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Intelligent Gels and Cryogels with Embedded Emulsions of Various Oils

Galina A. Komarova,[1,2] Sergey G. Starodubtsev,[1] Vladimir V. Lozinsky,[3] Irina R. Nasimova,[1] Alexei R. Khokhlov[1,2,3]

[1]Department of Physics, Moscow State University, Leninskye Gory 1, Moscow 119991, Russia

[2]Institute of Polymer Science, University of Ulm, Ulm D-89069, Germany

[3]A. N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, Vavilov str. 28, Moscow 119991, Russia

Correspondence to: G. A. Komarova (E-mail: komarova@polly.phys.msu.ru)

ABSTRACT: Thermo-responsive gels and cryogels with embedded microdroplets of Vaseline, olive, peanut, and linseed oils and their mixtures with hydrophobic dye Sudan 3 have been synthesized and studied. These composite gel matrices were obtained by the three-dimensional copolymerization of *N*-isopropylacrylamide and *N, N*'-bis(acryloyl)cystamine in the presence of oil emulsions stabilized with sodium dodecylsulfate or Span 80. Polymerization was performed at room temperature for conventional gels and at −15°C for cryogels. It was shown that all synthesized systems exhibit heat-induced collapse at temperatures higher then 34°C. For conventional gels prepared at room temperature shrinking lasts within 20 to 80 min in accordance with gel composition. No squeezing of oil droplets was observed. In the case of cryogels, shrinking was accompanied by release of oils and response time was significantly shorter, about tens of seconds. Collapse character and release of lipophilic phase did not depend on the chemical nature of oils, dissolved compounds, and surfactant used for emulsion stabilization. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

KEYWORDS: PNIPAM; filled cryogels; emulsions; drug release; collapse

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INTRODUCTION

Various matters, such as polymer particles, emulsions,[1] polymer micelles,[2] liposomes,[3] hydrogels[4] are widely used in medicine and veterinary as carriers of drugs.[5] The most common and long-term-used system in pharmacology and particularly in cosmetics are emulsions. Especially topical[6] application of emulsion systems is becoming with the advent of nano or microtechnology, which are directly related to the chemistry of colloids. Of greatest interest as media for drug delivery in a living body are the oil-in-water emulsions. Their specific feature is the ability of solubilizing and transporting hydrophobic substances through a continuous aqueous phase.[7] Potential application of emulsions includes their use as carriers of lipophilic drugs,[8] systems of prolonged action,[9] to ensure the targeting delivery of drugs,[10] increasing cell permeability to drugs, etc.

Recently, we have synthesized a series of gels filled with embedded oil droplets. It was demonstrated that composite gels that contain entrapped emulsions are effective absorbers of lipophilic organic substances. These systems are expected to be of significant practical interest as effective drug delivery systems.[11] Besides, these composites were used for the preparation of polyelectrolyte networks containing voids or channels, so called "swiss-cheese" gels.[12–14] As it was showed, polymer matrix of composite gels can be formed either by the network of cross-linked synthetic polymers, for instance, poly(acrylamide),[12,13] or by physical gels, for instance, agarose-based.[15] Composites containing entrapped emulsions of water-insoluble hydrocarbons such as tetradecane (TD) demonstrate very high stability and do not release the "oily phase" for at least several months.

During recent years a great interest was devoted to stimuli-responsive or so-called smart polymer gels. These gels undergo sharp volumetric transitions in the vicinity of specific critical points at small changes of external conditions, such as ionic strength, pH, temperature, the action of electric current, mechanical force, the presence of ionic surfactants, etc.[16–19] These features of smart gels make them increasingly attractive for biotechnology and medicine (See the monograph[20]). One might expect that stimuli induced collapse of smart gels that contain incorporated emulsions will lead to the release of emulsion droplets with dissolved drugs or other biologically

