Determination of the Optical Properties of PNIPAAm Gels Used In Biological Applications

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ABSTRACT

Poly(N-isopropylacrylamide) i.e. PNIPAAm is a temperature sensitive smart material which displays a lower critical solution temperature (LCST) at 33-35^{o}C. At the lower critical solution temperature, the gel changes from hydrophilic to hydrophobic which has significant consequences in cell culturing. The first known measurements of the optical properties i.e. absorption (μ_a) and reduced scattering (μ'_s) coefficients, as a function of temperature, of a series of crosslinked PNIPAAm gels, using an Integrating Sphere setup, is presented at a wavelength of 632.8 nm. These properties showed a direct correlation between the scattering coefficient and the crosslinker density for the gels. The absorption properties correlated well with the known absorption characteristics of these gels.

Keywords: absorption, reduced scattering coefficient, crosslinked PNIPAAm

1. INTRODUCTION

The Integrating Sphere (IS) instrumentation, the golden standard for the measurement of bulk in vitro tissue optical properties, is applied in this work to measure the optical changes that occur in the thermo-responsive hydrogel, poly(N-isopropylacrylamide) (PNIPAAm). PNIPAAm has been extensively exploited for cell culturing due to its unique non-invasive release mechanism for cells. When the temperature crosses over its lower critical solution temperature (LCST), the surface of PNIPAAm changes reversibly from hydrophilic to hydrophobic. The reverse change from hydrophobic to hydrophilic allows for cells to be spontaneously released, without requiring destructive enzymes such as trypsin. Monitoring the optical properties of the gel as it undergoes this phase change could facilitate a simple inline monitoring system that easily identifies the gel transition. This study also provides some fundamental properties on the nature of the gel and a means to evaluate quality control in the gel synthesis from the absorption, μ_a and reduced scattering, μ'_s coefficients.

2. MATERIALS AND METHODS

The set up, shown in figure 1, comprised of a 7.4 mW He-Ne laser (λ=632.8 nm, JDS Uniphase laser) coupled into a multimode fibre (core diameter 62.5 um) using a 10X (numerical aperture (NA) = 0.25) microscope objective such that at least 90% of the laser intensity was transmitted through the fibre-lens system. The beam was collimated to a diameter of 2-3 mm using a 40X (NA=0.65) microscope objective. A heating system was connected to the sample holders (diameter 25 mm) on the entrance and exit ports of the integrating sphere (Labsphere, 8 inch (203.2 mm) diameter). The heating system works on the principle of a voltage feedback loop such that adjustments in the voltage control the temperature of the sample holders. This was necessitated by the need to maintain a constant temperature for the samples during the measurements. Diffuse transmittance and diffuse reflectance as well as the total transmittance of each gel (see Table 1) was measured in triplicates on a spectrometer (Ocean Optics USB4000) coupled to the sphere using a fibre (diameter=600 um, Ocean Optics). The spectrometer was set to take an average of 10 measurements integrated over 10 ms. By fitting the measured data to a tissue simulating calibration model, μ_a and μ'_s were extracted using the multiple polynomial regression method and the Newton-Raphson algorithm [1].

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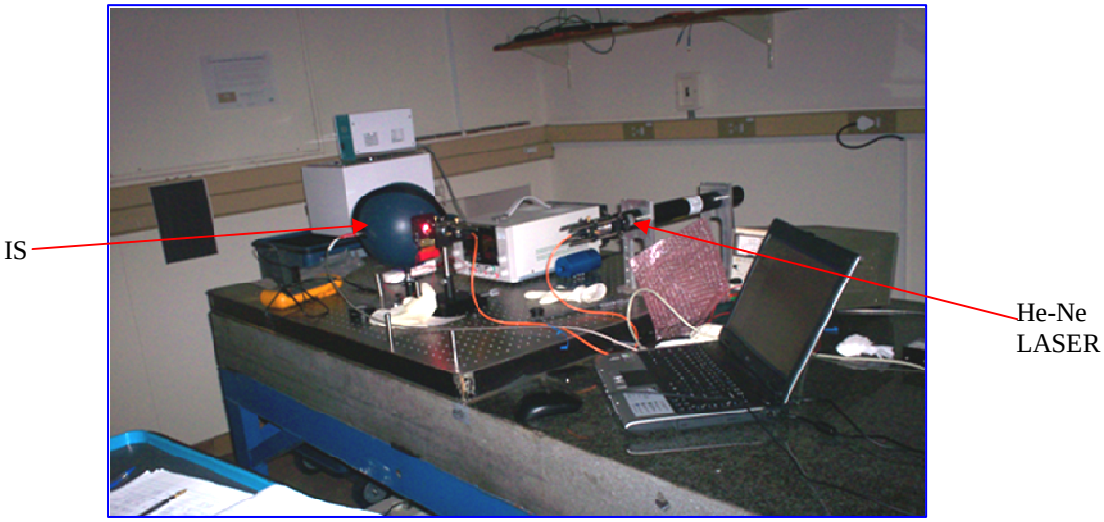

