

ADDITIVE-INDUCED ENHANCEMENT OF OPTICAL CLARITY OF POLYACRYLAMIDE HYDROGEL*

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Abstract The aqueous polymerization of acrylamide and crosslinking with *N,N*-methylenebisacrylamide afforded hydrogels displaying high levels of light scattering (poor optical clarity). Enhancement of the optical clarity within a polyacrylamide (PAm) hydrogel was accomplished through the implementation of “refractive index matching”. Water-soluble additives were utilised to better match the refractive index inhomogeneities throughout a given hydrogel. This resulted in lower light scattering within the system and hence improved clarity. Amino acids, sugars, polymers, and other water-soluble additives such as glycerol were investigated by this methodology. Most additives investigated displayed potential for effectively reducing the light scattering within a PAm hydrogel as a function of increased additive concentration. On increasing the refractive index of the water medium, the overall refractive index of a PAm hydrogel was also observed to increase. This provided a quantitative means of determining the effectiveness of a given additive for improving the optical clarity within a hydrogel.

Keywords Optical clarity, Polyacrylamide hydrogel, Refractive index matching, Bacteriorhodopsin, Optical memory device

INTRODUCTION

Crosslinked polymers formed via free-radical crosslinking copolymerization are used in many current and emerging applications. When designing a crosslinked polymer for specific applications it is important to understand the network formation and the resulting material properties as each application has specific material requirements. In the case of polymer gels, it has been recognized that the material properties such as mechanical strength^[1], visco-elasticity^[2–4], kinetics of swelling and deswelling^[5–7], permeability^[8], and the optical clarity^[9, 10], all depend on their spatial inhomogeneities.

Flory^[11] and Stockmeyer^[12] were the first to publish the classical gelation theory, based initially on step growth polymerization and of an idealistic homogeneous polymer network. Since then, it has been widely accepted that a kinetically controlled process dictated by the functionality, reactivity, and concentration of the monomeric components generally governs the formation of a three dimensional polymer network^[13]. Crosslinking monomers with a similar reactivity to the chain building monomer were traditionally selected to statistically form a polymeric network with a relatively even crosslinking density and high degree of gel homogeneity. However, discrepancies between the predicted and observed behaviour of polymer gels provided some evidence for the formation of an irregular structured polymer network^[14, 15]. Because chemically crosslinked polymer gels are usually prepared by copolymerizing monomers and crosslinkers, an inhomogeneous distribution of crosslinker is introduced into the gel, predominantly due to intramolecular cyclization or from differences in the reactivity of

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the monomers and pendant double bonds^[16, 17]. Therefore, variations in refractive index are produced and the heterogeneity becomes permanently built into the gel. Inhomogeneities within such crosslinked systems are found to increase with increasing crosslinker concentration^[18, 19]. It is these inhomogeneities in refractive index that diminish the optical clarity of a hydrogel and thereby limit its potential as an optical matrix.

Optical clarity, or the fraction of illumination transmitted through a gel is related by the Beer-Lambert relationship:

$$\lg I/I_0 = -AL \quad \text{and} \quad I/I_0 = e^{-AL}$$

where the fraction of illumination transmitted through the gel, I/I_0 , is dependent on the path length of light, L , and the absorptivity of the polymer at that wavelength of light, A . The percentage of light transmitted through a gel is also dependent on the amount of light scattered internally and at the surface of the material. Scattering of light is caused by random reflection on the surface irregularities and at the interface of small particles or voids embedded within the material, owing to changes in the direction of light as it passes through media of different refractive index. The scattering of light owing to inherent variations in refractive index of a given hydrogel is of great interest when considering such systems as candidate materials for optical applications.

We have previously reported^[20] that the optical clarity of a polyacrylamide (PAm) hydrogel matrix could be enhanced through the utilisation of the additive sucrose. This was proposed to occur through a method of refractive index matching, whereby the refractive index of the medium (water) was increased more closely to that of the forming polymer network. Improved optical clarity of the PAm hydrogel was observed with increasing sucrose concentration. This preliminary study outlined the potential for preparing hydrogels as optical matrices for use in a wide variety of applications including a bacteriorhodopsin (bR) 3-D optical memory device.

In this paper, PAm hydrogels were utilized in order to outline the potential of various biologically compatible additives as possible refractive index matching agents. These additives were screened and compared to sucrose for their ability to improve the overall optical clarity within a PAm hydrogel at various concentrations. Generally speaking, the methodology of RI matching was further extended and investigated as a means of preparing hydrogels capable of application to optical systems or devices.

