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STIMULI RESPONSIVE HYDROGELS: NIPAM/AAm/CARBOXYLIC ACID POLYMERS

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Abstract: Stimuli-responsive hydrogels (SRH) were prepared by using monomers (i.e. N-isopropyl acrylamide; NIPAM and acrylamide; AAm), co-monomers (i.e. methacrylic acid; MPA or mesaconic acid; MFA) and a crosslinker (N, N'-methylene bisacrylamide; N-Bis). SRH have been prepared by thermal free radical polymerization reaction in aqueous solution. Spectroscopic and thermal analyses such as Fourier Transform Infrared Spectroscopy, thermogravimetric analysis and differential scanning calorimetry analysis were performed for SRH characterization. The equilibrium swelling studies by gravimetrically were carried out in different solvents, at the solutions, temperature, pH, and ionic strengths to determine their effect on swelling characteristic of the hydrogels. In addition, cycles equilibrium swelling studies were made with the solutions at different temperatures and at different pH. NIPAM/AAm hydrogel exhibits a lover critical solution temperature (LCST) at 28 ^{o}C, whereas NIPAM/AAm-MPA and NIPAM/AAm-MFA hydrogels exhibit a LCST at 31 C and 35 ^{o}C, respectively, and the LCST of NIPAM/AAm-MFA hydrogel is close to the body temperature.

Keywords: Stimuli-responsive hydrogels, N-isopropyl acrylamide, Methacrylic acid, Mesaconic acid, Swelling.

Introduction

Hydrogels are crosslinked three-dimensional polymer networks that can absorb large amounts of water and remain insoluble. Depending on the

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nature of chemical units along the polymer chains, these materials can respond to certain external stimuli such as pH, temperature, ionic strength, electric field, light, magnetic field, and so forth. Therefore, hydrogel can be used in many fields such as advanced material for biomedical, environmental, catalysis, and sensor applications.[1-7]

In particular, hydrogels have proven themselves clever options in developing stimuli-responsive systems, because their chemistry permits modulating the properties by including responsiveness via sensitive chemical moieties.[8,9]

A large variety of hydrogels has been synthesized to response to physical stimuli (temperature, pH, light), chemical stimuli (various ‘‘signaling’’ molecules), or biological stimuli (enzymes). Stimuli-responsive hydrogels (SRH) undergo dramatic and abrupt physical and chemical changes in response to external stimuli. They are also termed ‘smart-’, ‘intelligent-’, or ‘environmentally sensitive’ polymers.[2,10-14]

One important feature of this type of material is reversibility, i.e. the ability of the hydrogel to return to its initial state upon application of a counter-trigger. In nature, biopolymers such as proteins and nucleic acids are all basic stimuli-responsive components of living organic systems and often remain stable over wide ranges of external variables but undergo drastic conformational changes abruptly at given critical points. These ‘natural’ stimuli-responsive polymers have led to the development of numerous synthetic hydrogels that have been designed to mimic their adaptive behaviors.[15-19]

By incorporating functional groups that are amenable to a change in character (e.g. charge, polarity and solvency) along a polymer backbone, the resulting relative changes in chemical structure will be amplified

synergistically, leading to dramatic transformations in macroscopic material properties.[15-19]

Typically, the 'response' of a hydrogel in solution alters its individual chain dimensions/size, secondary structure, solubility, or the degree of intermolecular association. In most cases, the present or destruction of secondary forces (hydrogen bonding, hydrophobic effects, electrostatic interactions, etc.), simple reactions (e.g., acid–base reactions) of moieties linked to the polymer backbone, and/or osmotic pressure differences are responsible for this response. Another type of 'response' is due to dramatic alterations in the polymeric structure, such as degradation of polymers upon the application of a specific stimulus by bond breakage in the polymer backbone or at pendant cross-linking groups.[15-19]

SRH can be designed either with a responsive polymer, or by combining a polymer with a responsive compound, the hydrogel serving only as a template/carrier for that compound.

In order to improve the swelling properties of NIPAM/AAm gels and to impart pH sensitivity it has been copolymerized for a monoprotonic carboxylic acid (methacrylic acid (MPA)), or a diprotonic carboxylic acid (mesaconic acid (MFA)). The resulting gel is responsive to both temperature and pH changes. By copolymerization with a suitable monomer, the hydration degree and swelling kinetics of the final hydrogel are easily tuned. Acrylamide (AAm) is a monomer of the class poly(acrylamides), which upon polymerization gives a water soluble polymer with no LCST. In general, acrylamide-based compounds are well known for their absorptive and medicinal properties. In line with our research interest in AAm based materials,[20-26] in this article we report the

copolymerization of NIPAM with AAm. The present work aims to develop N-isopropyl acrylamide/acrylamide; (NIPAM/AAm) based hydrogels with pH and temperature sensitivities and higher swelling properties. In order to easily compare the effects of the stimulating parameters investigated, MPA and MFA were selected as acidic comonomers which were very similar in chemical structure with only one excess of carboxyl group of unsaturated organic acids containing methyl group. The preparation and characterization of copolymer hydrogels, detailed analysis of their swelling behavior, and human serum albumin adsorption are presented in this report. A detailed understanding of the physical properties of these new ionizable hydrogels in relation to molecular heterogeneity is an important criterion in targeted applications such as temperature, pH, ionic strength and solvent responsive sorbent systems.

Results and discussion

- *Preparation of Hydrogels*

Among the monomers used for stimuli responsive hydrogels in this study, NIPAM was selected to give temperature sensitivity to the hydrogel, AAm monomer to give mechanical strength to the structure, and organic carboxylic acids containing double bonds such as MPA and MFA were chosen to give pH sensitivity to the polymer.

