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COMMUNICATION

A new method for centrifugal separation of blood components: Creating a rigid barrier between density-stratified layers using a UV-curable thixotropic gel†

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Current gels used in blood separation tubes create an imperfect barrier between the blood components because of their physical and thixotropic nature. As a result, blood components tend to leak into the gel layer or *vice versa* during transport and storage. To overcome these problems, we demonstrate the use of a UV-curable thixotropic gel composed of a sorbitol-based gelator in a diacrylate oligomer. Initially, the sample is a physical gel composed of weak, non-covalent bonds, and its thixotropic nature allows it to flow under centrifugation and form a barrier between the density-stratified layers of blood. Immediately afterward, the gel is chemically crosslinked by short exposure to UV light for 10–30 s. This results in a rigid, impenetrable barrier that is freeze-thaw stable. The gel is compatible with blood, allowing blood samples to be stored in the tube and analyzed over long times. We believe the present method is a significant advance in the practice of blood analysis for medical purposes.

Introduction

Blood tests performed for clinical diagnostic and disease monitoring purposes typically use only the cell-free fractions of whole blood (serum or plasma).[1,2] Commonly, plasma is defined as whole blood without the cellular fraction and serum is the fraction without the cells or clotting proteins. Important analytes of interest such as electrolytes, enzymes, and hormones are assayed in these solutions surrounding the blood cells. There is hence a need to separate serum or plasma from the blood cells and maintain a physical barrier between these phases. For this purpose, it is common practice to draw whole blood into an evacuated tube that contains a hydrophobic "separator" gel at the bottom. This gel is engineered to have a density between that of blood cells and the solution component of interest.[1,3] The tube is then centrifuged, whereupon the gel is reversibly liquefied and flows to a position between the heavier blood cells and the lighter supernatant by virtue of relative densities. After centrifugation, the separator reverts to a gel state, leaving a soft barrier between the cells and the test fraction (either serum or plasma) (see Fig. 1c).[4] The tube can then be transported and stored on a short-term basis until testing is performed.

A tremendous amount of science and engineering has been invested in the design of the "separator" gel used in blood separation tubes.[1,3] The gel-like nature is important because it ensures that prior to blood collection the material remains at the bottom of the tube during transportation, storage and handling, as shown in Fig. 1a (*e.g.*, the gel does not flow even if the tube is inverted or lightly shaken). After blood collection, it is the gel-like nature that provides a barrier between the blood layers following centrifugation, as shown in Fig. 1c. From a microstructural standpoint, a gel is a material that has a three-dimensional network of its constituent units, due to which it shows elastic or solid-like behavior.[5,6] However, it is important to note that the gel in a blood separation tube is necessarily a "physical" gel, *i.e.*, the bonds in its network are weak, physical bonds rather than covalent bonds. It is because the bonds are weak that the network can be disrupted by shear during centrifugation, allowing the gel to flow like a liquid. In other words, the gel is said to be *shear-thinning* and *thixotropic*, which implies that its viscosity decreases with shear rate and shear time, respectively.[3,4] Once the shear is stopped, the gel recovers its original network rapidly, allowing it to form a soft barrier between the blood components.

There are some critical disadvantages with the current physical gels used in blood separation tubes. The gel creates an imperfect barrier between the blood components because of its physical and thixotropic nature. That is, the separated blood components tend to leak into each other during long term storage or during freeze-thaw cycling. In addition, the gel contaminates pipette tips or laboratory analyzer probes when inserted into the tube to aspirate a sample. Lastly, the shear-thinning property of the gel results in disruption of the gel barrier when collected blood samples are shipped to reference labs. That is, the vibrations and impacts incurred during transport effectively liquefy the gel and compromise its adherence to the tube wall and thereby its barrier properties.