Figure 1: The Experimental setup

2.1 Gel Preparation

PNIPAAm-co-BIS gels were prepared by crosslinking NIPAAm with methylene-bis-acrylamide (BIS) in an aqueous medium using a solution free radical polymerization method [2]. The crosslink density (i.e R) of the gels varied between 91-30 by changing the NIPAAm:BIS molar ratio (Table 1). The gels were prepared in cuvettes (Figure 2) with a thickness of ~4 mm. Two microscope slides were used to sandwich a black spacer (Figure 2b) and the gel.

Table 1: Composition of the PNIPAAm gels ($R=n_{NIPAAM}/n_{BIS,}$ BIS is methylene-bis-acrylamide)

R	91	70	50	30
NIPAAm mass/g	1	1	1	1
BIS mass/g	0.015	0.019	0.027	0.045
NIPAAm mol%	98.91	98.62	98.06	96.80
BIS mol%	1.09	1.38	1.94	3.20

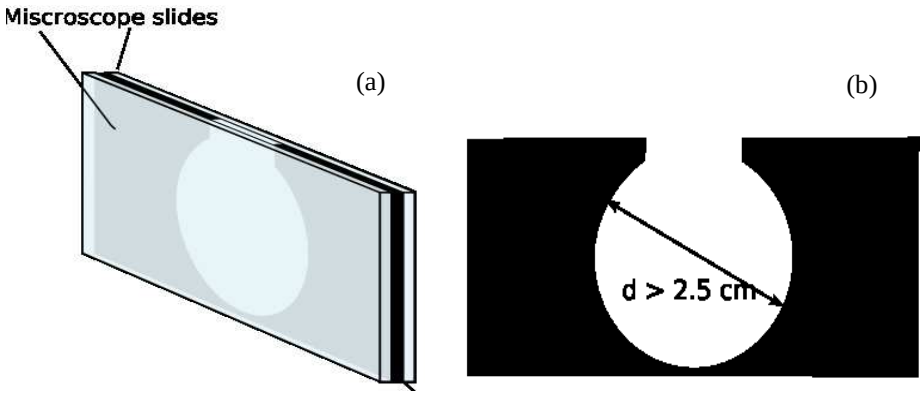

Figure 2: (a) The cuvettes (b) the spacer used

3. RESULTS AND DISCUSSION

3.1 Optical Property Measurements

The aim of this study was to determine the optical property changes occurring in PNIPAAm-co-BIS gels with R of 90-30 as a function of temperature. By studying the transmission and reflectance measurements (data to be published), a clear relationship with the phase transition of the gel was observed. This was then directly correlated to μ_a and μ'_s for the gels at different temperatures. The results, shown in Figure 3, indicate that for PNIPAAm-co-BIS gels with R91-R30, the scattering coefficient is temperature-dependent. At temperatures below 33°C all gels display a low μ'_s which oscillates around 0cm^{-1}. The negative values are treated as being zero indicating little to no scattering. However at temperatures > 33°C for gels R91-R50 and >36°C for R30 there is a rapid and sharp increase in μ'_s as the phase transition occurs. It is well known in literature that at temperatures below its LCST, PNIPAAm gels are transparent, form hydrogen bonding with water, and display a swollen coil conformation. However at temperatures above its LCST, the gels shrink, and become opaque, and change reversibly to a compact globule conformation, which is dominated by hydrophobic interactions. The observed increase in light scattering as the temperatures passes over the LCST can be attributed to the change in molecular conformation from coil to globule.