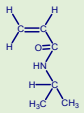
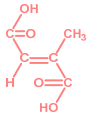
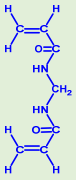
Hydrogels composed of NIPAM, AAm, and carboxylic acid unit(s) containing monomers (Table 1) were prepared by thermal free radical solution polymerization using N, N'-methylenebisacrylamide (NBis) as crosslinker. The polymerization mechanism is very well established and schematic representation is given in Figure 1. It is obvious that the

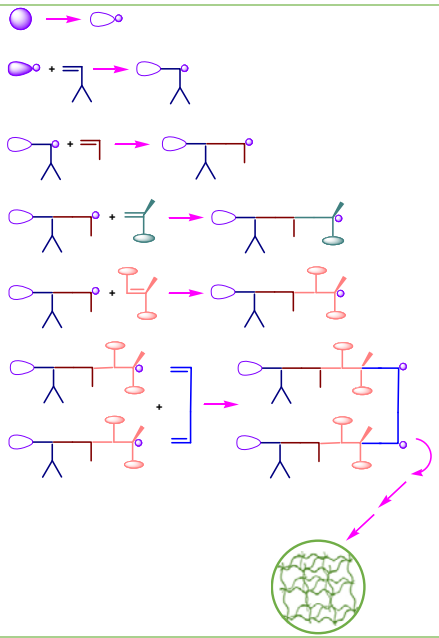
polymerization and crosslinking mechanism took place simultaneously. Although a gelatin was occurring in about 1 h, the reaction let it proceed for 24 h, and finally the hydrogel rods obtained in the plastic straws were cut into pieces of 3–4 mm length and washed with distilled water and dried in air and vacuum. Crosslinked copolymeric hydrogels were colorless and transparent.

- *Characterization*

FTIR Analysis. Typical spectra of SRH is shown in Figure 2. In the FTIR spectra of the hydrogels, the typical absorption bands for NIPAM, AAm and MPA or MFA units can be seen about 3600– 3100 cm^{-1} as broad bands for secondary NH amide, between 2980 and 2878 cm^{-1} for CH stretching frequencies for isopropyl groups, and at 1670 cm^{-1} a strong C=O amide I band, and at 1550 cm^{-1} for other strong amide II bands. The peak around 1400–1300 cm^{-1} belong to $-CH(CH_3)_2$ group in isopropyl groups, and the peaks about 1268–1000 cm^{-1} can be attributed to C−N bands in amide groups.[27,28] There are no bands appeared at 900-1000 cm^{-1} of the evidence of monomeric double bonds, so all components (NIPAM, AAm and MPA or MFA) were considered to be successfully polymerized. From this spectral analysis, it can be concluded that a polymeric network is formed, because of the functional groups of each component of the hydrogel units: NIPAm, AAm, and MPA or MFA monomers with NBis.

Table 1. The chemical structure and some properties of the monomers.

monomer	abbreviation	chemical structure	illustration	molar mass g mol^{-1}
N-isopropyl acrylamide	NIPAM			113.16
acrylamide (2-Propenamide)	AAm			71.08
methacrylic acid (2-methylpropenoic acid)	MPA			86.09
mesaconic acid (methylfumaric acid)	MFA			130.10
N, N'-methylene bisacrylamide (Bis-acrylamide)	NBis			154.17



free radicals formation

NIPAM addition (*initiation step*)

AAm addition (*propagation step/copolymerization*)

MPA addition (*propagation step/copolymerization*)

MFA addition (*propagation step/copolymerization*)

NBis AAm addition (*propagation step/crosslinking*)

SRH formation (*termination step/gelation*)

Figure 1. The plausible polymerization and crosslinking mechanism of the hydrogels

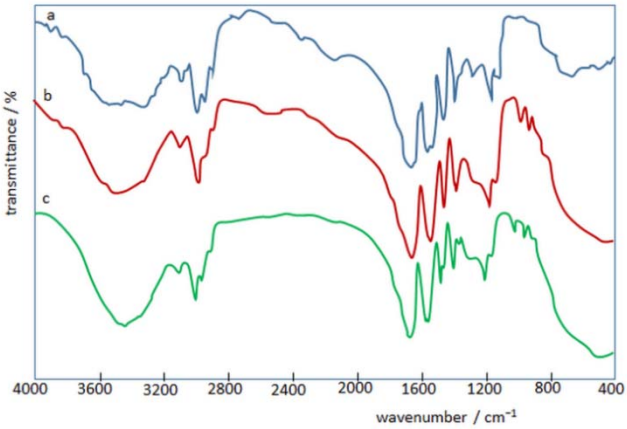


Figure 2. FTIR spectra of the hydrogels, **a** - NIPAM/AAm, **b** - NIPAM/AAm-MPA, **c** - NIPAM/AAm-MFA

Thermal Analysis

TGA Analysis. The thermal degradation values such as the initial degradation temperatures (T_i), the temperature of maximum rate (T_{max}), the degradation final temperature (T_f), the half-life temperature (T_h), and the maximum decomposition rate (r_{max}), and the amount of the substance at the maximum rate (C_{max}) values were found the thermograms of hydrogels (Figure 3) and were given Table 2.

As can be clearly seen, the thermograms of the hydrogels exhibit three distinct stages. The first one is in the range of 25–330/340 ^{o}C due to loss of water adsorbed both on the surface and in the pores of the hydrogel, and the other one is in the range of 330/350–400/425 ^{o}C that can be attributed to the thermal decomposition of the organic network, starting with the amide groups and carboxyl groups on the SRH network. The third region corresponds mainly to the degradation of polymer chains.[27,28] Above 400/425 ^{o}C for the SRH represents substantial mass loss and is normally attributed to main chain of hydrogel breakdown.

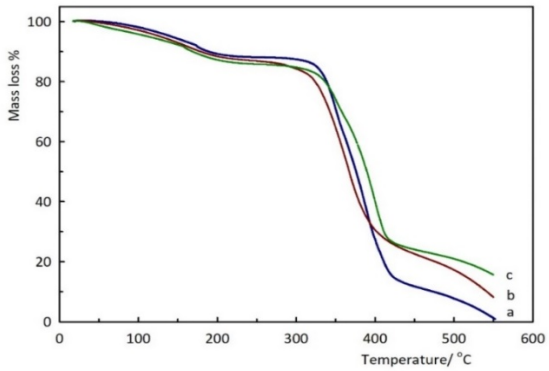


Figure 3. TG thermograms of the hydrogels, **a** - NIPAM/AAm, **b** - NIPAM/AAm-MPA, **c** - NIPAM/AAm-MFA

According to the all temperature values of NIPAm/AAm hydrogel, the temperature values of NIPAm/AAm-MPA were small and the temperature values of NIPAm/AAm-MFA were high. Accordingly, the incorporation of MPA containing monocarboxylic unit into the NIPAm/AAm hydrogel gives flexibility to the polymeric structure while the polymeric structure becomes more rigid with the addition of NIPAm/AAm-MFA containing the dicarboxylic unit. r_{max} values range from 0.76 to 0.85 mg min^{-1}, while C_{max} values range from 0.44 to 0.56 %.

