

pHEMA hydrogels

Synthesis, kinetics and in vitro tests

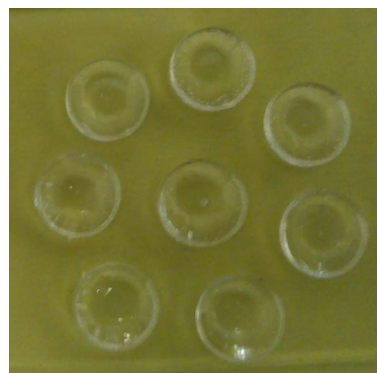
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Abstract Hydrogels of poly (2-hydroxyethyl methacrylate) (pHEMA) are suitable materials for medical applications. Synthesis, structural characterization, kinetic, physical properties and cell viability of these materials are reported in this article. The system was conducted via free radical polymerization in the absence of solvents or cross-linking agents. Hydrogels were obtained with excellent dimensional stability and high thermal stability (738 K), glass transition temperature of approximately 375 K and a degree of swelling of 66 ± 4 % in ethanol. The conversion of C=C double bond of the monomer 2-hydroxyethyl methacrylate was confirmed by Fourier transform infrared spectroscopy. Results showed dense and rough morphologies in the pHEMA, presence of high molecular interactions and good resistance to various organic solvents. In vitro tests showed excellent cell viability. The materials did not display cytotoxicity, and cells proliferated and adhered at a satisfactory rate on the hydrogel. These materials have great potential for biomedical application.

Graphical Abstract pHEMA hydrogels are materials receiving great attention among researchers due to their ease of synthesis and biomedical application. This article demonstrates a new method of obtaining pHEMA hydrogels by bulk polymerization, in a single processing step and

without the use of cross-linking agents. A kinetic study was evaluated, and the results of in vitro tests showed good cell proliferation.



Keywords pHEMA · Hydrogel · Biomaterial · Calorimetry · Kinetic · In vitro tests

Introduction

Hydrogels are a class of materials frequently applied in tissue engineering since the pioneering work of Witcherle and Lim [1]. The properties of hydrophilicity, biocompatibility and high water absorption without dissolving (>38 %) [2] have attracted great interest from researchers concerning these biomaterials. They can be considered as physical or chemical gels and can be of natural origin (e.g., chitosan, hyaluronic acid) or synthetic [e.g., polyethylene glycol (PEG), poly (2-hydroxyethyl methacrylate) (pHEMA), poly (lactic acid) (PLA)]. As physical or reversible gels, the three-dimensional network present in

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the polymer structure is linked by molecular entanglements and/or forces of secondary bonds such as hydrogen bonding, ionic or hydrophobic forces. Permanent or chemical hydrogels, in turn, have covalently cross-linked networks [3–5].

Many methods can be used to obtain hydrogels with different molecular structures: copolymerization [6], radiation [7], radical polymerization by atom transfer (ATRP) [8], among others. The molecular structures obtained include cross-linked networks or intertwined, from linear homopolymers, linear copolymers or graphitized blends or semi-interpenetrating networks [5].

The use of hydrogels in the medical field is extensive. They can be applied for regeneration of articular cartilage [9], contact lenses [10], regenerative medicine and controlled drug delivery systems [11]. Hydrogels have the ability to release therapeutic agents *in vivo* in chosen sites in a controlled space–time frame. The release of bioactive factors is a promising strategy to disease therapy and also enhances tissue regeneration [12]. The hydrogel of poly (2-hydroxyethyl methacrylate) (pHEMA) is one of the most studied polymers in this field of science. It has excellent biocompatibility, has physical properties similar to living tissue and contains hydroxyl functional groups on its surface that can be used for protein bioconjugation [13]. This work aimed to obtain pHEMA crosslinked hydrogels without solvents or cross-linking agents, in a totally clean and less costly process. In some cases, the use of solvents requires subsequent steps of purification of the final product to avoid toxicity. Furthermore, depending on the concentration and molecular structure of the cross-linking agent, hard and brittle hydrogels can be obtained and secondary reactions can occur more easily. The kinetics reaction was studied by the method proposed by Sbirrazzuoli and Vyazovkin [14] using differential scanning calorimetry (DSC). Morphology and physical and chemical properties of the materials were investigated by scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TG/DTG), absorption and solubility tests, proton nuclear magnetic resonance (^1H NMR) and DSC. The cytotoxicity of the hydrogels was examined by the colorimetric assay of MTT ((3-[4, 5 dimethylthiazol-2-yl])-2, 5-diphenyl tetrazolium bromide).