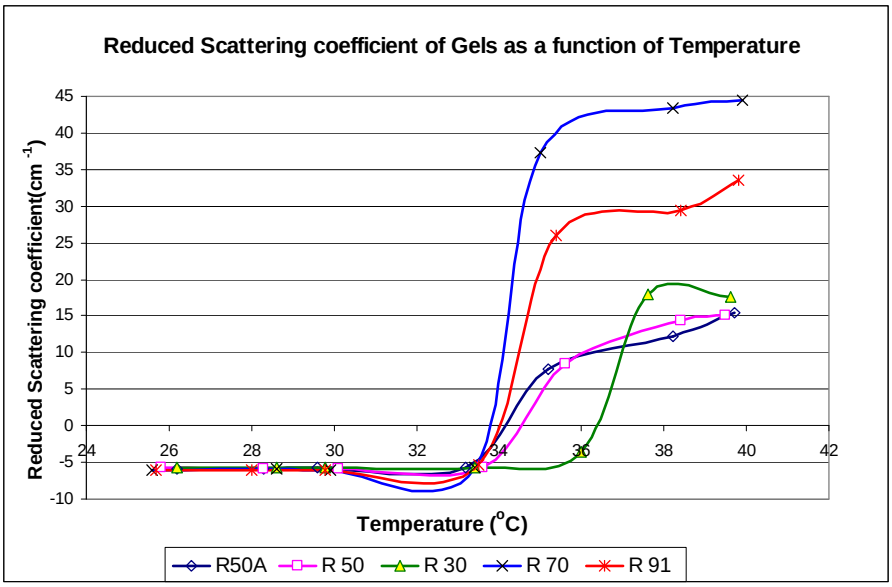

Figure 3: μ'_s as a function of temperature for the gels

This phase transition was observed to be more rapid and dominant for the gels with lower crosslink density (i.e R=91 and 70) than for gels with a higher crosslink density (i.e R=50 and 30). This could be attributed to the higher NIPAAm content in the former gels, which contain less crosslinker. At 40°C gels (R30) and (R50) reach a maximum μ'_s of (~15cm^{-1}) even though there is a difference in the initial value of μ'_s. This could be due to the close structural relationship between the two samples. The increase in the other two gels is more distinct and was found to remain different for the entire duration of the phase transition observed. This could be a means to distinguish between the different gels No comparative literature data could be found for the particular PNIPAAm-co-BIS gels investigated in this study. However measurements on linear PNIPAAm (prepared by a similar method but without BIS) was found to be between 3-5 cm^{-1} for μ'_s at temperatures between 26-40°C, and compared well to published data [3]. This sample was however not investigated in depth and is reported here with great uncertainty.

In the temperature range investigated (26-40°C), μ_a was in most instances shown to be negligible (varied between 0.05-0.12 cm^{-1}) as seen in Figure 4. The initial values for μ_a at temperatures before the phase change occurs is taken to be invalid for this particular method of evaluation due to the absence of scattering within the sample for this region. However once the phase transition has occurred μ_a is found to be negligible. This corresponded with the UV-VIS absorption spectrum of PNIPAAm (shown in Figure 5) that did not indicate an absorption peak at 632.8 nm. The early peak indicated in Figure 5 may be due to the instrument used and not necessarily a characteristic of the sample. However for this particular study that region was not an area of interest. The measurement of the absorption spectra of

the gels was a separate and independent study. The fluctuation between 0.1 and 0.18 cm^{-1} for gels (R=70 and 91) was taken to be invalid as it applies to the pre-phase transition phase but the values do tend to zero once the change occurs. However for all the gels at temperatures >33°C there is a sharp drop in absorption as the material crosses its LCST. The negative values predicted can again be assumed to be zero as it is possible the prediction of μ_a close to or on the boundary of the prediction model is less accurate than values falling within the matrix of the calibration model [4]. These changes occurring in the fundamental optical properties of the gels in this instance is evident of some structural changes occurring within the gel, which changes it from having minimal scattering to relatively strong scattering. The scattering properties are dependant amongst others on the refractive index of the sample.