The above problems stem from the fact that the gel is not solid or hard enough, because the substance is a physical gel formed from weak, non-covalent bonds, as opposed to a "chemical" gel based on

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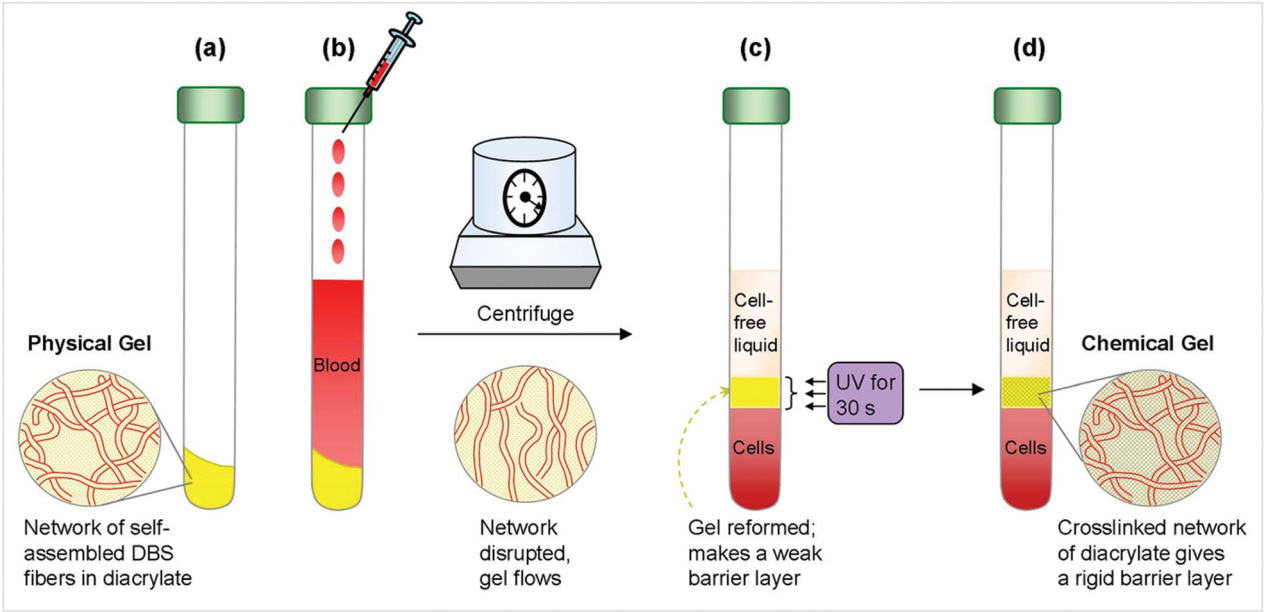

Fig. 1 Use of a UV-curable thixotropic gel in blood separation. The gel is formed by the self-assembly of DBS into nanoscale fibers within a diacrylate oligomer. Initially **(a)**, the gel is placed at the bottom of a centrifuge tube. Next **(b)**, a sample of whole blood is added to the tube on top of the gel. The tube is then placed in a centrifuge, whereupon the gel is liquefied as shear disrupts the fibrous network and aligns the fibers. Owing to its density, the liquefied gel flows to a position between the components of blood (cells and cell-free protein solution). When centrifugation is stopped, the network is re-established and the gel recovers its solid-like character; thus, it forms a weak barrier between the blood layers, as shown by **(c)**. The gel layer is then irradiated with a UV lamp, converting the material into a hard, chemically crosslinked network, as shown by **(d)**. In turn a rigid, inert, and permanent barrier is achieved between the blood layers.

strong, covalent crosslinks. Nevertheless, as stated above, the physical nature of the gel and its concomitant rheology (shear-thinning, thixotropy) are essential to the function of the gel within the centrifuge tube. Thus, an ideal solution would be to have a *physical gel prior to centrifugation*, followed by conversion to a *chemical gel after centrifugation*.[7] In this paper, we explore the above concept and demonstrate its feasibility for use in blood testing.

The materials created in our study are thixotropic gels that can be subsequently crosslinked by ultraviolet (UV) light (Fig. 1). The key constituents are a molecular gelling agent, 1,3:2,4-dibenzylidene sorbitol (DBS),[8–10] and a mixture of UV-curable liquid oligomer and photoinitiator. Chemical structures of the above three components are shown in ESI,† Scheme S1. DBS is well-known for its ability to form a network of nanoscale fibers in the liquid,[8–10] which entraps the liquid within and thereby gives rise to a physical gel (Fig. 1a). A DBS gel of the correct density is introduced into the blood collection tube. Blood is placed in the tube (Fig. 1b) and upon centrifugation, the gel liquefies and flows to the space between blood cells and serum (Fig. 1c). We then expose this physical gel layer to UV light for 10–30 s, whereupon the oligomers in the sample are induced to form chemical crosslinks. The physical gel is thereby converted to a hard, impenetrable chemical gel (Fig. 1d). Note that UV light is sufficiently transmitted through polycarbonate, which is the common material used for blood separation tubes. In this paper, we characterize the structure and rheology of the above gel and also show its biochemical compatibility with analytes in blood and with blood assay methods. We believe the present method can prove to be a significant advance in the practice of blood analysis in clinical laboratories.