Experimental

Materials

2-hydroxyethyl methacrylate (HEMA, 99 %), di-tert-butyl peroxide (TBPO, 98 %) and poly (2-hydroxyethyl methacrylate (pHEMA- standard, $M_v = 300,000$) were purchased from Sigma-Aldrich and used as received.

Synthesis

Poly (2-hydroxyethyl methacrylate) was obtained by free radical polymerization initiated thermally. Approximately 15 mg of HEMA and TBPO mixture, and 1 % m/m of the peroxide relative to the initial content of monomer was added to the DSC cell. The synthesis was conducted in an inert atmosphere with nitrogen flow set to 100 mL min^{-1} and a heating rate of 10 K min^{-1} . The DSC cell was sealed with a pierced and inverted cover.

Kinetics of polymerization

The radical polymerization kinetic of HEMA was studied using the method proposed by Vyazovkin [14] without requiring prior knowledge of the model reaction. DSC data were investigated by three dynamic curves, the heating rates of 5, 10 and 20 K min^{-1} . The curves were analyzed automatically using model-free kinetics. The conversion was estimated as a function of time. The activation energy was calculated as a conversion function and was compared with Kissinger's corrected kinetic equation [15, 16] (Eq. 1). The results were interpreted in terms of the physical phenomena that take place during the reaction of vinyl monomers microscale [17].

$$\ln\left(\frac{\beta}{T_p^2}\right) = \ln\frac{AR}{E_a} - \frac{E_a}{RT_p} \quad (1)$$

where β is the heating rate (K min^{-1}); A is the pre-exponential factor (s^{-1}); E_a is the activation energy (kJ mol^{-1}); T_p is the peak temperature (K); and R is the universal gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$). The slope of the curve obtained by Eq. (1) provided E_a .

Characterization

Differential scanning calorimetry (DSC)

Physical and thermodynamic properties were evaluated by DSC Mettler Toledo 823e equipment. DSC crucibles were subjected to a dynamic scan from 273 to 523 K (first heating cycle) at a heating rate of 10 K min^{-1} to eliminate the thermal history of the material, cooled from 523 to 273 K and reheated from 273 to 523 K (second heating cycle) to determine the glass transition temperature (T_g) under N_2 at 100 mL min^{-1} . The enthalpy of polymerization was obtained by integrating the area between the DSC curve and the base line. The samples were weighed in a standard $40\text{-}\mu\text{L}$ aluminum pan, with pierced and inverted lid and a sample mass between 10 and 20 mg. The fusion point and the enthalpy of the Indian chemical element were used for calibration of equipment.

Fourier transform infrared spectroscopy (FTIR)

The conversion of the double bond C=C of the HEMA monomer and the evaluation of the characteristic functional groups of pHEMA were analyzed by the IR spectrophotometer Nicolet 100 Thermo Scientific, 400–4000 cm^{-1} , resolution 4 cm^{-1} , direct transmittance mode (ATR).

Scanning electron microscopy (SEM)

LEO scanning electron microscope (Oxford/Leo 440i) at 500–2000 magnifications was used to evaluate the morphology of fracture of the pHEMA in the dried samples

Thermogravimetric analysis (TG/DTG)

The thermal stability of the pHEMA was evaluated using TA Instruments 2960 SDT V3.0F equipment. Nitrogen was used as carrier gas at 100 mL min^{-1} and a heating rate of 10 K min^{-1} . The data were reported in terms of mass change and the initial and final temperatures of thermal decomposition.

