Int J Thermophys (2008) 29:2036–2045
DOI 10.1007/s10765-007-0332-6

Measurement of the Sol–Gel Transition Temperature in Agar

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Published online: 8 January 2008

Abstract Aqueous solutions of agar show a sol–gel transition temperature in the range from 32 °C to 47 °C depending on colloid concentration; however, the width of the transition and the subsequent process occurring after it, are not well understood. In this work the sol–gel transition of agar is studied by a simple optical method. It consists of the illumination of the sample using a non-coherent light source and the monitoring of transmitted light using a photodiode. It is shown that during the sol–gel transition, the transmitted light decays over a broad range of temperature. Simultaneously, it is possible to observe that fluctuations in the transmitted light decrease along the sol–gel transition, and at a specific temperature, they become very small. Based on these observations, the sol–gel transition temperature for three different concentrations of agar (0.5, 1.5, and 2.5% w/v) is determined. These results are compared with the sol–gel transition temperature values provided by the conventional rheological method.

Keywords Agar · Gelling · Light transmission · Sol–gel transition

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1 Introduction

Agar is a phycocolloid, which is constructed from complex polysaccharide molecules (D-galactose and 3,6-anhydro-L-galactose units) that is extracted from certain species of red algae (Gelidium, Gelidiela, Pterocladia, Gracilaria, Graciliaropsis, and Ahfeltia). The most important property of this substance is its capability to form gels simply by cooling hot aqueous solutions without the need of acidic conditions or oxidizing agents. This characteristic gives agars the ability to perform a reversible gelling process without losing their mechanical and thermal properties [1]. The gelling process in agar is due to the formation of hydrogen bonds in a continuous way (Fig. 1) [1]. Another important property of agar is its significant thermal hysteresis, in which the temperature difference between gelling (T_{sg}) (32 °C–47 °C) and melting (80 °C–92 °C) is very wide. These properties make agar useful as a prototype model for all gelling systems and useful in a great variety of applications [1]. In the food industry, it is used as a gelling, thickening, and stabilizing additive. Pharmaceutical applications of agar range from laxant to excipient and release agent. In the bacteriology area, it is used as a substrate media in which microbial organisms can grow. Agar is also used in some molecular microbiology techniques to obtain DNA information [1,2].

In this work, a simple new methodology to study the gelling process of agar solutions by means of the increase in the optical dispersion and the subsequent decrease in the transmitted light, when the system cools, is presented. Based on our results, two methods are proposed to analyze the sol–gel transition in terms of the light transmission, one of them based on the kinetics of its decay and the other on the fluctuation of the signal registered by the photodiode. The results obtained for the sol–gel transition

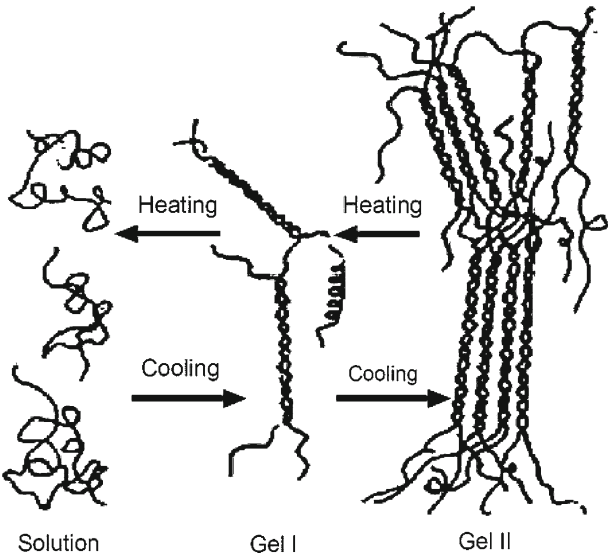

Fig. 1 Mechanisms of gel formation in agars are due to hydrogen bonding, which forces double helix formation

temperatures for the different agar solution concentrations are compared with corresponding values obtained by the traditional rheological method.

2 Experimental

2.1 Samples

Powder bacteriological agar extracted from marine algae of the genus Gelidium was used (BD Bioxon, Becton Dickinson). Three concentrations, 0.5, 1.5, and 2.5% w/v of agar dissolved in boiling distilled water, were prepared, in order to study the sol–gel transition process by means of the rheological method and the optical methods proposed in this work.

