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# Preparation of dextran cryogels for separation processes of binary dye and pesticide mixtures from aqueous solutions

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**Abstract**

We present mechanically strong macroporous, squeezable dextran cryogels as a column filling material for the removal and separation of binary organic dye and pesticide mixtures from aquatic medium. Dextran cryogels were prepared from aqueous solutions of dextran of various molecular weights (MWs) in the presence of 20 to 50 mol% divinyl sulfone (DVS) as a cross-linker at −18°C. The cryogels have interconnected irregular pores of 100 μm in sizes, and exhibit 69-84% reversible squeezability without damaging the 3D dextran network. Their total open pore volumes (6.3-10 mL $g^{-1}$), weight swelling ratios in water (1380%-2200%), and mechanical parameters could easily be adjusted by both DVS mol% and MW of dextran. Dextran cryogel with the highest modulus (3.8 ± 0.5 MPa), compressive stress (8 ± 2 MPa) and plateau stress (0.46 ± 0.04 MPa) was obtained at 50 mol% DVS using dextran with a MW of 15 to 25 kg·$mol^{-1}$. Dextran cryogels are hydrolysable at pH = 1 and 9 but stable at 7.4 independent on both the degree of cross-linking and MW of dextran. At below 50 mol% DVS, they are blood compatible and possess slight thrombogenic effect with blood clotting index value of 98% ± 5%. They are also capable to separate binary dye and pesticide mixtures from aqueous solutions via ionic interactions.

**KEYWORDS**

biopolymeric cryogels, dextran cryogel column, macroporous network, mechanical properties, pesticide removal, toxic molecule separation

## 1 | INTRODUCTION

Dextran, a microbial polysaccharide composed of glucose repeating subunits is a hydrophilic, biodegradable and non-toxic biopolymer with a large number of hydroxyl side groups.[1-3] Due to its antithrombotic properties, it is used as an anticoagulant in vascular surgery and in medical applications that replace blood plasma.[4,5] As the water solubility of dextran is high, it does not show cellular toxicity when administered into the body as a drug carrier. Due to its natural and biocompatible features, dextran is used in various applications in the food industry, biomedicine, nanomedicine, and for drug carrier/drug delivery.[6-12]

Cryogelation, in other words, cryo-structuration is a simple and versatile technique in the synthesis of macroporous gels, called cryogel prepared at subzero temperatures, and has exceptional mechanical properties such as a high toughness, rapid swelling/deswelling response, and almost complete squeezability under load

without damage.[13-16] Cryogels are usually prepared by cross-linking of linear polymers, or by free-radical cross-linking copolymerization of vinyl- and divinyl monomers in aqueous solutions at temperatures below the freezing point of the reaction systems. Cryogels are commonly preferred over the conventional brittle hydrogels in biomedical fields such as cells and drug delivery systems,[17-21] protein/antibody separation/absorption,[22-25] biomolecule separation,[26,27] wound dressing materials,[28,29] tissue engineering[30-33] and, ion exchanger,[34] antibacterial activity[35,36] and antioxidant activity.[36] In addition to biomedical applications, they are also a good candidate as a sorbent for the removal and separation of organic molecules such as pesticides and dyes from aqueous media.

Cryogelation of dextran has mainly been conducted after its functionalization with acrylate or methacrylate groups.[2,3,37,38] Reichert et al. prepared dextran cryogels at −20°C by electron-beam assisted free-radical crosslinking reaction of methacrylated dextran and hyaluronic acid.[37] Wu et al. prepared macroporous dextran by mixing aqueous solutions of oxidized dextran and chitosan followed by directional freezing at below −80°C.[38] To our knowledge, cryogels based on unmodified dextran was recently reported via photo-crosslinking of two high molecular weight dextrans (100-200, and 2000 kg $mol^{-1}$) at −20°C using *N*,*N*′-methylenebisacrylamide as a cross-linker.[39] It was shown that increasing concentration of dextran from 2 to 10 wt% increases the storage modulus up to around 1.7 kPa followed by a slight decrease to 1.2 kPa at 25 wt% dextran. Our preliminary experiments showed that cryogelation of dextran with a molecular weight of 15 to 25 kg $mol^{-1}$ can be conducted using divinyl sulfone (DVS) as a crosslinker at a concentration of 50 mol% based on the dextran repeating unit.[40]

Here, we investigate the effects of the molecular weight (MW) of dextran and the amount of DVS cross-linker on the mechanical properties, degradation behavior, and blood compatibility of dextran cryogels. The cross-linking of dextran of various MWs with various amounts divinyl sulfone (DVS) cross-linker, between 20 and 50 mol% in aqueous solutions at −18°C were used to determine it is effect on the prepared dextran cryogel physical and chemical properties. Dextran cryogels have interconnected irregular pores of 100 μm in sizes in both dry and swollen states, and exhibit 69% to 84% reversible squeezability without damaging the 3D dextran network. Their total open pore volumes (6.3-10 mL $g^{-1}$), weight swelling ratios in water (1380%-2200%), and mechanical parameters could easily be adjusted by both DVS content and MW of dextran. Dextran cryogel with the highest modulus (3.8 ± 0.5 MPa), compressive stress (8 ± 2 MPa) and plateau stress (0.46 ± 0.04 MPa) was obtained at 50 mol% DVS using dextran with a molecular weight of 15 to 25 kg $mol^{-1}$. To show the application of dextran cryogel, they used in the removal and separation of binary mixtures of dyes and pesticides from aqueous solutions. As organic dyes in excess amounts are known to be toxic and carcinogenic, and pesticides accumulating over time in the soil threaten the lives of humans and other living species.[41,42] Therefore, separation of these toxic substances using suitable sorbents is very important due to environmental, agricultural and food safety concerns. Therefore, we aimed to demonstrate that dextran cryogels are capable to separate binary dye and pesticide mixtures from aqueous solutions via different interactions such as ionic, hydrophilic and so on.

## 2 | EXPERIMENTAL SECTION

### 2.1 | Materials

Dextrans from *Leuconostoc* spp. (Sigma Aldrich) with molecular weights 15 to 25, 150, and 450 to 650 kg $mol^{-1}$, denoted by dextran A, B, and C, respectively, were used for the preparation of the cryogels. Divinyl sulfone (97%, DVS, Sigma-Aldrich) was used a cross-linker. Methylene blue hydrate (MB, 97%, Sigma-Aldrich), methyl orange (MO, Reag. Ph. Eu., Fluka), and 2,4-dichlorophenoxyacetic acid (2,4-D, 99+ %, Acros), 1,1′-dimethyl-4,4′-bipyridinium dichloride (paraquat, PQ, Fluka) were used as organic dyes and pesticides, respectively. Cyclohexane (≥99.5%, VWR), hydrochloric acid (HCl, 36.5%-37%, Sigma-Aldrich) and sodium hydroxide (NaOH, Merck) were used as received. All aqueous solutions were prepared using distilled water (DW, GFL 2108).