Table 2. Thermogravimetric parameters of the hydrogels

SRH	T_i / °C	T_{max} / °C	T_f / °C	T_h / °C	r_{max} / mg min^{-1}	c_{max} / %
NIPAm/AAm	330	382	415	374	0.85	0.44
NIPAm/AAm-MPA	322	360	396	368	0.76	0.56
NIPAm/AAm-MFA	337	389	424	387	0.80	0.49

The Thermal Decomposition Kinetics of the SRH. To determine the thermal decomposition of the samples, Freeman-Carroll and Jeres methods were used.[29]

Freeman-Carroll equation;

$$Y = n - \frac{E}{R}X$$

can be derived, where $X = \frac{\Delta\left(\frac{1}{T}\right)}{\Delta ln(1-c)}$, $Y = \frac{\Delta ln\beta\left(\frac{dc}{dT}\right)}{\Delta ln(1-c)}$.

In these equations, n; reaction order, E; activation energy of the reaction, R; ideal gas constant, T; absolute temperature, β; heating rate and c, mass fraction reacted, as symbolized.

In the Jeres method, the X and Y data of the Freeman-Carroll method corrected with the mathematical form of an application (Figure 4). According to the Jeres method, the decomposition kinetics parameters such as n and E are calculated for the following equations.

$$n = \frac{\bar{Y}}{1-Q\bar{X}} \text{ and } E = \frac{RQ\bar{Y}}{1-Q\bar{X}}$$

Where $\overline{X}$ and $\bar{Y}$ are mean values X and Y, and Q is given as

$$Q = \left[\frac{c'T_{max}^2}{1-c}\right]_{c'=max} = \frac{E}{nR}$$

Here, $c' = \frac{dc}{dT} = \frac{1}{\beta}\frac{dc}{dt}$ and DTG peak maximum transformation rate peak is calculated by reading.

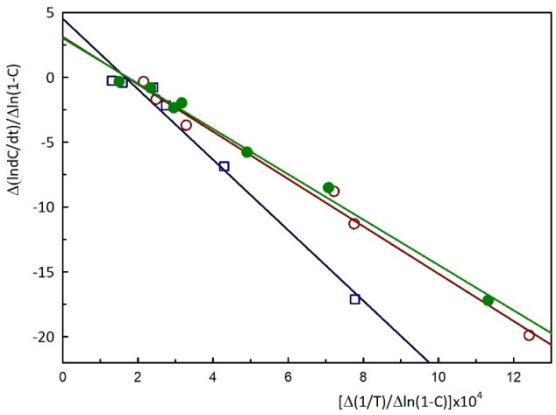


Figure 4. Freeman-Carroll graphs of RSH, □ - NIPAM/AAm, ● - NIPAM/AAm-MPA, o - NIPAM/AAm-MFA

n and E values calculated via both J and FC methods are presented in the Table 3.

Table 3. Kinetic parameters of thermal degradation of SRH

SRH	IDT/ °C	n_{FC}	n_J	E_{FC}/ kJmol^{-1}	E_J/ kJmol^{-1}
NIPAm/AAm	330	4.52	0.17	225.9	118.3
NIPAm/AAm-MPA	322	3.10	0.25	151.5	111.2
NIPAm/AAm-MFA	337	3.00	0.16	144.9	95.3

FC: Freeman-Carroll, J: Jeres

While the n_{FC} values vary between about 3.00 and 4.52, the n_J values are approximately zero for all hydrogels. The zero order is reasonable for the degradation of SRH, because the rate of degradation is independent of the amount of unreacted solid material according to the transition state theory given below.

The Thermal Decomposition Thermodynamics of the SRH. According to the transition state theory the degradation reactions generally can be represented as

solid reactant $\rightleftharpoons$ intermediates* $\longrightarrow$ solid residue + volatile product

The reaction rate is governed by the rate of decomposition of the intermediates, and the rate of formation of the intermediates is assumed to be so rapid that they are present in equilibrium concentration at all times.

$$\frac{dC}{dt} = k\,C^n$$

where C; the amount of the substance still to decompose at time t, n; the order of reaction, and k; the temperature-dependent rate constant. The temperature-dependence of k is given by the Arrhenius equation:

$$k = \mathrm{A}e^{-\frac{E}{RT}}$$

The k and A values are calculated by using the n_J and E_J values at the maximum decomposition rate temperatures, T_{max}.

By using E_J and n_J values from the Jeres method, the enthalpy of activation (ΔH^*), the entropy of activation (ΔS^*) and the Gibbs function of activation (ΔG^*) values at T_{max} were calculated from the following equations[29], and these values are given in Table 4:

$$\Delta S^* = R\left[\ln\left(\frac{hA}{kT}\right) - 1\right] \qquad \Delta H^* = E\text{-}RT \qquad \Delta G^* = \Delta H^* - T\Delta S^*$$

where k and h are Boltzmann's and Planck's constant, respectively.

Table 4. Thermodynamic parameters of the SRH

SRH	T_{max} / °C	ΔH^* / kJmol^{-1}	ΔS^* / Jmol^{-1}K^{-1}	ΔG^* / kJmol^{-1}
NIPAm/AAm	382	112.8	-113.5	187.2
NIPAm/AAm-MPA	360	105.9	-119.0	181.3
NIPAm/AAm-MFA	389	89.8	-150.9	189.7

The ΔG^* values are positive and this suggests that the formation of activated complex molecules doesn't proceed spontaneously, and ΔG^* values of the all SRH approximately were found approximately the same values. The ΔS^* values are negative and this suggests a high ordering of the transition state. The values of ΔH^* and ΔS^* of the hydrogels containing carboxyl groups were found low than neutral NIPAM/AAm polymer.