active compounds.[21] Such scenario would give an opportunity to localize the gel with incorporated emulsion containing drug in the proper place and to get the oil release in this place after the gel heating. To test this assumption, we have synthesized gels based on conventional thermosensitive polymer—poly(*N*-isopropylacrylamide) (PNIPAM) containing TD emulsion stabilized by sodium dodecylsulfate (SDS). PNIPAM gels exhibit volume phase transition at temperatures higher than 34°C. We expected that the collapse of TD-NIPAM composite gels under heating would cause the release of the oil droplets from the gel sample. However, as we found, that was not a case. Under the experimental conditions that were used the TD droplets kept entrapped in the conventional collapsed gel. To solve the problem of the oil release, we have proposed to use other type of gel systems, so-called cryogels.[22,23] The latter ones are formed via the rather famous cryotropic gelation technique,[22,23] which is known to produce macroporous gels with labyrinth-like interconnected large pores. The gelation process in a moderately frozen medium has the following characteristic features: first, it results in the crystallization of the major portion of the solvent, whose polycrystals in this case act as porogens for the future cryogel; second, considerable increase in solute concentration in the still unfrozen regions facilitates the gelation phenomena producing gel substances pierced with gross, often of capillary sizes, interconnected macropores after the system's thawing. The size and the structure of these pores depend on the composition and the chemical structure of the polymerizing mixture, the peculiarities of the freezing process, etc.[22–25] One of the evidences for the presence of such interconnected large pores in stimulus-responsive cryogels is a very high rate of their collapse, when the squeezed-out water is rapidly removed from the heterophase gel matter through a system of capillary-size "channels."[22–25] It was demonstrated that in contrast with the conventional gels the release of oil phase from TD-filled PNIPAM cryogels is significantly higher.

However, linear hydrocarbons like the TD are not the biocompatible substances and could not be used for medical applications. Taking this into account, the purposes of this study were as follows:

i. to synthesize PNIPAM cryogels with incorporated emulsions of natural oils;
ii. to investigate the influence of the oil nature and type of the surfactant that were used for emulsion stabilization on the release of oil phase and on the features of the composite gels collapse.

As surfactants rather hydrophilic anionic SDS and hydrophobic Span 80 were used. For visualization of the oil release hydrophobic dye Sudan 3 was dissolved in oil phase.

EXPERIMENTAL

Materials

N-isopropylacrylamide (NIPAM), *N, N′*-bis(acryloyl)cystamine (BAC), Sudan 3, ammonium persulfate (APS), *N, N, N′, N′*-tetramethylethylenediamine (TEMED), Span 80, TD, as well as linseed, peanut, and olive oils were purchased from Sigma (Germany). SDS was from Merck and Vaseline (VZ) was from VWR (Germany). The structural formula of Sudan 3 is listed below:

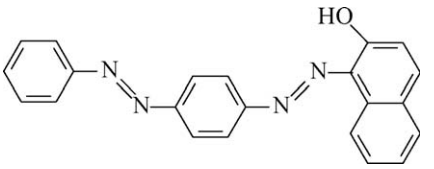

Table I. Composition of the Polymerizing Mixtures

Water (mL)	Oil (mL)	C_{BAC} (mol L^{-1})	C_{NIPAM} (mol L^{-1})	C_{SDS} (mol L^{-1})	$C_{Span\ 80}$ (mol L^{-1})
4.08	-	0.01	0.76	-	-
2.46	VZ[a], 1.56	0.01	0.76	0.032	-
2.46	VZ[a], 1.56	0.01	0.76	-	0.052
2.44	Olive[a], 1.56	0.01	0.76	0.032	-
2.44	Peanut[a], 1.56	0.01	0.76	0.032	-
2.43	Linseed[a], 1.56	0.01	0.77	0.032	-

[a]Concentration of Sudan 3 was 10^{-3} M.