Materials and methods

Materials

The aliphatic polyurethane diacrylate oligomer EBECRYL 230 and the photoinitiator Additol BDK (2,2-dimethoxy-1,2-diphenyl-ethan-1-one) were gifts from Cytec Industries. The molecular weight of the oligomer, as reported by the manufacturer, is ~5,000 Da. The organogelator 1,3:2,4-dibenzylidene sorbitol (DBS) (purity 98%), was a gift from Milliken Chemicals. Chemical structures of the oligomer, photoinitiator, and organogelator are provided in ESI,† Scheme S1. The stabilizer phenothiazine (purity ≥ 98%) was purchased from Sigma Aldrich.

Sample preparation

To prepare the gel, appropriate amounts of the oligomer, initiator, stabilizer, and gelling agent were combined in a vial and the mixture was homogenized at room temperature by a hand-held mixer (Tissue Tearor, Biospec Products Inc.). The sample was then centrifuged to remove bubbles. Density of the gel was measured by a volumetric flask. A known weight of gel was filled to a designated volume without bubbles and the density was thus obtained. UV-crosslinking of the samples for rheological studies was achieved using a Oriel 200 W mercury arc lamp. A dichroic beam turner was used to access the broadband (<400 nm) UV range of the emitted light.

Rheological measurements

Steady and dynamic rheological experiments were performed on an AR2000 stress-controlled rheometer or an RDA III strain-controlled

rheometer (TA Instruments). Samples were run on a parallel-plate geometry (25 mm diameter, 1 mm gap) or a cone-and-plate geometry (40 mm diameter, 4° cone angle). Experiments were performed 30 min after equilibrating the loaded sample. Dynamic frequency spectra were obtained in the linear viscoelastic regime of each sample, as determined by dynamic strain-sweep experiments.

Transmission electron microscopy (TEM)

The procedure used for TEM imaging of the structure in DBS gels is similar to that used by Spontak *et al.*[8,10] First, the DBS/diacrylate gel was combined with tetrahydrofuran (THF) to dissolve the oligomeric matrix around the fibrillar network. 10 μL of this solution was applied to a TEM grid with a micropipette. After evaporation of THF, the sample was annealed at 120 °C for 10 min. The sample was then immersed in diethyl ether to remove residual oligomer. The DBS fibers left on the grid were then examined by a JEOL CX200 TEM at 120 kV.

Clinical laboratory assays

Control plasma and serum tubes used for the comparison trial were commercially available serum separator tubes (SSTs), plasma separator tubes (PSTs) and plain serum tubes (BD Vacutainer Tubes, Becton Dickinson Inc.) Experimental tubes were made by adding approximately 1 mL of the UV-curable gel to each type of serum and plasma tubes above. Blood was collected by venipuncture from volunteers into syringes and immediately deposited into a set of control and experimental tubes. In parallel, all tubes were centrifuged according to standard protocol. Following centrifugation, the experimental tubes were exposed to 10 s of UV light from a spot-cure light source (RocketCure 225, Lightwave Energy Systems, Inc.) positioned 2 cm from the tube wall. All tubes were then processed according to standard procedures for the respective assays. Comprehensive metabolic panels were performed using the Synchron LX20 (Beckman Coulter) laboratory analyzer.

Results and discussion

Characterization of the gel (before/after UV)

A typical formulation of the gel was made as follows. We started with the EBECRYL 230 oligomer, which was a sticky, viscous liquid with a viscosity of 980 mPa s. In this liquid, we dissolved 1 wt% of the Additol BDK photoinitiator, 0.1 wt% of the phenothiazine stabilizer and 0.6 wt% of the DBS gelling agent. The resulting sample was transparent and, as is typical of strong gels,[11] it was capable of supporting its weight in an inverted vial (inset, Fig. 2a).