2.2 Rheological Method

There are various kinds of rheological techniques to measure the T_{sg} in gelling substances [3–5]. The most extended method, due to its simplicity, is the one in which a Pyrex glass sphere of 5 mm diameter is moving at the bottom of an assay tube filled with a solution of agar, above the temperature at which the sol–gel transition occurs [5]. When the sample is cooled, the temperature, at which the bead stops due to the gelling process, is identified as T_{sg}. The experiments for each concentration were performed in triplicate in order to verify repeatability and to determine the standard error of the mean (error bars) of the measured parameters.

2.3 Optical Method

A home-made Teflon® cylindrical cell (Fig. 2) was used. It contains a compartment in which the agar sample was placed. At one of the flat faces of the cylinder, a minilamp with a tungsten filament was installed (Radio Shack 272-1141A). At the opposite side, a glass optical bundle was mounted; its purpose was to collect the light transmitted through the sample and send it into a germanium photodiode (EG&G Judson J-16-5SP-R03M-SC). The signal was amplified using a home-made transimpedance amplifier and sent to a Keithley multimeter Model 2000.

In Fig. 3, the experimental setup used in this work is presented. The cylindrical cell and the temperature sensor are immersed in a high precision (less than 0.05 °C) temperature-controlled water bath regulated by a fully automated PID computer algorithm [6] developed in LABVIEW 6.0 ® and a Peltier thermoelectric element (TE Technology Inc. Model TE-2-(127-127)-1.15) placed at the bottom of the water recipient, and fed with a Sorensen DCS 40-25 programmable DC power supply. The system is able to perform linear temperature scans from 5 °C to 70 °C at a maximum rate of $0.5\,^{\circ}\mathrm{C} \cdot \mathrm{min}^{-1}$. The water-bath temperature was measured using a LM35DZ precision integrated-circuit temperature sensor, and registered with one of the channels of the same Keithley multimeter mentioned above.

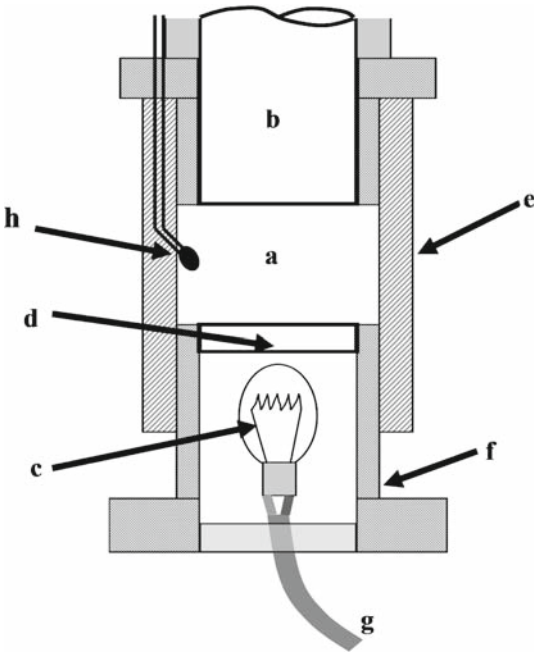

Fig. 2 Cross section of the optical cell used: (a) sample, (b) optical bundle, (c) non-coherent light source, (d) Plexiglas window, (e) Teflon cylinder, (f) light source mobile screw, (g) DC power line, and (h) NTC thermistor

To develop the experiment, the agar solution is heated to 85 °C, and then placed inside the measurement cell compartment at a constant 48 °C temperature. When the system reached thermal equilibrium, the linear decreasing temperature scan began, at a rate of $0.4\,^{\circ}\mathrm{C}\cdot\mathrm{min}^{-1}$. The temperature inside the cell was measured with a NTC thermistor (Betatherm Model 100K6A1ID), and the photodiode signal was registered by the multimeter and sent to a personal computer (Fig. 3). In order to analyze the complete sol–gel transition, the measurements were performed from 48 °C to 20 °C. The experiments for each concentration were performed in triplicate in order to verify repeatability and to determine the standard error of the mean (error bars) of the measured parameters.

3 Results

In Fig. 4, the dependence of the photodiode voltage signal (V) as a function of temperature for three typical different agar samples is presented. The transmitted light decays during the sol–gel transition due to the light dispersion induced by the gradual formation of a network of agar molecules joined by hydrogen bonding while the transition evolves (Fig. 1). The kinetics of the decay exhibits an asymmetric sigmoidal-like behavior.