### 2.2 | Synthesis of dextran cryogels

Dextran cryogels were prepared under cryogenic conditions via a method similar to that reported before for carboxymethyl cellulose (CMC) cryogels.[43] Briefly, a dextran solution was prepared by dissolving 5.00 g of dextran of various MWs in 100 mL aqueous 0.2 M NaOH solution under stirring at 500 rpm. After keeping the solution at −18°C for 3 minutes, different amounts of DVS, for example, 20, 40, and 50 mol% with respect to dextran repeating unit were added under stirring. The solution was then transferred to pipettes of 6 mm in diameter and kept in a deep freezer at −18°C for 24 hours for cryogelation. The cryogels thus formed were cut to about the same dimensions and washed five times with distilled water to remove unreacted chemicals. They were then dried to reach constant weight in a freeze-dryer and packed in double zip-lock bag for later use.

## 2.3 | Characterization of dextran cryogels

Functional groups in dextran cryogels were ascertained by Fourier transform-infrared spectroscopy (FT-IR, Thermo Fisher Scientific) employing 4 $cm^{-1}$ resolution with ATR technique in 4000 to 650 $cm^{-1}$ spectra range. Morphological analysis of cryogels was performed by FE-SEM (Field Emission Scanning Electron Microscopy, Hitachi Regulus 8230) and optical microscopy (Olympus BX53F, Japan). To acquire FE-SEM images, dried cryogels were placed on carbon tapes affixed to aluminum stubs and coated with gold under vacuum, followed by applying a voltage of 2.0 kV.

Thermal characterization of the cryogels was conducted on a thermal analyzer (TGA, SII TG/DTA 6300, Japan). To remove the moisture, the specimens of about 3 mg in mass were first subjected to heating and cooling cycles between 50°C and 100°C under $N_2$ gas atmosphere. The weight lost was then recorded between 50°C and 1000°C under 100 mL $min^{-1}$ flow rate of $N_2$.

Uniaxial compression tests were carried out at 23 ± 2°C on a Zwick Roell universal test machine equipped with a 500 N load cell using dry cryogel specimens of 6 mm in diameter and 3 mm in length. To minimize the effect of uneven specimen surface caused by cutting, a pre-load of 0.01 N was applied before the measurements. The nominal stress $\sigma_{nom}$, the force per per cross-sectional area of the virgin specimen, and strain $\varepsilon$ data were recorded at a constant strain rate of 10 mm $min^{-1}$. Young's modulus $E$ was calculated from the slope of $\sigma_{nom} - \varepsilon$ curves between 5% and 15% compression while the fracture stress $\sigma_f$ was determined from the maxima of true stress $(\sigma_{true}) - \varepsilon$ curves, as detailed before (Figure S1).[14]

The swelling behavior of the cryogels was determined by immersing dried dextran cryogel specimens of a known weight in 20 mL phosphate buffer (pH 7.4). Preliminary experiments showed that they all attain their equilibrium swollen states within 10 seconds. The equilibrium swelling degree $S\%$ and the water uptake capacity $W_u\%$ of the cryogels were calculated using the equations,

$$S\% = \left(m_{sw} - m_{dry}\right)/m_{dry} \times 100 \quad (1)$$

$$W_u\% = \left(m_t - m_{dry}\right)/m_{sw} \times 100 \quad (2)$$

where $m_{sw}$ and $m_{dry}$ are the masses of swollen and dry cryogel specimens, respectively, and $m_t$ is the mass of swollen cryogel specimen at time $t$.[30] The squeezability of the cryogels were determined by squeezing swollen cryogel specimens by hand for 30 seconds and then weighing the squeezed samples. The degree of squeezability Sq% was estimated by,

$$Sq\% = \left(m_{sw} - m_{sq}\right)/m_{sw} \times 100 \quad (3)$$

where $m_{sq}$ is the mass of squeezed cryogel specimens. In order to determine the total volume $V_p$ of open pores in the cryogels, the specimens were immersed in cyclohexane for 30 minutes which is a poor solvent for dextran. $V_p$ was calculated by,

$$V_p\% = \left(m_{ch} - m_{dry}\right)/\left(m_{dry} d_1\right) \times 100 \quad (4)$$

where $m_{ch}$ is the mass of specimen in cyclohexane and $d_1$ is its density. All the swelling tests were repeated at least three times and the average values are reported with standard deviations.

Moreover, the density and gel content of the prepared cryogels were determined. Densities of the cryogels were calculated by measuring the diameter and height a dry cryogel after weighing ($v$ is for volume), by dividing the cryogel specimen weight by volume, $d = m/v$. To determine the gel content of the prepared cryogels, the cryogels were freeze-dried just after preparation and then weighed, then thoroughly washed to remove the loose polymer chains and unreacted DVS molecules, after which the washed cryogels were freeze-dried and re-weighed again.

## 2.4 | Hydrolytic degradation of dextran cryogels

Water-swollen cryogel specimens of about 50 mg in mass were placed in three different pH buffers at pH 1 (citrate buffer), pH 7.4 (phosphate buffer), and pH 9 (phosphate buffer). The weight of the specimens was monitored during the course of the swelling process in these buffer solutions until their maximum swelling capacity was reached. The specimens were then placed in 50 mL of the corresponding buffer solutions at 37.5°C under stirring at 100 rpm. To examine the degradation of cryogels over time, swollen specimens at various time intervals were dewatered with filter paper to remove surface water and weighed. The weight loss of the cryogels was calculated using the equation,

$$\text{Weight Loss} = \left(1 - m_t/m_o\right) \quad (5)$$

where $m_o$ is the initial mass of the swollen cryogel and $m_t$ is the mass of the degraded cryogel at time $t$.

### 2.5 | Blood compatibility test for dextran cryogels

The blood compatibility of dextran cryogels was determined by in vitro hemolysis and blood clotting tests according to a procedure accepted by Canakkale Onsekiz Mart University Human Research Ethics Committee (KAEK-2016-27). The details of hemolysis and blood clotting tests are given in Supporting information text.