DSC Analysis. Glass transition temperature (T_g) of the SRH determined with the DSC thermograms (Figure 5). The T_g value of NIPAm/AAm hydrogel (171 °C) is approximately equal to the T_g value of the NIPAm/AAm-MPA hydrogel (173 °C), but it is greater than the Tg value of the NIPAm/AAm-MFA hydrogel (151 °C). The two –COOH groups in the structure of NIPAm/AAm-MFA polymer containing dicarboxylic acid monomer behaves like plasticizer causes the Tg value of this polymer to be lower than the T_g value of NIPAm/AAm hydrogel. Thus,

the dicarboxyl groups in SRH reduce the T_g value of the NIPAm/AAm hydrogel.[27,28]

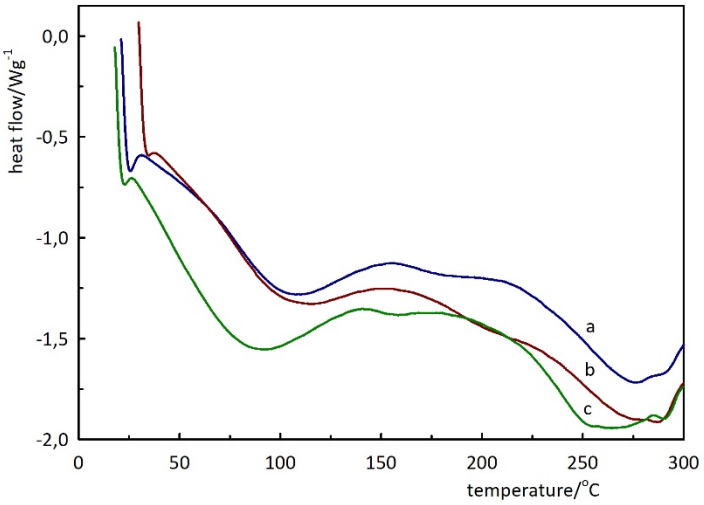


Figure 5. DSC thermograms of the hydrogels, **a** - NIPAM/AAm, **b** - NIPAM/AAm-MPA, **c** - NIPAM/AAm-MFA

- *Swelling Experiments*

The influence of the operating parameters (i.e. pH, temperature, solvent type, solvent concentration) on the SRH swelling performance was performed by measuring the percent of water retained in the hydrogel. The equilibrium swelling; (S%) was calculated using the following relation:

$$S\% = \frac{m_s - m_d}{m_d} \times 100$$

where m_s and m_o were of fully swollen and dried hydrogel mass, respectively.

Temperature sensitive swelling behavior

Swelling values of RSH at pH=3 or 8 and I=0.05 M were obtained at the temperatures in the range of 10-60 ^oC. Temperature dependent swelling graphs were drawn, and a representative graph is shown in Figures 6.

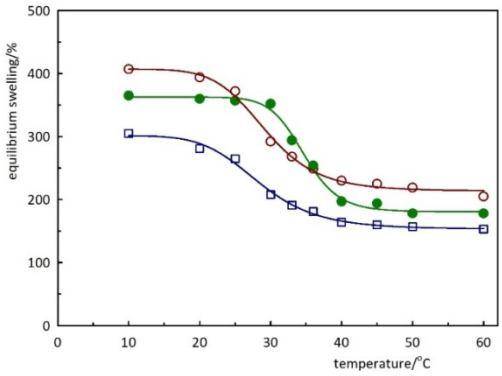


Figure 6. Temperature dependent swelling graphs of the SRH: □ - NIPAM/AAm, ● - NIPAM/AAm-MPA, o - NIPAM/AAm-MFA

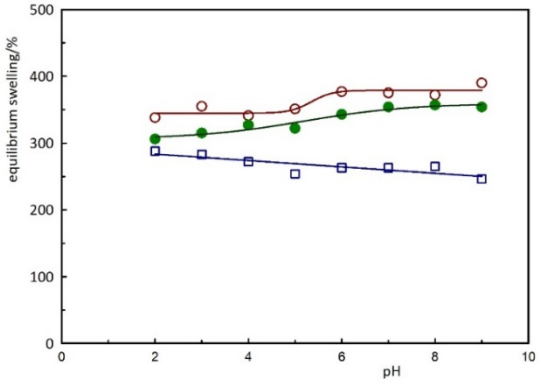


Figure 7. pH dependent swelling graphs of the SRH: □ - NIPAM/AAm, ● - NIPAM/AAm-MPA, o - NIPAM/AAm-MFA

The values of the LCST of the SRH were found by the first derivatives of the curves, and are presented in Table 5.

Table 5. LCST values of the SRH

SRH	pH=3 LCST / °C	pH=8 LCST / °C
NIPAm/AAm	27.65	27.70
NIPAm/AAm-MPA	31.30	28.25
NIPAm/AAm-MFA	34.80	34.70

These SRH swell upon cooling below LCST, and they collapse when heated above the LCST. With the increase in the number of ionizable groups, the volume change at the transition increases because of the

increasing electrostatic interaction between the similarly charged groups and the transition temperature increases accordingly.[27,30,31]

pH sensitive swelling behavior

The temperature-sensitive networks containing ionizable functional groups exhibit pH sensitivity. The effect medium pH on the swelling values of SRH at 25 ^{o}C between pH=2–9 with ionic strength of I=0.05 M is shown in Figure 7.

The values of the inflection point of the SRH were found by the first derivatives of the curves, and are presented in Table 6.

Table 6. Inflection point values of the SRH

SRH	inflection point at T=25 ^{o}C	inflection point at T=40 ^{o}C
NIPAm/AAm	–	–
NIPAm/AAm-MPA	5.05	5.05
NIPAm/AAm-MFA	5.50	5.60

The swelling behavior of the SRH containing carboxylic acid group(s) differs from that of NIPAm/AAm hydrogel at 25 ^{o}C, the nonionic NIPAm/AAm hydrogel is below its LCST and it swells, but there was not a big change in the swelling values with pH, as expected. In contrast, the swelling of the SRH containing carboxylic acid group(s) were strongly dependent on the pH value of the external medium. At low pH values, the degree of swelling was low because the carboxylic groups in the side chains were not ionized and intermolecular complexation via hydrogen bonds may occur (physical crosslinking). As the degree of ionization increases above the nominal pK_a values of carboxylic acid monomers (pKa=4.65 for MPA, pKa_1=3.09 and pKa_2=4.75 for MFA), the increased hydrophilicity resulted

in greater swelling values as reported in the literature for the similar structures.[27,28,32]

Ionic strength sensitive swelling behavior

Ionic strength can play an important role in the swelling behavior. The effect of ionic strength on swelling behavior was investigated at various NaCl concentrations at 25 °C as illustrated in Figure 8.