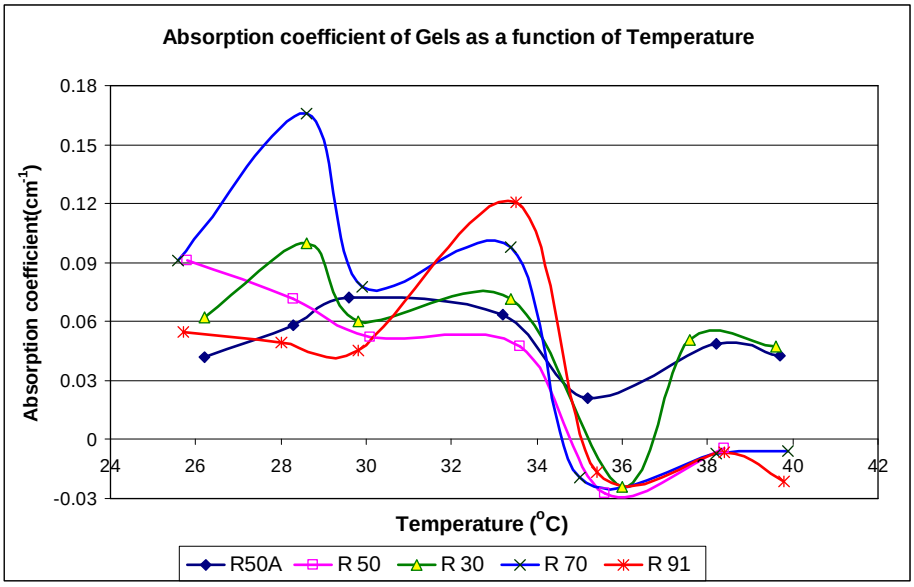

Figure 4: μ_a as a function of temperature for the gels

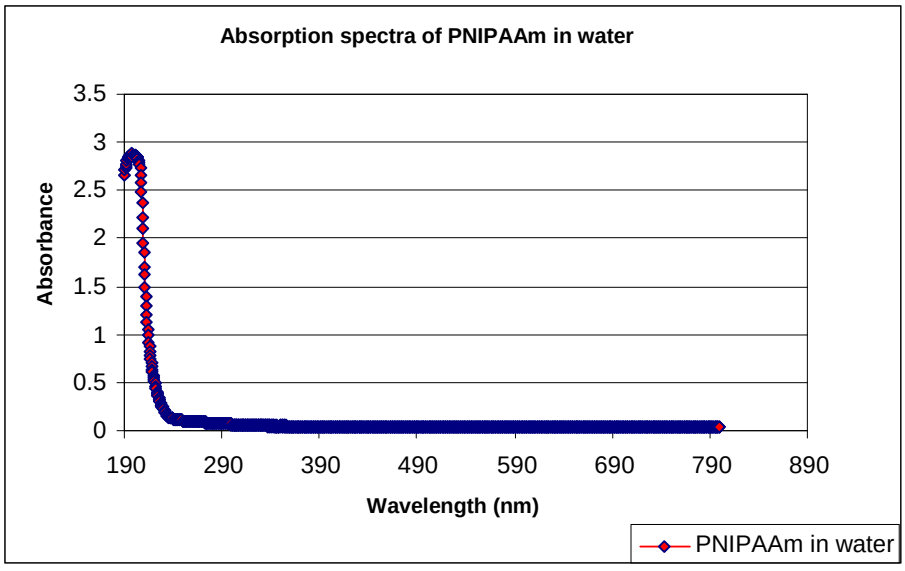

Figure 5: The Absorption spectra of PNIPAAm in water

3.2 Light Propagation through the Gels

Each μ_a and μ'_s was then input to the ASAP software to model the propagation of light through the gel. In this particular instance the absorption at different layers in the gel was modeled during the transition process.

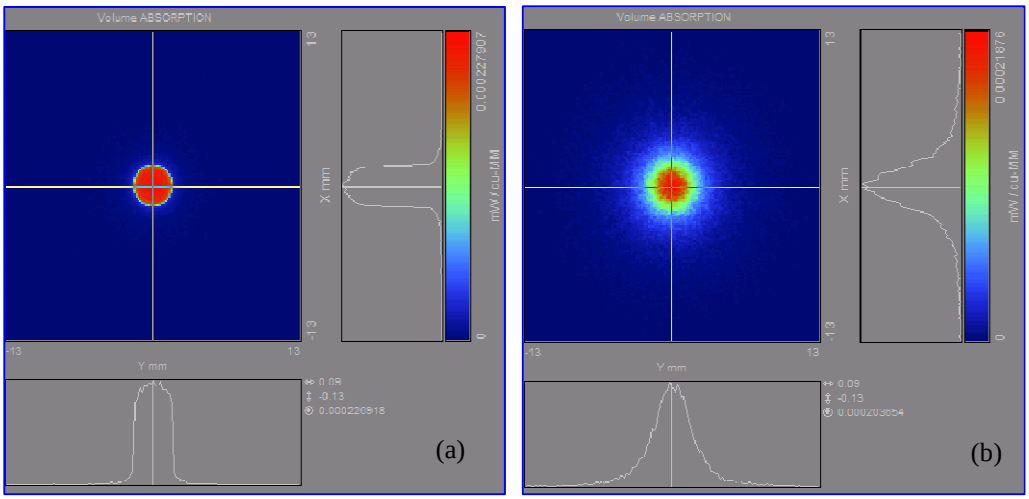

Figure 5: Absorption at a depth of 3.48 mm in gel(R=30) (a) at 36°C (b) at 39.6°C