In order to analyze the optical signal, the first derivative of the voltage ($\mathrm{d}V/\mathrm{d}T$) was obtained (Fig. 5a). It can be observed that $\mathrm{d}V/\mathrm{d}T$ shows a maximum at a temperature T_c. Considering that light dispersion increases due to the formation of new hydrogen bonds, T_c is a critical temperature at which the formation rate of bonding reaches a

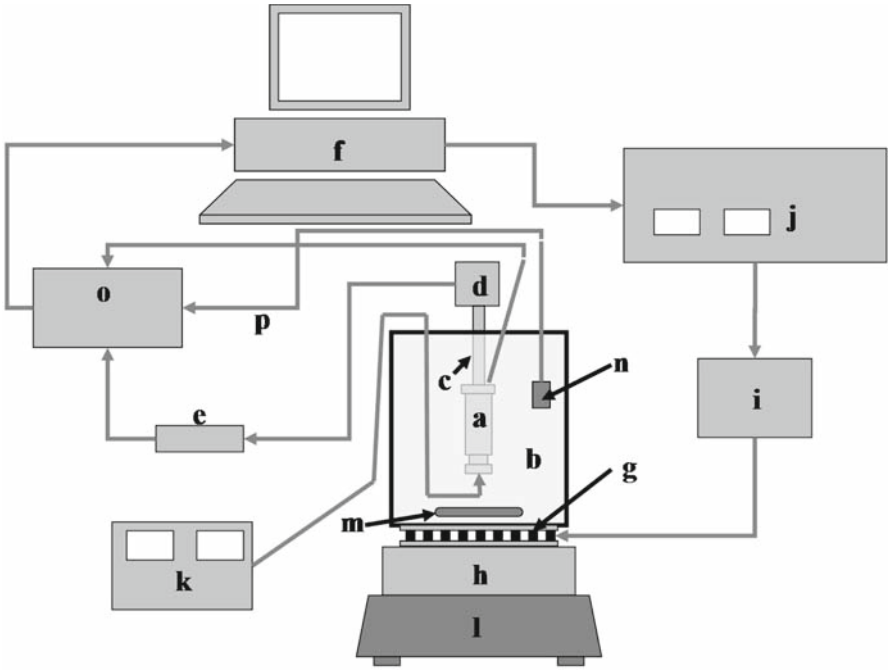

Fig. 3 Experimental setup: (a) sample cell, (b) water bath, (c) optical fiber, (d) photodiode, (e) photodiode amplifier, (f) Computer, (g) Peltier cell, (h) water radiator, (i) DC voltage polarizer, (j) programmable DC power supply, (k) low voltage DC power supply, (l) stirring device, (m) magnetic stirrer, (n) temperature sensor (for water bath temperature control), (o) multimeter, and (p) NTC thermistor temperature sensor (for cell temperature record)

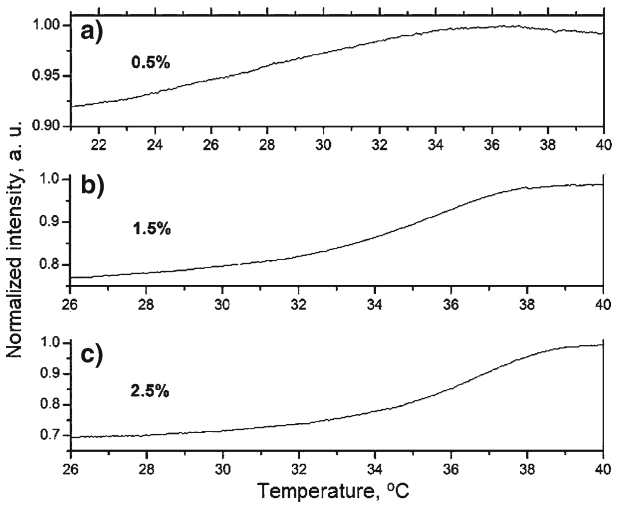

Fig. 4 Typical experimental results for the photodiode sensor voltage (with transmitted light mode) during the sol–gel transition. The different lines are for the three distinct agar concentrations analyzed (0.5, 1.5, and 2.5%)