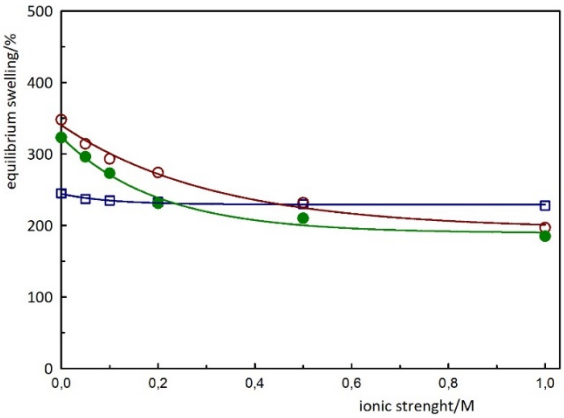


Figure 8. Ionic strength dependent swelling graphs of the SRH, □ -NIPAM/AAm, ● - NIPAM/AAm-MPA, o - NIPAM/AAm-MFA

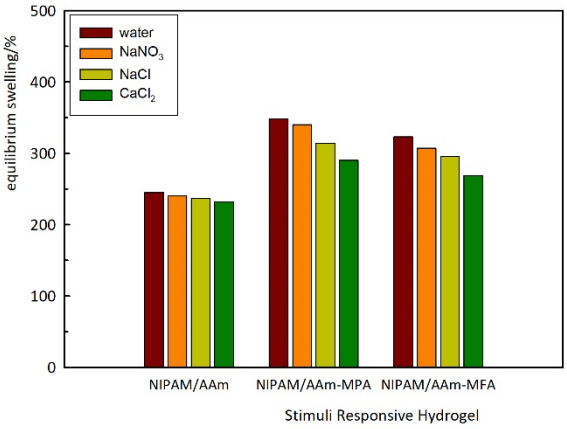


Figure 9. Ion and counter ion dependent swelling graph of the SRH

The swelling value of SRH containing carboxylic acid group(s) decreased as the ionic strength is increased. These phenomena can be

attributed to the electrostatic repulsion between charged groups on the network chain, and the concentration difference between mobile ions inside the hydrogel and external solution.[32-34]

To compare the influence of ion and counter ion for the SRH swelling ratios at equal ionic strength are shown in Figure 9. The swelling ratios decreased according to the following sequence water, $NaNO_3$, NaCl, $CaCl_2$. As the SRH containing carboxylic acid group(s) were swelled in saline solutions, the –COOH groups were neutralized by the cations in the external solution, and the swelling ratios were decreased. When the fixed charges on polymeric side chains were fully neutralized, SRH containing carboxylic acid group(s) showed nonionic behavior like NIPAm/AAm hydrogel. In various saline solutions, hydrogels showed a Donan effect when the charges on the polymeric side chain were neutralized, and then showed a salting-out effect with the gels going to a nonionic state.[35,36]

In the polymer chain, monovalent Na^+ ions interact with a $-COO^-$ unit, a divalent Ca^{2+} ions interact with the two $-COO^-$ units. NaCl solution and $CaCl_2$ solution than in the polymeric chain results in the increased neutralization will reduce the loads on. Consequently, uncharged polymer chains to act as the polymer chains and the swelling value decreases.

Also according to Cl^- ions NO_3^- ions stronger salting precipitant (salting out) due to the inducing action of water molecules around the polymer out of the degree of swelling of the polymer $NaNO_3$ > NaCl was in the form.

The Effect of Solvent on Hydrogel Swellings

Solvent-dependent swelling studies were done using organic solvent-water mixtures and pure organic solvents.

The effect of solvent concentration on hydrogel swellings. The swelling measurements were also carried out in dimethyl sulfoxide (DMSO)-water, and 2-propanol (PA)-water mixtures at 25 °C. The results are demonstrated in Figure 10 and 11, as the dependence of the swelling value in the external solution v/v%.

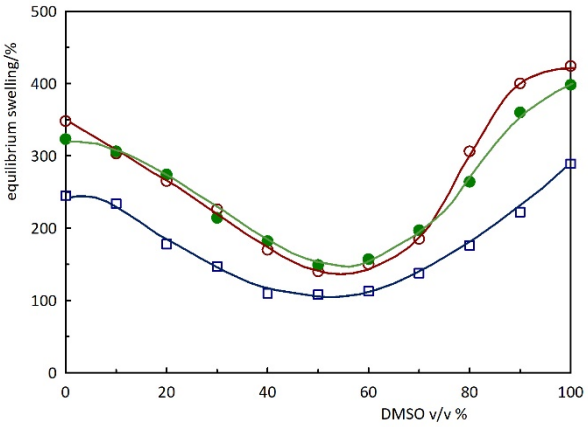


Figure 10. Dimethyl sulfoxide concentration-dependent swelling graphs of the SRH, □ - NIPAM/AAm, ● - NIPAM/AAm-MPA, o - NIPAM/AAm-MFA

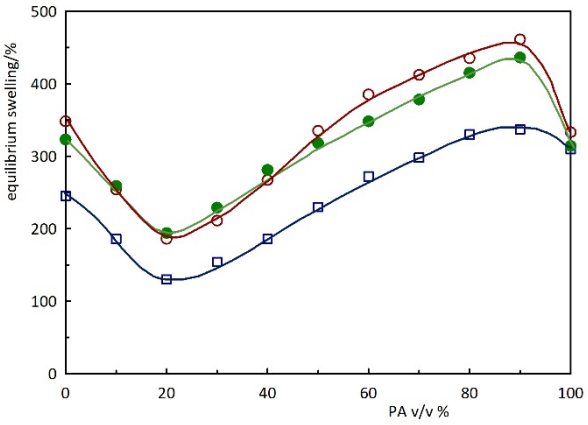


Figure 11. 2-Propanol concentration-dependent swelling graphs of the SRH, □ - NIPAM/AAm, ● - NIPAM/AAm-MPA, o - NIPAM/AAm-MFA