Synthesis of Thermo-Responsive Conventional Gels Filled with Oil Droplets

To obtain the initial emulsions, mixture of oil and surfactant SDS/Span 80 were placed in a plastic vial. After that the aqueous solution of NIPAM and BAC was added. The compositions of the polymerizing mixtures are listed in Table I. The oil and water mixture was treated with ultrasound (Ultraschall 450-D Branson, Germany) at a power 10% for 1 min. During the treatment the vial was immersed in the ice-water slurry. TEMED and APS were added after emulsion preparation to initiate the polymerisation. The vials were thoroughly shaken by jog and kept at 22°C or placed in the cryostat (Julabo Labortechnik Gmbh, Germany) at −15°C for 24 h. After polymerization the gels were immersed into distilled water and washed for 3 days. It should be noted that the fractions of NIPAM and BAC dissolved in VZ are very small and does not effect markedly the composition of polymerizing mixture.

The Emulsions Droplets' Size Investigation

Dynamic light scattering (DLS) studies were carried out on a static/dynamic compact goniometer ALV-CGS-5000/6010 (Langen, Germany). He-Ne laser with a power of 22 mW (wavelength, 632.8 nm) was used as the incident beam. The relaxation times and hydrodynamic radii distribution functions were calculated using the CONTIN data analysis package. All the DLS experiments were set up for 90° scattering angle. Before the measurements the obtained concentrated emulsions were diluted with water.

To control the effect of the ice formation on the size of the droplets, the initial emulsions were immersed in the cryostat at –15°C for 24 h. Then the samples were defrosted as was controlled by sight.

Measurement of the Temperature-Dependence of the Swelling Ratios and Deswelling Kinetics

For the temperature-dependence study of the swelling ratios, gels were equilibrated in distilled water at temperatures ranging from 22 to 60°C, covering the expected range of LCSTs of PNIPAM gels. The samples were allowed to swell in distilled water for at least

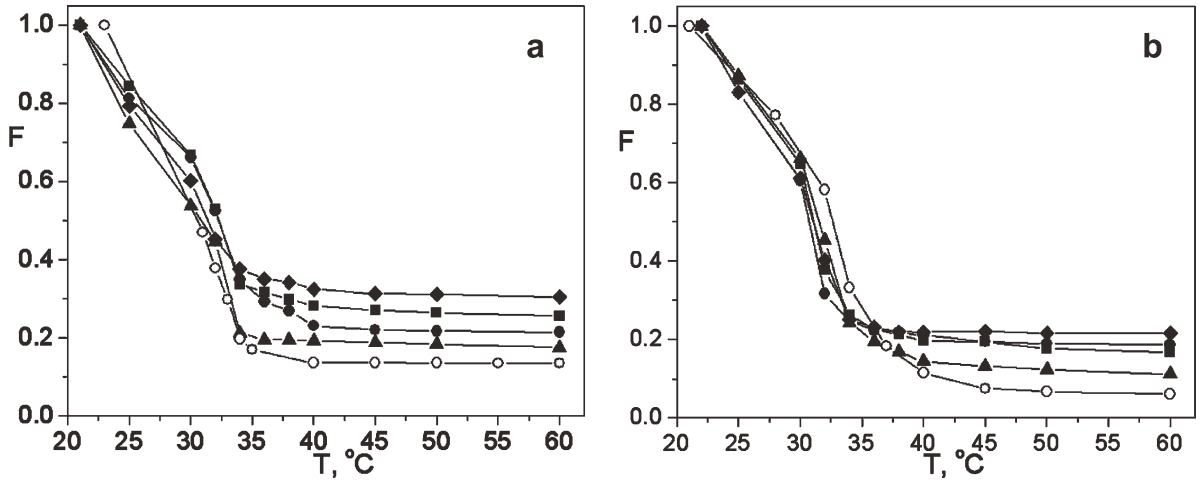

Figure 1. Temperature-dependences of the swelling ratios of the gels (a) and cryogels (b) filled with oil droplets stabilized by SDS (designations: –○– conventional PNIPAM gel/cryogel; –●– PNIPAM gel/cryogel filled with olive oil; –▲– PNIPAM gel/cryogel filled with VZ; –◆– PNIPAM gel/cryogel filled with linseed oil; –■– PNIPAM gel/cryogel filled with peanut oil).

