GELATION TIMER Application note: A05-001A

Use of the Techne Gelation Timer

Introduction

The “gel time” is an important parameter in the manufacture of adhesives and polymers as it gives a measure of the curing or setting time of the product. Fast curing products which are capable of setting at low temperatures and/or high humidity reduce down time and allow a more rapid production throughput. Examples of these are flooring compounds. Conversely, products with a long pot life (handling time) allow plenty of processing time for large objects. Indeed, the gelation timer itself was developed by the original founder of Techne, Dr. Norman de Bruyne, who is renowned for the adhesives he invented for the construction of aircraft in the 1930s and 1940s. One of these, “Redux” is still in use today.

Figure 1: Dr. Norman de Bruyne (1904-1997), who founded Techne Limited in 1948.

Gelation time is also an important factor in the food industry where the timer can be used to perform technological tests on food ingredients such as gelatin and other setting/thickening agents. Gelatin is also widely used in other industries such as the manufacture of pharmaceuticals (coatings on tablets) and photographic film.

Principle of operation

All liquids display both viscous and elastic properties to different relative extents, whereas a solid is characterised mechanically by its elasticity[1], or resistance to deformation rather than flow. A gel represents a physical state with properties between those of solid and liquid phases. The transition of a liquid polymer to a solid, or gel is preceded by a considerable rise in viscosity. An instrument to measure the time of gelation must be able to discriminate between a state of high viscosity and that of elasticity.

Figure 2: The Techne gelation timer

The design of the Techne gelation timer is based on these considerations. Work published by Hills in 1962[1] demonstrated that the instrument emphasises the elastic rather than the viscous properties by measuring piston falling times through a high viscosity liquid (silicone oil) and comparing them with those of a true gel (gelatin). The principle of operation of the gelation timer allows definition of a gel point of fundamental significance based on the mechanical properties of a gel.

A flat weighted disc or plunger is suspended from a linkage which is crank-driven by a synchronous motor. The plunger is pulled up on the upstroke by the synchronous motor and then falls under gravity in the polymer liquid. This allows the plunger to perform a vertical simple harmonic motion of fixed amplitude with a period of 1 minute or 1/10 minute, depending on the model. At the gel point the rigidity of the polymer is sufficient to support the weight of the plunger, causing the link

to be compressed and to close an electric circuit thereby stopping the timer.

The motion of the plunger is resisted by both elastic and viscous forces. However the size, weight and time have been selected so that the magnitude of a purely viscous force sufficient to trip the mechanism would be far greater than is observed in practice before gelation has taken place.

In this application note we investigate the gelling and polymerisation properties of some commonly used laboratory reagents to demonstrate the use of the Techne gelation timer.

Methods

The gelation time of various solutions of agarose, agar-agar and gelatin were measured. In addition, the effect of varying the initiator concentration used for the polymerisation of acrylamide was investigated.

Solutions of Agar-agar (Fisher Scientific, A/1080), Agarose I (Amresco, 0710) and NuSieve® GTG® agarose (Lonza Rockland, Inc.), a low melting point agarose, were prepared in 100ml distilled water. The solution was heated on a hotplate stirrer (Stuart model SD162) until the agarose or agar was completely dissolved then allowed to cool to 62ºC. Beef gelatin (SuperCook®, UK) solutions were prepared in the same way, except that the gelatin was dissolved before it reached 62ºC. Where repeated measurements were made, the same gelatin solution was re-melted by heating to 62ºC and used again.

Once dissolved/melted, the solution was transferred to a 150ml plastic container (Sterilin part code 165A) suitable for use with the gelation timer and transferred to a water bath set at 23ºC. Transfer of the solution resulted in a small loss in sample temperature so that the sample was approximately 60ºC when placed in the water bath. The plunger of the gelation timer (Model GT5) was inserted to the required depth and the timer started. The gelation time for each solution was recorded.

For measurement of acrylamide polymerisation, the solutions were equilibrated to room temperature (20ºC). Volumes of 40% acrylamide/ bis-acrylamide (Sigma A7168), tetramethylethylene-diamine (TEMED, Sigma T9281) and ammonium persulphate (APS, Sigma A3678) were mixed in a conical flask as described in Table 1 below.

The polymerisation was initiated by the addition of TEMED at which point the gelation timer was started on the HOLD position. The mix was transferred to a 150ml plastic container and the plunger of the gelation timer inserted. 3ml of the mix was placed in a UV plastic cuvette to monitor the polymerisation by recording the absorbance at 260nm in a UV-Vis spectrophotometer (Jenway Genova).

Gel (%)	Acrylamide/bis -acrylamide (ml)	TEMED (µl)	10% APS (µl)	H_2O (ml)
5	12.5	50	500	86.95
10	25.0	50	500	74.45
15	37.5	50	500	61.95

Table 1: Volumes of acrylamide solution, TEMED and APS used to prepare various percentage polyacrylamide gels.

