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Dedicated to Professor Alexandru T. Balaban on the occasion of his 85th anniversary

RHEOLOGICAL INVESTIGATION OF THERMOREVERSIBLE POLYURETHANE HYDROGELS

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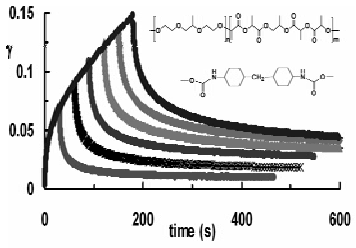
The aim of this study was to improve the knowledge concerning the thermoreversible polyurethane hydrogels in order to provide important insights for designing *in situ* gelling systems for future biomedical applications. Thereby, a polyurethane sample was synthesized by polyaddition reaction of poly(isopropyl lactate) diol (PILD) and poly(ethylene oxide)−poly(propylene oxide)−poly(ethylene oxide) diol (PPP) and 4,4'-methylene dicyclohexyl diisocyanate (H_{12}MDI). The chemical structure and molecular characteristics were investigated by ^{1}H-NMR, FTIR and GPC analysis and critical micelle concentration was determined by surface tension measurements. The viscoelastic properties of the polyurethane in aqueous medium were determined in dynamic oscillatory conditions as a function of temperature and the formation of the thermoreversible hydrogel was evidenced for very low heating rate. The shear flow and the creep-recovery behaviours of the hydrogel at 37 °C were also investigated.



INTRODUCTION

Hydrogel formation by physical or chemical crosslinking is a developing area of research toward biomaterials suitable for drug delivery or tissue engineering applications.[1-4] Thermoreversible polymers show reversible transition between micelle formation and dissociation due to changes in intermolecular interactions between the polymeric blocks at a critical temperature. Thus, these polymers have the ability to form low viscosity aqueous solutions at ambient temperature and gels at a critical temperature which could be close to that of the human body. This property makes these systems to be suitable as injectable formulations, widely used especially in the pharmaceutical applications.[5] Over the years, many thermoreversible hydrogels with various topologies, such as poly(N-isopropylacrylamide) based copolymers, poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymers, and so on, have been extensively explored.[6-8] On the other hand, polyurethane hydrogels belong to this class of biomaterials and are of actual interest due to the chemical versatility of the components used in their synthesis.[9-13]

The rheological characteristics of hydrogels are very important for their applications since the viscoelastic properties correlate with their microstructures and could provide useful information to modulate the hydrogel properties.[14,15] In the polyurethane structures, major molecular geometry changes occur at the

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body temperature, leading to an induced gelation and crystallization with water molecules. These polyurethane gels gave very good results *in vitro* and in non-invasive heart surgery.[3,12] Thus, the fundamental understanding of gelation conditions helps us to tailor new hydrogels based on polyurethanes for tissue repair and drug delivery. Thereby, this paper is focused on the design and rheological investigation of a thermoreversible polyurethane in aqueous solution.

RESULTS AND DISCUSSION

Synthesis and characterization of polyurethane

The polyurethane was synthesized by polyaddition reaction of polyols (PILD and PPP) and diisocyanate (H_{12}MDI). The chemical structure of polyurethane (Fig. 1) was verified by ^{1}H-NMR and FT-IR spectroscopy.

