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A colorimetric iron sensor based on the partition of phenanthroline complexes into polymeric hydrogels. Combinatorial synthesis and high throughput screening of the hydrogel matrix

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ABSTRACT

A combinatorial library of hydrogels is produced by radical polymerization and tested for retention of iron (II) complexes, using a simple colorimetric high throughput screening (HTS). Six acrylamide monomers are used to produce a library of 21 cross-linked hydrogel matrixes. The swelling of all hydrogels is determined and related with the structure of each monomer unit present. The HTS shows that some materials are able to retain the tris(phenanthroline)iron(II) ($Fe(Phen)_3^{2+}$) complex. The partition coefficient of $Fe(H_2O)_6^{2+}$ and $Fe(Phen)_3^{2+}$ in all hydrogels is determined by UV–vis spectrophotometry and related with the presence of anionic (e.g. $-SO_3^-$), hydrophobic (e.g. isopropyl) and hydrophilic (e.g. $-NH_2$ of acrylamide) groups. Hydrogels with sulfonic ($-SO_3^-$) groups show large values of partition coefficients of Fe^{2+} into the hydrogel (>500). Based on the data and HTS, poly(acrylamide-*co*-50%(2-acrylamido-2-methylpropansulfonic acid)) (PAAm-*co*-50%AMPS) is selected to build a colorimetric sensor. Since the phenanthroline could be released from the hydrogel, bathophenanthroline (BPhen) which is not released is used as ligand. The hydrogel is loaded with BPhen and exposed to Fe^{2+} in water. The visual detection of color gives a detection limit of 0.1 ppm. Little interference of different common ions is found. A special set-up is made which allows spectrophotometric measurement of the complex inside the gel. The detection limit is found to be of ca. 0.01 ppm by spectrophotometry. Using the sensor, the iron content in milk is measured directly demonstrating the use of the sensor to measure free iron in opaque liquids where solution spectrophotometry is not effective without complex sample pretreatments. The non-covalent retention of ligands and complexes inside polymeric hydrogels seems to be a suitable method for building specific ion sensors.

1. Introduction

Hydrogels are defined as hydrophilic three-dimensional polymeric networks. Due to hydrophilic groups present in their polymeric chains such as —OH, —COOH, $—NH_2$, $—CONH_2$, or $—SO_3H$, they can absorb water up to hundreds of times their dry mass. Some of them have the ability to change shape and volume in response to external stimuli and there are called smart hydrogels [1]. Smart properties allow to use these materials as drug delivery systems and water decontamination [2]. Hydrogels and composites have been employed as materials for the removal of metal ion species [3]. Anionic homopolymers (e.g. polyacrylic acids) or copolymers have been used for removing a variety of metals, such as Fe^{2+}, Zn^{2+}, Cu^{2+}, Co^{2+} and Cr^{3+} [4], retained trough coulombic intercations. Alternatively hydrogels containing cationic units have been used to remove anionic dyes [5] or toxic arsenic anionic species [6]. Although these methods are very effective, good selectivity is not achieved with respect to a particular species using simple coulombic interactions. The World Health Organization (WHO) recommends that iron concentration would be less than 0.3 mg/L for potable water [7]. Therefore, the building of colorimetric iron sensors with quick responsive and/or systems able to lower the iron content of water by retention are of great interest.

Ozay et al. [8] synthesized in a first stage a monomer based on Rhodamine ((Rhodamine-6G)lactam-N′acryloyl-ethylenediamine (RH6GAC)). This was copolymerized with non-ionic monomers as 2-hydroxyethylmethacrylate (HEMA) and acrylamide (AAm) in order to obtain PHEMA-*co*-RH6GAC and PAAm-*co*-RH6GAC hydrogels using *N,N′*-methylenebisacrylamide (MBA) as crosslinker. Despite these copolymers detect visually and selectively Fe^{3+}

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ions, the lowest detection limit achieved is 1 ppm. In addition, the method of obtaining the monomer is quite complex, since it involves several synthetic steps. Moreover, the amount of chelating introduced was limited to less than 5% mol ratio because an increasing in the component concentration alters the gelation or thermosensitivity.

In a similar way, Vallejos et al. synthesized a copolymeric acrylamide containing 8-hydroquinoline pendant groups and developed a colorimetric sensor for iron [9]. Again, the chemical synthesis of the monomer bearing the chelating group is quite complex. In both works only one non functionalized co-monomer is used. Therefore, possible effects of the main polymer component on the detection are not studied.

It is known that Fe^{2+} and Fe^{3+} form stable colored complexes with a small number of organic ligands being some of them poorly soluble in water. Those properties have been used to extract inorganic ions into organic solvents by biphasic system for the colorimetric detection of the ions concentration in water [10]. During the investigation of hydrogels for drug carrier/release applications we found that organic molecules and metal complexes could be partitioned inside polymeric hydrogels [2,11]. We could obtain high values of partition coefficients (Cp) hydrogel/water, likely due to hydrophobic interactions between the molecules/ions and the hydrogel matrix.

On the other hand, we have recently reported a novel alternative method to incorporate an conducting polymer (polyaniline, PANI) inside a poly(*N*-isopropylacylamide) (PNIPAM) hydrogel (by swelling of hydrogel) in a true solution of PANI [12] using *N*-methyl pyrrolidone as solvent. Being as PANI is slightly soluble in water or common organic solvents, we have chosen a solvent which dissolve PANI and also swell effectively the hydrogel. The swelling of a crosslinked matrix requires for mobile chains (between crosslinking points) to be soluble in the solvent. Otherwise, the solute will not load into the hydrogel. Therefore, a good organic solvent (different to water) for the polymeric chains are able to swell the hydrogel and allows the incorporation of organic molecules with low solubility in water, but which are soluble in the organic solvent. It is noteworthy that some hydrogels which are swollen in water could not be swollen in NMP (e.g. poly((3-acrylamidopropyl)trimethylammonium chloride)). Therefore a hydrogel/solvent pair should be found in each case. As discussed before, a helpful criterion is the solubility of the linear polymer, which gives the mobility to chains in the solvent. The same criteria could be used to incorporate an organic molecule inside hydrogel.

There is a wide selection of ligands which gives colored complexes [13,14] with Fe^{2+}. Moreover, some ligands are poorly soluble in water but soluble in organic solvents and used in liquid/liquid analytical extraction [15,16]. The formation of tris(phenanthroline)iron(II) inside a hydrogel based on methacrylic acid was studied before [17]. It was found that the complex could ionically crosslink the weakly crosslinked hydrogel. However, we are presenting a study more extensive about behavior of complex/hydrogel systems as colorimetric sensors.

Based on our results, we propose a new method of incorporation of chelating agents inside hydrogels and its use to detect trace of iron ions in polluted waters. A series of different hydrogels were obtained by combinatorial synthesis [18]. Combinatorial chemistry is a powerful tool to discover new chemical compounds [19] or materials [20], including polymers [21–23], with defined functions, therefore this has been applied successfully to the selection of materials in hydrogel libraries [24]. The library consists in an orthogonal array of copolymers obtained by copolymerization of six commercially available acrylic monomers (in a molar 1:1 feed ratio), and the respective homopolymers.

Subsequently the dry hydrogels were swollen in ethanol/water solution (containing phenanthroline (Phen)). In that way, the ligand agent/hydrogel matrix (LHM) was obtained. Then, the detection sensibility towards Fe^{2+} was screened by a HTS (High Throughput Screening) procedure [25], based on the colorimetric detection of the complex formation inside the gel and the determination of partition coefficients (Cp) of Fe^{2+} inside each of materials. The method allowed identifying those materials which exhibit the better properties of: I) retention of colored complex into the hydrogel matrix, II) high absorption values of metallic cation.