Absorption capacity

The study of polymer absorption levels was obtained by Eq. 2 [18]. Ethanol was used as a test solution. It is applied commercially to enhance the skin permeability in transdermal drug release applications [19]. W_s is the swollen sample mass and W_d is the dry sample mass. A vacuum oven was used to dry the samples for 6 h. The immersion in ethanol was done at 37 °C (body temperature) for 24 h.

$$\% = \frac{W_s - W_d}{W_s} \cdot 100 \quad (2)$$

Solubility

Methanol, chloroform and dimethyl sulfoxide (DMSO) were used to test the solubility of pHEMA in organic solvents at temperature of 45 °C for 48 h. The concentration used was 5 mg/0.6 mL. Tetrahydrofuran and dimethylformamide at 1 g L^{-1} were also visually evaluated for the purpose of determining the molecular mass of pHEMA by gel permeation chromatography (GPC).

Proton nuclear magnetic resonance ($^1\text{H NMR}$)

$^1\text{H NMR}$ spectrum of the pHEMA was evaluated using Bruker 250 MHz equipment and deuterated dimethyl sulfoxide (DMSO- d_6) at 25 °C.

Cell viability

Cell viability was estimated by the MTT assay described by Mosmann [20]. The methodology consists in the conversion of soluble chromogen [3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT)] in slightly soluble formazan via dehydrogenases present in viable cells. The formazan provides a dark color in the cells, and it may be subsequently read in a spectrophotometer at a wavelength of 570 nm, after DMSO solubilization.

Fibroblast human lung cells (MRC-5) were then cultured with the materials. Initially, the hydrogel was sterilized by autoclave, under water vapor pressure at 121 or 134 °C. The cell viability was measured at 24 h and 7 days after seeding of cells onto the materials. Phenol 1 % and polyethylene (PET) were used as controls for the viability assay.

Results and discussions

Polymerization kinetics

The vinyl monomer polymerization reaction is accompanied by the release of heat due to the addition reaction to the double bond of the monomer [17, 21]. The heat released is proportional to the enthalpy of polymerization and is given by the integral of the area under the curve. Figure 1 allowed the determination of the temperature values and enthalpy of polymerization of the vinyl monomer (HEMA). Average results were obtained at $427 \pm 3 \text{ K}$ and 273.59 J g^{-1} , respectively. The exothermic heat flow observed can be related to the energy balance between the opening of the C=C double bond and the formation of two single bonds during the polymerization chain [22].

The result of the heat release in normalized values versus temperature is shown in Fig. 2a at different heating

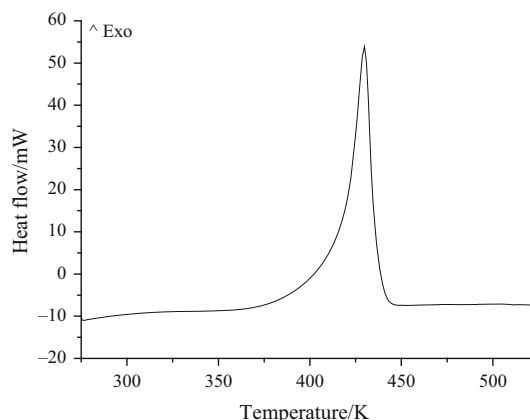


Fig. 1 DSC curve of pHEMA at 10 K min^{-1}

rates. In Fig. 2b, the conversion of the double bond of the monomer is plotted as a function of time for different temperatures. The activation energy in function of the conversion is shown in Fig. 2c. For a given conversion (α), it was also possible to estimate the temperature and reaction time (Fig. 2d). As observed in Fig. 2a, temperature shifts for the reaction arose for increasing heating rates, a phenomenon known as thermal lag effect [15]. Although the increase in heating rate increases speed and the yield of reactions, there is a difficulty in separating consecutive events.