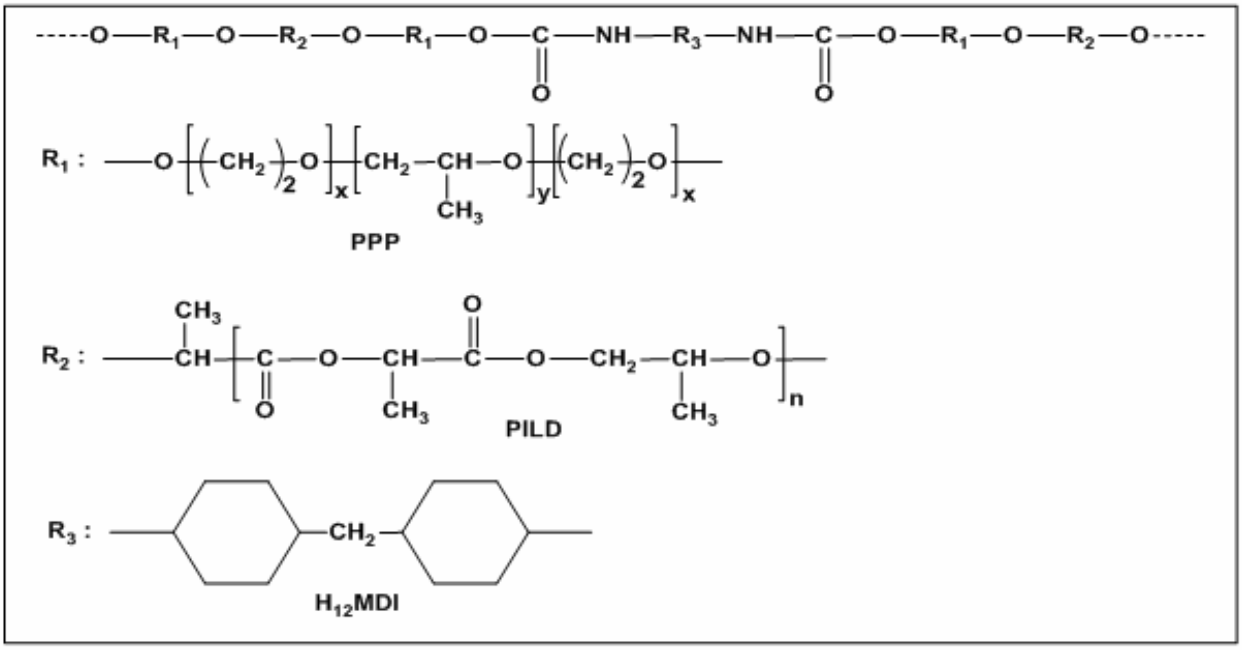

Fig. 1 – Chemical structure of PU-H_{12}MDI.

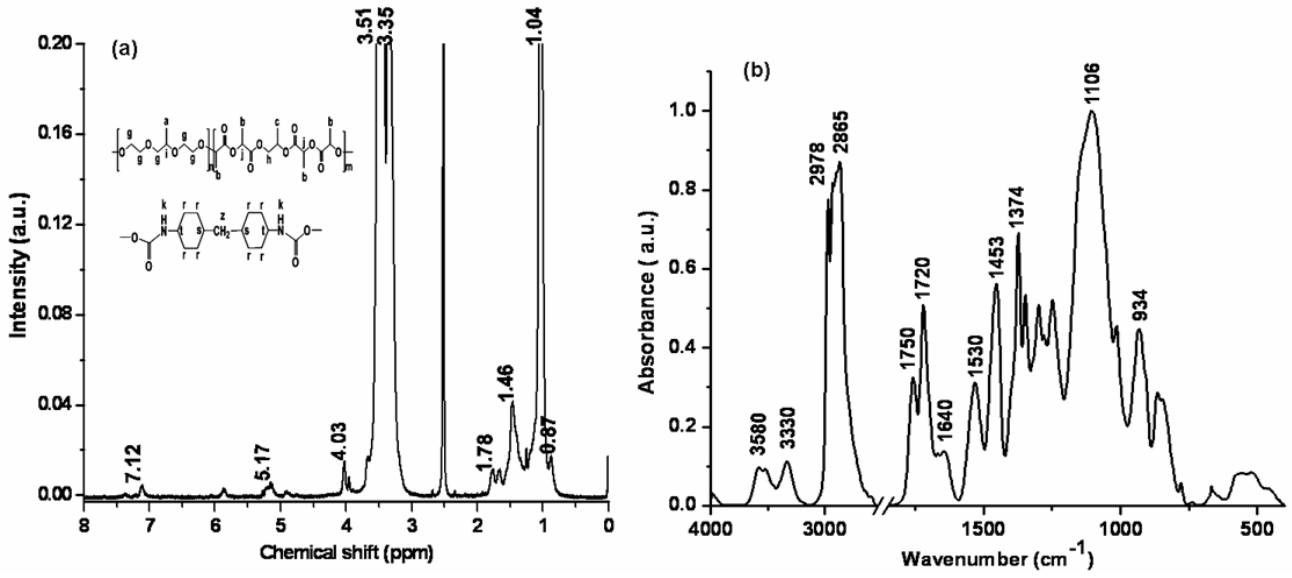

Fig. 2 – a) ^{1}H-NMR and b) FT-IR spectra of PU-H_{12}MDI.