Next, the best materials were characterized with respect to their ability to swell in ethanol/water solutions (from 0% v/v up to 99.5% v/v) to be able to load batophenanthroline (BPhen). The Cp values of Fe^{2+} inside hydrogels load with phenanthroline (Phen) and bathophenanthroline (BPhen) were determined by UV–vis spectroscopy through a peak at 510 nm to $[(Phen)_3Fe]^{2+}$. The hydrogels with 2-acrylamido-2-methylpropanesulfonic acid (AMPS) monomeric units in their structures (e.g. PNIPAM-*co*-50%AMPS, PAAm-*co*-50%AMPS and PAMPS) show larger partition coefficients of Fe^{2+} in aqueous medium. PAAm-*co*-50%AMPS was selected as the better material to build a visual sensor of iron with a detection limit of 0.1 ppm. Using spectrophotometry, the detection limit is extended to 0.01 ppm. The sensor is shown to be able to determine directly the iron content in milk, where direct solution colorimetry of the complex could not be performed due to the opacity of the sample. The simple and versatile procedure should allows detecting other ions by changing the chelating agent and, if necessary, the hydrogel matrix.

2. Materials and experimental methods

2.1. Hydrogel combinatorial synthesis

2.1.1. Monomers

Hydrogels are synthesized by free radical polymerization using the following monomers: *N*-isopropylacrylamide (NIPAM) (Scientific Polymer Products), acrylamide (AAm) (Fluka), 2-acrylamido-2-methylpropanesulfonic acid (AMPS) (Scientific Polymer Products), acrylic acid (AA)(Aldrich), *N*-hydroxyethylacrylamide (HEAA) (Aldrich), and (3-acrylamidopropyl)trimethylammonium chloride (APTMAC) (Aldrich). Scheme 1 shows the respective chemical structures.

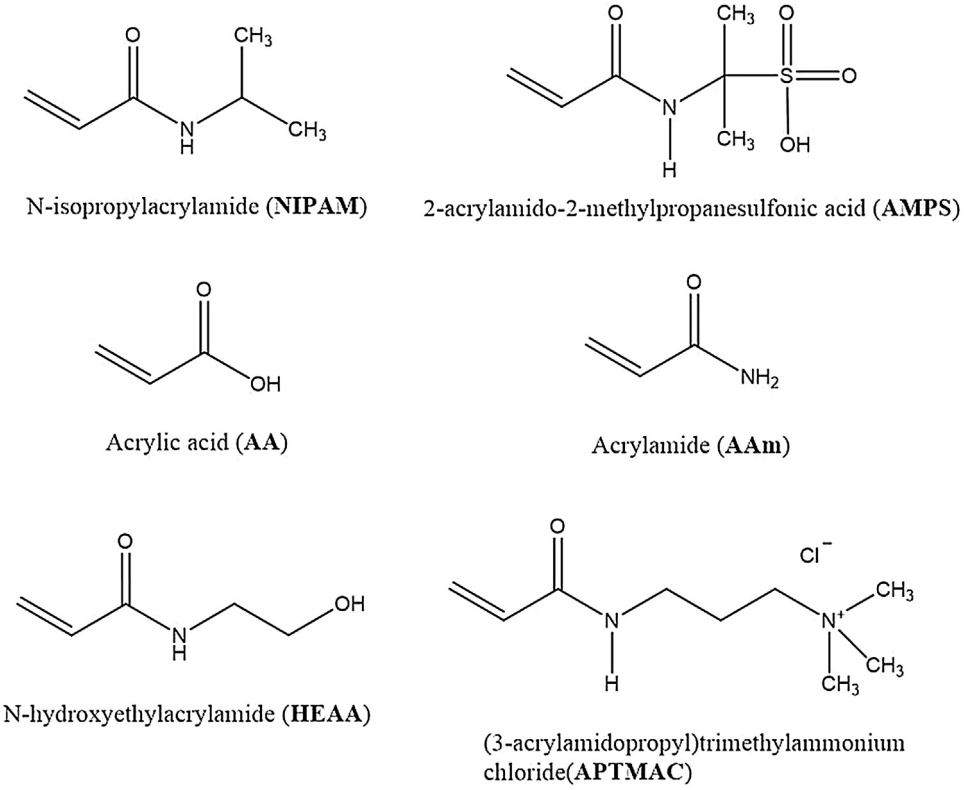
Since a single polymerization procedure was not able to produce hydrogels with all monomers, the free radical polymerization were also initiated using either a thermal initiators (2,2 '-azo bis [2-(2-imidazolin-2-il)propano] dihydrochloride (VA044) and 2,2′-Azobis(2-methylpropionamidine) dihydrochloride (V50)) which works at temperature above 60 °C or a redox initiator (Ammonium persulfate (APS) and *N,N,N′,N′*-tetramethylenediamine (TEMED)) which works at room temperature. In all cases, 2% (mole ratio regarded total monomers) of *N,N′*-methylenbisacrylamide (BIS) (Aldrich) is added as crosslinker.

Table 1 presents the combination different of monomers to synthesized hydrogels.

The order of the monomers is unimportant since these were synthesized in molar ratio 1: 1. Combinatorial synthesis gives rise to a lot of materials in a short period of time, for that the studying of property must do quickly and automatically. A library of 21 hydrogel matrixes was created. Synthesized materials are ordered in Table 1, considering firstly, synthesized homopolymers from 1 to 6, and synthesized copolymers from 7 to 21.

2.1.2. Polymerization by a thermal initiator system

The radical polymerization was initiated thermally by 2,2′-azobis [2-(2-imidazolin-2-il)propano] dihydrochloride (VA044)



Scheme 1. Chemical structure of the monomers used.

Table 1
Monomeric concentrations and type of initiators used in the combinatorial synthesis of hydrogels.

Monomers	N°	Concentration [M]	INITIATOR
NIPAM	1	0.5	APS/TEMED
AAm	2	0.5	APS/TEMED
AMPS	3	2	V50
AA	4	2	V50
HEAA	5	0.5	APS/TEMED
APTMAC	6	2	APS/TEMED
NIPAM-AAm	7	0.5	APS/TEMED
NIPAM-AMPS	8	2	APS/TEMED
NIPAM-AA	9	0.5	APS/TEMED
NIPAM-HEAA	10	0.5	APS/TEMED
NIPAM-APTMAC	11	2	APS/TEMED
AAm-AMPS	12	2	V50
AAm-AA	13	2	APS/TEMED
AAM-HEAA	14	0.5	VA044
AAm-APTMAC	15	2	V50
AMPS-AA	16	2	V50
AMPS-HEAA	17	0.5	VA044
AMPS-APTMAC	18	0.5	APS/TEMED
AA-HEAA	19	0.5	VA044
AA-APTMAC	20	2	V50
HEAA-APTMAC	21	0.5	VA044

(Aldrich) and 2,2′-Azobis(2-methylpropionamidine) dihydrochloride (V50) (Aldrich). The azo initiators decompose at temperatures of 44 °C y 56 °C respectively, generating radical initiators.

The monomers and cross-linker were dissolved in buffer solution pH 10 (H_3BO_3/Na(OH)/KCl) until final volume of 4 mL. The prepolymeric solution was purged by bubbling with N_2 gas and then the initiator system (0.003 g/mL) was added. The feed composition of each homopolymer and copolymer is shown in Table 1. The polymerization was carried out in a sealed glass tube into a water bath at 60 °C during 15 min to allow the initiation of polymerization. The tube was left in the bath until the polymerization was completed. The monolithic hydrogels were then immersed in distilled water at room temperature during 48 h. The water was renewed several times in order to remove unreacted chemicals. Then, the hydrogel pills were dried in an oven at 50 °C.

