A Convenient and Minimally-Invasive Method to Study Gel Formation by Surface Tension Measurements

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***Abstract*- Hydrogels provide a three-dimensional polymeric network and are useful as matrices for the delivery of therapeutics or as scaffolds for tissue engineering. However, the sol-gel transition of hydrogels can be difficult to quantify and methods commonly used to study gelation kinetics such as visual inspection, rheology or light scattering have their limitations. Using polyacrylamide, gelatin and poly (hydroxyethyl) methacrylate gels as model systems, we now show that the surface tension of a gelling system can be used to monitor its transition from an initially free-flowing solution state into a gel capable of supporting its own weight in an inverted container. This method is automated, minimally-invasive and samples can be easily recovered after measurements. It can potentially be applied to other gel systems to provide a rapid, simple and objective comparison of gelation kinetics.**

Keywords- Hydrogel; Gelation Kinetics; Surface Tension; Gel Formation

I. INTRODUCTION

Hydrogels are essentially water-swollen structures that are useful in the field of drug and gene delivery [1], or as three-dimensional scaffolds for tissue engineering applications [2]. Our group is interested in the development of novel materials to form hydrogels and has recently identified several short peptide sequences (3-7 residues) capable of self-assembly into fibers that ultimately result in hydrogel formation [3, 4]. However, the gelation process is usually not instantaneous and the gelation time can be subjective among observers, making it difficult to quantify. We therefore sought to develop a rapid and convenient method that allows us to objectively compare the gelation kinetics of hydrogels under various gelling conditions such as monomer concentration, salt, pH, etc. Ideally, the method should be minimally invasive so as not to interfere with gel formation. It should also be non-destructive so that we can easily recover the sample by lyophilisation for cost savings.

One method commonly used to determine gelation time is by visual inspection, i.e., by regularly tilting or inverting the vial containing the sample [5]. This method is simple but is also labour-intensive, subjective and is not feasible if gelation occurs over a long time. Furthermore, tilting or inverting places stress on the system and interfere with natural gel formation. An alternative method involves rheology. Such a method assumes that hydrogels are viscoelastic materials and works by tracing the temporal variation of the storage (G') and/or loss modulus (G'') of the sample. Gel formation is then usually defined as when G' intercepts G'' [6]; or when G' has reached a plateau or some empirically defined value [7]. While this method can be automated and provides an objective platform to compare gelation kinetics, it is ultimately invasive as strains are applied to the system. Samples are also not easily recoverable at the end of experiments. The method of light scattering, on the other hand, depends on the inhomogeneity of gels and can be easily performed [8]. However, the question remains if laser-point measurements accurately reflect macroscopic properties. This method is also not suitable for systems susceptible to aggregation, such as some of our peptides [3, 4]. Here, we propose that the surface tension (SFT) changes as a gel transitions from its initial free-flowing solution state into a gel capable of supporting its own weight in an inverted container. SFT can thus be a useful parameter to study the kinetics of gel formation.

II. METHODS, RESULTS AND DISCUSSION

To monitor the SFT as a function of time, we made use of a force tensiometer. We selected force tensiometry over other forms of tensiometry (e.g., optical, volumetric or bubble tensiometry) because it does not require the formation of droplets or air bubbles which interfere with gelation. This method is also automated, requires little optimization and the specimen can be easily recovered for further use. Two of the more commonly used experimental setups in force tensiometry involve either a Wilhelmy plate [9, 10] or a du Noüy ring [11]. In the former, a platinum-iridium plate is lowered slightly below the surface of a solution and the force exerted on it by the wetted surface is measured. In the latter, the ring moves through the liquid layers and the force needed to raise the ring from the surface is measured. Since the Wilhelmy plate remains static, it is less invasive than the du Noüy ring method and is preferred for the monitoring of gelation kinetics in this study.