MATERIALS AND METHODS

Sample Preparation

Acrylamide (Am), N,N' -methylenebisacrylamide (MBA), ammonium persulfate (APS), N,N,N',N' -tetramethylethylenediamine (TEMED), sucrose, glucose, trehalose, cysteine, glycine, histidine, proline, threonine, phenylalanine, glycylglycine, glycyllucine, phenylglycine, gelatin, glycerol, and poly(vinyl pyrrolidone) ($M_w \sim 10,000$) were all purchased from Sigma-Aldrich Inc. and used as received. Ficoll 70, a polymer of sucrose, was purchased from Pharmacia Fine Chemicals and used as received. Hydrogels were prepared at room temperature (20°C) as follows; Stock solutions of 60 W/V% Am, 2 W/V% APS, 20 W/V% TEMED, and 5 W/V% MBA were prepared using distilled/deionized H₂O and all solutions were *purged* for 10 min using nitrogen.

Into each 4 mL polymethacrylate (UV grade) cuvette, 0.50 mL of Am solution, 0.120 mL MBA solution, 0.050 mL TEMED solution and 2.27 mL distilled/deionized H₂O were introduced. For gels containing RI matching additives, volumes from stock solutions of additive comprising 2.27 mL or less were used to yield the appropriate concentration. If less than 2.27 mL was utilized, the difference was made up to 2.27 mL with distilled/deionized water. Finally, 0.060 mL of APS was added to the polymerization solution, whereupon the cuvette was mixed quickly, sealed with parafilm and set aside to allow complete polymerization. For each gel prepared, a total volume of 3 mL was used.

Light Scattering Measurements

All light scattering measurements were performed on a Shimadzu RF-1501 spectrofluorometer, using water as a blank. The detector within a spectrofluorometer is 90° to the incident beam of light, and thus any light detected under the conditions employed would be a result of light scattering within the material. The detection of light

scattering in these experiments was only in one quadrant and was relative to other samples. All sample gels were measured on an emission setting, meaning the system was excited at 700 nm and the emission spectrum was detected over the range of 650–800 nm. The 700 nm wavelength was chosen for these experiments as all writing and reading of information within the bR based 3-D optical memory system previously described^[20, 21] takes place in this region of the light spectrum.

Refractive Index Measurements

All refractive index (RI) measurements for the aqueous solutions of additives were performed using a Fisher LR45302 refractometer. A blank of water was used in each case. All refractive index measurements on PAm hydrogels were performed on a J.A. Woollam Co. Inc. M-44 ellipsometer and modeled against data for a provided Cauchy material. The gels in this case were prepared as a thin film (≈ 0.3 cm thickness) between two glass plates and analyzed on the ellipsometer in free-standing form. All gels were investigated over a wavelength range of 400–1000 nm, and values of RI at 700 nm were recorded.

RESULTS AND DISCUSSION

The effects of RI matching on the optical clarity within PAm hydrogels were investigated through the incorporation of various water soluble additives into the polymerization solution prior to the onset of hydrogel formation. Various additives displaying water solubility and biological compatibility to compounds such as proteins were investigated. Sugars tend to display high water solubility and are common compounds in biological systems. Thus, a few representative sugars were investigated for their ability to lower the light scattering characteristics within a PAm hydrogel via refractive index matching. Glucose and trehalose were compared to the sugar additive sucrose that was previously described^[20]. The values for relative light scattering of PAm hydrogels containing the same weight percent concentration (15%) of these three additives can be seen in Fig. 1. These additive containing hydrogels were compared to a PAm gel containing no additive. All gels containing sugar additives were found to display a reduction in light scattering characteristics when compared to that of a gel without additive.

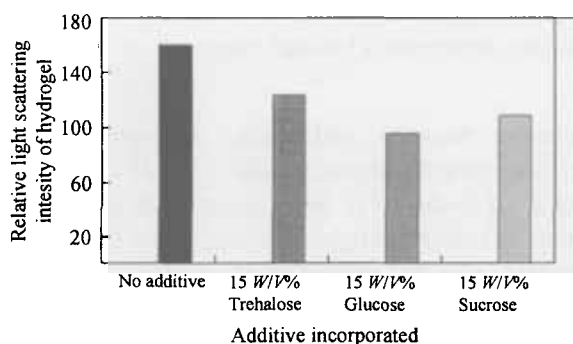


Fig. 1 Light scattering comparison of PAm hydrogels prepared with no additive, 15 W/V% trehalose, 15 W/V% glucose, and 15 W/V% sucrose