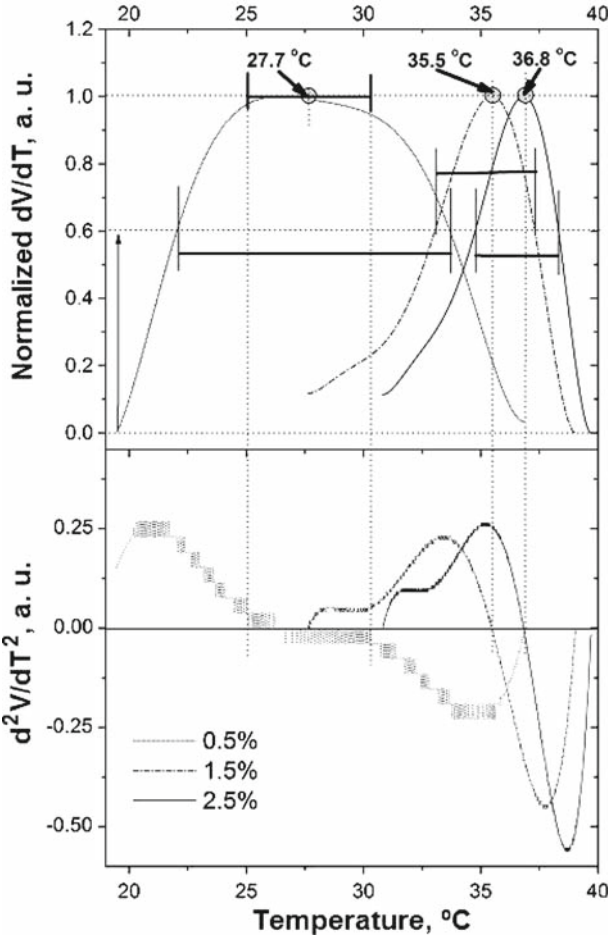

Fig. 5 (a) Derivative of the photodiode sensor voltage for the agar samples during the gelation process and (b) second derivative of the photodiode sensor voltage

maximum, and for lower temperatures, the rate of formation of those bonds begins to decrease. Therefore, the temperature T_c separates two different stages of the transition and can be considered as a useful definition of the sol–gel transition temperature T_{sg}.

It can also be observed from Fig. 5a, that samples with higher concentrations (2.5% and 1.5%) show clearly the temperature at which the first derivative of the voltage (dV/dT) has a maximum. However, the samples with low agar concentration (0.5%) show in the middle of the temperature range, around 27.7 °C, a nearly constant value. In order to have a better understanding of this behavior, the second derivative of the signal (d^2V/dT^2) (see Fig. 5b) was obtained. It can be observed that the second derivative of the voltage is nearly constant and equal to zero in the interval from 25 °C to 30 °C for 0.5% agar concentration. This indicates that in this range, it is not possible to resolve the precise temperature at which the first derivative exhibits a maximum and,

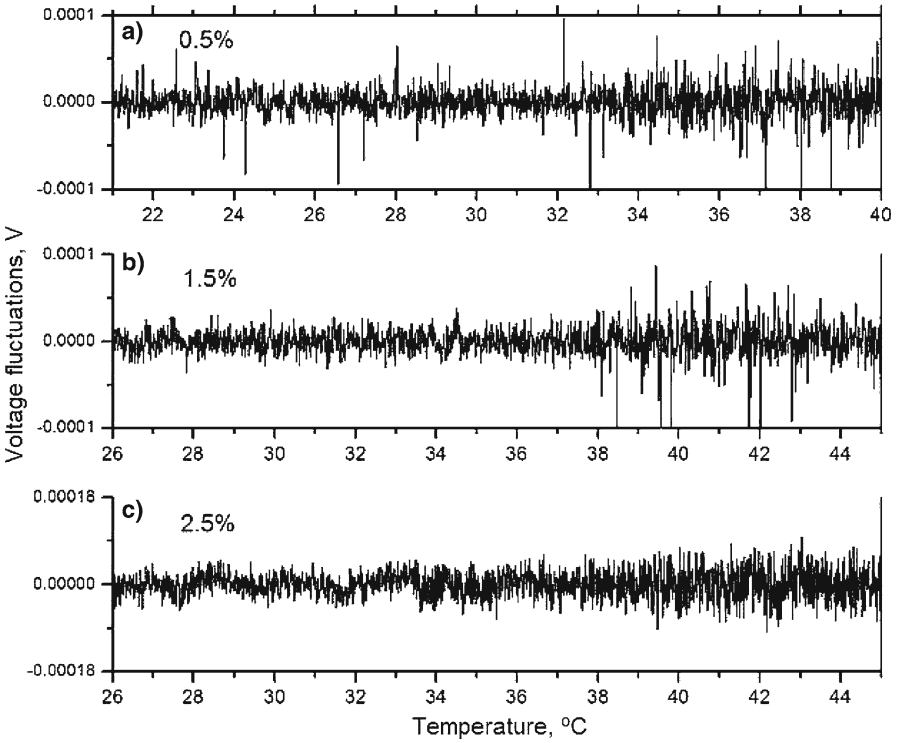

Fig. 6 Spontaneous fluctuations of the transmitted light as a function of temperature. The fluctuations decrease when the sol–gel transition proceeds due to the decrease in temperature