To demonstrate the general applicability of this method, we monitored the SFT variations of several commercially available gel systems. We started with polyacrylamide gels where acrylamide monomers were polymerized and crosslinked with bis-acrylamide into a covalently connected network – i.e., a "chemical" gel – upon the addition of ammonium persulfate (APS) and varying amounts of tetramethylethylenediamine (TEMED). In this experiment, 10% acrylamide solutions were prepared by adding 5 mL of 40% acrylamide/bis solution (19:1, Bio-Rad Laboratories Inc., CA, USA) to 15 mL of 10× Tris-Borate-EDTA (TBE,

pH8.3, 1st Base, Singapore) buffer. 200 µL of freshly prepared APS solution (10%, Bio-Rad Laboratories Inc.) was then added, followed by 15 to 30 µL of TEMED (Invitrogen, Singapore). The mixture was homogenised by vortexing for 3 s and then immediately loaded into a DCAT 21 tensiometer (DataPhysics Instruments GmbH, Filderstadt, Germany) fitted with a TV70 temperature control unit. Unless otherwise stated, the external sink surrounding the sample holder was always maintained at 22°C with a circulating water bath to ensure consistency between experiments. Data were collected every 10 s with a Wilhelmy plate (10.00 mm by 9.95 mm with a thickness of 0.02 mm). Prior to each experiment, the Wilhelmy plate was always cleaned by burning over an open flame until red-hot. The SFT value obtained with our in-house de-ionized (DI) water at 25°C was 71.5 ± 0.2 mN/m and was routinely used to validate the readings obtained from each experiment [12].

Figure 1a illustrates the SFT variation of various formulations of polyacrylamide gels. In general, all curves started with similar baseline values before increasing to their respective maxima. Each experiment was replicated and the results overlaid in the same graph. Upon visually comparing with parallel samples left at identical conditions, it was observed that the time when the SFT value initially departed from its baseline value reliably and reproducibly coincided with the time by which a gel was formed. Here, a gel was defined to be formed when it could support its own weight in an overturned container, as depicted in the inset of Figure 1a. For polyacrylamide gels formed under such conditions, it was observed that there was always a small amount of unincorporated liquid which did not become part of the gel network even after 24 hours of polymerization. Figure 1b offers a closer view of the region where the curves initially increased from their baseline values. As can be seen, the curves are extremely reproducible and as expected, increasing the amount of TEMED significantly decreased the length of time taken for the gel to form. For instance, solutions containing 30 µL of TEMED gelled the fastest (3.7 ± 0.1 mins) (mean ± standard deviation), followed by when 25 µL (5.1 ± 0.3 mins), 20 µL (7.4 ± 0.1 mins) and 15 µL (10.5 ± 0.1 mins) of TEMED were added. We accept that there must be post-gelation events, e.g., gel hardening, shrinkage or expansion that can account for the shape of the curves after the formation of a gel. However, unlike the transition from solution to gel, these phenomena are complex and are not visually obvious after the gel has formed. We currently cannot fully account for the shape of the curves. Having said that, we note that this study aims to develop a convenient method to objectively compare gelation kinetics; and this is adequately fulfilled by reproducible, well-defined endpoints within the initial segments of the curves.

We next evaluated this method using gelatin, which is an amphipathic, collagen-derived polymer that forms a "physical" gel (i.e., its polymeric network is held together by non-covalent forces) upon cooling. Here, gelatin solutions (Type A from porcine skin, Sigma, Singapore) ranging from 5% to 15% were prepared in 20 mL of DI water and heated using microwave to 85-90°C. The solution was then loaded into the tensiometer and its temperature was manually monitored with a thermometer. SFT readings were initiated once the temperature of the sample had cooled to 60°C.