### 2.6 | Use of dextran cryogels for separation and adsorption purposes

Two organic dyes, methylene blue (MB) and methyl orange (MO), and two pesticides, 1,1′-dimethyl-4,4′-bipyridinium dichloride (paraquat, PQ) and 2,4-dichlorophenoxyacetic acid (2,4-D) were used in adsorption and separation experiments. Dextran cryogel columns were prepared by squeezing water-swollen cryogel specimens and then placing into glass columns of 6 mm in internal diameter and 120 mm in height. For each column, around 15 ± 2 mg of swollen cryogel specimen was used that produced a height of 8 mm in the column. Five milliliters of dye solutions in DI water containing 25 ppm MO, 25 ppm MB, and MO/MB mixture each 25 ppm were then passed through the columns under gravity. The separation capacity of dextran cryogels for MO/MB mixtures was determined via UV-vis spectroscopy by using their maximum absorptions at 464 and 664 nm, respectively The spectra of the dyes were recorded before and after passing the dye solutions through the column, and the amount of absorbed dye per gram of cryogel was calculated from a calibration curve that was prepared for reference aqueous MB solution in DI water at 664 nm. The separation (SE) efficiency was calculated using $SE = (1 - C/C_o)$, where $C$ and $C_o$ are the dye concentrations after and before separation, respectively. Similarly, 5 mL of aqueous pesticide solutions containing 20 ppm PQ, 20 ppm 2,4-D, and PQ/2,4-D mixture each 20 ppm were used for adsorption and separation of the pesticides as described above. The maximum absorption wavelengths of PQ and 2,4-D at 257 and 234 nm, respectively, were employed for determination of pesticide concentrations. Moreover, to quantify the selectivity of dextran cryogels for PQ and 2,4-D, the distribution coefficient $K_{d(i)}$ was calculated as,[44]

$$K = \frac{K_{d(PQ)}}{K_{d(2,4D)}}$$

$$K_{d(i)} = \frac{(c_0 - c)V_s}{Cm} \quad (6)$$

where $i$ = PQ or 2,4-D, $C_o$ and $C$ are the initial and final concentrations of the species $i$, $V_s$ is the solution volume and $m$ is the cryogel mass. The distribution coefficient $K_{d(i)}$ at equilibrium refers to the relative affinity of a sorbent when exposed to a solution containing different molecules.[44,45]

The selectivity coefficients $k$ are calculated from the Equation (7) in the presence of the species with higher distribution coefficient.

$$K = \frac{K_{d(PQ)}}{K_{d(2,4D)}} \quad (7)$$

## 3 | RESULTS AND DISCUSSION

### 3.1 | Preparation and characterization of dextran cryogels

Dextran cryogels were prepared in aqueous solutions at 5 wt/vol % dextran concentration using DVS as a cross-linker at −18°C. Two sets of experiments were carried out: In the first set, the amount of DVS was varied between 20 and 50 mol% with respect to the dextran repeating units at a fixed MW of dextran (15-25 kg $mol^{-1}$). In the second set, dextran MW was varied between 15 to 25 and 450 to 650 kg $mol^{-1}$ at 20 mol% DVS. The synthesis parameters and densities of the cryogels are compiled in Table 1. The structural and thermal characterization of the cryogels were performed in their dried state via FT-IR and TGA analysis, respectively. In the FT-IR spectra, the characteristic peaks for dextran in the 3362 to 3316 $cm^{-1}$ range for —OH groups, and at 2922 and 1643 $cm^{-1}$ for —CH groups remain unchanged after cryogelation while a new peak appears at 1313 $cm^{-1}$ which belongs to the stretching vibrations of the sulfone (S═O) groups (Figure S2). The intensity of the S═O peak increased with increasing DVS content from 20 to 50 mol% indicating incorporation of increasing amount of DVS cross-links into the cryogel network. As can be seen from the FT-IR spectrum of pure DVS in Figure S2, the characteristics S═O group peak is between 1380 and 1308 $cm^{-1}$ and the vinyl group peaks (—C═C—) with between 1741 and 1610 $cm^{-1}$ is disappeared. Moreover, no significant difference in the thermal degradation of dextran before and after cryogelation was detected from TGA curves (Figure S2). They all start to degrade at about 239°C to 272°C with a weight loss of about 5%; the degradations of

**TABLE 1** Synthesis conditions, and the equilibrium swelling ratio $S$%, water content $W_u$%, squeezability $Sq$%, total pore volume $V_p$, densities and gel content of dextran cryogels

| Dextran type | MW (kg mol$^{-1}$) | DVS (mol%) | $S$% | $W_u$% | $Sq$% | $V_p$ (mL g$^{-1}$) | Density (g mL$^{-1}$) | Gel content% |
|---|---|---|---|---|---|---|---|---|
| A | 15–25 | 20 | 2200 ± 100 | 90 ± 1 | 78 ± 5 | 10 ± 2 | 0.52 ± 0.01 | 79 ± 0.04 |
| | | 40 | 1510 ± 30 | 87 ± 2 | 84 ± 2 | 10 ± 1 | 0.59 ± 0.04 | 85 ± 0.02 |
| | | 50 | 1380 ± 12 | 85 ± 1 | 84 ± 1 | 6.5 ± 0.9 | 0.73 ± 0.02 | 88 ± 0.07 |
| B | 150 | 20 | 1500 ± 30 | 87 ± 2 | 71 ± 2 | 9.2 ± 0.2 | 0.46 ± 0.04 | 79 ± 0.01 |
| C | 450–650 | 20 | 1460 ± 40 | 85 ± 1 | 69 ± 1 | 6.3 ± 0.3 | 0.26 ± 0.03 | 80 ± 0.02 |

virgin dextran and its cryogels continue at higher temperatures up to around 800°C with about 99% and 96% weight losses, respectively.

The equilibrium weight swelling ratio $S$% and water content $W_u$% of the cryogels were determined in water at pH = 7.4 and listed in Table 1. They all swell about 1380% to 2200% with respect to their dry states, and hence their water uptake capacity is about 14 to 22 folds of their dry states. The gel content of the prepared cryogels were also determined and given in Table 1. It is obvious from Table 1, by increasing the DVS content from 20 to 50 mol%, the gel content is increased to 88 ± 0.07 from 79 ± 0.04 for the same MM of dextran, 15 to 25 kg mol$^{-1}$. On the other hand, the gel content for the cryogels prepared from higher MW of dextran dos not change significantly change as about 79 ± 0.01 and 80 ± 0.02% gelation was measured for the cryogels prepared using dextran with MW of 150 and 450 to 650 kg mol$^{-1}$, respectively. Therefore, it obvious that regardless of MW of the used dextran, when 20% DVS is used about 79% or 80% gelation is attained suggesting that almost all the DVS is reaction taking place in the crosslinking of dextran chains. Moreover, the cryogels exhibit superfast swelling kinetics in that they attain equilibrium swelling capacities in water within 10 seconds. This swelling behavior of dextran cryogels is very similar to the other cryogels prepared from synthetic monomers or polymers,[46] and biopolymers such as casein, gelatin, ovalbumin[42] and so on.[47,48] Increasing DVS content at a fixed dextran MW decreases the swelling ratio of the cryogels which is attributed to their increasing cross-link densities. A similar behavior was observed when dextran MW is increased at a fixed DVS content: The higher the MW of dextran, the lower is the swelling capacity of the resulting cryogels. This can be explained with decreasing number density of polymer chains with increasing molecular weight of the chains leading to a larger number of DVS cross-links per chain and hence lower swelling ratios. The densities ($d$) of the cryogels were calculated by measuring the diameter and height (for volume, $v$) after weighing the dried cryogels, $d = m/v$. The d values of the cryogels is increased with the increase in amount of used crosslinker for the same molecular weight of dextran. For example, the $d$ values of 0.52 ± 0.01, 0.59 ± 0.04, and 0.73 ± 0.02 g mL$^{-1}$ was determined for 20%, 40%, and 50% crosslinked dextran with MW of 15 to 25 kg mol$^{-1}$. As anticipated, when the MW of dextran is increased for the same amount of DVS crosslinker usage (ie, 20%), the d values were reduced significantly as the d values of 0.46 ± 0.04 and 0.26 ± 0.03 g mL$^{-1}$ was calculated for dextran cryogels that are prepared by using 20% DVS crosslinking for dextran molecules with MW of 150 and 450 to 650 kg mol$^{-1}$, respectively. Moreover, in contrast to the high weight swelling ratio of all cryogels, they exhibited negligible volume swelling in water. For instance, the images in Figure 1A show a cryogel specimen with 50 mol% DVS during swelling by the addition of water. When water is dropwise added on the top of the dry specimen, it absorbs 1380-fold water within 10 seconds without changing visually its volume. This indicates that the swelling mainly occurs by filling the pores within the cryogels with water without expansion of the 3D dextran network.