24 h at each predetermined temperature. Gravimetric analysis was used to study each gel's swelling ratio. After immersion in distilled water at a predetermined temperature, the gels were removed from the water, soaked with a filter paper to remove the excess of water from the gel surface and then weighed. After these measurements, the gel was reequilibrated in distilled water at another predetermined temperature and its wet weight was determined thereafter. The swelling ratio F_T was defined as follows:

$$F_T = m_T/m_0 \quad (1)$$

where m_0 is the equilibrated mass of the gels in distilled water at 22°C, m_T is the mass at the temperature T. The results of the experiments were presented as dependences of F_T on T.

The deswelling kinetics of the gels was measured gravimetrically at 40°C after wiping off the excess water from the gel surface using moistened filter paper. Before the measurement, the samples were allowed to swell to equilibrium in distilled water at 22°C the mass changes of gels were recorded at regular time intervals. The deswelling kinetics was defined as time-dependent swelling ratio F_t:

$$F_t = m_t/m_0 \quad (2)$$

where m_t is the mass of the PNIPAM gel at time t at 40°C.

UV-Spectrophotometric Analysis of Sudan 3 Release from the Cryogels

Sudan 3 release from the cryogel samples was controlled by UV-spectrophotometer (GENESYS 10, Thermo Fisher Scientific, USA). Oil was colored with water-insoluble dye Sudan 3 for the release visualization. Sudan 3 has absorption maxima at 513 nm and extinction coefficient 19,300 in TD. Cryogels samples with incorporated oil phase containing Sudan 3 equilibrated in the water at 22°C were put in the air thermostat in glass tubes containing ca. 5 mL of water equilibrated at 40°C. Diameter of the tubes was 2 cm. On the water surface 1 mL of TD was injected. Due to its lower density (0.76 g cm^{-3}) the hydrocarbon formed an oil layer over water. The tubes were closed and incubated in the air thermostat at 40°C for 60 min. The droplets of the oil contained Sudan 3 were released from the composite cryogel, floated up and dissolved in TD-layer. The concentration of the dye in the organic layer c was estimated by UV-spectroscopy using the determined extinction coefficient. The measurements were made one hour after the temperature jump. The amount of the released oil was calculated as: $c \cdot V$, where V is the volume of the organic phase.

RESULTS AND DISCUSSION

Temperature-Dependent Swelling Ratio of Conversional PNIPAM Gels and Cryogels Filled with Oil Droplets

Figure 1 shows the classical temperature-dependent swelling behavior of the conventional PNIPAM-based hydrogels when the temperature of the aqueous media increased from 22 to 60°C. As expected, the conventional gels exhibit transition into the collapsed state at around 35°C, and thereupon the gel's volume hardly slightly changed till 50°C. At higher temperature the values F_T are not practically changing. Analogous dependences are observed for cryogels. However, the shrinking of the filled cryogels is much more pronounced than that of the conventional gels. One explanation of this difference is the release of the oils from the cryogels during the collapse. Figure 2 shows photos of the composite PNIPAM gel and the cryogel filled with Sudan 3-colored VZ droplets during the collapse in water at 40°C for about half a minute. It should be noted that the initial diameters of these both gel disks equilibrated in water at 22°C were the same. The images clearly demonstrate that the collapse and release of the oil from the cryogel proceed very quickly, within tens of seconds.

Deswelling Kinetics of Conventional PNIPAM Gels and Cryogels Filled with Oil Droplets Stabilized by SDS

Figure 3 shows the deswelling kinetics of the conventional oil-filled gels (a) and cryogels (b) during the collapse at 40°C. The obtained results clearly demonstrate that collapse of the filled cryogels proceeded much faster than that of the conventional filled gels.