2.1.3. Polymerization using a redox initiator system

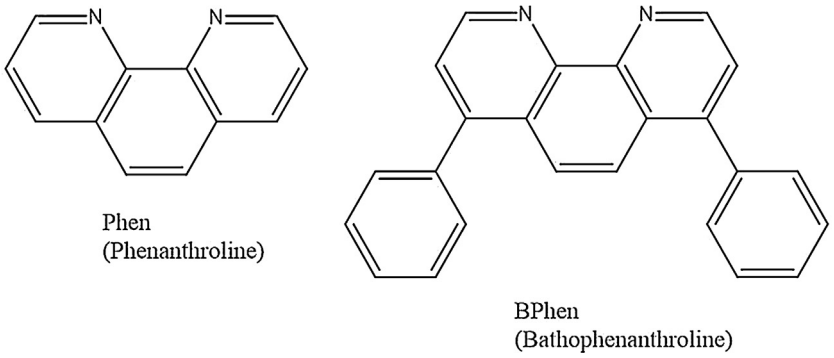
Ammonium persulfate (APS) (Aldrich) and *N,N,N′,N′*-tetramethylenediamine (TEMED) (Aldrich) was used as the initiator system of polymerization [26]. Monomers and cross-linker agent (BIS) were dissolved in aqueous solution until final volume of 4 mL. Next, the solution was purged by bubbling with N_2 gas and polymerization initiator system (APS- (0.001 g/mL)) and (TEMED- (10 μL/mL)) was added. The polymerization was carried out in a sealed glass tube at room temperature (20 °C) for 3 h. When the polymerization was completed, the hydrogels were immersed in distilled water at room temperature during 48 h and the water was renewed several times in order to remove unreacted chemicals. Then, pills of hydrogel were dried in oven at 50 °C.

2.2. Measurements of swelling capacity in water

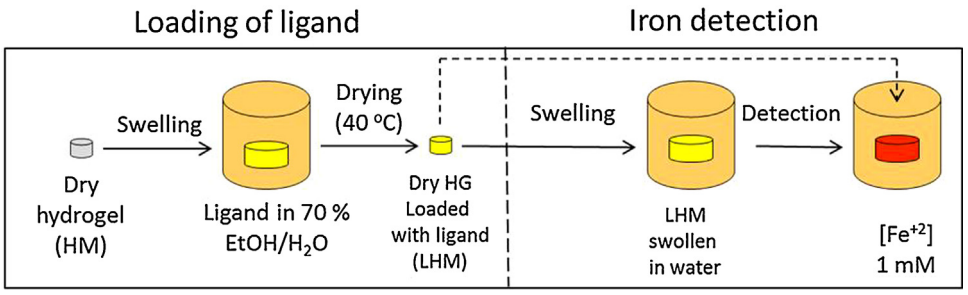
Firstly, dry hydrogel samples previously washed and weighed was placed in water at room temperature. After 24 h (necessary time to achieve the swelling equilibrium condition) the sample was removed from solution, was superficially dried with tissue paper, and was weighed in an analytic balance. The measurements were repeated until sample achieved the constant weight. The swelling percentage (% Sw) is calculated as:

$$\%Sw = \frac{(Weq - Wd)}{Wd} * 100 \quad (1)$$

Where Weq represents the weight of swollen hydrogel in equilibrium state and Wd is the weight of dry hydrogel. Every data of% Sw obtained was the averaged of five measurement with absolute



Scheme 2. Chemical structure of the ligands (L) used.



Scheme 3. Experimental steps for ligand loading and Fe^{2+} complexation into hydrogels.

error around ±150. This characterization was made to the complete library of hydrogels.

2.3. Measurements of swelling capacity in ethanol/water

Measurements of the volume swelling ratio (Sw_v) at 20 °C were made for PNIPAM, PNIPAM-co-50%AMPS, PAAm-co-50%AMPS and PAMPS in ethanol/water solution different from 0%v/v up to 99.5%v/v of ethanol. Dried sample of hydrogel, previously weighed (Wd), was placed in solvent mixtures of concentration different (x) at room temperature. After 48 h, necessary time to achieve the swelling equilibrium condition, was recorded the wet gel weight ($W_{eq,x}$) placing the gel into a ground joint vial with hermetic closure to prevent evaporation of ethanol.

The equilibrium volume swelling ratio of the hydrogels is expressed as the volume of ethanol/water mixture that able to absorb the gel respect at water volume that incorporates the same pill when is immersed in pure water. It is calculated as (Eq. (2)):

$$Sw_v = \frac{V_x}{V_w} = \frac{\left(W_{eq,x} - Wd\right)/\rho_{x,T}}{\left(W_{eq,w} - Wd\right)/\rho_{w,T}} \quad (2)$$

Where Weq,x represent the mass of the swollen gel in the ethanol/water mixture (x),Weq,w is the weight of wet gel in pure water, $\rho_{x,T}$ is the ethanol/water mixture (x) density [27] and $\rho_{w,T}$ is water density at 20 °C.

The volume of water (V_w) incorporated by the gel matrix could be estimated from the swelling percentage in water (%Sw) (Eq. (3)).

$$V_w = \frac{\%Sw * Wd}{100 * \rho_{w,T}} \quad (3)$$

2.4. Ligand loading process and subsequent absorption of Fe^{2+} by hydrogels

Phenanthroline (Phen) and Bathophenanthroline (BPhen) were the ligands used to complex Fe^{2+}. Scheme 2 shows the chemical structures.

Phen (Anedra) was the first ligand used to determinate the partition coefficient screening on the hydrogels library created. Phen is moderately soluble in water, for that it was dissolved in ethanol/water solvent (70:30) previous to load.

The dry gel was swollen in 1 mM of Phen solution for 48 h and then was dried in stove at 40 °C to remove the solvent. The dry ligand/hydrogel was then swollen during 48 h in Fe^{2+} aqueous solution [1 mM] ($SO_4Fe(H_2O)_7$ (Mallinckrodt)) with excess of hydrazine to avoid the oxidation of Fe^{2+} to Fe^{3+}. Scheme 3 shows a representation of the complete process.

Then, a small group of hydrogels with $-SO_3^-$ groups was selected to study the retention of iron from the incorporation of other water insoluble ligand, bathophenanthroline.

The loading of ligand takes place by equilibration with the solution of ligand in $EtOH/H_2O$:

$$HM + L \rightleftharpoons HML \quad (4)$$

In all cases the absorption process of Fe^{2+} was studied by three different ways:

i) absorption by hydrogel matrix without ligand (**HM**)

$$HM_{dry} + Fe^{2+}{}_{aq} \rightleftarrows HM_{sw}/Fe^{2+} \quad (5)$$

ii) direct absorption of ion by the ligand/hydrogel matrix (**HML**$_{dry}$) during swelling

$$HM_{dry} + Fe^{2+}{}_{aq} \rightleftarrows HML_{sw}/Fe^{2+} \quad (6)$$

iii) swelling of the dry ligand-hydrogel in water (**HML**$_{dry}$), followed by diffusion of the ion inside the swollen ligand/hydrogel matrix (**HML**$_{sw}$)

$$HM_{dry} + H_2O \rightleftarrows HML_{sw} \quad (7)$$

$$HM_{dry} + Fe^{2+}{}_{aq} \rightleftarrows HML_{sw}/Fe^{2+} \quad (8)$$

2.5. High throughput screening of $Fe(Phen)_3{}^{2+}$ retention inside HML

The High-Throughput Screening (HTS) [28] is an experimental process used to identify the desired materials according to defined properties. Detection of the capacity of the hydrogels to absorb Phen-Fe^{+2} complex was qualitatively tested analyzing the complete hydrogel library. Pieces of dry hydrogel previously loaded with ligand (HML_{dry}), were swollen in an aqueous solution of Fe^{2+} (1 mM) at 25 °C during 24 h (Scheme 3). Next, photographs were taken to record the coloration of the hydrogels.

2.6. Measuring the partition coefficients of Fe^{2+} and $Fe(Phen)_3{}^{2+}$ inside the synthesized hydrogels

In order to determinate quantitatively the partition of Fe^{+2} between hydrogel and water, the determination of partition coefficients Fe^{2+} were carried out in hydrogel matrixes (HM) library with Phen incorporated previously [2].