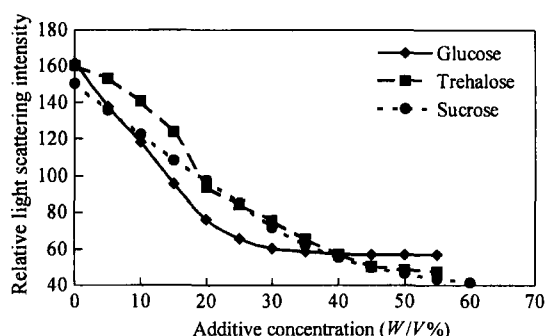


Fig. 2 Relative light scattering intensities for hydrogels containing the additives sucrose, glucose and trehalose over a range of concentration

The trehalose and glucose sugar additives were investigated over a range of concentration within the hydrogel system and compared to sucrose (Fig. 2). For the additive glucose, after initial reductions in light scattering with increased concentration, the additive reaches a plateau at approximately 40 W/V%. This is likely due to the fact that glucose is a reducing sugar and may act to disrupt acrylamide polymerization at increased concentrations, possibly by quenching propagating radical species. Trehalose and sucrose are both non-reducing sugars and exhibit a similar trend in light scattering reduction for a PAm hydrogel, the exception being at lower concentrations. Trehalose also displayed a slight plateau in reduction beginning at about 45 W/V%.

In addition to sugars, amino acids display variable water-solubility and have known compatibility with

biological compounds such as proteins. Several amino acids and amino acid derivatives were investigated for their RI matching potential. Amino acid solubility in water is limited when compared to the sugar additives, however, effective reductions in inherent light scattering of a PAm hydrogel were observed for all the amino acid and amino acid derivatives investigated. These enhanced gels were compared to a hydrogel containing no additive and a hydrogel containing 30 *W/V*% sucrose, as seen in Fig. 3. In this figure, the PAm hydrogel containing no additive was used as a reference such that its inherent amount scattering was defined as being 100% (not enhanced). All hydrogels prepared containing amino acid based additives were observed to display improved optical clarity and were thus compared in a relative manner as a percentage of the total light scattering observed within the hydrogel containing no additive. These amino acid based additives yielded hydrogels with similar or improved optical clarity when compared to a hydrogel containing sucrose at 15 times the additive concentration.

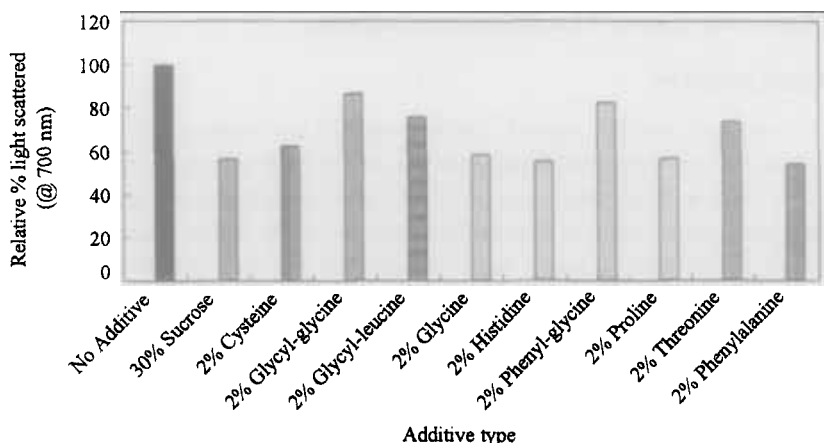


Fig. 3 Observed % light scattered by PAm hydrogels containing various amino acids, and amino acid derivatives (2 *W/V*%)
These gels are compared to a hydrogel containing no additive and a hydrogel containing 30 *W/V*% sucrose

The additives proline, histidine, cysteine, phenylalanine, threonine, and glycine displayed the most promising results and were thus investigated over a greater range of additive concentration (Fig. 4). Glycine and threonine act to improve the optical clarity of a PAm hydrogel gradually as their concentration is increased. Proline, histidine, phenylalanine, and cysteine were observed to reduce the light scattering within the hydrogel rapidly over the lower ranges of concentration. It is interesting to note that simpler amino acids (*i.e.* glycine and threonine) displayed a similar trend in light scattering reduction (Fig. 4), while the more complex amino acids displayed similarities amongst themselves. The additive cysteine was observed to inhibit the gelation reaction at concentrations greater than 4 *W/V*%. This can be explained by the fact that cysteine contains a sulfur group that likely acts as a chain transfer agent. The formed reactive sulfur species preferentially quenches with itself and thereby would result in the termination of chain propagation within the free radical polymerization reaction.