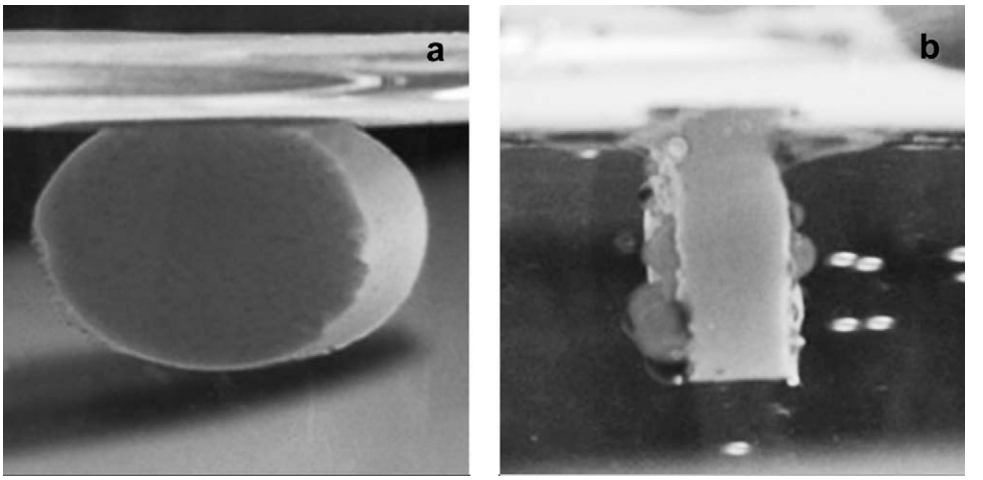

Figure 2. Pictures of the PNIPAM gel (a) and cryogel (b) filled with VZ droplets containing Sudan 3 during the collapse in 30 s after immersion in water at 40°C.

Such large difference between the shrinking rate of the composite conventional gels and cryogels can originate from two reasons. First, the presence of the system of interconnected macro pores in cryogels makes it possible for the fluids in the pores the flow mechanism of the release under the action of pore walls shrinking. Second, it is well known that for the gels synthesized in homogeneous conditions the response rate is typically slow due to the formation of a dense skin layer during the collapse transition.[1] It is unclear what happens with the pore channels at the cryogel surface during the collapse. If the channels are closing fast the large fraction of oil droplets will be hold in them in the gel body. On the other hand, local surface gel collapse needs two-dimensional deformation of the surface layer due to kinetics reasons (the rate of water release from the inner part of the gel is limited); in this case the fast surface closing of the pores is questionable. Anyway, the determination of the fraction of the oils released from the cryogels during the collapse was an important next step of this study.

Sudan 3 Release From the PNIPAM cryogels with Embedded Oil Droplets Stabilized by SDS During the Collapse

As described above, amounts of released dye from the filled cryogels were determined using UV-spectrophotometer. The fraction of water-insoluble dye Sudan 3 released from the cryogels during the collapse is listed in Table II. In spite of different chemical nature of the oils (olive, peanut, linseed, and VZ) the released fractions of dye are rather close. Taking into account that the hydrophobic dye can be released only with oil droplets one can assume that the fractions of the released dye and of oil should be equal. Thus, oil release from the cryogels during the collapse weakly depends on the chemical nature of the oil.

Since it could be expected that oil release should depends on emulsion droplet size the average hydrodynamic radii R of droplets of the initial emulsion were obtained using DLS technique (data are listed in Table III). As it can be seen, droplets of emulsions of natural vegetable linseed, peanut, and olive oils have very close values of R varied in the range 235−256 nm.