The mass balance of Fe^{+2} in the initial and the equilibrium conditions, allows us to determine the partition coefficient (Cp) defined by Eq (9) as the molal concentration of metal into the hydrogel relative to molality of metal in solution, once equilibrium is reached. Knowing also the swollen gel mass could be calculated the Cp values of Fe^{2+} with the follow equations:

$$Cp = \frac{[M^{+n}]\,hydrogel}{[M^{+n}]\,solution} \quad (9)$$

$$\left[M^{+n}\right] = \frac{m_{hydrogel}}{W_d} * 1000 \quad (10)$$

$$m_{hydrogel} = mol_{initial} - mol_{final} \quad (11)$$

$$mol_{initial} = C_{initial} * V \quad (12)$$

$$mol_{final} = C_{final} * \left[V - \left(W_{equilibrium} - W_d\right)\right] \quad (13)$$

Where $\boldsymbol{m_{hydrogel}}$ represents the metal moles inside hydrogel, $\boldsymbol{mol_{initial}}$ is the initial metal moles in solution, $\boldsymbol{mol_{final}}$ is the final metal moles when equilibrium is reached, $\boldsymbol{V}$ (L) is solution volume, $\boldsymbol{W_{equilibrium}}$ represents the weight of swollen hydrogel when equilibrium is reached, $\boldsymbol{W_d}$ is the weight of dry hydrogel, $\boldsymbol{C_{initial}}$ and $\boldsymbol{C_{final}}$ are initial and final metal concentration (mol/L) in the solution, respectively.

Experimentally, a piece of dry HM or dry HML of known mass was immersed in 3 mL of Fe^{2+} solution at defined concentrations. After 24 h of immersion, the concentration of remaining Fe^{2+} in the solution was determined by addition of Phen excess to formation of a red complex very stable $[(Phen)_3Fe]^{2+}$ of stoichiometry 3:1. By UV–vis spectroscopy (Hewlett-Packard-8453 UV–visible Spectrophotometer) the absorbance of $(Phen)_3Fe^{2+}$ complex at 510 nm was measured and the initial and final Fe^{2+} concentration were determined. The complex solution obeys Beer's law, its intensity is independent of pH from 3 to 9 although a low pH (2.9–3.4) ensures fast color development. A calibration curve of absorbance of $(Phen)_3Fe^{2+}$ versus concentration was built at the chosen wavelength (at λ=510 nm) (Supplementary information-Fig. S1).

2.7. Visual detection limit and response times of Fe^{2+} by PNIPAM/BPhen and PAAm-co-50%AMPS/BPhen sensor systems

PNIPAM and PAAm-co-50%AMPS disks were loaded with BPhen (1 mM in 70% EtOH/H_2O) and then exposed to Fe^{2+} solutions of different concentrations. The response times was recorded when the first color appear and photographs were taken after 15 min of exposition.

2.8. In-situ spectrophotometric study of Fe^{2+} absorbed inside PAAm-co-50%AMPS/BPhen

PAAm-co-50% AMPS were pre-loaded with BPhen (1 mM in 70% EtOH/H_2O) and putted in holder with Fe^{2+} solutions of different concentrations: 0.1 ppm, 2 ppm, 5 ppm and 10 ppm. It was absorbed while the optical spectrum is measured. Since the extinction coefficient of free iron(II) ion is much smaller than the one of the complex, the absorbance measured is taken as only due to the complex formation. A special device to hold the gel in position to measure absorbance was designed and built using a 3D printer (CODEX 2020, Argentina) in black ABS (acrylonitrile-butadiene-styrene). The holder is made of two pieces which assemble and fits exactly into a plastic optical cell (1 cm of path length), allowing the light beam shine directly across the gel, blocking the scattering light. The set-up is described in the Supplementary information- Scheme S1.

2.9. Measuring the interfering effect of different ions on the visual detection of Fe^{2+}

It is possible that other ions present in sample could interfere on the visual detection of Fe^{2+}. Therefore, the interfering several effects were study in PAAm-50%AMPS system. Hydrogel disks were placed in 0.1 ppm of Fe^{+2} aqueous solutions during 1 h to form a clear color and then were put in contact with metals solution different that could be interfering in sensed: Ca^{2+}, Mg^{2+}, Mn^{2+}, F^-, Zn^{2+}, Ni^{2+}, Cr^{3+}, Cd^{2+} (Each one 50 ppm) Co^{2+}, Cu^{2+} (20 ppm) and ClNa (20000 ppm). After 1 h the hydrogels were photographed.

2.10. Measuring iron content in a milk sample

Quantitative dilutions (0.5/10, 1/10, 1/2, undiluted) of a commercial milk sample (1.5% half fat milk) were prepared with tridistilled water in calibrated 10 mL flasks. Then, equivalent cylinders of PAAm-50%AMPS/BPhen were immersed in the different solutions and left for 1 h. Next, all samples were gently washed with tridistilled water and the colorations observed in each of systems were compared with that of the PAAm-50%AMPS/BPhen immersed in a 0.1 ppm solution. The real concentration of iron in the original milk sample corresponds to 0.1 ppm multiplied by the dilution coefficient of the solution where iron is detected.

3. Results and discussions

3.1. Swelling of the hydrogels in water

The swelling capacity of the hydrogel is related to the inner porosity and control de mass transport of ions [29]. The swelling capacity (% SwExp) is strongly affected by the presence of hydrophilic groups and net charges, due to the osmotic pressure from the mobile counterions [30]. However, this effect will favor the ingress of ions (e.g. Fe^{2+}) in the case of hydrogels bearing fixed negative charges but will excludes the same ions when the gel have fixed positive charges. In Fig. 1 are shown the swelling values at equilibrium state for the hydrogel library, representing the experimental measurement of% swelling at equilibrium state and the calculated

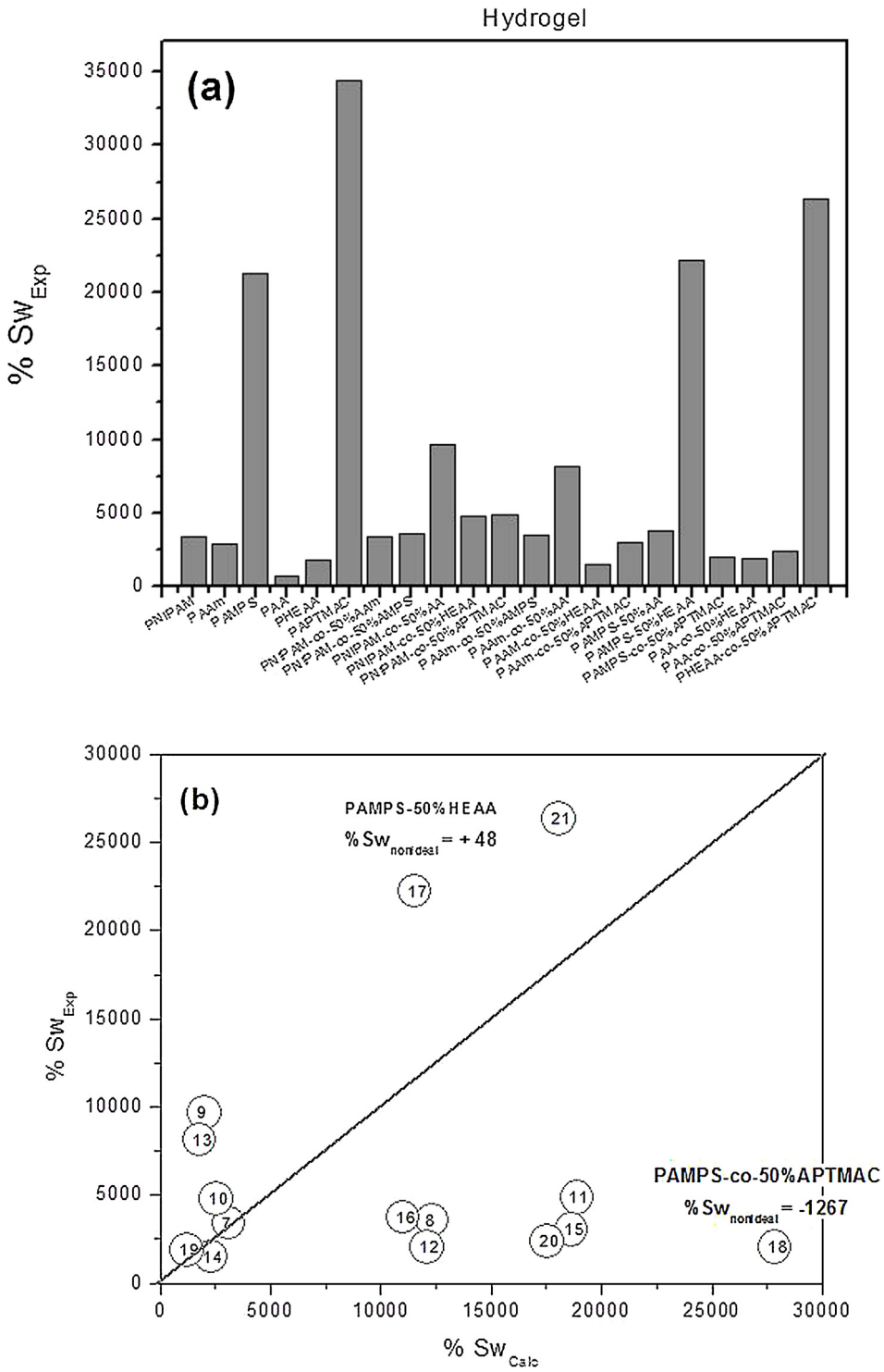


Fig. 1. a) Swelling of hydrogels produced combinatorially in water. b) Relationship between the experimental swelling ($\%Sw_{Exp}$) and the calculated swelling ($\%Sw_{Calc}$). The gray straight line represents the ideal case where the experimental swelling is directly proportional to the calculated arithmetic mean. The numbers in the graph correspond to the hydrogel library in Table 2.