The controlled diffusion phenomenon acting on the kinetics of radical polymerization was evidenced in Fig. 2b, and the activation energy profile as a function of the conversion (Fig. 2c) supports this finding. At low conversions (first polymerization stage), there is a nearly linear dependence of conversion with time, indicating a purely chemical control of the polymerization as demonstrated Achilias [17]. Diffusion of the monomer and initiator molecules by the classical kinetic mechanism of free radical polymerization, consisting of three phases, is observed: initiation, propagation and termination.

The phenomenon known as the self-accelerating effect, or gel effect (controlled diffusion), occurs at conversions above 20 %, primarily evident at temperatures above 399 K. At this stage, there is an increase in system viscosity, due to the passage of a viscous liquid to an elastic gel. The increase in cross-linking reduces the mobility of the chain due to a rise in reticulation density. A change in the curvature of conversion versus time is then observed

[17]. The reaction subdues almost completely before monomer conversion completion. At temperatures of 355 and 377 K, it is possible that the period of 1800s (30 min) was not sufficient to achieve equilibrium conversion. It is found that increasing the polymerization temperature leads to higher conversions at shorter reaction times. The maximum equilibrium conversion obtained was 99.76 % after a period of 175.49 s at a temperature of 465 K.

The overall activation energy (E_a) was calculated according to Eq. (1) and resulted in a value of 56.73 kJ mol⁻¹. This value is lower than that reported by Achilias [17] for pure pHEMA (89 ± 3.1 kJ mol⁻¹), but similar to values obtained by Huang et al. [23] (56.5 to 78.3 kJ mol⁻¹), both values measured based on DSC data. The experiences that were gained from the methodologies employed in these different studies should be investigated further. Moreover, it is possible that systems with greater mobility of the chains (monomers and macroradicals) result in lower activation energies. The rate of reaction can be enhanced by applying higher process temperatures and a lower reticulation density.

Inspection of the graph for activation energy versus conversion (Fig. 2c) by the isoconversional method establishes an initial value of E_a to 47.07 kJ mol⁻¹, with a maximum obtained at about 20 % conversion (77.73 kJ mol⁻¹). Then, the activation energy drops to a minimum of 48 kJ mol⁻¹ (20–56 % conversion). After this period, there is a marked increase in activation energy reaching the value of 102.264 kJ mol⁻¹ and final conversion of 98.85 %. These events are possibly associated with the chemical reaction governed by the steps of initiation, propagation and termination and with the controlled diffusion phenomenon (gel effect).

The effect of temperature on the reaction time at a given conversion is shown in Fig. 2d. According to Chan and Gleason [24] for chemical vapor deposition reactions (CVD) of vinyl monomers, the temperature of the heated wire should be 473–573 K [25]. By graphic inspection, it is still possible to infer that the polymerization of HEMA in CVD systems may occur at relatively high reaction rates (26–62 s), as long as the same analysis conditions are utilized. Chan and Gleason [24], for example, obtained pHEMA homopolymers at reactor residence times of 5 s.

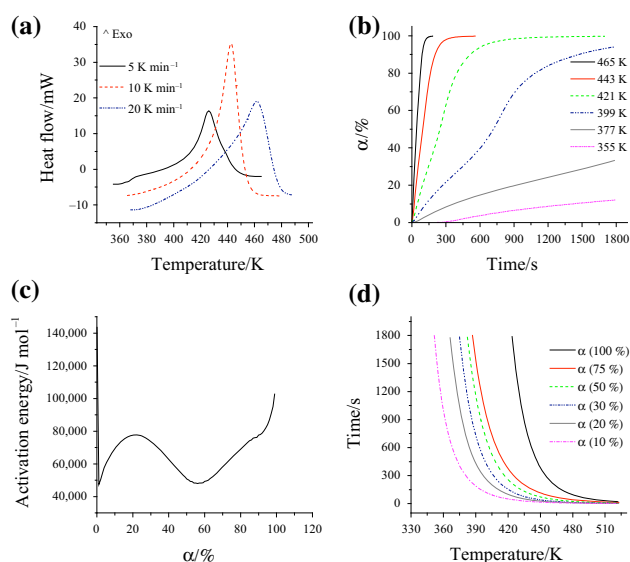


Fig. 2 **a** Heat flow versus temperature for different heating rates; **b** conversion of the double bond C=C as a function of time; **c** activation energy as a function of conversion; **d** temperature and reaction time for a given conversion