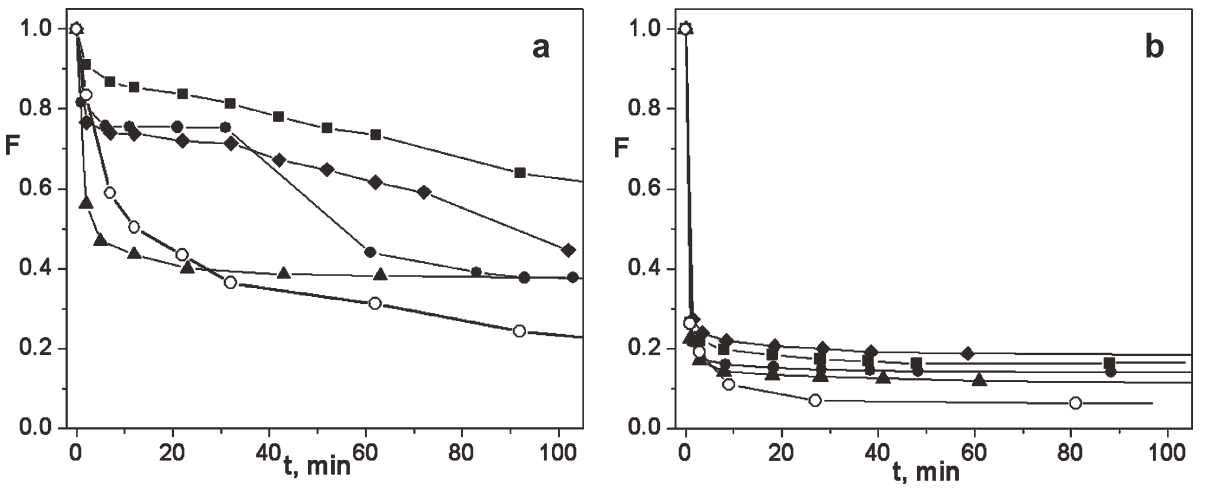

Figure 3. Shrinking kinetics of PNIPAM gels (a) and cryogels (b) with embedded oil droplets during the collapse at 40°C (designations: –○– conventional PNIPAM gel/cryogel; –●– PNIPAM gel/cryogel filled with olive oil; –▲– PNIPAM gel/cryogel filled with VZ; –◆– PNIPAM gel/cryogel filled with linseed oil; –■– PNIPAM gel/cryogel filled with peanut oil).

Table II. Sudan 3 Release from the Cryogels Filled with Oil Droplets Stabilized by SDS During the Collapse at 40°C

Name of the oil	Release of Sudan 3 (wt %)
VZ	49
Olive	60
Peanut	49
Linseed	46

While, in spite of the equal content of the components in all emulsions during their preparation the value of R for the VZ is fourfold higher in comparison with the other emulsions. This result can be explained by the chemical nature of the VZ oil, which is a product of purified oil fraction and consist of nonpolar hydrocarbons only. In contrast with it, natural oils used in this study contain lipids with polar head groups and these oils are amphiphilic by nature. Hence, one can assume that the surface tension in the case of VZ would be higher and the size of the droplets greater. Absolute viscosities of linseed, olive, peanut, and VZ oils were measured and their values at 20°C are equal ~ 0.08, 0.09, 0.09 and 0.20 Pa.s, respectively. Obtained results evidence the size VZ emulsion droplets must be greater than emulsion droplets of other oils at the same conditions of preparations.

The comparison of the data listed in Tables II and III demonstrate the absence of correlation between the initial size of the emulsion droplets and the fraction of the oil released during the collapse.

Effect of Freezing on the Emulsions "Oil-in-Water"

To explain the independence of oil releases from the cryogels during the collapse the effect of freezing on the emulsions. We found that the emulsions have undergone significant changes caused by the freeze-thaw influence. The images of the defrosted emulsions of the oils are shown in Figure 4. As it can be seen from the images, after the freezing all the emulsions exfoliate. The obtained results clearly demonstrate that freezing significantly enhances the segregation of the emulsions and leads to a demulsification. Since emulsions break by freezing we suppose emulsions break in the cryogel's pores after defrost too. In particular, the emulsions break may be the cause of the relatively high fraction of VZ oil remaining in the samples after their collapse. Thus we assume that the size of the oil droplets into the pores arbitrary increases, and it can be concluded the size of the emulsion droplets does not affect the oil release.