A sampling of other water-soluble additives was performed, investigating their ability to improve the optical clarity of a hydrogel through RI matching. The additives studied were glycerol, poly(vinyl pyrrolidone) (PVP) ($M_w \approx 10000$), gelatin, and ficoll 70, a polymer of sucrose. Figure 5 outlines the relative light scattering observed within PAm hydrogels prepared at 5 *W/V*% of additive concentration. With the exception of glycerol, all the other additives displayed light scattering values higher than the hydrogel containing no additive, likely owing to microphase separation of the two different polymers.

The additive glycerol was further investigated over a range of additive concentrations. PAm hydrogels were prepared containing 0–50 *W/V*% of glycerol. The inherent light scattering measurements for these gels are depicted in Fig. 6. Glycerol shows a gradual but constant decrease of light scattering within the hydrogels as a

result of increased additive concentration ($R^2 = 0.9908$). This trend in light scattering reduction is similar to that observed for sucrose^[20].

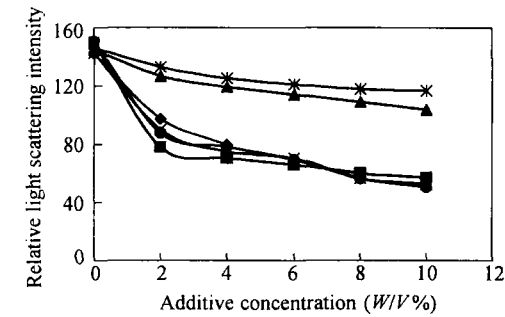


Fig. 4 Relative light scattering intensity of PAm hydrogels prepared with various amino acid additives incorporated in a concentration range of 0–10 W/V%

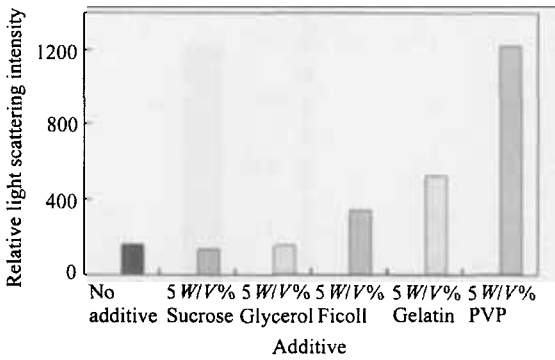


Fig. 5 Relative light scattering intensity of PAm hydrogels containing 5 W/V% glycerol, ficoll, gelatin, PVP, and sucrose as compared to a hydrogel containing no additive

The principle behind refractive index matching is to minimize the difference in refractive index between the materials in question. In the case of a hydrogel, it is between the forming polymer network and that of water. By dissolving additives in the polymerization medium, the refractive index of water is increased, minimizing this difference and improving the optical clarity of the gel. Thus, it becomes quite obvious that if this is actually the case, the more effectively a given additive increases the RI of water, the better that additive should function in yielding optically enhanced hydrogels. In order to test this methodology, aqueous solutions of various additives were prepared over a range of concentration and their refractive index was recorded and plotted as seen in Fig. 7. The aqueous additive solutions are plotted against the refractive index of water and the calculated refractive index of PAm as outlined by Swallen^[22].

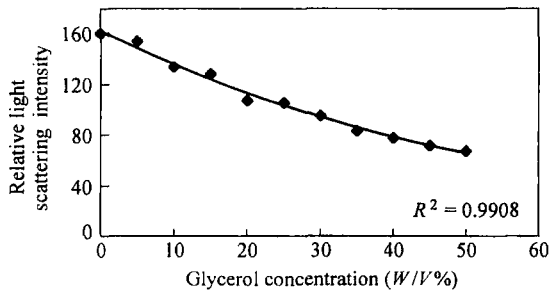


Fig. 6 Relative light scattering intensity of hydrogels prepared with the additive glycerol over a range of concentration from 0–50 W/V%

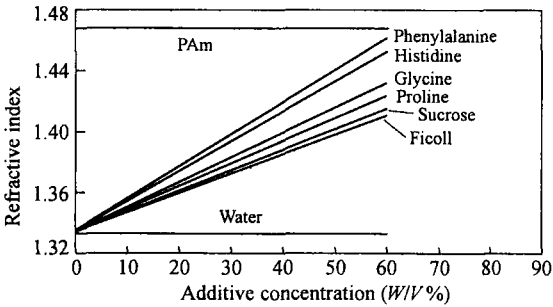


Fig. 7 Refractive index measurements for various aqueous solutions of additives taken over a range of concentration and compared to those of water and PAm