It is a point to note that distribution of the absorbance for the 2 temperatures in the same gel is very different. There is more absorbance at the lower scattering (36°C) than at the higher scattering (39.6°C) as illustrated in Figure 5. These observations correspond to a change of 0.02 cm^{-1} for the absorption coefficient and ~16 cm^{-1} for the scattering coefficient. These observations illustrate how the optical properties of a sample influence the interaction and propagation of light within the sample. A similar distribution is seen for Gel (R91) as shown in figure 6. It must be noted that the distribution once the transition has occurred is similar but the actual absorption maximum through the sample differs as indicated in Figures 5 and 6.

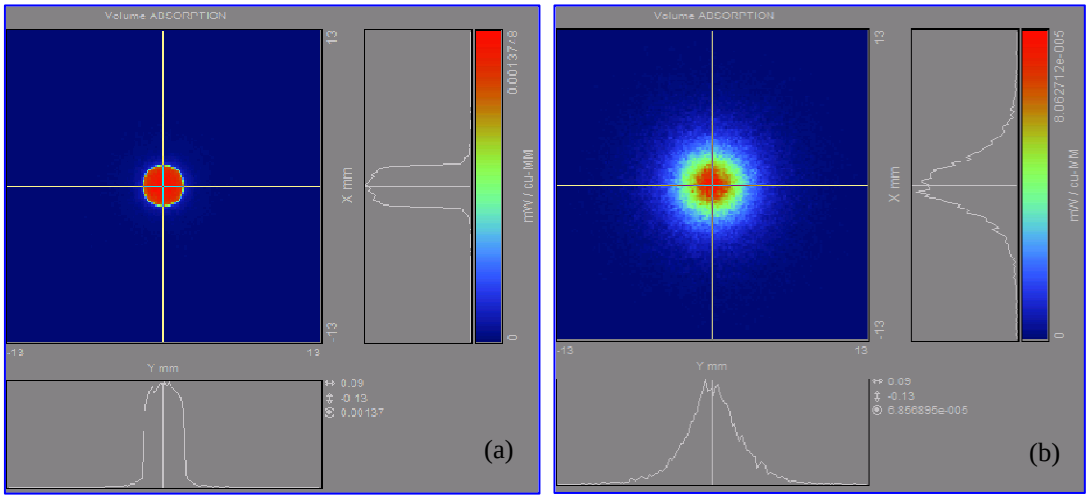

Figure 6: Absorption at a depth of 3.47 mm in gel(R=91) (a) at 33.5 °C (b) at 39.9°C

Having studied the pattern of the distribution of the absorption, the average absorption at different depths within the gel samples was investigated using the experimentally determined values for μ_a and μ'_s (see Figure 7). At the first temperature where a distinct observation was made with regard to the optical properties of the different gels, the absorption through the samples was found to be inversely related to the crosslinker density i.e. Gel (R91) > Gel (R70) > Gel (R50) > Gel (R30). The absorption appears to be constant throughout the gel. However a peak appears at the centre of the sample. This observation is a matter of great interest even if the absorption is considered negligible and will need

to be studied further. This peak was not evident for the other temperatures evaluated. The model was also assessed further to determine if this was a result of the model. However this examination revealed that the results were not due to the model. It was_interesting that the absorbance of the gels was found to be directly related to the molar ratio of NIPAAm: BIS (R). With an increasing molar ratio of NIPAAm to BIS, the crosslink density decreases and the absorbance increases. This could be attributed to the presence of more freely mobile chains in the less crosslinked gel R91 compared to the higher crosslinked gels which are more tightly packed.