Table III. Average Radii R of Emulsion Droplets of Different Oils Stabilized by SDS Before Cryopolymerization

Name of the oil	R (nm)
VZ	1019 ± 50
Olive	256 ± 18
Peanut	258 ± 5
Linseed	235 ± 2

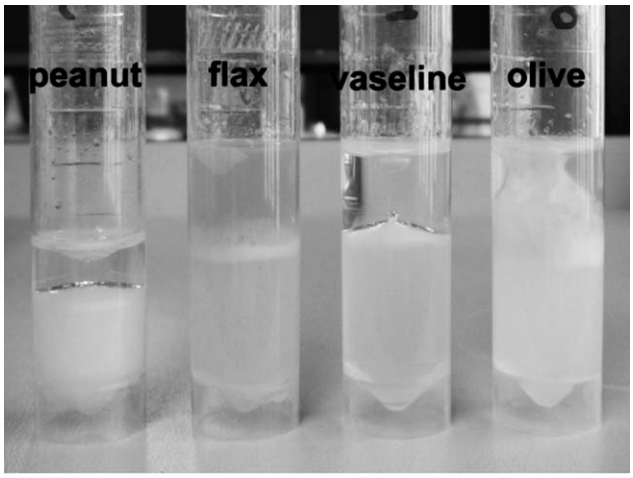

Figure 4. Pictures of peanut, linseed, olive, and VZ emulsions stabilized by SDS after freezing and follow thawing.

Effect of the Surfactant Nature on the Collapse of Conventional PNIPAM Gels and Cryogels Filled with VZ

The section describes a swelling and shrinking of the conventional PNIPAM gels and cryogels filled with VZ droplets stabilized with hydrophobic surfactant Span 80. The formation of the cryogels structure with incorporated emulsions of different oils is a complicated process depending on the peculiarities of the freezing process (propagation of the temperature gradient, the size of the sample, etc) and on the composition of the polymerizing mixture. In particular, the chemical nature of surfactant can affect the structure of the cryogels with embedded emulsion, the kinetics and the amplitude of the collapse and the amount of the released oil. Figure 5 demonstrates the shrinking of the composite PNIPAM cryogels with embedded VZ droplets as a temperature-dependence of the swelling ratio F_T. The emulsion incorporated in the conventional gel was stabilized by SDS, while the emulsion in the cryogel by hydrophobic surfactant Span 80. As expected, the conventional gel transformed into the collapsed state at around 34–35°C, and thereupon the gel's

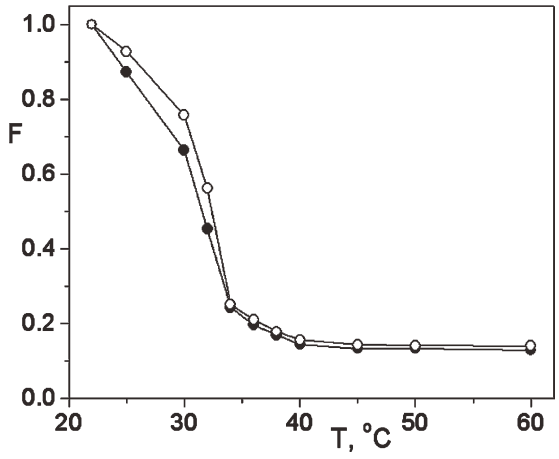

Figure 5. Temperature-dependences of the swelling ratio F_T of the PNIPAM cryogel filled with VZ emulsion stabilized by SDS (—●—) and by Span 80 (—○—).

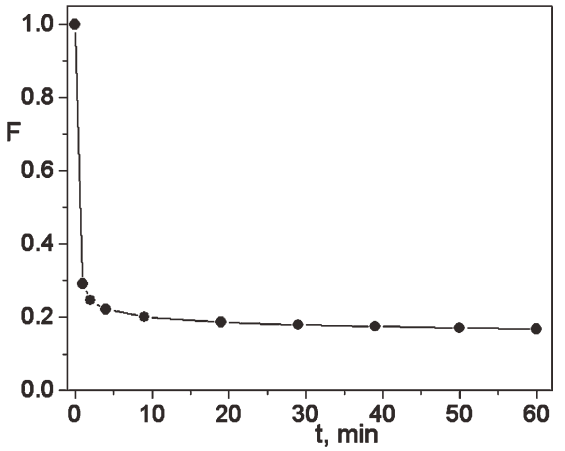

Figure 6. Shrinking kinetics of PNIPAM cryogels with embedded VZ droplets stabilized by Span 80 during the collapse at 40°C.