arithmetic mean of the swelling values for the homopolymers made of the co-monomeric units present in each copolymer.

The hydrogels containing hydrophilic groups, neutral (HEAA) or charged (AMPS, APTMAC) show large swelling values. As it can be seen in Table 2, swelling at equilibrium range is from 703 (PAA) to 34403 (PAPTMAC). As it can be seen in Fig. 1a, the experimental swelling can be changed extensively by copolymerization of monomers bearing different groups. From the practical point of view, a larger swelling allows the incorporation of more analyte solution by swelling the dry hydrogel inside the aqueous solution. Moreover, the production of an extensive library of related hydrogels allows ascertaining the structure-property relationship between the functional groups present in the comonomers and the swelling of the copolymers.

On the other hand, if the effects of groups will be additive, that is the copolymer being and ideal mixture of homopolymers, the

Table 2
Swelling of hydrogels combinatorially.

Hydrogel	N°	% Sw_{Exp}	% Sw_{Calc}[a]	% $Sw_{nonideal}$
PNIPAM	1	3355	3355	0
PAAm	2	2863	2863	0
PAMPS	3	21317	21317	0
PAA	4	703	703	0
PHEAA	5	1757	1757	0
PAPTMAC	6	34403	34403	0
PNIPAM-*co*-50%AAm	7	3421	3109	9
PNIPAM-*co*-50%AMPS	8	3577	12336	−245
PNIPAM-*co*-50%AA	9	9660	2029	79
PNIPAM-*co*-50%HEAA	10	4780	2556	47
PNIPAM-*co*-50%APTMAC	11	4875	18879	−287
PAAm-*co*-50%AMPS	12	2019	12090	−499
PAAm-*co*-50%AA	13	8144	1783	78
PAAm-*co*-50%HEAA	14	1508	2310	−53
PAAm-*co*-50%APTMAC	15	3033	18633	−514
PAMPS-50%AA	16	3749	11010	−194
PAMPS-50%HEAA	17	22221	11537	48
PAMPS-*co*-50%APTMAC	18	2038	27860	−1267
PAA-*co*-50%HEAA	19	1868	1230	34
PAA-*co*-50%APTMAC	20	2407	17553	−629
PHEAA-*co*-50%APTMAC	21	26339	18080	31

[a] Arithmetic mean of the swelling values for the homopolymers made of the monomeric units present in the copolymer.

swelling of 1:1 copolymers should be equal to the arithmetic mean of the swelling value of the homopolymers:

$$\%Sw_{Calc}(poly(Cm_x - co - 50\%Cm_y) = \frac{\%Sw_{Exp}(Cm_x) + \%Sw_{Exp}(Cm_y)}{2} \quad (14)$$

where Cm_x and Cm_y are two comonomers of the library. When x = y the material is a homopolymer.

However, as it can be seen in Table 2, the calculated values differ strongly to the mean suggesting that interactions between different groups affect strongly the swelling. To account for that, a normalized difference between the experimental swelling value and the arithmetic mean is calculated:

$$\%Sw_{nonideal} = 100(Sw_{Exp} - Sw_{Calc})/Sw_{Calc} \quad (15)$$

The values are shown in the leftmost column of Table 2.

In general, copolymer reactivity ratios are usually close to one [31,32], therefore the copolymer ratio is directly proportional to the feed ratio. In an ideal copolymer, the swelling of the 1:1 copolymer (e.g. PAA-*co*-50%HEAA) should be directly related to the arithmetic mean (Column 4 to the left in Table 2) of the swelling of each homopolymer (e.g. PAA and PHEAA).

Moreover, in Fig. 1b it is shown the relationship between the experimental swelling and the calculated arithmetic mean. It can be seen that in most cases the copolymer is not equivalent to a simple mixture of monomers units but a novel polymeric nature is evidenced. A clear case is the copolymer of AMPS (bearing $-SO_3^-$ groups) with APTMAC (bearing $-NR_4^+$ groups): PAMPS-co50%AMPTAC (No 18) where the large swelling due to each charged group, related with ion-dipole interactions, is nullified due to the formation of an inner salt. Accordingly, the departure to ideality is quite large (1267%). However, there are less obvious cases, like PAAm-co-50%HEAA (No 17) where the swelling is larger (+49%) than the calculated one and suggest a synergic effect of the neighboring groups.

3.2. Swelling capacity of hydrogel based on AMPS in ethanol-water mixture

While some ligands can be loaded inside a hydrogel by swelling of dry hydrogel in aqueous solution with ligand, most large organic ligands are poorly soluble in water. On the other hand, if the metal-ligand complex is insoluble in water it will be retained irreversibly inside the hydrogel allowing clear detection of the metal ion and/or complete elimination of the ion from the aqueous solution. Ethanol-water mixtures could favor the solubility of the ligand (e.g. BPhen), but the hydrogel should swell in the solvent mixture to load the ligand. Therefore, the swelling of hydrogels in ethanol/water mixtures should be determined. While some data has been reported in the literature [33], it has only been measured for homopolymeric hydrogels. We have measured the swelling behavior of several homopolymers and copolymers in mixed solution ethanol/water. The swelling degree does not monotonically change with the amount of ethanol present in the solution (Supplementary information-Fig. S3). This is likely due the specific interactions of ethanol and water with the functional groups in the mobile polymer chains. In an ideal solution, no specific interactions exists and the molecules of ethanol and water solvate the chains, while in this case the best solvent is enriched inside the gel swelling the material in a way non directly proportional to the relative concentration in the solution. From a practical point of view, the solvent mixture for loading the organic ligand is chosen as the one which produces the larger swelling while been able to dissolve the ligand.

Based on the results observed, 70% ethanol/water mixture is selected as the best mixed solvent in the following studies, because it is able to dissolve the ligands and hydrogels have good degree of swelling in this media.

3.3. High throughput screening (HTS) of hydrogel systems for retention of the colored $[Fe(Phen)_3]^{+2}$ complex

A visual colorimetric assay was applied to the retention of the iron complex inside the hydrogels listed on Table 1. Hydrogel pieces were located in Fe^{2+} solutions in order to analyze if the $[Fe(Phen)_3]^{+2}$ complex (orange-red colored) will be formed inside matrix. Fig. 2 shows images of Phen/HM systems after 24 h in Fe^{2+} solutions. It is possible to observe that in case of the hydrogels containing anionic sulfonate ($-SO_3^-$) groups (3, 8, 12, 16 and 17) a dark orange colour (see colour image in the web version) is developed suggesting that the coloured complex is retained inside the hydrogel. It seems that the negative charge present at the polymer chains interact coulombically with the positive charges in the complex. Indeed, some copolymers where acrylic acid is the only anionic bearing monomer (PAA-*co*-50%HEAA, 19) do not show orange complex formation, while others (PAA-co-50%APTMAC, 20) suggesting that not only the anionic group is important but also the environment related to the co-monomeric unit. It is noteworthy that PAA-co-50%APTMAC contains both anionic and cationic groups while PAA-co-50%HEAA contains anionic and neutral groups. In the case of hydrogels loaded with Phen, partition equilibrium is established between the hydrogel and the external solution where soluble complex can be clearly seen in the solution. Therefore, it could not be used in analytical systems (ex. biological media) where the dye could contaminate the analytical system. Obviously, this is the same disadvantage that solution based colorimetric detection.