Synthesis and characterization

Synthesis

pHEMA was obtained by radical polymerization using a bifunctional initiator in the absence of reticulation agents and solvents. A disk with 5 mm diameter and 1 mm thick was obtained. The samples showed good dimensional stability, transparency and flexibility (Fig. 3).

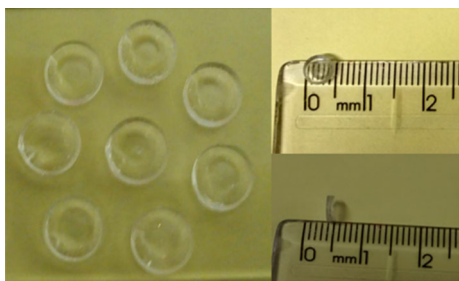


Fig. 3 pHEMA hydrogels obtained by radical polymerization in DSC cell

Characterization

The chemical structure of pHEMA and conversion of the C=C double bond was confirmed by FTIR (Fig. 4). FTIR analysis suggested high conversions of the HEMA monomer. FTIR spectrum showed characteristic bands in the range of 3200–3600 cm^{-1} attributed to the stretching of the hydroxyl (OH) groups; 2847–2948 cm^{-1} related to CH stretching of methyl groups ($-\text{CH}_3$) and methylenes ($-\text{CH}_2-$); 1720.75–1720.98 cm^{-1} band relating to the stretch of the ester carboxyl groups (C=O); and further, the band to 1451 cm^{-1} , attributed to bending of $-\text{CH}$ [26–30]. Traces of unreacted HEMA monomer (1650 cm^{-1}) were not detected. The spectral shape in the O–H and C=O stretching region and the absence of the band assigned to the free OH present on hydrogen bonds at 3640 and 3624 cm^{-1} are evidence for hydrogen bonds through the hydroxyl groups on the chain terminal pHEMA, as OH–OH and C=O–OH [31].

Results obtained from the DSC curve (Fig. 5) showed the presence of monomers and volatile residues in the first heating. These impurities had evaporated after the thermal history of the material had been eliminated (2nd heating). The formation of an amorphous polymer was also observed. The endothermic peak in heat flow during cooling is not observed, demonstrating the absence of crystallinity in the material. The glass transition temperature (T_g) was 375 ± 2 K and is related to the flexibility of the amorphous pHEMA chain. This result is much higher than other similar polyacrylates [32, 33]. The value obtained was similar to the results presented by Holmes et al. [29] using γ -radiation polymerization and greater than those reported in studies reported in the literature using commercial atactic pHEMA (353 K) [32], by free radical polymerization (360 K) [34] and in the absence of solvents (363 K) [35]. Although in the preparation of the HEMA, diester ethylene glycol dimethacrylate (EGDMA) may be present, working as a reticulation agent [23], the controlled processing conditions suggest the formation of