volume hardly changed till 50°C; at higher temperature the values of F_T practically did not change. Similar dependence was observed also for the oil-filled cryogel. It should be noted that in spite of the difference in the methods of the gels synthesis and in the chemical nature and hydrophobicity of the surfactants the points of the collapse for both gels coincided.

Figure 6 shows the shrinking kinetics of the cryogel with incorporated VZ droplets stabilized by Span 80. The curve has no features in comparison with the other cryogels. The cryogel collapses rapidly within less than 1 min after immersing in warm water.

The release of VZ droplets stabilized by Span 80 from the oil-filled cryogel after the collapse was also studied. Figure 7 shows the kinetics of Sudan 3 release after the jump-wise increase of temperature from 22 up to 40°C. The greater fraction of the dye releases during the fast collapse of the composite cryogel. The total fraction of the released oil runs up to 59%. Comparison of this result with the data of Table II shows that at the same network composition, content of oil and the chemical nature of the surfactant plays a minor role in the ability of the cryogels to release oils.

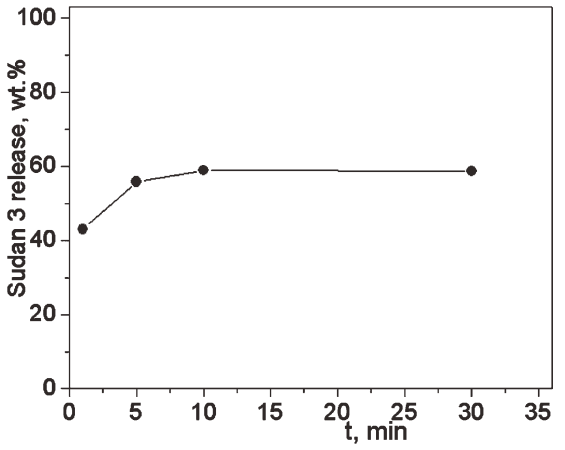

Figure 7. Kinetic of Sudan 3 release from the cryogels filled with VZ droplets stabilized by Span 80 during the collapse at 40°C.

CONCLUSIONS

Incorporation of lipophilic emulsions in the matrix of polymer conventional hydrogels gives rise to the opportunity for their new applications as drug delivery systems, especially in the delivery of lipophilic or amphiphilic drugs. The gel matrix can be passive in the sense that it does not change its water content under the action of the body heat, acidity, etc. In such a case the medicines or other biologically active substances just diffuse from the emulsion droplets through an aqueous medium of the swollen polymer network. Another type of such delivery "devices" is the medicine-loaded composite gels possessing the stimuli-responsive polymer matrix capable of releasing the entrapped constituents in response of external influence. In this work, we have studied the gels with an active thermo-responsive matrix of PNIPAM polymer. It was demonstrated that at the temperature close to mammal body the conventional gels with incorporated emulsions collapse. However, they do not release entrapped oil emulsions due to low effective size of their gel's pores. In contrast, the cryogels having the same composition release a large fraction of oils together with different substances dissolved in the emulsions. Different vegetable oils can be used as filler for the cryogels. Their chemical nature only lightly affects the amount of the released oil during the gel collapse. The chemical structures of the surfactant used for stabilizing of the emulsions also do not influence strongly the resulting release of oils from the thermo-responsive cryogels. The cryogel films with incorporated emulsions containing drugs and other biologically active substances can be used at the external use in medicine and cosmetics as medical applications that contains known amount of biologically active substance (drug) and can be easily removed after the use. An important advantage of such system is the absence of thickening agents which are usually rather toxic compounds.

ACKNOWLEDGMENTS

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