It is seen that all SRH exhibit reentrant conformational transitions in these solvent mixtures. This demonstrates that the isopropyl groups on the network chains are responsible for the observed reentrant phenomena of the hydrogel in DMSO-water mixtures. Thus, water and DMSO taken separately are good solvents for the network. However, in mixtures, the attractive water-DMSO interactions seem to dominate over water–hydrogel or DMSO-hydrogel interactions so that the gel deswells in DMSO-water mixtures. While in the shrunken state, there appears a minimum on swelling curves at V_{DMSO} %=0.50. Figure 11 also shows that both the collapse and recollapse transitions occur earlier, if DMSO is replaced with 2–propanol (V_{PA}%=0.20). The variation of the reentrant phase transition regions depending on the type of the solvent can be explained with higher extent of hydrogen bonding interactions between 2–propanol and water compared to those between DMSO and water. In the presence of a hydrophobic monomer such as NIPAM in the hydrogel, water molecules form clusters around the hydrogel due to the hydrophobic interaction. When 2-propanol is added to water, 2-propanol molecules like to stay in the solution due to the strong interaction between 2–propanol and water, which reduces the gel swelling. The decrease in the gel swelling will increase the intramolecular hydrophobic interactions of the isopropyl groups, which may promote further shrinkage of the hydrogels. At high 2-propanol concentration, attractive polymer-propanol interactions dominate over the 2-propanol-water interactions due to the increased number of contacts between 2-propanol molecules and NIPAm segments. As a result, 2-propanol enters into the gel phase and results in gel swelling. As the 2-propanol concentration is decreased, water-propanol interactions start to dominate so that the gel deswells. Thus, the competing attractive interactions between

water–solvent and polymer–solvent result in the reentrant conformational transitions in the network.[37-39]

The effect of solvent type on hydrogel swellings. The hydrogels were immersed in large amounts of solvents (benzene, tetrahydrofuran, acetone, 1-butanol, 1-propanol, ethanol, methanol and ethanolamine) having increased solubility parameter values at 25 °C until equilibrium was attained. Swelling ratio of the hydrogels ($Q=V_s/V_d$) were calculated by assuming additively of volumes, where V_s and V_d are the volumes of swollen and dry gel samples, respectively. The relation between the swelling ratio of the hydrogels and solubility parameter of various solvents are given in Figure 12.

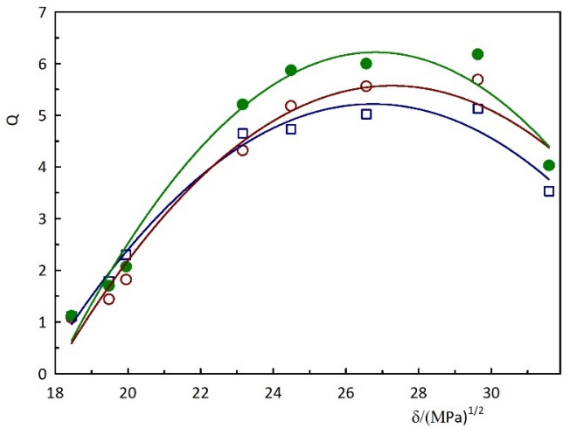


Figure 12. Solvent type dependent swelling graphs of the SRH, □ - NIPAM/AAm, ● - NIPAM/AAm-MPA, o - NIPAM/AAm-MFA

The solubility parameters[40,41] of SRH which were found the maximum of the curves in Figure 12 are given in Table 7. The solubility parameters of SRH are found to be in the range 27.58–28.19 $(MPa)^{1/2}$.

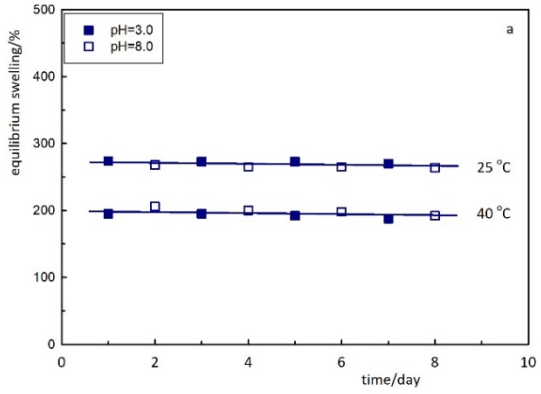
Table 7. Solubility parameters of used organic solvent[40] and hydrogels

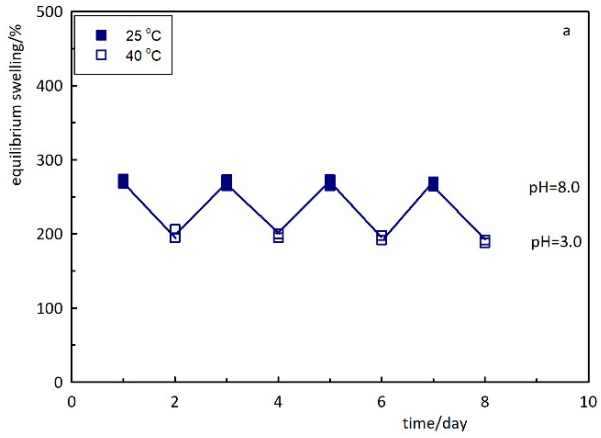
Solvent	$\delta_{solvent}$ / $(MPa)^{1/2}$	hydrogel	$\delta_{hydrogel}$ / $(MPa)^{1/2}$
benzene	18.45	NIPAm/AAm	27.58
tetrahydrofuran	19.48	NIPAm/AAm−MPA	27.62
acetone	19.95	NIPAm/AAm−MFA	28.19
1-Butanol	23.16		
1-Propanol	24.49		
ethanol	26.56		
methanol	29.63		
ethanolamine	31.59		

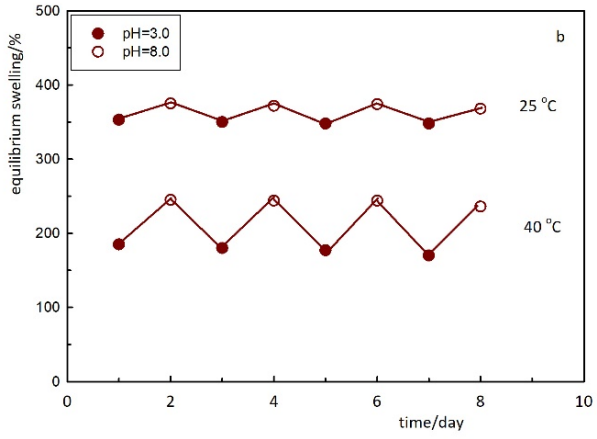
Reversible Swelling Behavior

Reversible swelling experiments for hydrogels were also performed at two extreme pH (below and above the dissociation values of carboxyl units, 3.0 and 8.0, respectively) and at the two extreme temperature (below and above the LCST values, 25 and 40 ^{o}C, respectively). Further, the selected high temperature value is slightly above the body temperature, while the selected high pH value is slightly above the pH value of most body fluids.