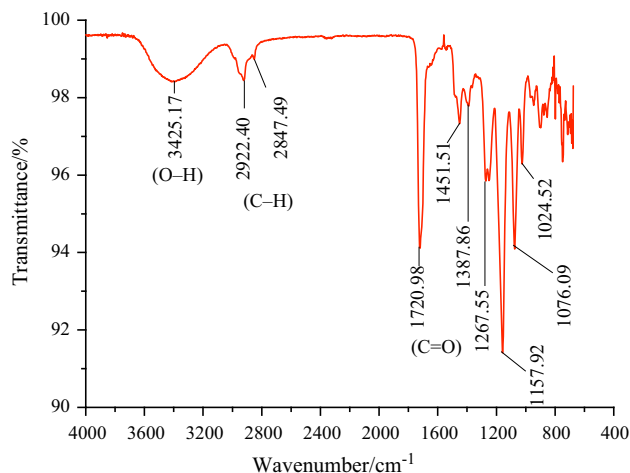


Fig. 4 FTIR spectrum of pHEMA

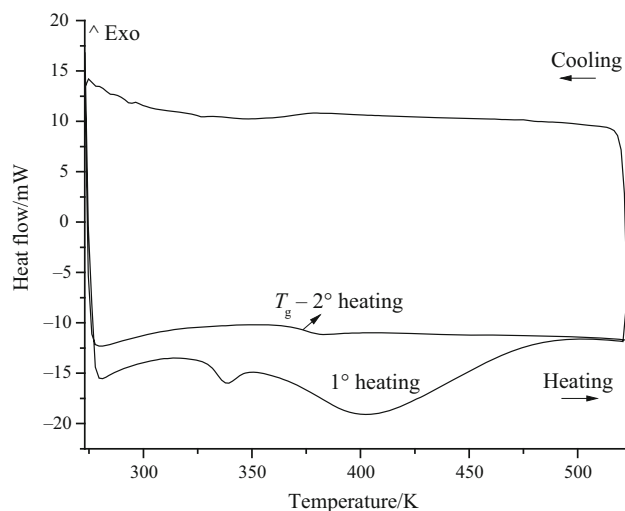


Fig. 5 DSC curve of pHEMA under heating and cooling at 10 K min^{-1}

strong interactions between the hydroxyl groups in the polymer chain.

Figure 6 shows the curves of mass loss (TG) and its derivative (DTG) of the pHEMA sample obtained by radical polymerization (a) and of the commercial pHEMA sample (b) (Sigma-Aldrich). Three levels were observed on both materials: the first threshold may be associated with evaporation of water physically adsorbed on the hydrogel network (373 K) as well as the presence of residual monomers; the second and third levels can be related to the break of the polymer chains. The initial temperature of the mass loss of commercial pHEMA (Fig. 6b) was 352 K and maximum at 728 K. For pHEMA obtained by radical polymerization (Fig. 6a), these values are 345 and 738 K, respectively. These results suggest a higher content of residual monomers and/or adsorbed water in the pHEMA

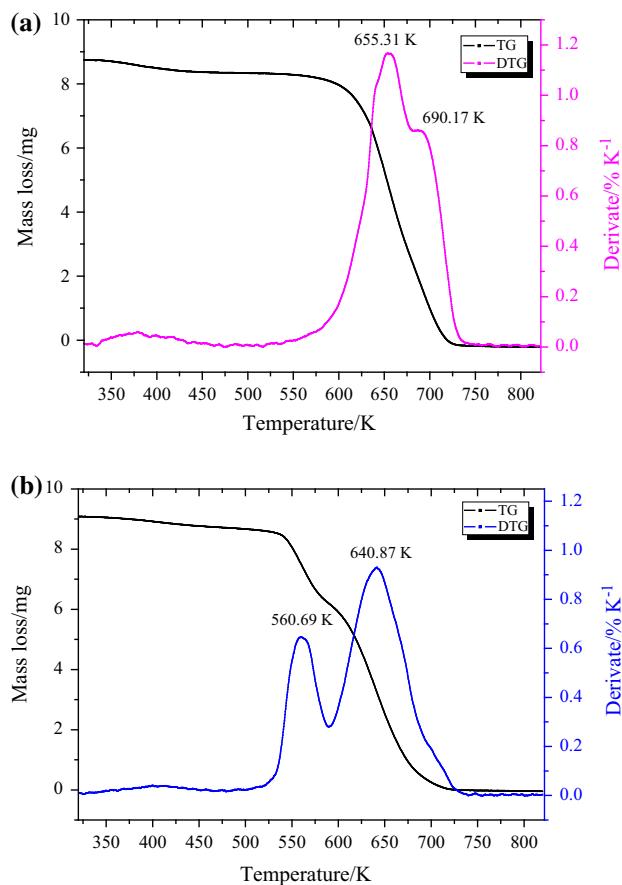


Fig. 6 Thermogravimetry (TG) and derivative thermogravimetry (DTG) curves of **a** pHEMA obtained by radical polymerization and **b** commercial pHEMA (Sigma-Aldrich)

chain from this study (Fig. 6a) as well as a higher thermal stability, relative to commercial pHEMA (Fig. 6b) [31].