3.4. Partition coefficient of Fe^{2+} in the absence and presence of phenanthroline for the complete library

While the discovery process in combinatorial chemistry only needs the high throughput screening, followed by detailed characterization of the lead material, a more detailed investigation of the whole library allows ascertaining the structure-property relationship of the specific parameter.

For the application in sight, partition coefficients of ions are quite relevant. The values of Cp for Fe^{2+}/HM and Fe^{2+}/LHM (where L is Phen) are shown in Fig. 3a and Table 3. As it can be seen, the structure of the copolymer has a strong influence on the partition

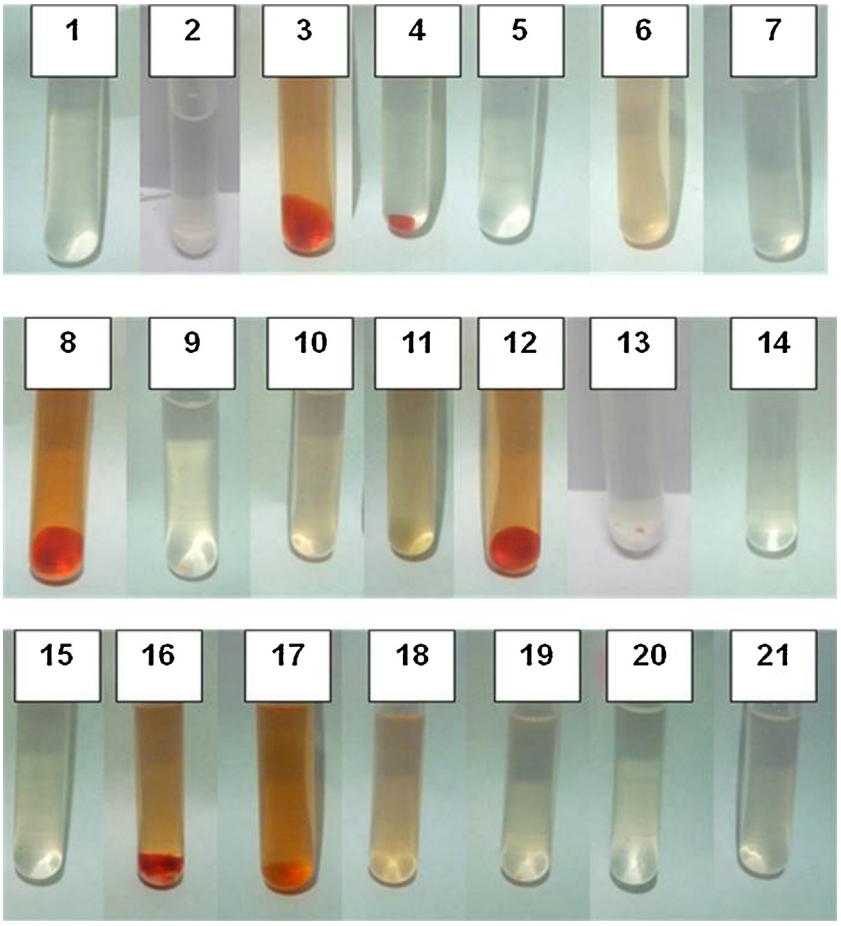


Fig. 2. Photography of hydrogels loaded with Phen and then immersed in Fe^{2+} aqueous solutions during 24 h. (For interpretation of the references to colour in the text, the reader is referred to the web version of this article.)

Table 3
Partition coefficients in hydrogel/water for: Fe^{2+}/HM (absence of Phen) and Fe^{2+}/LHM (presence of Phen).

Hydrogel	N°	$CpFe^{2+}$	$CpFe^{2+}{}_{(Phen)}$	%ΔCp
PNIPAM	1	13.9	−25.9	−286
PAAm	2	60.8	33.9[a]	−44
PAMPS	3	2086	2035	−2
PAA	4	51.9	23.3	−55
PHEAA	5	22.3	15.7	−30
PAPTMAC	6	84.1	63.1	−25
PNIPAM-*co*-50%AAm	7	18.5	24.6	33
PNIPAM-*co*-50%AMPS	8	428.4	489.9	14
PNIPAM-*co*-50%AA	9	14.1	86.7	515
PNIPAM-*co*-50%HEAA	10	49.1	28.8	−41
PNIPAM-*co*-50%APTMAC	11	83.5	25.8	−69
PAAm-*co*-50%AMPS	12	1009	911.4	−10
PAAm-*co*-50%AA	13	38.5	−112.7[a]	−393
PAAM-*co*-50%HEAA	14	41.5	25.5	−39
PAAm-*co*-50%APTMAC	15	43.8	−12.8	−129
PAMPS-50%AA	16	1040	1123	8
PAMPS-50%HEAA	17	453.6	382.1	−16
PAMPS-*co*-50%APTMAC	18	25.9	9.4	−64
PAA-*co*-50%HEAA	19	132.9	131.3	−1
PAA-*co*-50%APTMAC	20	36	−14.9	−141
PHEAA-*co*-50%APTMAC	21	49.5	31.5	−36

[a]Low swelling capacity in 70% ethanol/water mixture. Therefore, Phen was incorporated from aqueous solution at lower concentration.

coefficient both of the free iron and of the complex. A question is the influence of the hydrophobicity and size of the phenanthroline complex compared with the hydrophilicity and size of the $Fe(H_2O)_6{}^{2+}$ aqueous complex. The partition coefficients of the aqueous iron and the complex correlate with each other very well at large Cp values. This is reasonable since all the hydrogels shown in the large Cp range (Fig. 3c) contain the sulfonate group which coulombically interacts with cations, both the $Fe(Phen)_3{}^{2+}$ and the $Fe(H_2O)_6{}^{2+}$ complexes. On the other hand, the hydrogels in the low Cp range (Fig. 2b) show Cp values for both ions that roughly correlate.

However, some hydrogels show Cp values which depart from the correlation. Specifically, two hydrogels containing acrylic acid (AA) differs from PAA (No 4) which fall in the linear fit. PNIPAM-co-50%AA shows a Cp for the complex much larger while PAAm-co-50%AA shows a Cp for the complex much smaller than expected from the correlation. This is reasonable since AAm is very hydrophilic while NIPAM is hydrophobic. Therefore, the hydrophobic complex is excluded from the AAm containing hydrogel and specifically retained in the NIPAM containing hydrogel. It is noteworthy that the measurements were made at a pH value (<3) where the acrylic acid is protonated and no negative charges are present.

The last column presents the change of partition coefficient (%ΔCp), due to the presence of the ligand, which is calculated as:

$$\%\Delta C_p = \frac{(CpFe^{2+}{}_{(Phen)} - CpFe^{2+})}{CpFe^{2+}} x100 \qquad (14)$$

Where $\mathbf{CpFe^{2+}}_{(Phen)}$ and $\mathbf{CpFe^{2+}}$ are the partition coefficients of Fe^{2+} in presence and absence of Phen, respectively.