Based on the data presented in Fig. 7, a given concentration of phenylalanine increases the RI of water better than any of the other additives presented at the same concentration. Thus, at a given concentration, phenylalanine should yield a hydrogel displaying improved optical clarity over any of the other additives at the same concentration. Figure 8 outlines the relative light scattering of PAm hydrogels prepared at 10 W/V% of the additives presented in Fig. 7. The general trend observed in Fig. 8 for PAm hydrogel light scattering coincides with the data presented in Fig. 7. Incorporation of 10 W/V% phenylalanine additive results in a hydrogel with the greatest optical clarity, while incorporation of 10 W/V% of ficoll additive results in a hydrogel displaying the

least optical clarity (most light scattering).

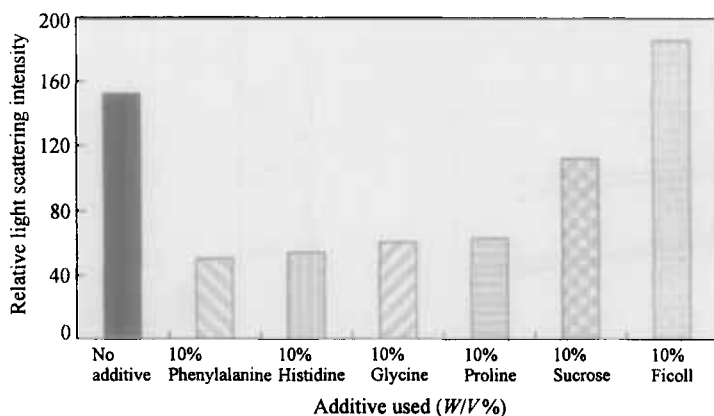


Fig. 8 Relative light scattering of hydrogels containing 10 W/V% of the various additives outlined in Fig. 7

Further verification of the successful application of refractive index matching within PAm hydrogels comes from overall RI measurements on the hydrogel systems containing each additive in question. The RI value for a given hydrogel can be simplistically viewed as an average refractive index of all the components in the system (polymer + water). Thus, as the refractive index of the water is increased via additive incorporation, the RI difference between the two components in the system is reduced but the overall RI of the hydrogel should increase. This increase of RI in the hydrogel would be a function of the additive concentration and of the ability a given additive has of increasing the RI of water. Table 1 displays the measured RI of PAm hydrogels containing different additives at a 10 W/V% concentration as compared to the overall RI of a hydrogel containing no additive. All RI measurements for the hydrogels were carried out using an ellipsometer and displayed mean standard error (MSE) values below 8.0, indicating the model developed was in good agreement with the experimental data. As expected, the hydrogel containing phenylalanine displays the greatest increase in overall RI when compared to the RI of a PAm hydrogel containing no additive. The data presented in Table 1 is in agreement with that presented in Fig. 7 and Fig. 8. By monitoring the changes an additive induces in the overall refractive index of a hydrogel, a quantitative estimate can be made of the effectiveness of light scattering reduction associated with that additive.

Table 1. Overall refractive index measurements for PAm hydrogels containing either no additive, 10 W/V% sucrose, 10 W/V% glycine, or 10 W/V% phenylalanine

Additive incorporated into PAm hydrogel	Measured refractive index of hydrogel (700 nm)
No additive	1.3791
10% W/V Sucrose	1.4010
10% W/V Glycine	1.4119
10% W/V Phenylalanine	1.4224

CONCLUSIONS

It is evident from the results presented within this paper that through the utilization of a suitable water-soluble additive, the overall optical clarity of a PAm hydrogel can be enhanced. The PAm hydrogel system has provided a means of identifying various additives and additive types that can be incorporated into other hydrogel networks for improved optical clarity. Various additives have been shown to affect the overall optical properties within a formed hydrogel to different extents. Thus, certain RI matching additives can be incorporated at much lower concentrations yet still yield the optically enhanced gels previously reported with sucrose^[20]. The methodology of refractive index matching has proven to be useful throughout this investigation and provides a foundation for the

development of other hydrogel-based optical materials. Such optically improved hydrogels have already been applied to a bacteriorhodopsin-based 3-D optical memory storage device^[20-21]. Basically, any system or device requiring a high degree of optical transmission and some degree of biocompatibility would be candidate applications for these enhanced hydrogels.

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