We conducted rheological studies under dynamic and steady shear on this sample. Dynamic rheological data are shown in Fig. 2a as a plot of the elastic (G') and viscous (G'') moduli as functions of the frequency ω. At low frequencies, G' is about 10-fold higher than G''. Such rheology is characteristic of a gel because it indicates the elastic nature of the sample.[5] The frequency-independence of the moduli at low frequencies implies that the sample does not relax over long timescales. Note that the value of G' as $\omega \rightarrow 0$ is the shear modulus of the gel and here it is ~20,000 Pa, which is indicative of a strong gel.[5,11]

Fig. 2b shows the rheology of the sample under steady shear: in this case, data for the apparent viscosity are plotted as a function of the shear rate. Note that the viscosity is extremely high (~10^6 Pa s) at

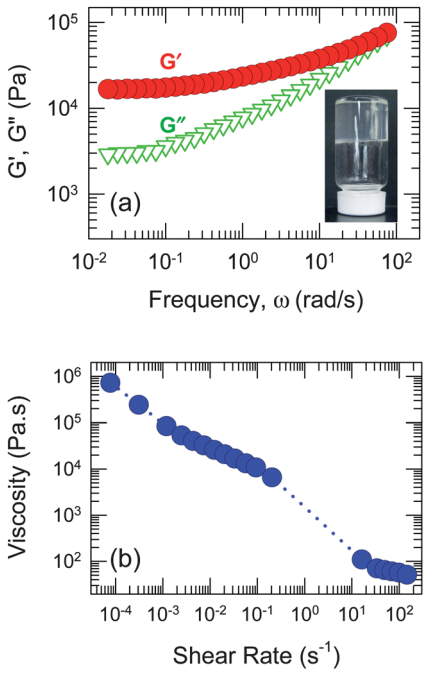

Fig. 2 Rheology of the physical gel of DBS (0.6 wt%) in the diacrylate oligomer. **(a)** Dynamic frequency spectra showing the elastic (G') and viscous (G'') moduli as functions of the angular frequency ω. **(b)** Steady-shear rheological data for the apparent viscosity as a function of the shear rate. A photograph of the gel is also shown as an inset in (a).

low shear rates, while it continuously decreases with increasing shear rate. The above response is characteristic of a physical gel and it shows that the gel is shear-thinning and thixotropic.[5,6] The shear-thinning behavior reflects the physical nature of the bonds in the gel network, *i.e.*, the bonds are not strong enough to withstand shear.[6,11] However, following cessation of shear, the bonds are re-formed and the network is re-established: thus, the gel recovers its elastic character.

The mechanism for gelation of liquids by DBS is known to involve the self-assembly of DBS molecules into nanoscale fibers, which in turn form an entangled network that entraps the liquid.[9,10] Evidence for such nanoscale network formation was obtained in the present case using TEM, and typical micrographs are shown in Fig. 3. As expected, the micrographs reveal a dense network of DBS nanofibrils, with the fibril diameter ranging from 10 to 50 nm. Similar

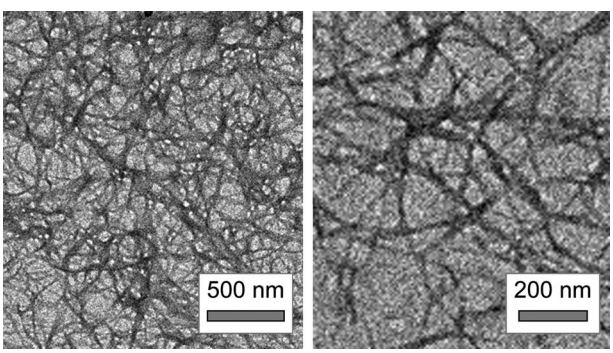

Fig. 3 TEM micographs of the DBS nanofiber network in the diacrylate oligomer. Images at two levels of magnification are shown.

micrographs have been published in previous studies on DBS gels.[8–10] The self-assembly of DBS molecules into nanofibers is known to be mediated by a combination of hydrogen-bonding interactions between the hydroxyl groups on DBS as well as π–π stacking of the aromatic rings.[9,10]