Results

Agar and its derivatives agarose and NuSieve® GTG® agarose all form polysaccharide gels which are widely used in life science applications. Agar is derived from various species of *Gelidium* and *Gracilariae* seaweed[2]. It consists of a mixture of agarose and agaropectin, the latter a mixture of small molecules which gels only poorly. Agarose, purified from agar, is a linear polymer of 1,3-linked β-D-galactopyranose and 1,4-linked 3,6-anhydro-α-L-galactopyranose[3]. In solution it forms a random coil structure which folds into a double helix during the initial stages of gelation and then to bundles of double helices in the final stage. The resulting gel is a matrix with an average pore size of 100 to 300nm depending on the concentration.

NuSieve® GTG® agarose is native agarose derivatised to hydroxyethyl agarose then partially depolymerised[4]. This forms a product which has a lower melting temperature than standard agarose allowing easier recovery of samples from the gel and facilitates in-gel reactions. The gelation times for different % solutions of each of these reagents are shown in Figure 3.

The differences in gelation time of standard agarose compared to the low melting point derivative are quite apparent. Agarose produces a firm gel of high strength and for most applications concentrations of 1% or less are generally used. The NuSieve® GTG® agarose gels are much less rigid and are generally prepared at a concentration of 4% for use in molecular biology applications. Indeed a 0.5% solution did not form a firm enough gel to register a gelation time even after more than 1000 minutes. The ability to prepare gels of increased concentration makes this type of agarose ideal for separation and resolution of low molecular weight DNA

fragments. The gelation times of the natural agar fell in between the two.

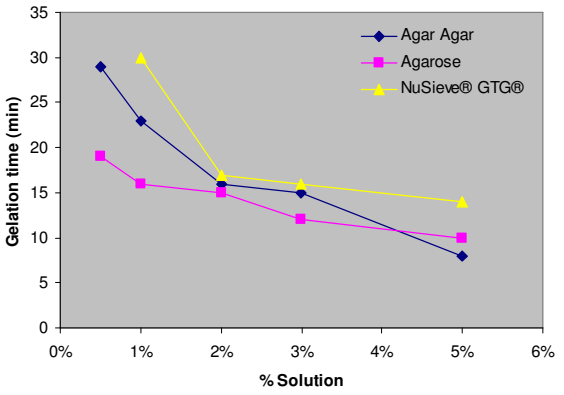

Figure 3: Gelation times of agar, agarose and NuSieve® GTG® agarose solutions.

In contrast to agar and agarose which are polysaccharides, gelatin is a protein derived from animal collagen that is widely used in the food and many other industries. Its structure contains repeating sequences of glycine–X-Y triplets where X and Y are frequently proline and hydroxyproline residues[5]. This sequence is responsible for the triple helical structure of gelatin when it forms a gel.

Gelatin produces gels of much more elastic properties than the equivalent % weight of agarose as shown by the gelation time given in Figure 4. Indeed, a 2% solution failed to form a firm enough gel at 23ºC to register a gelation time even after more than 1000 minutes.

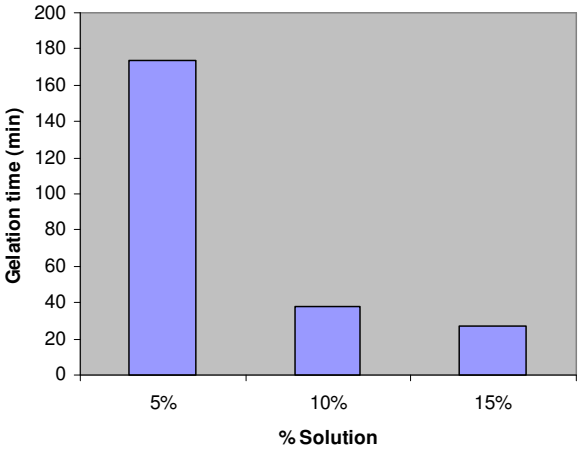

Figure 4: Gelation times of different gelatin solutions.

One of the main properties of gelatin is the reversibility of the gelling process on warming the solution. To demonstrate this, the 15% gelatin solution was repeatedly melted and re-gelled. The gelation times were measured after each melt and showed good repeatability with an average of 26.8 minutes and standard deviation of 0.8. A slight decrease in gelation time in the last couple of repeats may be due to loss of some water by evaporation.