The ^{1}H-NMR spectrum reveals the coexistence of the PILD and PPP segments at 0.87-1.25 (-CH_3), 1.45-1.78 (-CH_2-) and 3.35-3.51 (-CH-) ppm. Also, the protons from the urethane moiety (−NH) were found at ~7.12 ppm (Fig. 2a).

The FT-IR absorption spectrum of PU-H_{12}MDI is shown in Fig. 2b and demonstrates the presence of the expected functional groups. Thus, the peak at 3580 cm^{-1} has been assigned to the O−H stretching vibration (υ(O-H)) from hydroxyl-terminated PILD and PPP, and at 3523 cm^{-1} from water of hydration (crystallization).[12] The peak at 3330 cm^{-1} is characteristic to the H-bonded N−H stretching vibration (υ(N-H)) of urethane structures. Between 3000 and 2700 cm^{-1} are four characteristic bands due to the asymmetric and symmetric stretching vibration (υ(C-H)) of −CH_2 and −CH_3 groups. At 1750 cm^{-1} is the peak that corresponds to the stretching vibration of free carbonyl υ(C=O) from lactate structure, and at 1720 cm^{-1} is the peak attributed to H-bonded carbonyl from the ester and urethane structures. The peak with maximum at 1640 cm^{-1} could be assigned to the deformation vibration (δ(O-H)) of water.[12] At 1530 cm^{-1} is the characteristic peak of δ(N−H) which overlaps with ν(C−N) (amide II). Between 1400−1300 cm^{-1} appear the absorption bands attributed to the vibration deformation of δ(CH_2) and δ(CH_3) groups. In the region 1328-1207 cm^{-1} are the characteristic peaks of δ(N−H), which overlaps with ν(C−N) (amide III). The 1200−900 cm^{-1} region is attributed, especially, to the stretching vibration ν(C−O−C) from esteric and etheric functional groups.

The molecular weights were determined using gel permeation chromatography (GPC). In Table 1 are given the number average molecular weight (M_n), z-average molecular weight (M_z) and the molecular weight distribution of synthesized PU-H_{12}MDI. M_n is around 6×10^4 Da and the polydispersity index is 1.35.

Critical micelle concentration (CMC) determination was carried out by surface tension measurements and from the plots of the surface tension versus the concentration of the polymer (Table 1). PU-H_{12}MDI self-assemble into micelles at a lower CMC of 3.26×10^{-7} mol/l, compared to the CMC of PPP (4.4×10^{-6} mol/l).[16] This is in accordance with the literature data, which shows that the lower is the CMC value of a given amphiphilic polymer, the more stable are the micelles.[17]

Rheological investigation

Influence of the heating rate on the viscoelastic behaviour

In order to investigate the thermal induced gelation, the evolution of viscoelastic properties was followed in temperature sweep tests at different heating rates and constant oscillation frequency (ω = 1 rad/s), in the linear viscoelastic regime (shear stress of 1 Pa). Thus, Fig. 3 illustrates the dependence of the viscoelastic parameters (G′ - the elastic modulus, G″ - the viscous modulus and the loss tangent, tan δ = G″/G′) as a function of temperature for PU-H_{12}MDI sample at two different heating rates: 0.3 °C/min and 1 °C/min. It is observed that the heating rate has a significant influence on the sol-gel transition temperature as well as on the magnitude of the viscoelastic moduli.

At very low heating rate (0.3 °C/min, Fig. 3a), below 8 °C, the system shows a liquid-like behaviour: G′ < G″ and tan δ > 1. Above 8 °C, the viscoelastic moduli start to increase and at 11.1 °C, considered the sol-gel transition temperature (t_g), G′ = G″ and tan δ = 1. Up to 20 °C, it can be observed a sharp increase of the viscoelastic moduli, when the network formation occurs: G′ > G″ and tan δ < 1. Between 20 °C and 40 °C, this network structure remains nearly unchanged, G′ is approx. ten times higher than G″. Above 40 °C, a small decrease of G′ and G″ is registered, but the network structure is preserved; the low heating rate allows the macromolecules to form strong physical interactions which are not destroyed at further increase of the temperature.