We now consider the conversion of the physical gel into a chemical gel. Chemical crosslinking of the diacrylate groups of the oligomer can be induced by irradiating the sample with UV light. The photoinitiator used here, *i.e.*, Additol BDK, has strong absorbance in the UV range of 320 to 400 nm, with a peak in the absorbance around 340 nm. We irradiated the physical gel with broadband UV (*i.e.*, all wavelengths <400 nm) from a mercury arc lamp. The irradiation was done on a disk-shaped sample, 1 mm thick and 25 mm in diameter. In the process, the physical gel was UV-cured into a hard solid within 30 s. A dynamic rheological experiment (G', G'' *vs.* frequency ω) was conducted on the hardened gel, and the results are shown in Fig. 4. As expected, we find that both moduli are again frequency-independent, but their values are much higher than those in Fig. 2. Specifically, the gel modulus G' is now ~0.5 MPa, which is indicative of a densely crosslinked covalent network.[5] Also, this network is no longer a shear-thinning or thixotropic material because the covalent bonds in the network are strong and insensitive to shear. Fig. 4 also shows a photograph of a thin cured gel, which is strong enough to be held between two fingers.

Use of the gel in serum separation

The DBS/acrylate gel was then tested in serum separation tubes (SSTs). The density of the gel was 1.045 g cm^{-3}, which is intermediate between that of blood cells (1.125 g cm^{-3}) and serum (1.025 g cm^{-3}) and thus appropriate for serum separation.[2] The gel was introduced into a commercial SST (BD Vacutainer tube) above the BD gel at the bottom of the tube. Whole blood (6 mL) was added on top, as shown in Fig. 5a. After centrifugation, the gel formed a colorless layer between the yellowish serum phase and the dark-red blood cell phase. The DBS gel contained no visibly entrapped red cells which showed that it was sufficiently hydrophobic and that it did not interact adversely with the blood cells – this is a desirable feature for accuracy in lab testing and sample stability. At this point, the DBS gel provided a soft barrier between the blood fractions, much like current separator gels.

We then exposed the DBS/acrylate gel to UV light from a spot-source for 10 s. Fig. 5b shows the tube immediately after UV irradiation. The chemically crosslinked DBS gel is still transparent. Importantly, no perceptible shrinkage is observed, *i.e.*, the gel remains adhered to the walls of the tube. At this stage the DBS/acrylate gel barrier is hard and impenetrable to wooden applicator sticks or pipette tips, leaving no residue that can be aspirated by suction as used in laboratory analyzer probes. The physical properties of the cured gel barrier must also be resistant to freeze-thaw cycling. To test this, we subjected a tube containing the cured gel to 20 freeze-thaw cycles between a freezer at −20 °C and a water bath at room temperature. At the end of this cycling, no changes were found in the cured gel and it continued to remain firmly anchored to the walls of the tube. Note that identical results were obtained if the DBS gel was introduced into an empty tube rather than a tube containing the BD gel.

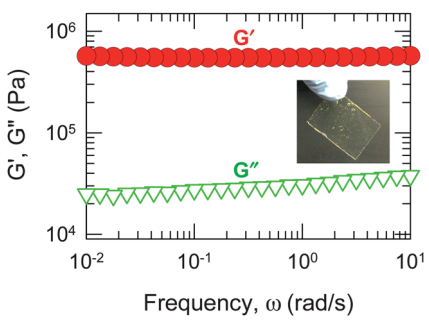

Fig. 4 Dynamic rheology of the UV-crosslinked gel of DBS (0.6 wt%) in the diacrylate oligomer. The elastic (G') and viscous (G'') moduli are shown as functions of the angular frequency ω. A photograph of a crosslinked gel film is shown as an inset.

