoms suitable for ophthalmic ultrasound calibration and testing.

Future work will focus on longitudinal monitoring of acoustic parameters during the dehydration period to assess temporal changes in performance. Extended usability trials beyond the three-week period are planned to evaluate long-term stability under varying storage conditions. Further investigations will examine the influence of a broader range of sulphuric acid concentrations on ionic resistance and conductivity, enabling a more complete understanding of saturation effects. In addition, alternative chemical agents with similar water-binding mechanisms will be explored to determine their efficacy in enhancing hydration stability and preserving acoustic stability in hydrogel-based retinal phantoms.

V. REFERENCES

[1] Mencarelli M., Puggelli L., Virga A., Furferi R., and Volpe Y.,. "Acoustic velocity and stability of tissue-mimicking echogenic materials for ultrasound training phantoms," J Mater Sci, 59, 6509–6524. 2024. https://doi.org/10.1007/s10853-024-09610-8

[2] Yashwanth N. K., Singh Z., Wang Y. N., Schade G. R., Kreider W., Bruce M., *et al*, "Development of Tough Hydrogel Phantoms to Mimic Fibrous Tissue for Focused Ultrasound Therapies", Ultrasound in Medicine & Biology, Vol 48, Issue 9, 2022, Pages 1762-1777, ISSN 0301-5629,

[3] Pogue B.W., Patterson M.S., "Review of tissue simulating phantoms for optical spectroscopy, imaging and dosimetry," J. Biomed. Opt. 11(4) 041102. 2006. https://doi.org/10.1117/1.2335429

[4] Lamouche G., Kennedy B. F., Kennedy K. M., Bisaillon CE., Curatolo A, Campbell G., *et al*.,, "Review of tissue simulating phantoms with controllable optical, mechanical and structural properties for use in optical coherence tomography," Biomed. Opt. Express, 3, 1381-1398. 2012.

[5] Ho TC, Chang CC, Chan HP, Chung TW, Shu CW, Chuang KP, Duh TH, Yang MH, Tyan YC. Hydrogels: Properties and Applications in Biomedicine. Molecules. 2022 May 2;27(9):2902. doi: 10.3390/molecules27092902. PMID: 35566251; PMCID: PMC9104731.

[6] Ahmed E. M., "Hydrogel: Preparation, characterization, and applications: A review", Journal of Advanced Research, Volume 6, Issue 2, 2015, Pages 105-121, ISSN 2090-1232, https://doi.org/10.1016/j.jare.2013.07.006.

[7] El-Sherbiny IM, and Yacoub MH., "Hydrogel scaffolds for tissue engineering: Progress and challenges." Glob Cardiol Sci Pract. 2013 Nov 1;2013(3):316-42. doi: 10.5339/gcsp.2013.38. PMID: 24689032; PMCID: PMC3963751.

[8] Athanassiadis A.G, Ma Z., Moreno-Gomez N., Melde K., Choi E., Goyal R., and Fischer P., "Ultrasound-responsive systems as components for smart materials", Chemical Reviews, vol 122, Issue 5, ISSN 5165-5208, https://doi.org/10.1021/acs.chemrev.1c00622

[9] Zhang X., Li D., Yang X., *et al.* 'Hydro-locking in hydrogel for extreme temperature tolerance', Science 387, 967-973(2025). DOI:10.1126/science.adq2711