Figure 1B-D shows typical optical microscopy (first and second rows) and FE-SEM images (last row) of dextran cryogels with 20 (B), 40 (C), and 50 mol% DVS (D). The optical images in dry and swollen states reveal existence of irregular pores of 100 μm in sizes in both states. Swelling results in the disappearance of small pores in favor of larger ones and decreases the irregularity of the pore morphology. The morphology of dry cryogels observed from SEM images also exhibits irregular pores with a wide range of sizes between 100 and 300 μm. Similar morphologies were also observed when MW of dextran is varied at 50 mol% DVS. However, how the average pore size varies depending on the DVS content and MW of dextran could not be calculated due to the irregular size of the pores. In contrast, the total pore volume $V_p$ of the cryogels was found to decrease with increasing DVS mol% or MW of dextran (Table 1). For example, $V_p$ decreases from 10 to 6.5-6.3 mL g$^{-1}$ with increasing DVS content from 20 to 50 mol%, or with increasing MW of dextran from 15-25 to 450-650 g mol$^{-1}$. The dependence of the total pore volume on the synthesis

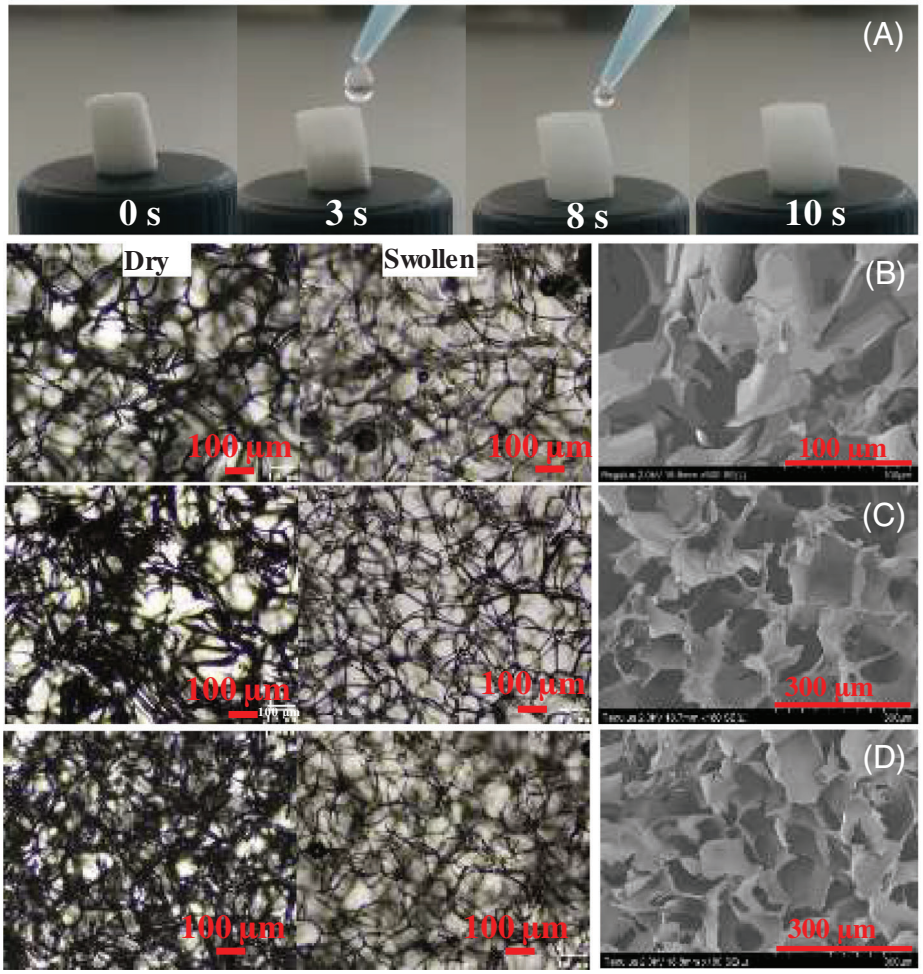


**FIGURE 1** A, Optical images of a dry cryogel with 50 mol% DVS during swelling by dropwise addition of water. The time of swelling is indicated. Dextran MW = 15 to 25 kg mol$^{-1}$. B-D, Optical microscopy (first and second rows) and FE-SEM images (last row) of dextran cryogels with 20 (B), 40 (C), and 50 mol% DVS (D). Scale bars are 100 μm [Color figure can be viewed at wileyonlinelibrary.com]

parameters thus exhibits a similar trend as that the equilibrium swelling ratios (Table 1). Because decreasing degree of swelling reflects increasing cross-link density of the cryogel network, it is obvious that the porosity decreases with increasing cross-link density of the cryogels, that is, by increasing DVS content or MW of dextran.

Other characteristics of water-swollen dextran cryogels is their squeezability by hand during which 69% to 84% water inside the pores could be reversibly removed without damaging the dextran network (Table 1). Immersion of squeezed cryogels in water recovers their initial water contents within 10 seconds. The easy and reversible squeezability of the cryogels reflects the highly elastic nature of their networks forming the pore walls. To highlight the mechanical performance of the pore walls in the absence of water, uniaxial compression tests were performed on the cryogels in their dried states. Figure 2A presents typical compressive stress-strain curves of dry cryogels prepared at various DVS contents (upper panel) and MWs of dextran (bottom panel). The insets are zoom-in to the curves below 40% strain. The general trend of the stress-strain curves is the existence of a linear, elastic regime at below 15% strain, followed by a near-plateau regime during which the instantaneous modulus significantly decreases, that is, the cryogels easily deform up to around 60% strain. At higher strains, strain-hardening behavior appears until they fracture under around 90% compression. The mechanical parameters of the cryogels, namely Young's modulus $E$, fracture stress $\sigma_f$, and plateau stress $\sigma_p$ are shown in Figure 2B as functions of DVS mol% and MW of dextran. $\sigma_p$ was calculated from the point of intersection of the straight-lines extending from the tangents of the stress-strain curves in the linear and plateau regimes. Both $E$ and $\sigma_f$ increase with increasing DVS content of the cryogels from 20 to 50 mol% at a fixed MW of dextran. For instance, the modulus $E$ and the fracture stress $\sigma_f$ increase from $0.8 \pm 0.1$ to $3.8 \pm 0.5$ MPa and from $4 \pm 1$ to $8 \pm 2$ MPa with increasing DVS content from 20 to 50 mol%, respectively. Similar to the effect of the cross-linker DVS, the MW of dextran also affects the mechanical properties of the cryogels, especially between 15 to 25 and 150 kg mol$^{-1}$.