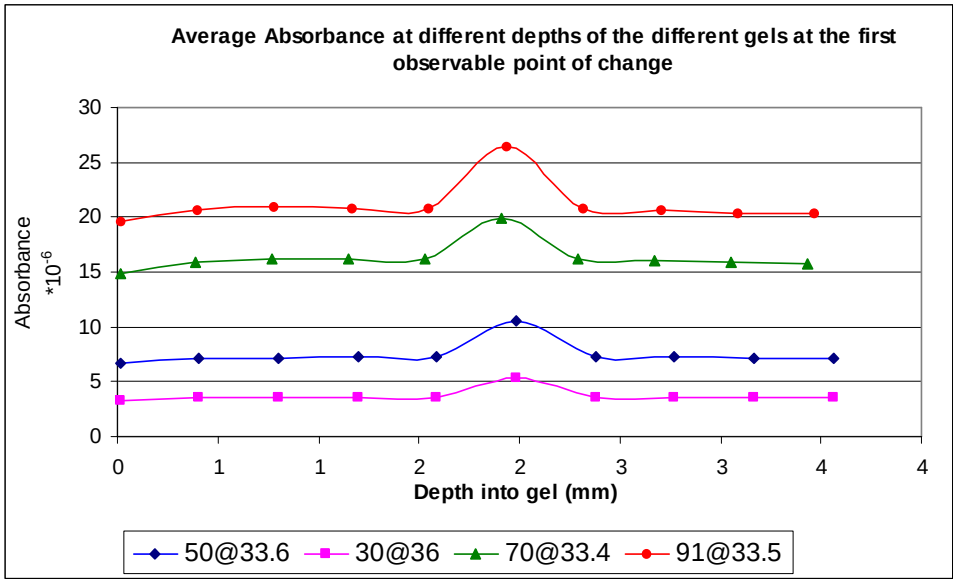

Figure 7: Average absorption at the first observable point of change for the different gels

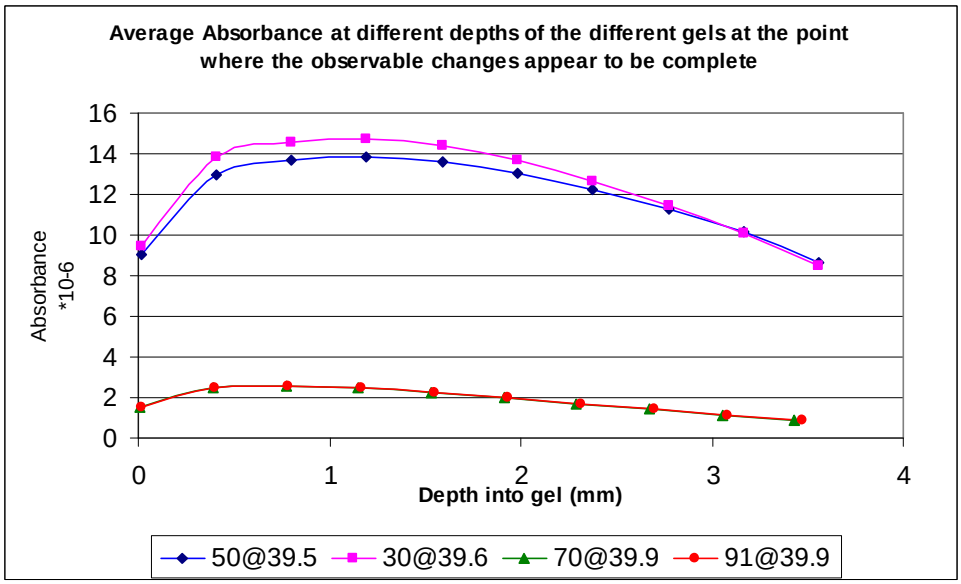

Figure 8: Average absorption at the final observable point of change for the different gels

The opposite pattern was noticed for the average absorption once the change was complete (Figure 8). There was a close relationship between Gel (R91) and Gel (R70) and between Gel (R30) and Gel (R50). There was a slight increase with the first few mm into the gel, most probably due to the backscatter light from the deeper levels, and then the absorption decreases as the light penetrated the gel further. The average absorption as shown in Figure 8 is lower for Gel (R30) and

Gel (R50) than in the first instance (Figure7) and higher for Gel (R70) and Gel (R91). This would be a consequence of the optical and structural changes occurring within these gels as stated previously.

4. CONCLUSIONS

The first known absorption and scattering coefficients of the series of gels with different crosslinker densities has been successfully determined. Furthermore they have been used to model the absorption of light through the gel for the different conditions and at the different depths using ASAP software (Breault Research Organization, Inc.). The results for the absorption fit well with the known absorption characteristics of the gel.

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