Table 1

Molecular characteristics of PU-H_{12}MDI

Sample code	M_n (Da)	M_p (Da)	M_z (Da)	M_w/M_n	CMC (mol/l)
PU-H_{12}MDI	5.96×10^4	7.55×10^4	1.11×10^5	1.35	3.26×10^{-7}

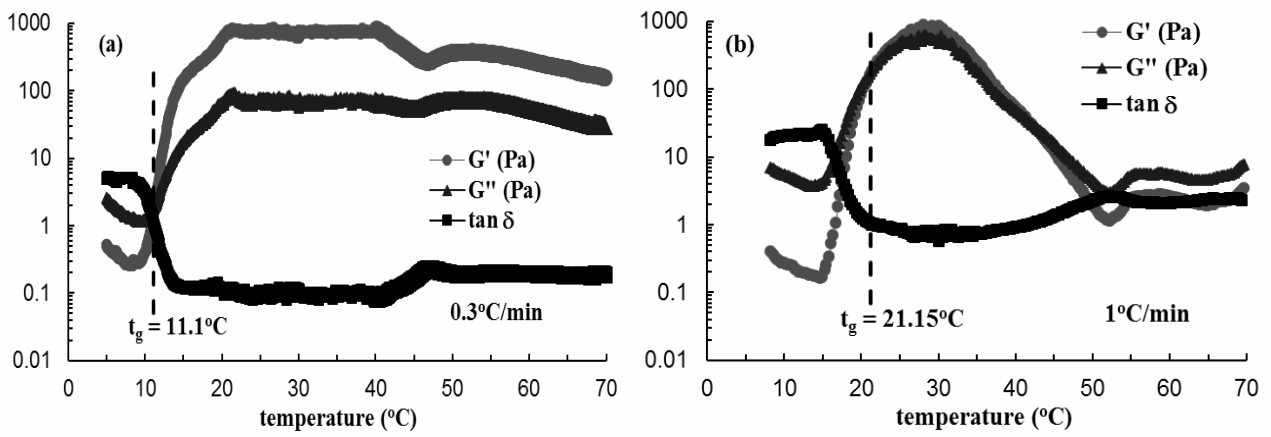

Fig. 3 – Evolution of viscoelastic parameters at increasing temperature with different heating rates: (a) 0.3 °C/min and (b) 1 °C/min.

At a higher heating rate (1 °C/min, Fig. 3b), the viscoelastic behaviour is very different. Up to 14 °C, the system shows preponderant viscous behaviour: $G' < G''$ and $\tan \delta > 1$. Above 14 °C, a sharp increase of the viscoelastic moduli is observed and, above t_g = 21.15 °C, G' slowly exceeds G'', but the values of the two moduli are very close, as a consequence, the loss tangent is close to unity. A maximum is observed around 29 °C and, at further increase of the temperature, G' and G'' decrease up to 51 °C. Above this temperature, only small changes in values of G' and G'' are observed. In addition, at 40 °C, G' becomes again equal to G''. This behaviour suggests that a weak network structure, known also as "critical gel", is formed between 21.15 °C and 40 °C and this structure is "melted" above 40 °C.

The thermoreversible behaviour is fully reversible; at 4 °C, the hydrogel formed at high temperatures is completely dissolved and after that a homogeneous solution is obtained.

Flow behavior at 37 °C

The variation of the apparent viscosity as a function of shear rate for PU-H_{12}MDI at 37 °C shows a pseudoplastic behaviour (Fig. 4). The slope of this dependence changes around a shear rate of 0.25 s^{-1} from 0.9 to 0.7, suggesting a change of the flow mechanism. The rest structure which contains polymicelles is still preserved at low shear rate. Above 0.25 s^{-1}, the shear force increases and the interactions between micelles are partially destroyed and, as a consequence, the flow determines a lower decrease of viscosity with the shear rate.