Each hydrogel was placed in a buffered solution at different pHs for 24 h, and its equilibrium swelling values were determined. Figure 13 shows the plot obtained from such swelling experiments.




25 °C
40 °C
a
pH=8.0
pH=3.0
equilibrium swelling/%
time/day
0
100
200
300
400
500
0
2
4
6
8
10


pH=3.0
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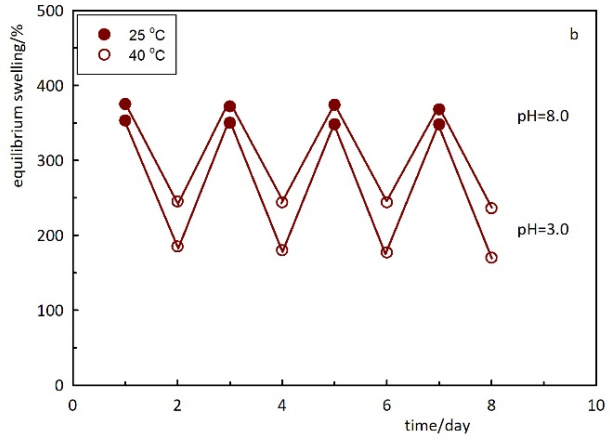
25 °C
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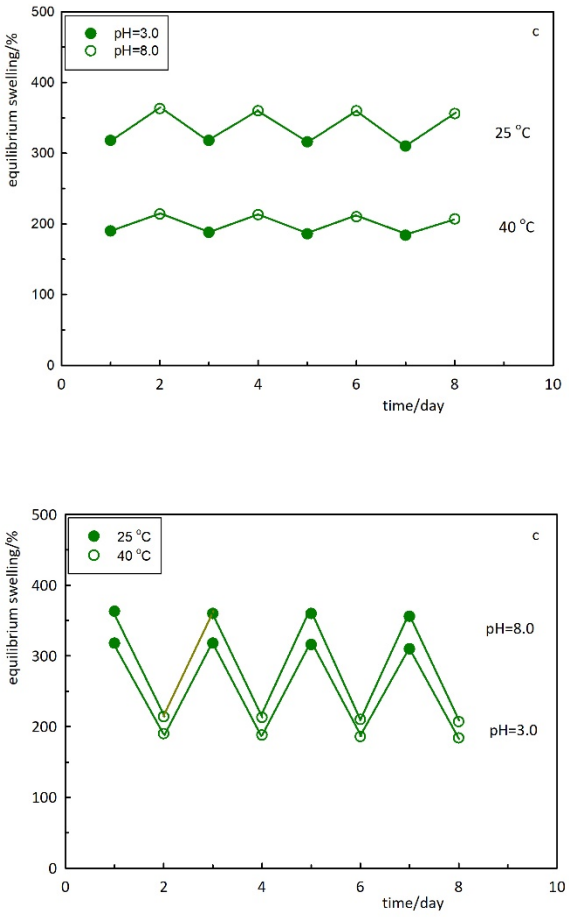

Figure 13. Reversible swelling profiles of SRH: a. NIPAM/AAm, b. NIPAM/AAm-MPA, c. NIPAM/AAm-MFA.

In the reversible swelling of neutral NIPAm/AAm hydrogel with extreme pH and temperatures, there is no change in the swelling behavior. Because of carboxylic acid unit(s) on NIPAm/AAm-MPA and NIPAm/AAm-MFA hydrogels, they show the swelling behavior of pH-dependence. At pH=8.0, negatively charged hydrogel increases electrostatic repulsion between the chains, and at pH=3.0, uncharging/discharging decrease the swelling. So, the hydrogels swell high at high pH, show relatively shrinkage at lower pH of the medium. It is important to note that

hydrogels retained its shape and integrity during the whole period of the experiments (8 days). From this result, it can be presumed that hydrogels are mechanically the durable.

In addition, the temperature and pH responsibility of the SRH were found from the difference in equilibrium swelling values and these values are given in Table 8.

Table 8. Temperature and pH responsibility of the SRH

SRH	Temperature responsivity		pH responsivity	
	At pH=3	At pH=8	At T=25 ^{o}C	At T=40 ^{o}C
NIPAm/AAm	78	65	7	7
NIPAm/AAm-MPA	163	128	23	64
NIPAm/AAm-MFA	119	149	44	24

By taking the advantage of temperature responsivity of N-isopropyl acrylamide monomer, and pH and ionic strength responsivity of carboxylic acid containing monomers, and the mechanical strength of acrylamide monomer, a model multi stimuli responsive hydrogels can be prepared as durable, homogenous appearance materials in cylindrical geometry as a *stimuli responsive* hydrogel or a *smart* polymer.

- *Adsorption of Albumin*

Commercial human serum albumin (HSA) and human blood serum were used in the albumin adsorption experiments. The amount of albumin in human blood serum was found as 3.8 g dL^{-1}. Commercial HSA and albumin and albumin in human blood serum concentration of 10 mg L^{-1} were prepared and in universal buffer solution, pH=3.0. Into these solutions, 0.1 g of dry RSH were placed and incubated in a water bath for 24 h at 40 ^{o}C. The amount of adsorbed albumin from human blood serum and commercial HSA solution (Adsorption %) were calculated using the equation;

$$Adsorption\ \% = \frac{C_o - C_e}{C_o} \times 100$$

where C_o and C_e were the initial and equilibrium concentration of albumin in mg L^{-1}, respectively.[27,42-44]

The bar graphs of albumin adsorption onto SRH are presented in Figure 14.

HSA adsorption from human blood serum was less than that of commercial available one. Because of the complex structure of human blood serum, albumin adsorption of this solution is lower. The adsorption of albumin in human blood serum, and all the SRH are almost identical, i.e., do not change too much with the type of carboxylic acid monomer in the hydrogel structure. However, hydrogels that stimuli-responsive can be used as chromatographic separations and can be prefer in different formulations e.g., NIPAm/AAm-MFA hydrogel for commercial HSA, and human blood serum. In any case these types of material can be used in biomedical fields for various purposes.