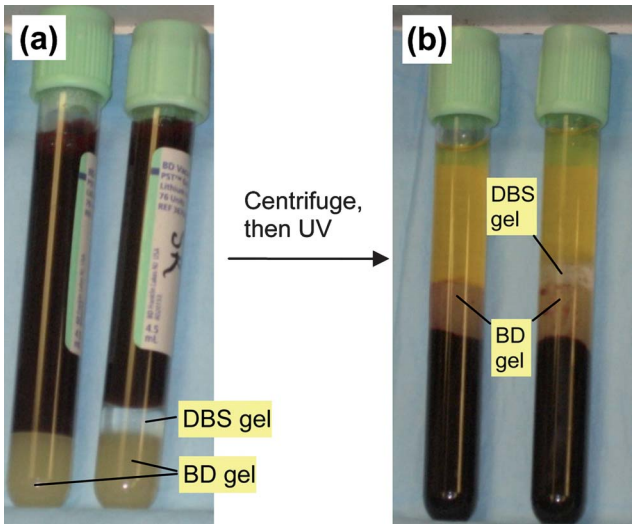

Fig. 5 Photographs demonstrating the use of the UV-curable DBS/diacrylate gel in serum separation. **(a)** Two BD tubes are shown: on the left, a control with BD gel, and on the right a tube with the DBS gel on top of the BD gel. **(b)** After centrifugation and UV spot-curing, the tube on the right has a hard, impenetrable barrier due to the DBS gel between blood cells and serum. The tube on the left only has a soft barrier due to the BD gel.

We then evaluated the biochemical compatibility of the UV-curable gel with blood and its components. Specifically, we wanted to verify that the gel did not interfere with the analytes of interest in blood, nor the assay methods in widespread use. In the present study, we show results from the most commonly ordered set of blood tests, which is the Medicare-approved Comprehensive Metabolic Panel (CMP). Blood samples were separated and analyzed in tubes containing UV-curable gel ("UV tubes"), while as a control, the same samples were analyzed using commercially available blood collection tubes (BD Vacutainers). The CMP includes tests for sodium, potassium, carbon dioxide, chloride, glucose, urea nitrogen, creatinine, calcium, alkaline phosphatase, alanine amino-transferase, aspartate aminotransferase, albumin, total protein, and total bilirubin.

The data from the CMP (ESI,† Table S1) show that the UV and BD tubes give comparable results (within the coefficients of variation for the assays). Thus, contact with the UV-curable gel and the UV curing process did not alter the analytes or interfere with the assays. Tubes were stored for up to eight days and some were subjected to freeze/thaw cycling. The data show that the analytes are stable in the

UV tubes, and as compared to the BD tubes, certain analytes such as glucose and potassium are more stable on longer-term storage (ESI,† Fig. S1) These analytes are particularly problematic for clinical laboratories. Thus, the UV-curable gels provide an improvement in performance over currently available tubes by demonstrating prolonged analyte stability upon storage and freeze-thaw cycling.

Finally, it is useful to summarize the various properties that have been engineered into the above gels for use in blood separation. First, the gel was ensured to have the right density (intermediate between cells and serum/plasma).[1,3] Second, the constituents of the gel were selected to be hydrophobic, but compatible with the biochemical components of blood. From a rheological standpoint, the gel was designed to be strongly thixotropic and shear-thinning at the outset, allowing it to flow quickly under centrifugation to its location between cells and serum. Then, we ensured that the physical gel could be quickly UV-cured (within 30 s) to a hard solid. There was negligible release of heat and negligible shrinkage during cure; both these properties were facilitated by the use of a less-reactive diacrylate oligomer rather than a monomer of lower molecular weight. We also added a stabilizer to the formulation to avoid premature cure during transit or storage. The lack of shrinkage upon cure results in good adhesion of the cured gel to the tube walls, and additionally, the material was stable to repeated freeze-thaw cycling. Lastly, we note that the gel was formulated using relatively inexpensive commercially available materials, which means that the material can be readily scaled up for application.

Conclusions

We have demonstrated a new gel that presents considerable advantages for use in blood separation tubes. The gel was formed by the self-assembly of DBS molecules into a nanofibrous network within a polyurethane diacrylate oligomer. The initial gel was shear-thinning and thixotropic, allowing it to flow to the precise density-stratified layer during centrifugation. This resulted in a weak barrier between the cell-containing and cell-free fractions of blood. The physical gel was then irradiated with UV light for <30 s to allow chemical crosslinking of the diacrylate. In turn, the gel was transformed into a hard solid, thereby forming a permanent, rigid barrier between the blood layers. The gel was shown to be biochemically compatible with blood analytes and assay methods. We believe the presence of a permanent barrier between blood layers is a dramatic advantage for the practice of routine blood analysis in clinics and laboratories.