The higher $CpFe^{2+}$ corresponds to hydrogels with $SO_3{}^-$ groups due to effect of electrostatic attraction between the positive ions and negative charged fixed groups. PAMPS is the homopolymer having higher $CpFe^{2+}$ presents. In the case of copolymers of AMPS

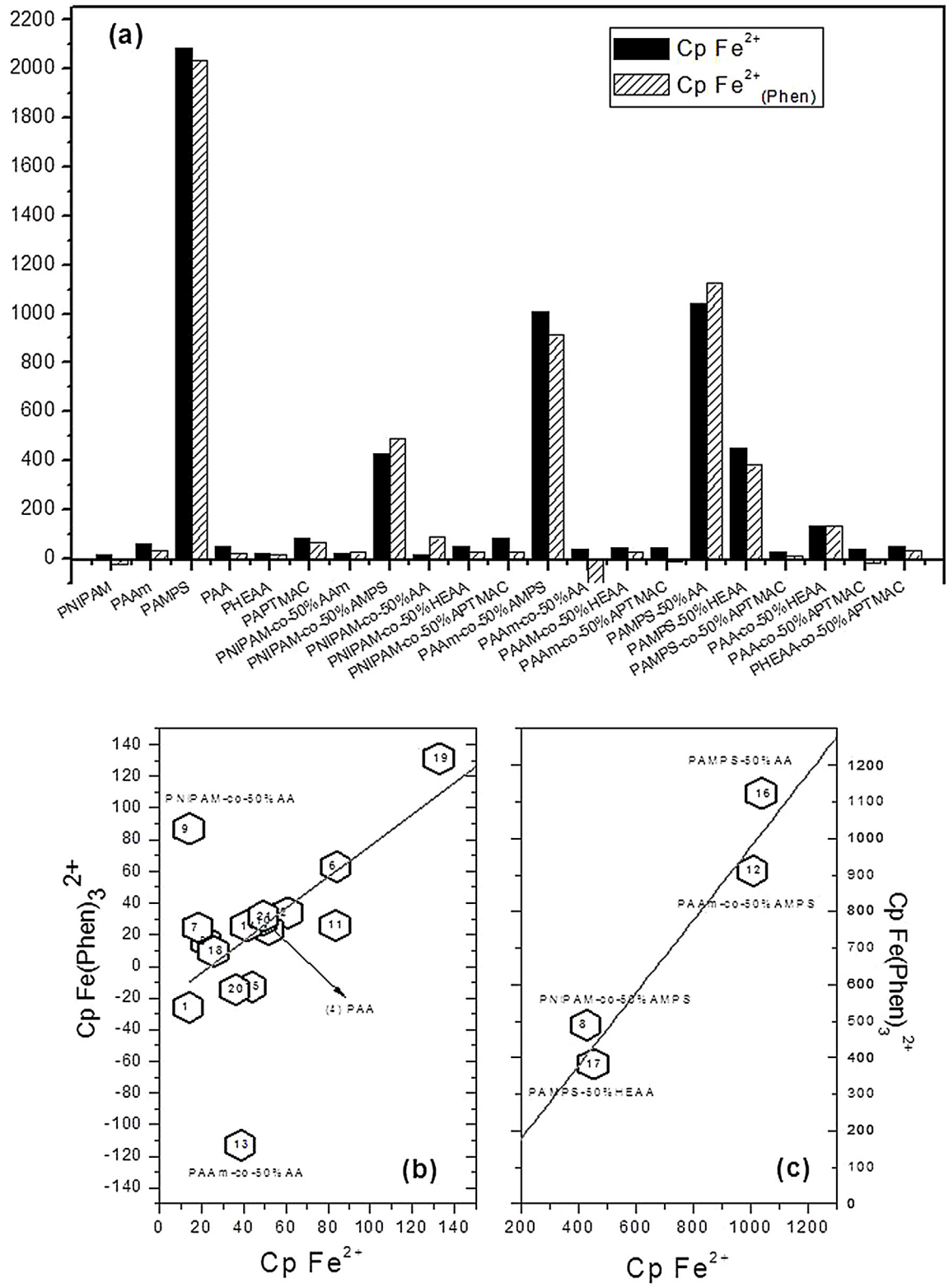


Fig. 3. a) Partition coefficients (Cp) of Fe^{2+}/HM and Fe^{2+}/LHM (L is Phen) in the hydrogels of the combinatorial library. b) Relationship between the Partition coefficients (Cp) of Fe^{2+}/HM and Fe^{2+}/LHM (low Cp range). c) Relationship between the Partition coefficients (Cp) of Fe^{2+}/HM and Fe^{2+}/LHM (large Cp range). The numbers in graphs (b) and (c) correspond to those in the hydrogel library listed in Table 3. The gray line is the linear fit of all the data with the same slope for graphs (b) and (c).

with more hydrophobic monomers, the $CpFe^{+2}$ decreases compared with the homopolymer but are still large. It is noteworthy that PAMPS-co-50%APTMAC shows a low $CpFe^{2+}$ possibly due to permanent internal electrostatic interactions between $-SO_3^-$ and $-N(CH_3)_3^+$ groups which make the hydrogel a zwitterion without net charge. Therefore, Fe^{+2} is not used as mobile counterion and the $CpFe^{2+}$ value is low.

Since the hydrophobic/hydrophilic nature of the hydrogels depends strongly on the groups present, the hydrophobic interactions with the large organic ligands (e.g. phenantroline) would

Table 4
Partition coefficients of Fe^{2+} in HM/water and LHM/water.[a]

Hydrogel	Hydrogel $CpFe^{2+}$	Hydrogel + Phen $CpFe^{2+}_{(Phen)}$	Hydrogel + BPhen $CpFe^{2+}_{(BPhen)}$
PNIPAM	14	−26	25
PNIPAM-*co*-50%AMPS	428	490	552
PAAm-co-50%AMPS	1009	911	1019
PAMPS	2086	2035	2450

[a] The data of Cp are the average of five measures.

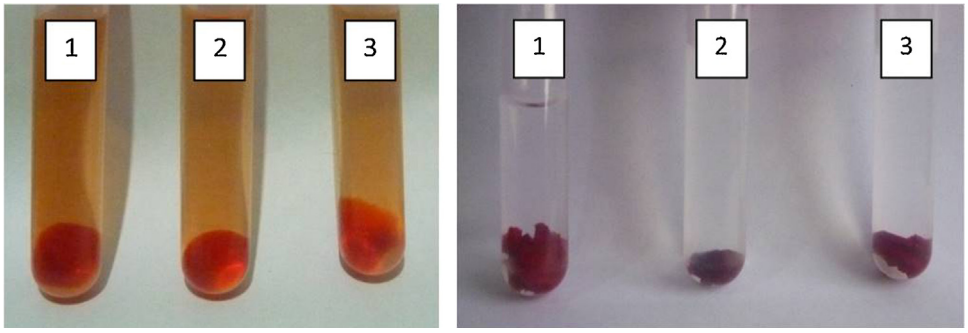


Fig. 4. Photographs of PNIPAM-co-50%AMPS (1), PAAm-co-50%AMPS (2) and PAMPS (3) hydrogels loaded with Phen (left) and BPhen (right) and then immersed in 1 mM Fe^{2+} for 24 h. (For interpretation of the references to colour in the text, the reader is referred to the web version of this article.)

depend on the groups present in the hydrogel matrix. Accordingly, several hydrogels show smaller partition coefficients for the iron-phenanthroline complex than for free iron ions. This is translates to negative values of%ΔCp (Fig. 3b). On the other hand, in some cases seems that the hydrophobic interactions with the large organic ligands makes the partition coefficient of the complex larger than the free ion. Such behavior occurs for PNIPAM-co-50%AAm, PNIPAM-co-50%AMPS, PNIPAM-co-50%AA and PAMPS-co-50%AA. On the other hand, the presence of hydrophobic groups alone does not induce large Cp of the phenanthroline complex, compared with the aqueous complex, as it is clearly shown by PNIPAM where the Cp of the complex is lower than the Cp of the aqueous iron. Therefore, a more complex model of structure-property relationship should be applied to understand the phenomena and to be able to design hydrogels which absorb specifically the complex. For the purpose of this work, the possible colorimetric detection by retention of iron in the form of complex is the parameter of choice.

Looking to the partition coefficients described above suggest that some materials (e.g. PAAm-co-50%AMPS) could be used both to retain free iron and to detect iron through the formation of the colored iron-phenanthroline complex, due to large Cp.

3.5. Comparative studies of partition coefficients of Fe^{+2} in hydrogels with Phen and BPhen

Hydrogels were loaded with ligands in 70% ethanol/water mixture previously. Then, partition coefficients of Fe^{2+} were determinate in water to hydrogel without ligands (HM) and with Phen and BPhen to compare. UV–vis spectroscopy shows the absorption bands of $(Phen)_3$ Fe^{2+} and $(BPhen)_3$ Fe^{2+} complexes inside hydrogels after 24 h in 1 mM Fe^{2+} solution (Supplementary information-Fig. S4).