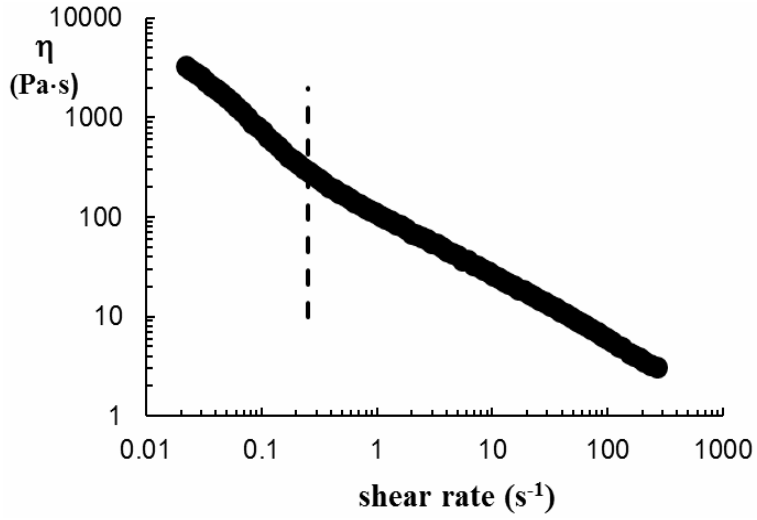

Fig. 4 – Flow curve for PU-H_{12}MDI hydrogel after a long storage (2 h) at 37 °C.

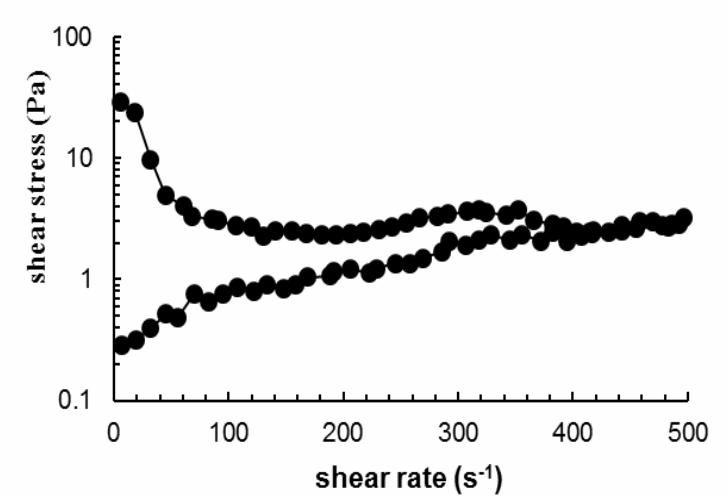

Fig. 5 – Thixotropic behaviour of the hydrogel sample after a long storage (2 h) at 37 °C.

As it was previously evidenced for the structured samples,[18,19] the hydrogel shows a thixotropic behaviour (Fig. 5) and the hysteresis area was determined as being 1440.4 Pa s^{-1}.

Creep-recovery behavior at 37 °C

In order to understand the long-term viscoelastic behaviour of the polyurethane hydrogel, creep-recovery tests were performed. Thus, Fig. 6 illustrates the creep-recovery curves at 37 °C, when a constant stress (τ) is applied during the creep and the time-dependent strain (γ) is measured. The relationship between these two parameters is the following:

$$\gamma(t) = J(t) \cdot \tau \qquad (1)$$

where J(t) represents the creep compliance.

As it is shown in Fig. 6, the creep curves consist of three parts: the instantaneous elastic strain, the delayed elastic strain, and the viscous strain. The recovery process starts after the applied shear stress is removed, and first the instantaneous strain is recovered in several seconds, then the delayed component, and finally the viscous part remains.

A high elasticity of the polyurethane gel was observed and the recovery deformation represents 80–90% from the maximum value reached by the deformation in the creep test. The origin of elasticity of this system is correlated with the structure and molecular composition of polymer.

The creep tests at different times of applying a constant shear stress, followed by recovery, have shown that the viscoelastic behaviour is independent of the shear history. The measurements were carried out successively and the creep curves were reproducible, as can be seen in Fig. 6. This suggests the fact that the hydrogel conserves its mechanical characteristics after it is sheared, important aspect for practical applications.