The immersion of pHEMA samples in different organic solvents also highlighted the possible formation of chemical reticulation in the material. All samples tested were insoluble in solvents (methanol, chloroform, dimethyl sulfoxide, tetrahydrofuran and dimethyl formamide). A significant swelling of 0.7 mm of the original diameter of the pHEMA was observed after immersion in DMSO. The material maintained its shape and flexibility. It is possible that the pHEMA obtained in DC cells has undergone a single solubilization stage: swollen gel. Although the diffusion of solvent molecules into the polymer mass has occurred, the disintegration of the polymer did not occur. This fact can be explained by the presence of cross-links, hydrogen bonds or polymer–polymer interactions greater than the polymer–solvent interactions [36]. Reticulated polymers are insoluble and infusible. Consequently, ^1H NMR analysis to confirm the identification of the molecular structure of pHEMA could not be performed. Moreover, commercial pHEMA showed solubility in methanol

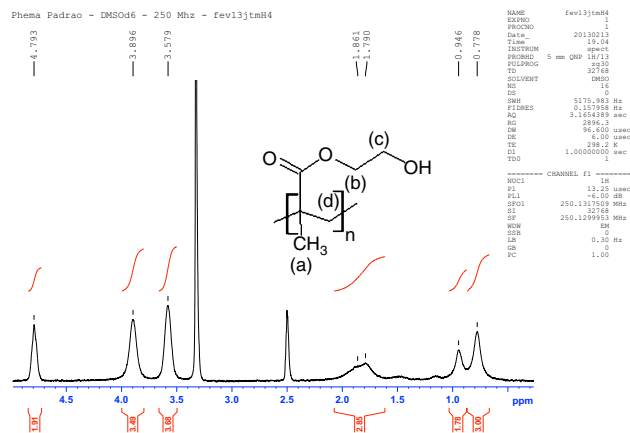


Fig. 7 ^1H NMR typical spectrum of commercial pHEMA (Sigma-Aldrich)

and DMSO. ^1H NMR spectrum showed that the commercial pHEMA polymer matrix contains protons of the methyl group (a) 0.778 and 0.946 ppm; methylenes groups (b) and (c) at 3.896 and 3.519 ppm, respectively; β -methylenes groups (d) between 1.861 and 1.790 ppm; and 4.793 ppm of the hydroxyl group (Fig. 7).

The uptake of ethanol in the samples was $66 \pm 4\%$, which is higher than the results shown by Sun et al. [19] ($<20\%$) and close to the values obtained for swelling tests in water (66 and 67 %) [35, 37]. The samples, when swollen, increase the spacing between their polymer chains. It is possible that ethanol acts as a penetrating agent and expands the space between the networks [19]. This will allow solutes to easily penetrate the pHEMA. Hydroxyl groups in ethanol may furthermore be interacting with pHEMA's carbonyl groups and hydroxyl groups, forming strong molecular interactions. On the other hand, chain mobility tends to be lower the greater the number of reticulations between the networks, and the degree of absorption may consequently become limited.