References

1 V. Bush and R. Cohen, *Lab. Med.*, 2003, **34**, 304–310.
2 A. Maton *Human Biology and Health, 3rd ed.*; Pearson Prentice Hall, 1997.
3 F. C. Lin, R. Cohen, R. Losada and V. Bush, *Lab. Med.*, 2001, **32**, 588–594.
4 A. R. Zine US patent 3,852,194, Dec. 3, 1974.
5 C. W. Macosko *Rheology: Principles, Measurements and Applications*; VCH Publishers: New York, 1994.
6 P. Terech and R. G. Weiss, *Chem. Rev.*, 1997, **97**, 3133–3159.
7 J. F. Emerson US patent 7,674,388, March 9, 2010; US Patent 7,673,758, March 9, 2010; US Patent 7,775,962, August 17, 2010; US Patent 7,780,861, August 24, 2010.
8 J. R. Ilzhoefer and R. J. Spontak, *Langmuir*, 1995, **11**, 3288–3291.
9 M. Fahrlander, K. Fuchs and C. Friedrich, *J. Rheol.*, 2000, **44**, 1103–1119.
10 E. A. Wilder, C. K. Hall, S. A. Khan and R. J. Spontak, *Langmuir*, 2003, **19**, 6004–6013.
11 S. R. Raghavan; B. H. Cipriano in *Molecular Gels*; Weiss, R. G., Terech, P., ed.; Springer: Dordrecht, 2005; -pp 241–252.

Electronic Supplementary Material (ESI) for Journal of Materials Chemistry

Supporting Information for:

A novel method for centrifugal separation of blood components: Creating a rigid barrier between density-stratified layers using a UV-curable thixotropic gel

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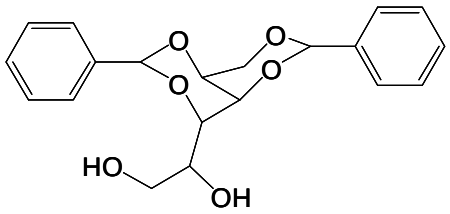
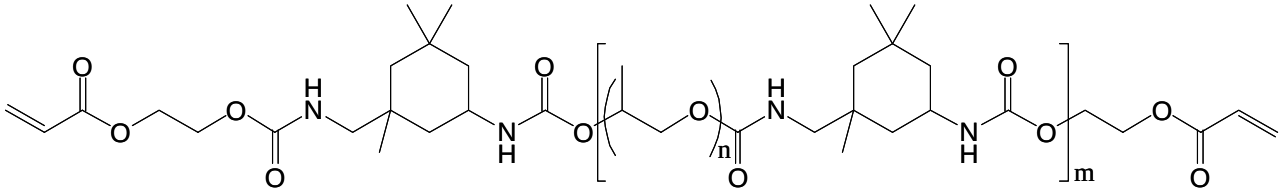
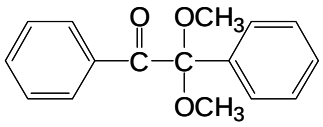


(a) Organogelator:
1,3:2,4-di-O-benzylidene-D-sorbitol (DBS)



(b) Oligomer:
Aliphatic polyurethane diacrylate (EBECRYL 230)



(c) Initiator:
2,2-dimethoxy-1,2-diphenylethan-1-one (Additol BDK)

Scheme S1. Structures of the organogelator, UV-crosslinkable oligomer, and UV initiator used in our study.