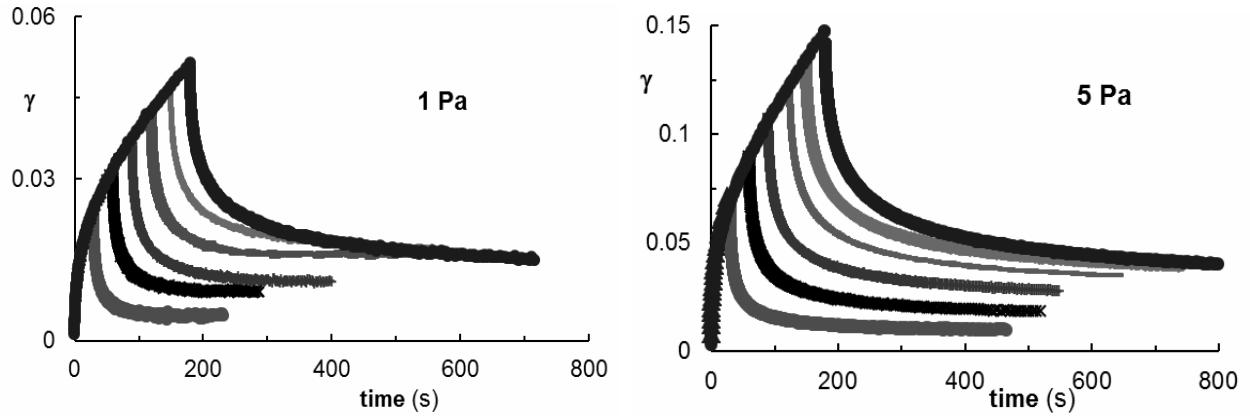

Fig. 6 – Creep-recovery curves at 37 °C; the stresses of 1 Pa and 5 Pa were applied at different period of times. Before measurement the hydrogel sample was kept 2 h at 37 °C.

EXPERIMENTAL

Materials

Poly(ethyleneglycol)-block-poly(propylene glycol)-block-poly-(ethylene glycol) (PPP) with M_n of approx. 5.8×10^3 Da was purchased from Sigma-Aldrich and used as received. Poly-(isopropyl lactate) diol (PILD) with hydroxyl number 56 mg KOH/g, acid number 0.01mg KOH/g and M_n $2x10^3$ Da was prepared in our laboratory.[9] 4,4'-Methylene dicyclohexyl diisocyanate 90% (H_{12}MDI) was supplied by Aldrich and was freshly distilled before synthesis. All the other chemicals and reagents were of the highest purity available and used without further purification.

Synthesis of polyurethane

The polyurethane was synthesized by bulk polymerization method, as was previously reported,[10] using PPP, PILD and H_{12}MDI. Briefly, PPP and PILD was dehydrated 4 h in a three-neck glass reactor equipped with a mechanical stirrer, under vacuum (0.2 mmHg) at 80 °C. Then, the diisocyanate was added dropwise, so that the molar ratio NCO:OH was 0.8:1, and the reaction took place for 8 h under stirring and dry nitrogen atmosphere at 60 °C. The resulting polymer was abbreviated PU-H_{12}MDI and the structure of this polyurethane is shown in Fig. 1.

Characterization methods

^{1}H-NMR spectrum was recorded at room temperature on a Bruker Avance DRX-400 spectrometer (400 MHz). The sample was dissolved in deuterated dimethyl sulfoxide (DMSO-D_6).

FTIR spectra were recorded using a Bruker Vertex 70 type spectrometer (U.S.), equipped with a diamond ATR device (Golden Gate, Bruker) and provided with software for spectral processing. The sample surface was scanned in the range 400−4000 cm^{-1}, at a 45° angle. The FTIR spectra were recorded at 25 °C.

The average molecular weight was determined by gel permeation chromatography (GPC) at 25 °C using a GPC PL-EMD 950 evaporative mass detector instrument. The sample was eluted with THF at 0.7 mL/min. Calibration was performed with narrow polydispersity polystyrene standards (Polymers Laboratories Ltd.).

Critical micelle concentration (CMC) was determined from surface tension measurements of initial 0.1% aqueous solution of polymer at room temperature, using Wilhelmy method and a Sigma 700 tensiometer (KSV Instruments, Finland).

Rheological investigations were carried out with a Bohlin CVO rheometer equipped with a Peltier device for the temperature control. The measurements were performed by using parallel plate geometry, the upper plate having a radius of 30 mm (gap of 500 μm). The behavior of the sample in temperature sweep tests was followed from 4 to 80 °C. 15% hydrogel aqueous solution was prepared and was then stored at 4 °C for 24 h before rheological measurements were performed.