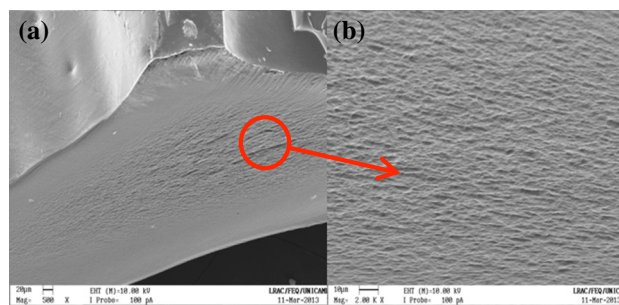


Fig. 8 Scanning electron microscopy of pHEMA: **a** oblique view of the pHEMA sample showing smooth and compact regions. High magnification of **a** showing high roughness of the pHEMA sample in central region

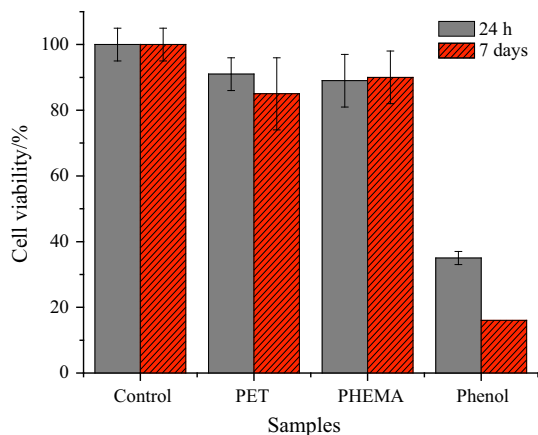


Fig. 9 Cell viability of MRC-5 cells on pHEMA disks after 24 h and 7 days of culture. $p < 0.001$ $n = 3$

Figure 8 shows pHEMA images at magnifications of 500 (a) and 2000 (b). Smooth and compact regions are observed on the surface of the material (a), alongside areas with high roughness in the central region (b).

Cell viability

As shown in Fig. 9, the pHEMA did not promote the death of cells at 24 h and 7 days after cell seeding. Cell viability was 89 ± 8 and 90 ± 8 % (24 h and 7 days) with pHEMA and 91 ± 5 and 85 ± 11 % (24 h and 7 days) with PET. There was no significant difference of viable cells in 7 days between pHEMA (90 ± 8 %) and the control group (100 ± 5). Phenol at 1 % led to cell viability 35 ± 2 and 16 ± 1 % after 24 h and 7 days, respectively. In all pHEMA samples, proliferation of MRC-5 cells on the hydrogel was observed, indicating the high affinity of the cells to the polymer. This demonstrates the ability of pHEMA for cell adhesion and growth.

Conclusions

pHEMA hydrogels were successfully synthesized by radical polymerization of 2-hydroxyethyl methacrylate monomers using DSC cells. The synthesis technique was effective and “clean.” TG/DTG data showed material’s excellent dimensional stability and high thermal stability (738 K). The glass transition temperature was approximately 375 K, in agreement with literature data. Diffusion of the monomer and initiator molecules by the classical kinetic mechanism of free radical polymerization (initiation, propagation and termination) was observed using DSC without requiring prior knowledge of the model reaction. Kinetic studies showed an overall activation energy (E_a) of $56.73 \text{ kJ mol}^{-1}$ and a thermal lag effect at the temperature of the reaction

with increasing heating rates. At low conversions (first polymerization stage), there was a nearly linear dependence of conversion of the reaction over time, indicating a purely chemical control of the polymerization. The phenomenon of self-accelerating or gel effect (controlled diffusion) occurred at conversions above 20 %.

Strong molecular interactions (intra and intermolecular) were observed even in the absence of cross-linking agents and chemical solvents and evidenced by swelling tests, solubility and FTIR. The optical transparency, the high degradation temperature, the chemical resistance to organic solvents and the absorption of ethanol molecules suggested the existence of chemical and physical reticulations in the hydrogel. Cell viability tests demonstrated the absence of cytotoxicity and good adhesion and proliferation of cells on the pHEMA biomaterial. These results demonstrate the feasibility of synthesis using DSC cells without the addition of solvents in a single processing step. Herein, pHEMA hydrogels are demonstrated to be suitable materials for biomedical applications, tissue engineering and regenerative medicine.

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