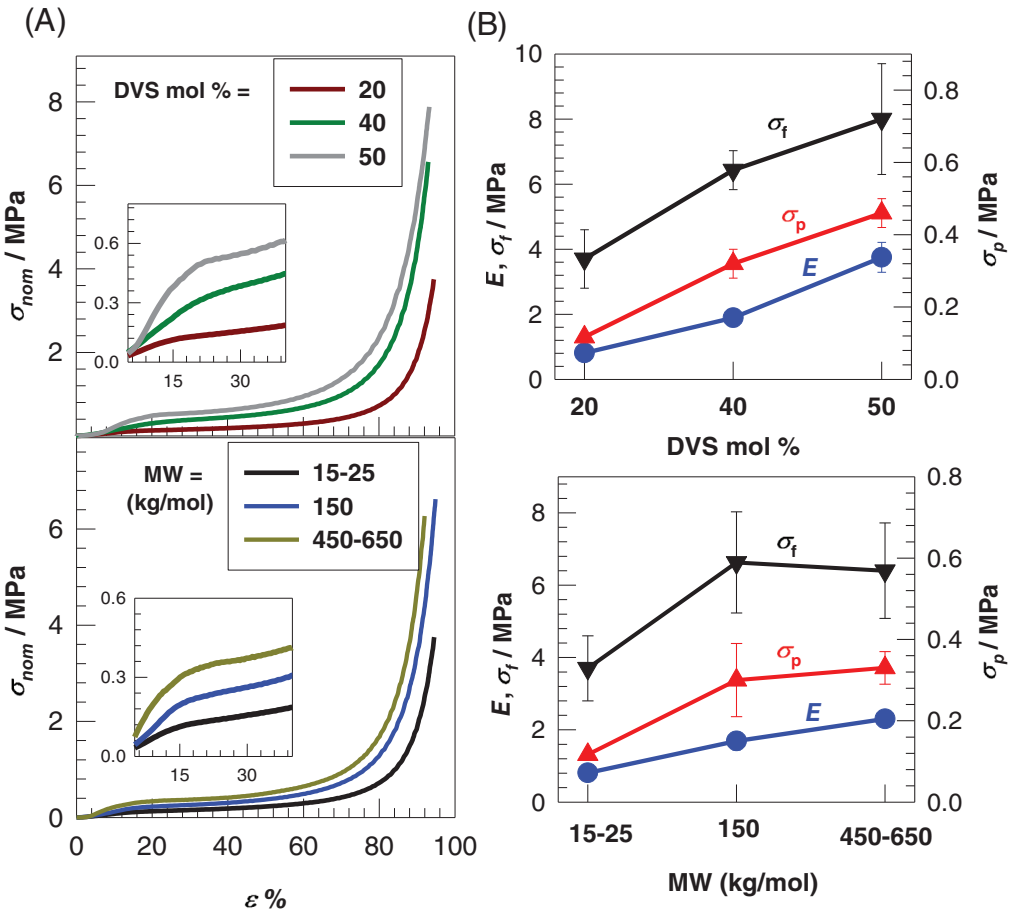


**FIGURE 2** A, Typical stress-strain curves of dextran cryogels prepared at various DVS mol% (upper panel) and MWs of dextran (bottom panel). The insets are zoom in to the curves below 40% strain. B, Mechanical parameters of the cryogels plotted against DVS mol% and MWs of dextran [Color figure can be viewed at wileyonlinelibrary.com]

As MW is increased from 15-25 to 450-650 kg mol$^{-1}$, the modulus $E$, and compressive fracture stress $\sigma_f$ increase from $0.8 \pm 0.1$ to $2.3 \pm 0.2$ MPa, and from $4 \pm 1$ to $6.4 \pm 1.3$ MPa, respectively. These values representing the stiffness and strength of dextran cryogels are the highest reported so far for dextran cryogels. Previous works show that the plateau stress $\sigma_p$ of the cryogels is correlated with the mechanical stability of the porous structure.[14,49] This is due to the fact that the pores start to collapse at the onset of the plateau regime so that the cryogels deform easily along the plateau until disappearance of the pores. Thus, the higher the strength of the 3D network structure forming the pore walls, the higher is the stress at the plateau regime. As seen in Figure 2B, $\sigma_p$ increases with both DVS mol% and dextran MW (from around 0.1 to 0.5 MPa) revealing increasing mechanical stability of the porous structure of dextran cryogels. Moreover, the strain-hardening behavior after the plateau regime in stress-strain curves is likely due to the compression of nonporous dextran network under high strain conditions. The mechanical test results thus show that both DVS content and MW of dextran are important parameters determining the mechanical performance of the cryogels. Increasing DVS mol% or MW of dextran results in an increase in the cross-link density of the cryogels leading to lower weight swelling ratios and porosities but higher modulus, fractures stress, and higher stability of the porous structure. Dextran cryogel with the highest modulus ($3.8 \pm 0.5$ MPa), compressive stress ($8 \pm 2$ MPa) and plateau stress ($0.46 \pm 0.04$ MPa) was obtained at 50 mol% DVS using dextran with a MW of 15 to 25 kg mol$^{-1}$. It is obvious that as the density of cryogel is increased, the compression modulus E is also increased almost linearly as it is given in Figure 2B for dextran cryogels prepared from dextran with MW of 15 to 25 kg mol$^{-1}$ (A) is in agreement with Table 1 in terms of gelation and density.[50]

Hydrolytic stability and blood compatibility are important parameters in biomedical applications of dextran cryogels. The hydrolytic degradation of dextran cryogels was investigated at 37.5°C in three different