CONCLUSIONS

In this work, we studied the thermoreversible behaviour of a polyurethane sample in water. Thus, the polymer synthesis was realized by polyaddition reaction of PILD, PPP and H_{12}MDI . The chemical structure and molecular characteristics of this polyurethane were confirmed by ^{1}H-NMR, FT-IR and GPC analysis. This polyurethane presents a very low critical micelle concentration compared with that of PPP, as determined by surface tension measurements.

The viscoelastic behaviour as a function of temperature has shown that the thermoreversible hydrogel structure is formed only in conditions of thermal equilibrium, for a very low heating rate of the aqueous solution kept at 4 °C or by a long time storage of the sample at 37 °C. The hydrogel presents tixotropy and high elasticity as evidenced by creep-recovery tests, conserving its mechanical characteristics after it is sheared.

Therefore, the present investigations bring a new insight into the rheological properties of thermoreversible polyurethanes which cover a large area of biomedical applications.

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REFERENCES

1. S. J. Buwalda, K. W. M. Boere, P. J. Dijkstra, J. Feijenc, T. Vermonden and W. E. Hennink, J. *Control. Release,* **2014**, *190*, 254–273.
2. S. C. Lee, I. K. Kwon and K. Park, *Adv. Drug Deliv. Rev*., **2013**, *65*, 17–20.
3. L.M. Gradinaru, C. Ciobanu, F. Quaini, C. Frati, D. Madeddu, S. Vlad and O. Petreus, "Thermoreversible Injectable Polyurethane Hydrogels: Future Challenges for Biomedical Applications", in "Polymer Materials With Smart Properties", Ed. M. Bercea, Nova Science Publishers, New York, 2013, p.37-66.
4. M. Bercea, S. Morariu and D. Rusu, *Soft Matter*, **2013,** *9*, 1244–1253.
5. T. Vermonden, R. Censi, and W. E. Hennink, *Chem. Rev*., **2012**, *112*, 2853-2888.
6. E. M. Ahmed, *J. Adv. Res*., **2015**, *6*, 105–121.
7. M. Bercea, R. N. Darie, L. E. Nita and S. Morariu, *Ind. Eng. Chem. Res.,* **2011**, *50*, 4199–4206.

8. M. Bercea, R. N. Darie and S. Morariu, *Rev. Roum. Chim.*, **2013**, *58*, 189–196.
9. M. Ding, J. Li, H. Tan and Q. Fu, *Soft. Matter*., **2012**, *8*, 5414-5428.
10. L. M. Gradinaru, C. Ciobanu, S. Vlad, M. Bercea and M. Popa, *Ind. Eng. Chem. Res.,* **2012**, *51*, 12344−12354.
11. L. M. Gradinaru, C. Ciobanu, S. Vlad, M. Bercea and M. Popa, *Cent. Eur. J. Chem*., **2012**, *10,* 1859-1866.
12. C. Ciobanu, L. M. Gradinaru, M. Drobota, F. Quaini, A. Falco, C. Frati, G. Graiani, D. Madeddu, C. Lagrasta, S. Vlad, M. Bercea and L. Sacarescu, *J. Hydrogels*, **2015**, *1*, 12–25.
13. C. Ciobanu, L. M. Gradinaru, M. Drobota, S. Vlad, M. Bercea and M. Popa, *J. Hydrogels*, **2015**, *1*, 41–49.
14. L. Weng, X. Chen and W. Chen, *Biomacromolecules*, **2007**, *8*, 1109–1115.
15. C. Yan and D. J. Pochan, *Chem. Soc. Rev*., **2010**, *39*, 3528–3540.
16. M. Y. Kozlov, N. S. Melik-Nubarov, E. V. Batrakova and A. V. Kabanov, *Macromolecules*, **2000**, *33*, 3305−3313.
17. V. P. Torchilin, *Cell. Mol. Life Sci.*, **2004**, *61*, 2549−2559.
18. S. Morariu and M. Bercea, *Rev. Roum. Chim.*, **2013**, *58*, 145–152.
19. M. Bercea, L. E. Nita, S. Morariu and A. P. Chiriac, *Rev. Roum. Chim.*, **2015**, *60*, 787–795.