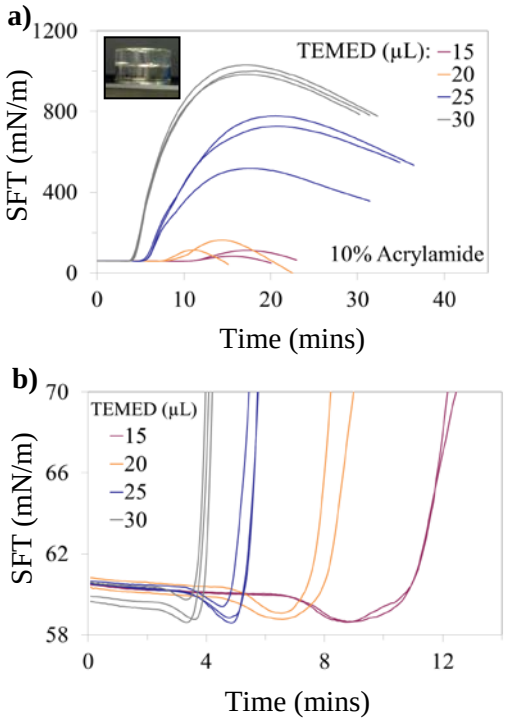

Fig. 1 SFT variation of polyacrylamide gels

20 mL of 10% acrylamide solutions were prepared, to which 200 µL of 10% APS and varying volumes of TEMED were added. SFT measurements were immediately started upon the addition of TEMED. a) The SFT of various polyacrylamide formulations were monitored over time. A gel would have been formed, as judged visually, by the time the SFT value exceeded its initial baseline value. All experiments were replicated, as indicated by the overlaying of curves for each condition. A representative post-gelation picture obtained when 20 µL of TEMED was added can be seen in the inset. b) Close-in view of the curves where the initial increase in SFT from their baseline values reliably and reproducibly indicated the completion of gelation. As expected, a faster gelation was achieved with the addition of increasing amounts of TEMED.

Unlike the polyacrylamide system, the SFT values of a 5% gelatin solution did not exhibit a maximum even after close to 12 hours of incubation (Figure 2a). Instead, the SFT increased monotonically within this timeframe. Interestingly, the G' value of fish-derived gelatin was also observed to increase continuously without reaching an equilibrium in another study and this was suggested to be due to the continuous rearrangements of gelatin helices after gelation [7]. Gelatin solutions of various weight percentages were then prepared and subjected to SFT measurements (Figure 2b). By comparing with parallel samples left at identical conditions, we observed that the minima before the monotonic increase coincided with the completion of gelation. Figures 2c and 2d offer a closer view of the curves

around their minima and show that results are highly reproducible. Accordingly, solutions of 15% gelatin gelled by 14.1 ± 0.3 mins, 10% gelatin by 14.7 ± 0.3 mins and 5% gelatin by 22.7 ± 0.4 mins. We further note that various experimental conditions like the material and geometry of the container, volume of solution used and ambient temperature can significantly affect gelation speed. This is particularly true for systems that gel upon cooling where the dynamics of heat transfer play a crucial role. These are therefore important considerations when parallel experiments are conducted to relate the results of SFT measurements to physical reality.