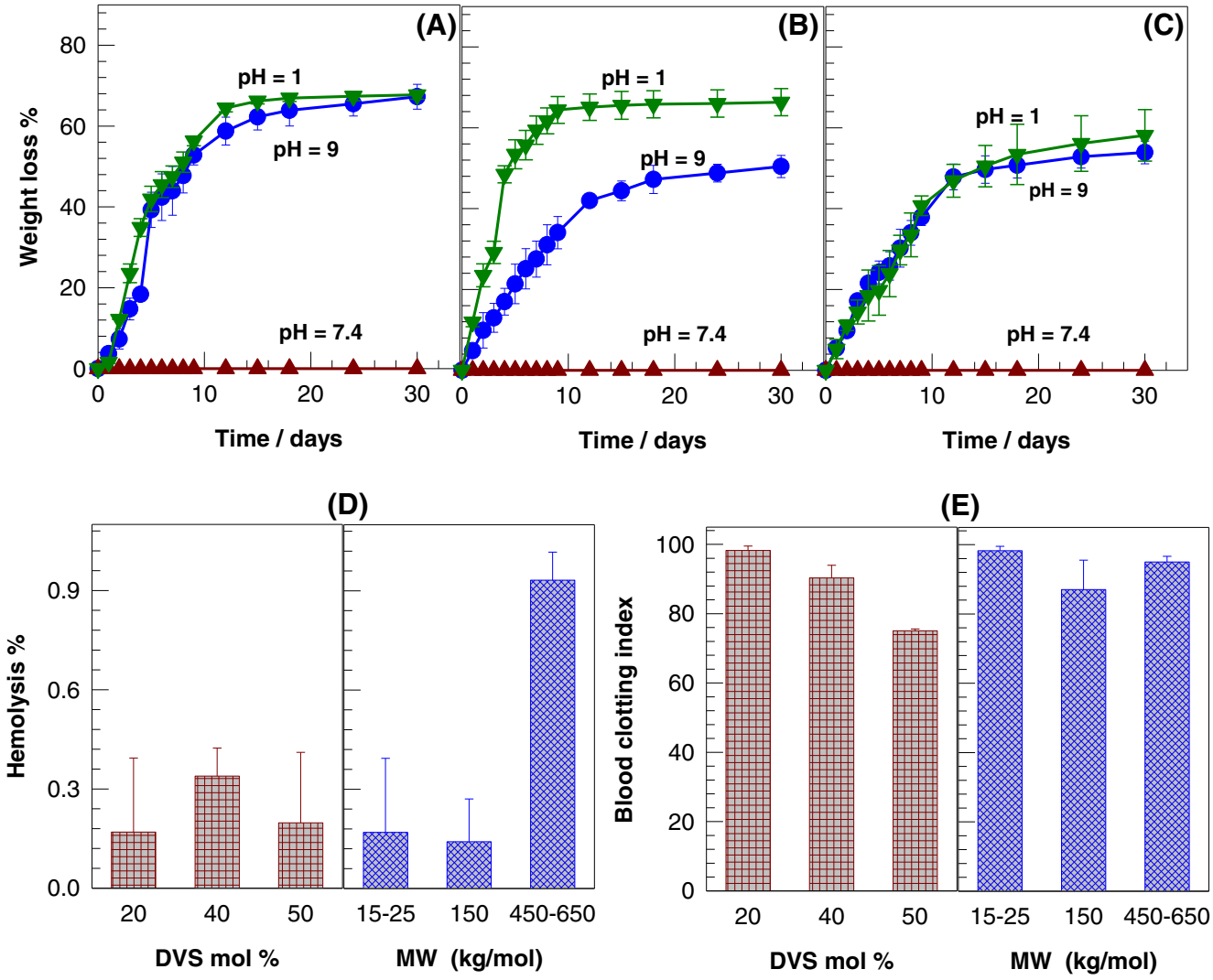


**FIGURE 3** A-C, The degradation profiles of dextran cryogels at 37.5°C in different pH buffer solutions as indicated. The cryogels were prepared at 20 (A), 40 (B), and 50 mol% DVS (C). MW of dextran = 15 to 25 kg $mol^{-1}$. D and E, Hemolysis (D) and the blood clotting indices (E) of dextran cryogels prepared at different DVS mol% and MW of dextran [Color figure can be viewed at wileyonlinelibrary.com]

buffer solutions at pH 1.0 (stomach pH), pH 7.4 (physiological pH), and pH 9.0 (about intestine pH). Figure 3A-C shows the weight loss of dextran cryogels formed at 20, 40, and 50 mol% DVS, respectively, plotted against the contact time with the buffer solutions. Independent on the DVS content, all cryogels are stable at neutral pH (7.4) for at least 1 month whereas they degrade in acidic or basic medium. At pH 1.0 and pH 9.0, they degrade almost linearly up to around 10 days followed by a slow degradation period until approaching a plateau after 30 days. The initial fast degradation period at pH 1.0 and pH 9.0 results in 53% ± 13% weight loss after 10 days, which increases to about 61% ± 8% at the end of the slow degradation period. Another characteristic of the degradation profiles is slower degradation rate of the cryogels at pH 9.0 as compared to pH 1.0, and slightly decreasing weight losses with increasing DVS content of the cryogels. Thus, dextran cryogel is stable in neutral conditions, whereas the acidic conditions produce higher degradation than in basic conditions.

The blood compatibility of dextran cryogels were investigated by hemocompatibility tests of hemolysis and blood clotting index to estimate their potential for use in blood contacting biomedical use. The hemolysis of dextran cryogels prepared at various DVS contents between 20 and 50 mol% and using different MWs of dextran show that they all are non-hemolytic (Figure 3D). The blood clotting indices of dextran cryogels prepared using 20, 40 and 50 mol% DVS are 98 ± 1, 90 ± 5 and 75 ± 1%, respectively, indicating that the cryogel with 50 mol% DVS may cause some blood clotting (Figure 3E). Moreover, all cryogels prepared using 20 mol% DVS and at