consequently, the sol–gel transition temperature. Therefore, the result for T_{sg}, obtained for the sample with 0.5% agar concentration shown in Fig. 5, is $(27.7 \pm 2.6)\,^{\circ}\mathrm{C}$.

The optical transmission method shows that the gellification process occurs over a range of temperature. The width of the transition can be defined as the temperature range whose lower and upper limits are the pair of temperatures T_1 and $T_2 (T_1 < T_2)$ at which the magnitude of the derivative of the temperature $(\mathrm{d}V/\mathrm{d}T)$ falls to 40% of its maximum (see Fig. 5a). The sol–gel transition takes several degrees to settle down, becoming wider when the agar concentration decreases. The values obtained for the width of the transition are 3.6 °C for 2.5%, 4.2 °C for 1.5%, and 11.6 °C for 0.5%.

In addition to the treatment presented above for the optical transmission method, the sol–gel transition can be studied by a fluctuation analysis of the optical signal [7]. In order to analyze the temperature at which noise decreases, a polynomial fit of the curves for the optical signal (shown in Fig. 4) was performed. After this, the difference between the experimental data for the optical signal at each temperature and the value generated by the fitting polynomial, at the same temperature, was determined. The results of the differences as a function of the temperature are shown in Fig. 6. In order to analyze these data, the standard deviation of them (in groups of 20 consecutive values) is calculated. In Fig. 7, the standard deviation data are shown. They were fitted using a Boltzmann sigmoidal equation (continuous line in Fig. 7). The temperature

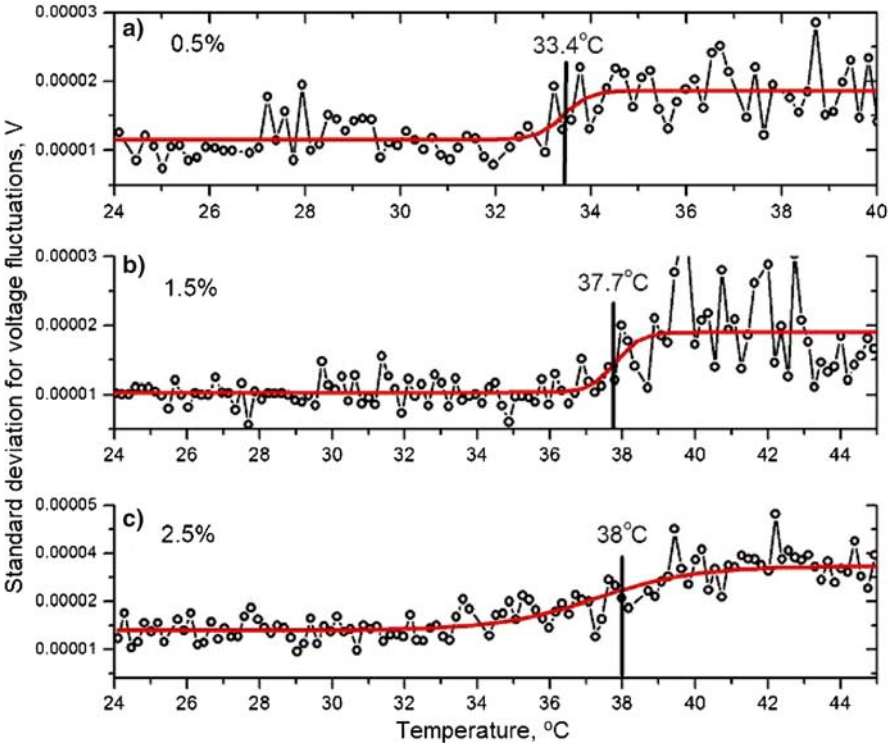

Fig. 7 Fluctuation standard deviation (in groups of 20 data). Continuous line corresponds to a sigmoidal fit

T_d, where the fluctuations show a critical change, corresponds to the inflection point of the sigmoidal fitting curves (i.e., the middle point in the function step).