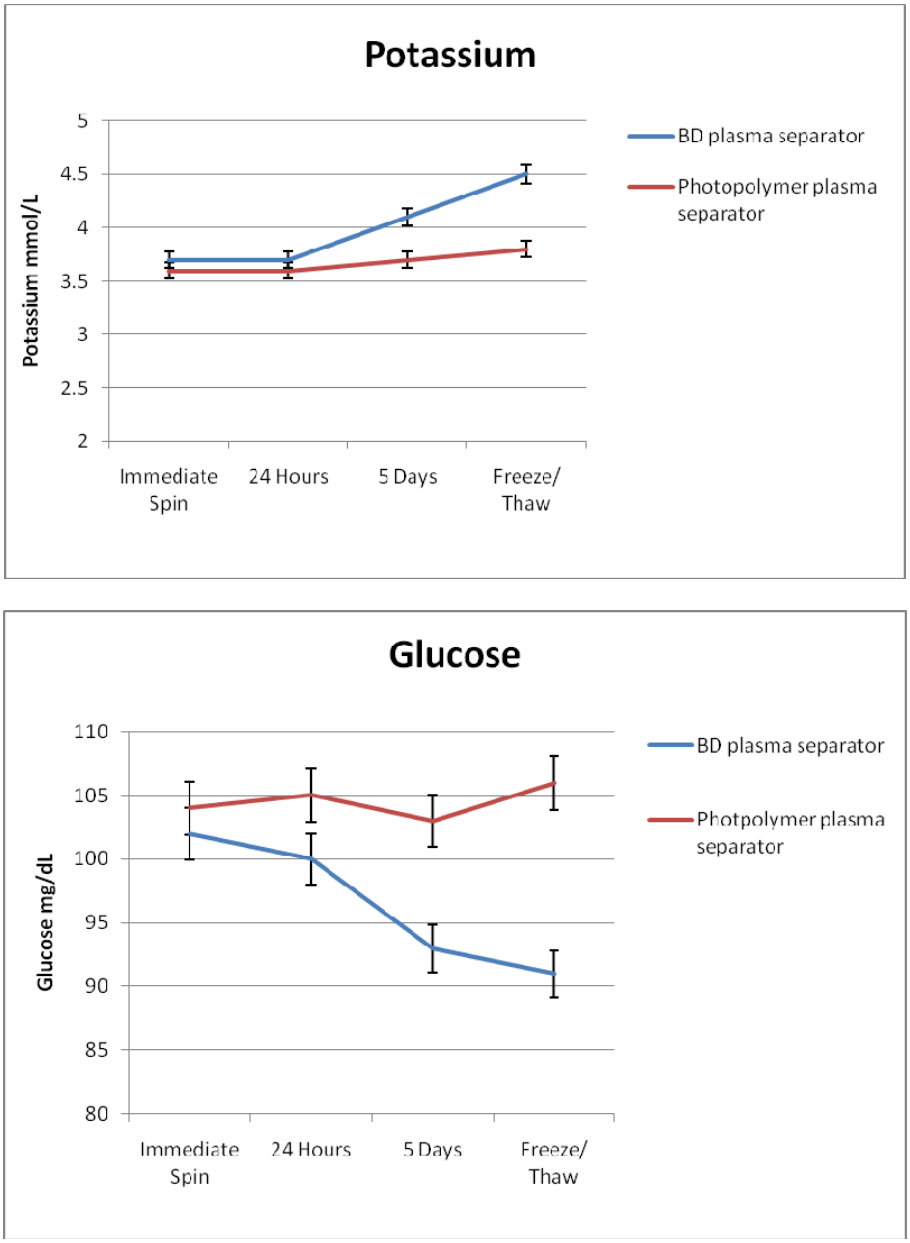


Figure S1. Comparison of analyte stability after storage and with a freeze thaw cycle. Immediate spin represents the value obtained with the primary tubes loaded onto the analyzers immediately after centrifugation. The primary tubes were then stored at 4°C and re-assayed at 24 hours and 5 days.

Measured Analyte	Serum Separator Tubes		Photopolymer Separator Tubes	
sodium (mmol/L)	141	141	141	141
potassium mmol/L	4.4	4.4	4.4	4.3
chlorides (mmol/L)	107	106	105	107
CO2 (mmol/L)	27	27	26	26
glucose (mg/dL)	96	95	97	97
urea nitrogen (mg/dL)	12	12	13	12
creatinine (mg/dL)	0.8	0.9	0.8	0.9
calcium (mg/dL)	9.4	9.4	9.2	9.4
total protein (g/dL)	7	6.9	7	6.9
albumin (g/dL)	3.6	3.6	3.7	3.7
alkaline phosphatase (U/L)	124	123	124	124
aspartate aminotransferase (U/L)	34	36	35	34
alanine aminotransferase (U/L)	43	41	43	42
total bilirubin (mg/dL)	0.5	0.3	0.5	0.4

Table S1. Comprehensive metabolic panel measurements obtained in duplicate in BD serum separator tubes and photopolymer separator tubes from the same subject.