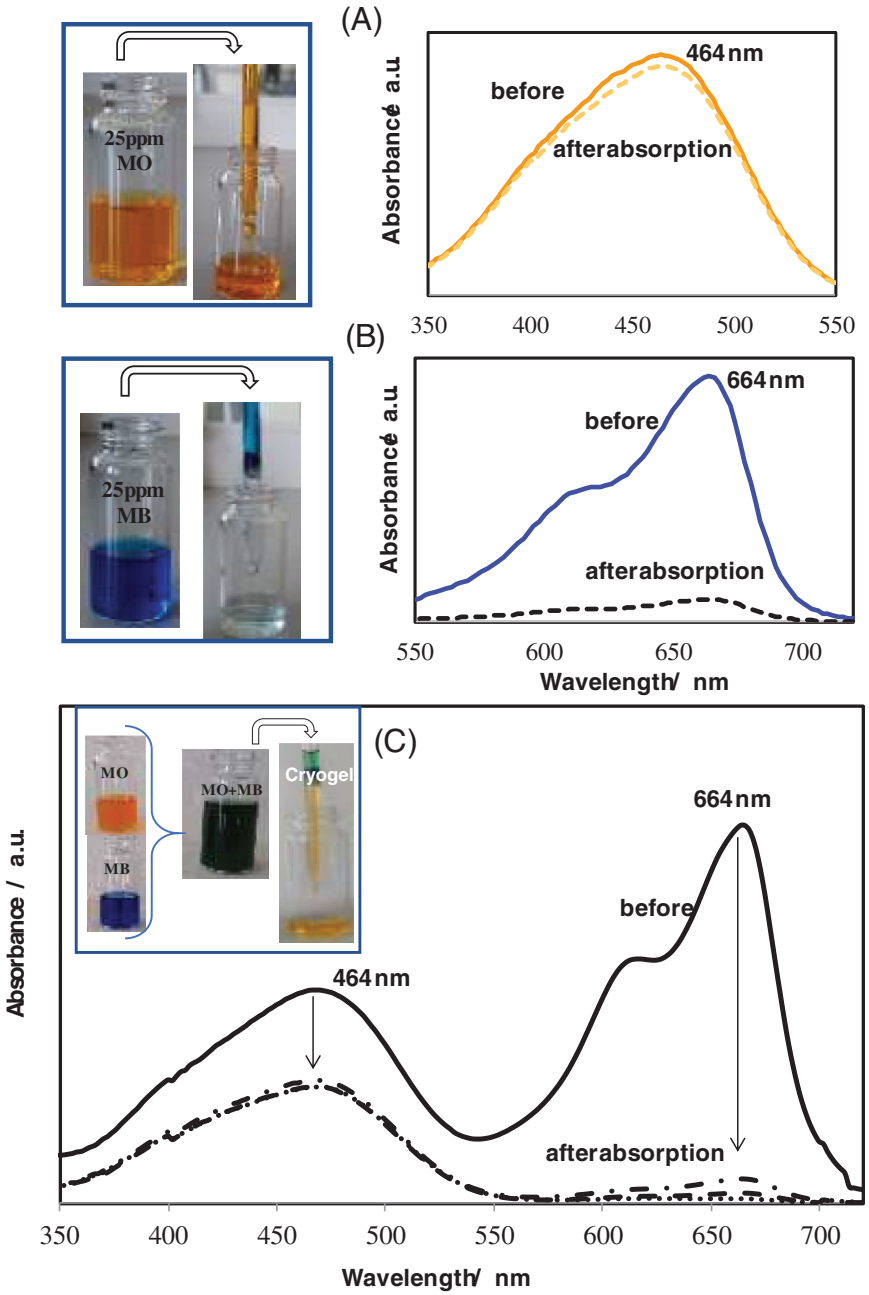


**FIGURE 4** Digital camera images and UV-vis spectra of MO (A), MB (B), and MB/MO mixture before and after passing through a glass column containing dextran cryogel as filling material. DVS content of the cryogels: 20 (dash-dot-dash line), 40 (dashed line) and 50 mol% (dotted line) [Color figure can be viewed at wileyonlinelibrary.com]

various dextran MWs induce no blood clotting indices. Consequently, regardless of MW, dextran cryogels prepared at below 50 mol% DVS are blood compatible and can be safely used in blood contacting applications.

## 3.2 | Separation of binary organic dyes and pesticides from aqueous solutions

Dextran cryogels were used as column filler material for the separation of binary organic dyes and pesticides from aqueous solutions. Methylene blue (MB)/methyl orange (MO), and 1,1′-dimethyl-4,4′-bipyridinium dichloride (paraquat, PQ)/2,4-dichlorophenoxyacetic acid (2,4-D) were used as the binary dyes and pesticides, respectively. For dye separation experiments, 5 mL of aqueous dye solutions containing (a) 25 ppm MB, (b) 25 ppm MO, and (c) MB/MO mixture each 25 ppm were passes through glass columns under gravity, each containing 15 ± 2 mg of swollen cryogel with different DVS contents. Figure 4A-C shows the images and UV-vis spectra of MO (a), MB (b), and MO/MB mixture (c) before and after passing through the dextran columns. The maximum absorption wavelengths of MO and MB at 464 and 664 nm, respectively, are indicated. The images in Figure 4A,B reveal that the orange color of MO remains visually unchanged while the blue color of MB disappears after passing through the column. The corresponding spectra indeed show an

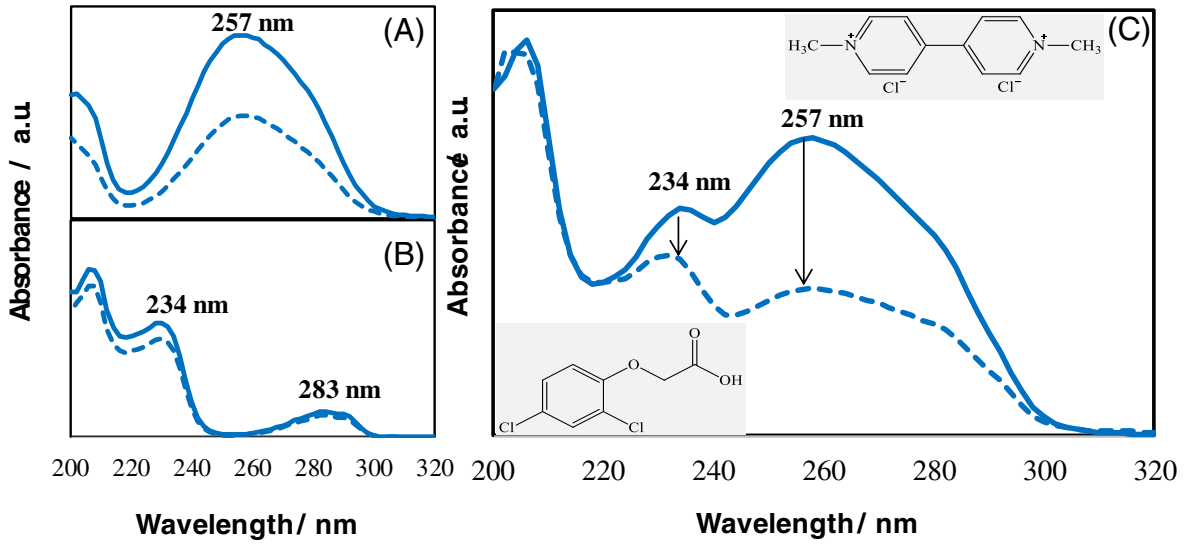


**FIGURE 5** UV spectra of aqueous solutions of PQ (A), 2,4-D (B), and PQ/2,4-D mixture (C) before (solid lines) and after passing through a dextran cryogel column (dashed lines). DVS = 50 mol%. The insets to C show the chemical structures of PQ and 2,4-D pesticides [Color figure can be viewed at wileyonlinelibrary.com]