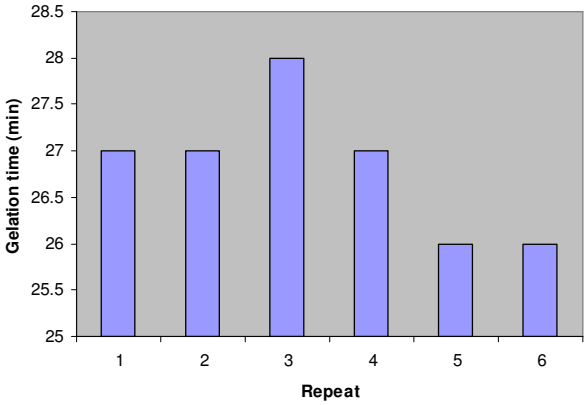

Figure 5: Gelation times of a 15% gelatin solution repeatedly melted.

Polyacrylamide gels are formed by co-polymerisation of acrylamide and bis-acrylamide and are most often used for the separation of proteins, either by size or by electrical charge. The reaction requires initiation by free-radicals which are generated in the reaction by the action of TEMED on APS. Unlike the setting of gelatin, this is an irreversible process as new chemical bonds rather than hydrogen bonds are formed. Gels can be prepared of different % concentration depending on the size of molecules to be separated.

In addition to measuring the gelation time, the rate of polymerisation was also monitored by measuring the absorbance of the solution at 260nm[6]. As acrylamide polymerises, the UV-absorbing double bonds are eliminated resulting in a decrease in absorbance as the reaction proceeds.

Although the gelation times of 5%, 10% and 15% polyacrylamide gels were recorded within a few minutes of each other: 20, 18 and 15 minutes respectively, the actual polymerisation process continued for much longer as shown by the UV absorbance measurements in Figure 6. The rate of polymerisation for the 5% gel was significantly slower than that for the 10% and 15% gels. For this reason it is recommended that gels are allowed to polymerize for around 2h before use to ensure maximum reproducibility in gel pore size[6]. Interestingly, the beginning of the drop in

absorbance correlated quite closely with the measured gelation time for each solution. This would suggest that the gelation point indicates some critical step in the acrylamide polymerisation process.

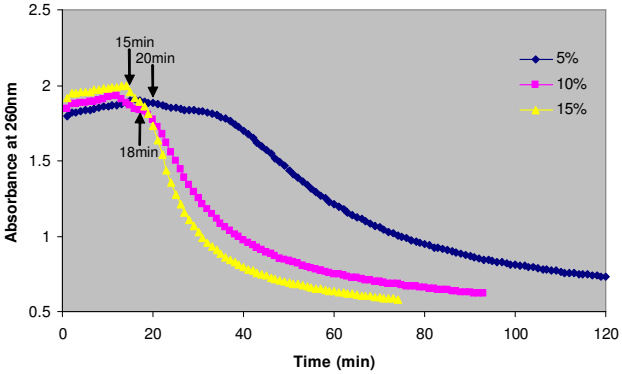

Figure 6: UV absorbance at 260nm measured during polymerisation of 5%, 10% and 15% polyacrylamide gels. The arrows show the measured gelation time for each solution, 20 min for 5%, 18 min for 10% and 15 min for 15%.

Many factors can influence the polymerisation process including the purity of the reagents, temperature, oxygen and the concentration of initiator. The latter was investigated by measuring the gelation time of 5% polyacrylamide gels using different concentrations of APS; the results are shown in Figure 7.

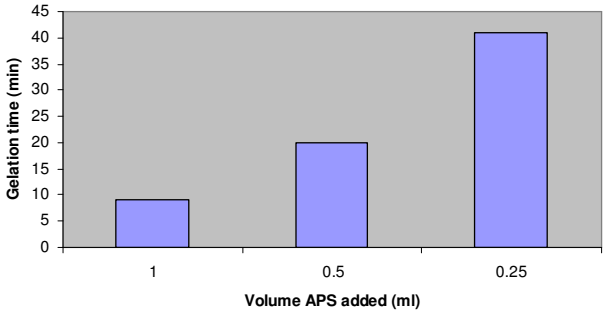

Figure 7: Effect of initiator (APS) concentration on gelation time of a 5% polyacrylamide gel.

Doubling the amount of initiator halved the gelation time and adding half the amount of APS doubled the time. A reaction adding 100µl APS was also tested but this failed to give a gelation point even after 130 minutes of polymerisation.

Conclusions

The Techne gelation timer is a simple device for determining the gelation point of a wide range of compounds with high accuracy and reproducibility. Indeed, the British Standard for testing the gelation time of polyester and epoxide resins[7] was written around its use. Also manufacturers of unsaturated polyester resin systems are required by BS 3532 : 1990[8] to publish the gelation time of the product in the specification.

Two models of gelation timer are available: the GT5, as used in this application note, with a reciprocation period of 60s and the GT6 with a reciprocation period of 6s. The GT6 is ideal for materials with short gelation times of between 5 and 20 minutes[7] as the timer displays to the nearest tenth of a minute.

References

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