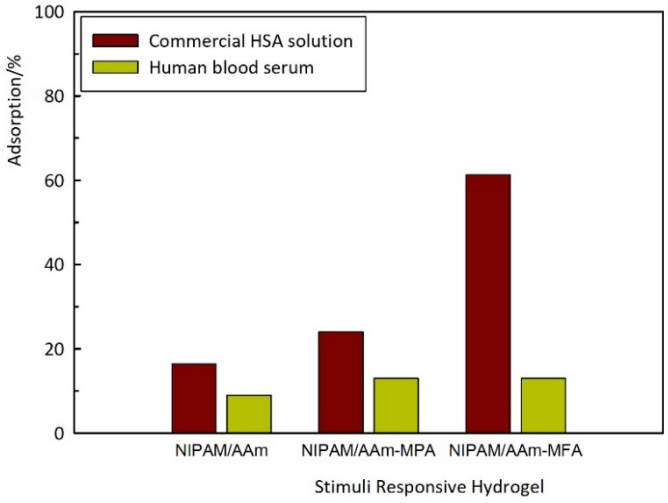

Figure 14. The bar graphs of albumin adsorption onto SRH

Experimental

- *Chemicals*

N-isopropyl acrylamide (Aldrich, Milwaukee, USA), acrylamide (Merck Darmstadt, Germany), methacrylic acid (Sigma, St. Louis, USA), and mesaconic acid (Aldrich, Milwaukee, USA) as monomer or co-monomer, N,N'-methylenebis acrylamide (Merck, Schuchardt, Germany) as crosslinkers, ammonium persulfate (Merck, Schuchardt, Germany) as redox initiator and N, N, N', N'-tetramethylethylenediamine (Sigma, St. Louis, USA) as catalyst were analytical grade, and were used as received. Double-distilled water was used for all the experiments.

- *Preparation of Hydrogels*

Hydrogels composed of NIPAm, AAm and carboxylic acid group containing monomers were prepared by thermal free radical solution polymerization in the presence of a crosslinker, NBis. An aqueous solution of monomers containing NBis with APS and TEMED were mixed and placed in PVC straws of 3 mm in diameter. The amount of substance used in the preparation of the hydrogels (in moles) were given in Table 9.

Table 9. The amount of substance used in the preparation of hydrogels (in moles)

SRH	NIPAM	AAm	MPA	MFA	Bis	APS	TEMED
NIPAm/AAm	8.1	1.0	-	-	0.5	0.1	0.1
NIPAm/AAm-MPA	8.1	1.0	0.9	-	0.5	0.1	0.1
NIPAm/AAm-MFA	8.1	1.0	-	0.9	0.5	0.1	0.1

The hydrogels were prepared in thermostated water bath at 70 ^{o}C for 24 h. The resulting hydrogel rods were cut into pieces of 3–4 mm length and washed with distilled water and dried in air and vacuum, and stored. The

hydrogels were named as NIPAM/AAm, NIPAM/AAm-MPA and NIPAM/AAm-MFA, respectively.

- *Characterization*

FTIR spectra were recorded with FTIR Nicolet-520 spectrofotometer in the 4000–400 cm^{-1} range, on grinded hydrogel pelled with KBr, and 30 scans were taken at 4 cm^{-1} resolutions.

Thermogravimetric analysis was carried out using TG and DSC (Shimadzu-50 model Thermal Analyzer). Thermogravimetric analyses were performed employing 10 mg samples in a platinum pan heating up to 600 ^{o}C under nitrogen gas flow rate of 25 mL min^{-1} with a heating rate of 10 ^{o}C min^{-1}.

- *Swelling Measurement*

Swelling studies of the hydrogels were done gravimetrically as equilibrium, and reversible swellings in different aqueous media. For the temperature dependent swelling studies, first, hydrogels were swollen in NaOH solution at pH 8.0 and heated to various temperatures to determine the range of the LCST phase transition. For the pH-dependent swelling studies, hydrogels were swollen in the different pH of solutions using HCl and NaOH ranging from 2 to 9 at 40 ^{o}C. The total ionic strength of solutions was fixed at 0.05 M NaCl. The effect of ionic strength on swelling behavior was investigated using various NaCl concentrations (I=0.05–1.0 M) at 25 ^{o}C. The influences of various ions for the hydrogels swelling were examined at equal ionic strength using NaCl, $NaNO_3$, and $CaCl_2$ solutions. The effects of different organic solvents such as benzene, tetrahydrofuran, acetone, 1-butanol, 1-propanol, ethanol, methanol, ethanolamine, 2-propanol and dimethylsulfoxide on hydrogel swelling were inspected. The incubation time of 24 h at 25 ^{o}C were chosen. The amount mass increase was calculated by removing hydrogel from the swelling medium and weighing. The effects of temperature and pH on swelling behavior of the hydrogels

were investigated by reversible swelling experiments in which hydrogels were swollen to their maximum swelling values consecutively between pH 3 and 8 at 25 ^{o}C for 24 h, respectively, before weighing. The same procedure was repeated at 40 ^{o}C that is above LCST.

Conclusions

- A new series of hydrogels (NIPAm/AAm, NIPAm/AAm-MPA, NIPAm/AAm-MFA) was synthesized in aqueous solution by the thermal free radical polymerization method and investigated in terms of swelling.
- Spectroscopic and thermal analyses were performed for the structural and thermal characterizations of the hydrogels.
- The thermal and swelling properties of the hydrogels depend on the number of carboxyl acid units and the isopropyl groups in the polymer network.
- LCST values of hydrogels varied with the number of carboxyl acid units in the hydrogel.
- LCST value of the NIPAm/AAm-MFA hydrogel is near body temperature.
- Reversible equilibrium swelling studies have shown that hydrogels mechanically stable for a long time.
- The NIPAm/AAm, NIPAm/AAm-MPA, NIPAm/AAm-MFA hydrogels can be defined as stimuli responsive hydrogels because they are sensitive to temperature, pH, ionic strength, salt type, solvent concentration and solvent type.

In conclusion, the NIPAm/AAm, NIPAm/AAm-MPA, NIPAm/AAm-MFA stimuli responsive hydrogels can be used in applications such as biocompatibility, immobilization of proteins or biologically active molecules, drug release, and the environmental applications.

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