**TABLE 2** Distribution coefficients $K_{d(i)}$ for PQ and 2,4-D pesticides and the selectivity coefficient $k$ of dextran cryogels

| Cycle number | $K_{d(PQ)}$ | $K_{d(2,4D)}$ | $k$ | PQ removal % | 2,4-D removal % |
|---|---|---|---|---|---|
| 1 | 442 ± 15 | 132 ± 18 | 3.55 ± 0.02 | 52 ± 7 | 29 ± 1 |
| 2 | 217 ± 5 | 87 ± 16 | 2.5 ± 0.1 | 76 ± 2 | 43 ± 1 |
| 3 | 197 ± 25 | 73 ± 24 | 2.70 ± 0.05 | 84 ± 2 | 54 ± 1 |
| 4 | 184 ± 35 | 72 ± 20 | 2.54 ± 0.08 | 89 ± 3 | 62 ± 1 |
| 5 | 153 ± 19 | 63 ± 16 | 2.40 ± 0.06 | 91 ± 5 | 68 ± 2 |

insignificant reduction in the peak intensity at 464 nm while that at 664 nm drastically reduces. 90% of MB could be removed from the solution within 7 minutes by passing 5 mL of 25 ppm MB dye through the column containing 15 ± 2 mg dextran cryogel in three cycles. It is obvious that the negatively-charged dextran network adsorbs the positively-charged MB dye via electrostatic interactions. Moreover, as seen in Figure 4C, the dark green color of MB/MO dye mixture turns into orange color of MO after passing the aqueous dye mixture through the column. The spectra recorded before and after separation through the column reveals that the peak at 664 nm almost disappears whereas the peak at 464 nm belonging to MO remains almost the same, confirming the separation on the dyes. Thus, the anionic dextran cryogel adsorbs the cationic dye MB while the anionic MO readily passes through the pores of the cryogel without adsorption.

To further demonstrate the applicability of dextran cryogel for separation purposes, aqueous solutions of binary PQ/2,4-D pesticide mixtures were used. PQ has a maximum absorption wavelength at 257 nm whereas 2,4-D has three absorption wavelengths at 206, 234, and 283 nm (Figure 5A,B). As the peak of PQ at 257 nm does not overlap that at 234 nm of 2,4-D pesticide, they both were employed for quantification of the separation results. As described above, glass columns were filled with dextran cryogels each 15 ± 2 mg, and 5 mL of 20 ppm pesticide solutions were passed three cycles through the columns and 52 ± 7% of PQ could be removed from solution in about 8 minutes. Figure 5A,B show UV-vis spectra of PQ and 2,4-D solutions, respectively, before (solid lines) and after passing through the columns (dashed lines). The peak intensity at 257 nm significantly decreases as compared to that at 234 nm, implying significant adsorption of the positively-charged pesticide PQ by the cryogel.

Moreover, as seen in Figure 5C, when aqueous solutions of PQ/2,4-D binary mixture each 20 ppm were passed through the columns, the peak at 257 nm for PQ is decreased to almost 52% while the peak of 2,4-D at 234 nm decreases to 29%.

Table 1 showing the distribution coefficients $K_{d(i)}$ of the pesticides reveals that $K_{d(PQ)}$ is 442 during the first cycle as compared to $K_{d(2,4D)} = 132$ while they both slightly decrease with increasing cycle number. As the distribution coefficient is higher for PQ, the selectivity coefficients $k$ for the binding of PQ in the presence of the competitor 2,4-D was calculated by using Equation (7).

Table 2 also shows the selectivity coefficients $k$, and removal percentages of aqueous PQ/2,4-D mixtures for five successive cycles. The selectivity coefficient for the dextran cryogel is $3.55 \pm 0.02$ in the first cycle and $2.40 \pm 0.06$ in the fifth cycle. Moreover, the removal percentages for PQ and 2,4-D pesticides are $52\% \pm 7\%$ and $29\% \pm 1\%$, respectively in the first cycle and they increase to $91\% \pm 5\%$ and $68\% \pm 2\%$, respectively, at the end of the fifth cycle. The reason for the increase in removal percentage of PQ and 2,4-D pesticide as the cycle numbers is increased can be assumed the increase in the extent of accessibility sites that are available for interaction of these molecules with the functional groups of dextran cryogel. Additionally, as cycle number is increased the concentration of PQ and 2,4-D pesticides is reduced in solution whereas their adsorbed amounts are increased within dextran cryogels, so pi-stacking of PQ and 2,4-D pesticide in dextran cryogel is also plausible. Thus, an almost complete separation of PQ from PQ/2,4D mixture via ionic, hydrophilic and pi-stacking interactions are realized by multiple passes through a column filled with dextran cryogel.

## 4 | CONCLUSION

We presented macroporous cryogels based on unmodified dextran with tunable porosities, swelling and mechanical properties. Dextran cryogels were synthesized from aqueous solutions of dextran of various MWs in the presence of 20 to 50 mol% DVS as a cross-linker at −18°C. Increasing the MW of dextran from 15-25 to 450-650 kg $mol^{-1}$, or increasing DVS content from 20 to 50 mol% decrease both the swelling degree and the total pore volume of the cryogels while improve their mechanical performances. The highest degree of weight swelling and total pore volume, $2200 \pm 100\%$ and $10 \pm 2$ mL $g^{-1}$, respectively, were obtained using dextran MW of 15 to 25 kg $mol^{-1}$ and 20 mol% DVS cross-linker. It was found that the increase in DVS mol% at a dextran MW of 15 to 25 kg $mol^{-1}$ leads to around 5- and 2-fold increase in Young's modulus $E$ and fracture stress $\sigma_f$, of the cryogels, respectively. Dextran cryogels with the highest Young's modulus, $3.8 \pm 0.5$ MPa, compressive stress, $8 \pm 2$ MPa and plateau stress, $0.46 \pm 0.04$ MPa were obtained using dextran of MW = 15 to 25 kg $mol^{-1}$ using 50 mol% DVS. The hydrolytic degradation tests revealed that, irrespective of dextran MW and DVS content, all dextran cryogels are hydrolytically degradable in aqueous solutions at pH 1 and 9 while they are stable at neutral pH (7.4) at least 30 days. The degradation at both pH 1 and 9 occurs for up to 12 days linearly with about 40% to 60% weight loss while it continues slowly up to 30 days. Furthermore, the blood compatibility tests showed that the cryogels are hemocompatible, do not destroy blood cells while showing slight thrombogenic effect at 10 mg concentration regardless of the amounts of DVS and MW of dextran used. The synthesized dextran cryogels are capable to separate MB from binary MB/MO mixture in aqueous medium because the anionic dextran network adsorbs the cationic MB while repels the anionic MO. Dextran cryogels can also be successfully used in the adsorption and separation of binary PQ/2,4-D pesticides from aquatic environments. In this case, dextran absorbs cationic PQ but repels anionic 2,4-D so that they can readily be separated with a high efficiency. Macroporous dextran cryogels presented here with good mechanical performances, hydrolytic degradability and blood compatibility, as well as organic toxic pollutant and biomolecule separation and purification capabilities offer great opportunities for versatile applications in macromolecular bioscience.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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