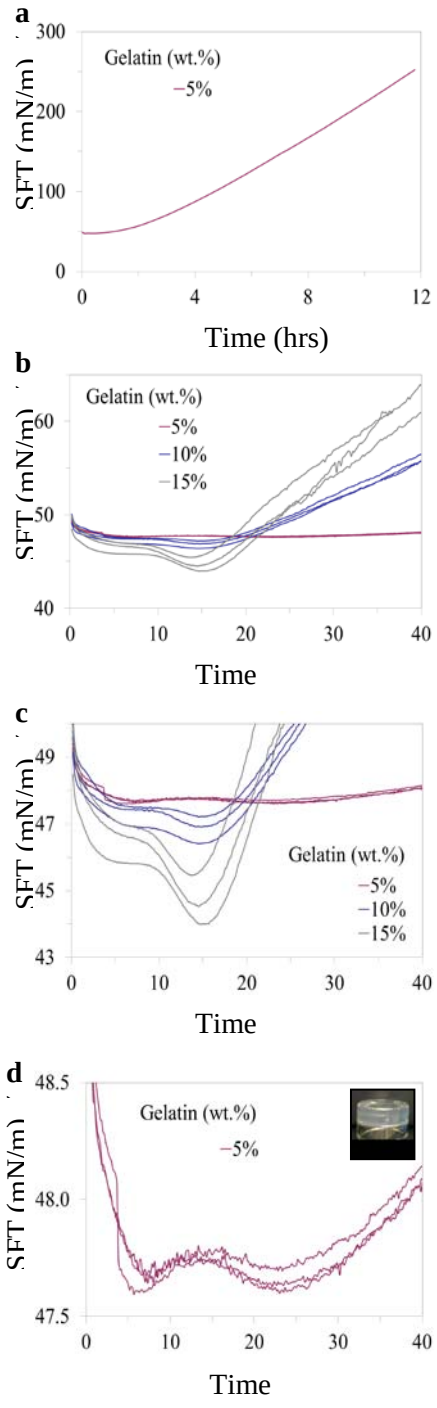

Fig. 2 SFT variation of gelatin gels

Varying weight percentages of gelatin solution were prepared in 20 mL of DI water and heated to 85-90°C. SFT measurements were started when the solution had cooled to 60°C. a) The SFT value of a 5% gelatin solution was observed to increase monotonically over 12 hours, although gelation was completed much earlier. b) The SFT of various gelatin solutions were monitored over time and c-d) are close-in views of the minima exhibited by the curves before the monotonic increase. It was observed that the minima repeatedly correlated with the time by which a gel was formed. All experiments were triplicated, as indicated by the overlaying of curves for each condition. A representative post-gelation picture obtained with 5% gelatin was provided in the inset of d). Results showed that solutions with higher weight percentages of gelatin took less time to form gels.

Finally, we tested this method on poly(hydroxyethyl)methacrylate (polyHEMA) gels. HEMA (99%, Sigma) was used as the monomer while ethylene glycol dimethacrylate (EGDMA, 98%, Sigma) was the crosslinker. 10% stock solutions of HEMA and EGDMA were first prepared in DI water and ethanol respectively. 40 µL of EGDMA was added to 4 mL of HEMA (1:100) before varying amount of freshly prepared APS (10%) was added. 10 µL of TEMED was finally added to initiate polymerization and the solution was immediately loaded into the tensiometer for SFT measurements. TEMED was essential for the initiation of crosslinking, without which, no polymerization occurred even after 24 hours – i.e., the SFT value failed to increase significantly from the baseline values (data not shown). Conversely, in the presence of TEMED, it was observed that the SFT values of HEMA gels containing 10 µL of APS increased initially before reaching a plateau (Figure 3a). An opaque gel (inset) was, however, already formed before the plateau region of the curve. We then varied the amount of APS used and triplicated each experiment (Figure 3b). The curves were again very reproducible and upon comparison with parallel experiments, it was empirically determined that a SFT value of 44 mN/m can be chosen to represent the completion of gelation. We acknowledge that the need for an empirically determined endpoint is a shortcoming for this particular system. Nonetheless, this is still in line with the practices of other groups who used empirically determined G' values to define the end-point of gelation [7]. In this case, HEMA gels polymerized with 50 µL of APS gelled the fastest (12.6 ± 0.1 mins), followed by 25 µL (26.1 ± 0.1 mins) and then 10 µL (83.6 ± 9.6 mins). While the gelation end-point for HEMA gels is not as clearly defined as earlier systems, it can still be used to objectively compare the gelation kinetics of different preparations of polymer. Like the polyacrylamide gels, there was some residual liquid that remained unincorporated into the HEMA network even after 24 hours of incubation.