As can be observed from Figs. 6 and 7, fluctuations decrease strongly close to the temperature T_d and maintain a small value after that. These fluctuations in the voltage are caused by the thermal convection of randomly localized distributed regions with different optical densities in the agar system. As the movement of the molecules decreases during the sol–gel transition, the viscosity increases, and as a consequence, the fluctuations of the optical signal decrease [7]. Due to this fact, the temperature T_d could be a useful definition of the sol–gel transition temperature (T_{sg}).

The results for the sol–gel transition temperature, obtained using the three different methods (rheological, optical dispersion, and fluctuation), are presented in Fig. 8. It is shown that the sol–gel transition temperature increases, in all cases, with agar concentration, consistent with literature results [2]. The difference in the results of the sol–gel transition temperature for the three different techniques is due to the fact that the same process is being monitored from different perspectives in each case. In a very wide transition, as the gelling process presented by agar, this result should be expected. During the sol–gel transition, the hydrogen bonding of the molecules makes the system stiffer and more dispersive to light. At a certain stage, the sample has reached a threshold viscosity that can stop the movement of the bead in the

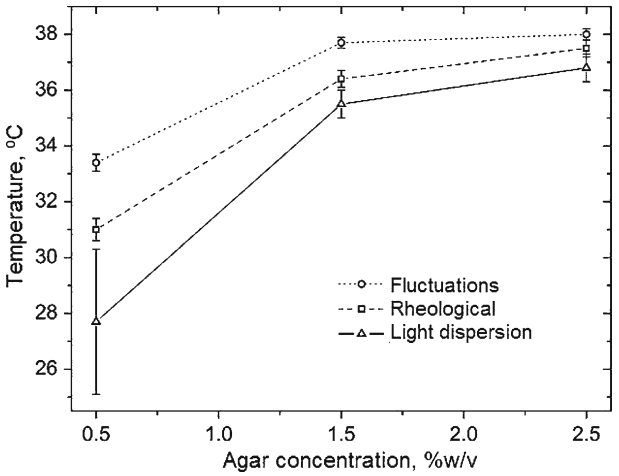

Fig. 8 Sol–gel transitions temperatures T_c in agar (at 0.5, 1.5, and 2.5% of agar concentration), measured with the rheological, optical light dispersion, and optical fluctuation methods

rheological method. However, the transition still continues; this can be observed by the fact that the decrease in light transmission persists at lower temperatures. As a consequence in the transmittance optical method, the inflection point of the observed transition (Figs. 4 and 5) is displaced to lower temperatures providing a lower value for T_{sg}. In contrast, the fluctuations of the optical signal are strongly dependent on the viscosity, the stage at which the bead has stopped (in the rheological method) corresponds to the final stage of the fluctuations, and therefore, the values provided for T_{sg} by the fluctuations method are the highest of the three methods investigated here.

The rheological method, widely used because of its simplicity, depends on the mechanical movement of a bead; this movement interferes, partially retarding the gelation process due to the collision of the moving bead with the bonding agar molecules. In contrast, the optical methods presented in this work, provide non-destructive and non-invasive characterization of the sol–gel transition in agar. On the one hand, the optical transmission method permits observation of the whole evolution of the transition process, but with a large error in the measurement of T_{sg}, mainly for low agar concentrations. On the other hand, the optical fluctuation method provides a precise measurement of the sol–gel transition temperature for all agar concentrations analyzed.

4 Conclusions

The sol–gel transition of three different concentrations of agar in aqueous solution has been studied by a novel optical light transmission technique. It has been shown that the transition can be observed as a continuous decrease of the transmitted light. The monitoring of the light transmission and its fluctuation are useful to determine

the sol–gel transition temperature. The results for T_{sg} are compared with the corresponding values obtained by the traditional rheological method. The results obtained using the transmitted light fluctuation and rheological methods, which depend on the change of viscosity during the transition, provide higher values of T_{sg}, as compared with the light transmission method.

The light transmission method can be used to monitor a wider range of temperature, being useful to monitor the transition dynamics for even lower temperatures at which the viscosity related methods are not reliable.

Acknowledgments This work was partially supported by the CONACYT 38493-U Project. The authors want to express their acknowledgments to M.S.J. Bante and M.S.D. Aguilar for their valuable help in the cell and electronic construction, and E. Ernesto Villaescusa from Betatherm.

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