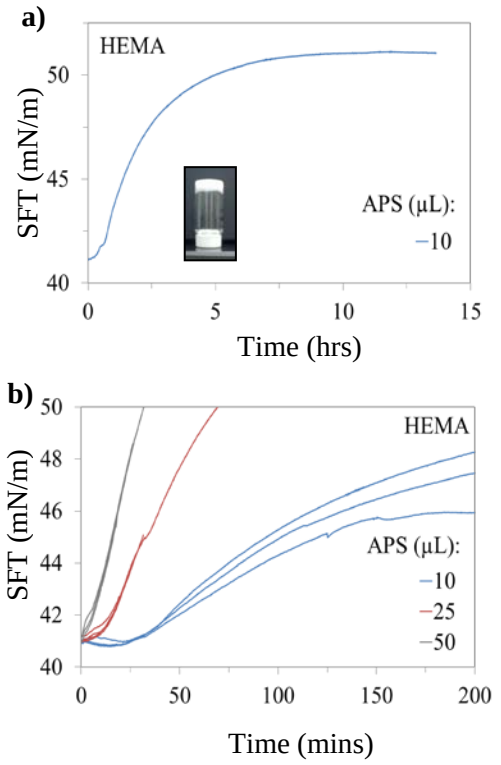

Fig. 3 SFT variation of HEMA gels

10% solutions of HEMA and EGDMA were first individually prepared and mixed at 100:1 (monomer:crosslinker) to give a final volume of 4040 µL. Varying volumes of 10% APS was then added, followed by 10 µL of TEMED. SFT measurements were started immediately upon the addition of TEMED. a) The SFT variation of a HEMA gel with 10 µL of added APS was monitored over time and observed to plateau off after an initial increase. b) The SFT variation of various HEMA preparations was then investigated and it was empirically determined that a SFT value of about 44 mN/m coincided with the time by which a gel was formed. All experiments were triplicated, as indicated by the overlaying of curves for each condition. A representative post-gelation picture obtained when 25 µL of APS were added can be seen in the inset of a). Data indicated that the addition of increasing volumes of APS reduced the amount of time taken to form gels.

We have proposed an alternative method to study the gelation kinetics of gels via SFT measurements. The experiment is simple to setup, non-destructive and minimally invasive towards the gelation process. We evaluated our method on several gelling systems and demonstrated that this method yielded highly reproducible results. Nonetheless, different systems exhibit characteristic SFT profiles and thus require customized interpretation. For instance, the increase in SFT from its baseline value reliably indicates the completion of gelation for polyacrylamide gels; while for gelatin, it is the minimum before the monotonic increase. For HEMA gels, empirical observations are needed to determine a representative SFT value for the completion of gelation. As discussed, visual inspections are initially needed to give physical meaning to the SFT variation. However, this is only required for the first few samples, after which, this method allows for the automatic and objective comparison of gelation kinetics for a given polymeric system. Usefully, it can be employed as a rapid and convenient screening tool to study the effects of different conditions (e.g., amount of monomer, catalyst, crosslinker, etc.) on gelation. It can also lend itself to the quality-check process during manufacturing when the gelation kinetics of different batches of polymer has to be verified in the research or industrial setting.

A concern may arise over the validity of using SFT readings to monitor gelation, a phenomenon which concerns the entire mass of the solution. Since hydrogels consist of highly interconnected three-dimensional networks, there will be interactions not only between molecules at the surface but also throughout the whole system. Importantly, our data showed that there was a good correlation between SFT and gelation kinetics and SFT measurements could reliably and reproducibly indicate the end of gelation. Nonetheless, we acknowledge that this method, although experimentally simple, is conceptually complex. For instance, SFT depends on temperature, solute concentration and the nature of the gelling system, amongst others. Any swelling and shrinkage of the gel will also affect SFT readings. The final graph obtained is thus a superimposition of various effects. While a full physical interpretation of the various curves currently eludes us, we stress that this study aims only to provide a rapid, convenient, minimally-invasive and objective method to compare the speed of gelation. Very importantly, the data were reproducible and shed light on physical reality. Therefore, this method can still be a valuable empirical tool for the monitoring of gelation kinetics.

III. CONCLUSIONS

The primary objective of this study is to report an alternative method to study gelation kinetics. We showed that the method of SFT measurements could be used for such a purpose and that it was simple, convenient, minimally invasive and non-destructive. Importantly, this method provides a platform to objectively compare gelation profiles. This method is also highly reproducible and samples can be recovered for further use. We believe this method can be extended to other gel systems – e.g., gels of biological origin, DNA, polysaccharides, peptides and proteins – to provide a rapid and objective comparison of gelation speed.

ACKNOWLEDGMENT

This work is supported by the Institute of Bioengineering and Nanotechnology (Biomedical Research Council, Agency for Science, Technology and Research, Singapore).

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