Table 4 presents Cp values of hydrogels selected. It is possible to observe that the retention capacity of Fe^{+2} by HM and Phen/HM load are similar, i.e. the adsorption of Fe^{2+} inside matrix is mainly given by electrostatic attraction. However, the retention capacity of Fe^{2+} is even greater in hydrogels load with BPhen. In this case, Fe^{2+} could be retained by electrostatic and complexation effect.

In Fig. 4 can be observed at left a deep orange color by presence of $(Phen)_3Fe^{2+}$,while at right $(BPhen)_3Fe^{2+}$ show a red-pink coloration. It is possible to observe that most of $(Phen)_3Fe^{2+}$ complex is retained inside the hydrogel while a few of the soluble complex in equilibrium is observed in the solution. On the other hand, the $(BPhen)_3Fe^{2+}$ is moderately soluble in water but all the complex is retained inside matrix.

Therefore, both Phen and BPhen complexes show large Cp values and could be used as sensors. Additionally, $(BPhen)_3Fe^{2+}$/HM could be used in systems where the presence of the complex in solution could affect the analyzed solution. Moreover, $(BPhen)_3Fe^{2+}$/HM seems more useful as indicator of saturation for iron in water decontamination systems or end point indicator in iron complexometric titrations.

While the value of Cp in some hydrogels (e.g PAMPS) is larger than PAAm-co-50%AMPS, we choose the later for further study because it has enough transparency to use it in optical and spectrophotometric measurements.

3.6. Visual limit of detection and response time

To measure the visual limit of detection and the response time, flat samples of PNIPAM and PAAm-*co*-50%AMPS loaded previously with BPhen, were placed inside solutions of Fe^{2+} different concentrations (Fig. 5).

As it can be seen both materials show coloration in the presence of Fe^{2+} at certain time. PNIPAM/BPhen matrix shows coloration above 1 ppm while the PAAm-*co*-50%AMPS/BPhen shows coloration above 0.1 ppm. Visually it was possible to detect changes of color in PAAm-co-50%AMPS/BPhen after a few seconds for 50 ppm of Fe^{2+}, 2 min 30 s to 1 ppm and 15 min at 0.1 ppm. Instead the response times for PNIPAM/BPhen were: 35 s/50 ppm, 4 min/1 ppm and it was not possible to detect visually concentrations lower than 1 ppm at least even at 1 h of exposure. At low concentrations the diffusion is slower. Therefore it is possible to visually detect very low concentrations as 0.1 ppm using a hydrogel with high concentration of anionic groups (PAAm-co-50%AMPS). Since the WHO recommends 0.3 ppm as acceptable concentration in drinking water, the simple system is able to detect water in compliance to the guidelines of the WHO [34].

The sensitivity of this simple system is also able to measure the range covered by the U.S. Environmental Protection Agency (EPA: 0.3 ppm) [35,36] and European Union (EU: 0.2 ppm) [37], drinking water standards for iron. Additionally, the typical iron con-

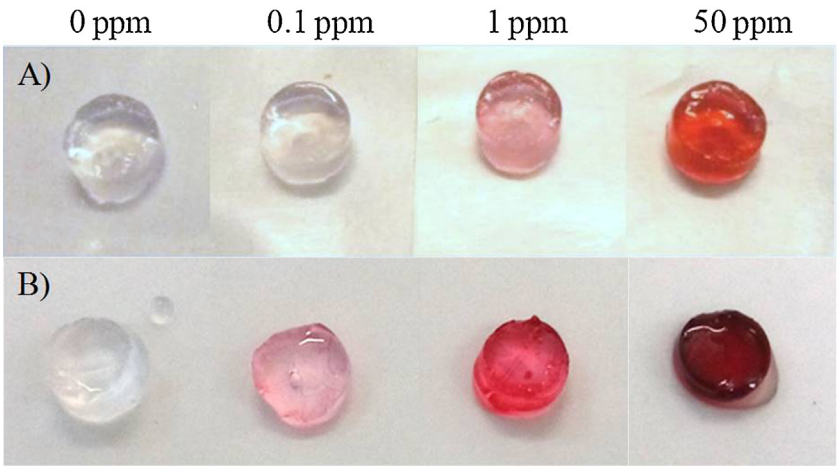


Fig. 5. Photographs of PNIPAM (A) and PAAm-co-50%AMPS (B) hydrogels loaded with BPhen, taken at 15 min of exposition in Fe^{2+} solutions. Concentrations of Fe^{+2} solutions: 0 ppm, 0.1 ppm, 1 ppm and 50 ppm.

tent in wines (1 to 10 ppm)[38], the normal range of free iron in human blood serum (0.8–1.8 ppm) [39], or the iron content in milk (0.3 ppm) [40], can be measured using quantitative dilutions.

3.7. Spectrophotometry in the gel phase for the detection of iron (II)

A holder was built which allows direct spectrophotometric measuring of the complex optical absorption inside the hydrogel (experimental part). In that way, the formation of the colored complex inside the hydrogel can be quantified in-situ for different Fe^{2+} solution concentrations in function of exposition time (Fig. 6). Since the actual complex formation is fast reaching equilibrium concentration in less than 2 s [41], the absorbance change is controlled by the diffusion of Fe^{2+} inside the hydrogel piece.

Fig. 6 The evolution of the absorbance with time depends on the mass transport of the ion inside the hydrogel. In a diffusion controlled system, where the absorbance obeys the Lambert-Beer law and it is proportional to concentration, the absorbance (*Abs(t)*) will obey the equation [42]:

$$F = \frac{Abs(t)}{Abs_{\max}} = \frac{4}{r}\sqrt{\frac{D_0}{\pi}}\sqrt{t} \qquad (15)$$

Where $Abs_{\max}$ is the saturation value, r is the radius of the disk and D_0 is the diffusion coefficient inside the hydrogel.

A plot of F vs $\sqrt{t}$ should render a straight line. As it can be seen in Fig. 6b, this is the case (correlation coefficients > 0.99) for concentrations larger than 0.1 ppm, while in the case of 0.1 ppm an anomalous behavior is observed. It is likely that the reaction rate for the formation of the complex is low (due to the low concentration) and the equilibrium is not established up to long times, affecting the relationship between complex absorption and ion concentration. The mean diffusion coefficient obtained from the mean slope of the straight lines is $D_0 = 0.56\,10^{-7}\,cm^2/s$, which is comparable with the one measured before for a methylene blue in poly(NIPAM-co-maleic acid) ($Do = 1.32\,10^{-7}\,cm^2/s$) [43].

From the final equilibrium value (at a fixed time of 13.5 min), the Lambert/Beer relationship of the $[(BPhen)_3Fe]^{+2}$ inside the hydrogel is calculated. A value of molar absorption coefficient, $\varepsilon = 7998\,M^{-1}cm^{-1}$ (optical path length 3 mm) which is lower to the value in pure aqueous media ($\varepsilon = 21186\,M^{-1}cm^{-1}$ Supplementary information- Fig. S2) likely due to the difference in the dielectric constant between free water and water bounded to the hydrogel. Using the linear relationship (Fig. 6c) and given the instrumental error of 0.05 absorbance units, concentration values down to 0.01 ppm.

3.8. Sensor specificity: interference effect by others metals

The formation of colored complex between metal-organic ligands can be useful to detect metal ions but could be interfered by the formation of stable complexes with other metallic ions present in the same solution. In the case of solution colorimetry, it is known that Cu^+ ion interferes strongly and Ni^{2+}, Co^{2+}, Zn^{2+} and Cd^{2+} interferes slightly [44]. To find out how specific is the sensor for Fe^{2+}, the colorimetric detection was performed in the presence of interfering ions. PAAm-50%AMPS/BPhen disks were placed in 0.1 ppm of Fe^{2+} aqueous solutions during 1 h to form a clear color and then were putted in contact with metals solution different that could be interfering in sensed. The final photography was taken after 1 h of exposition to the interfering ion (Supplementary information- Fig. S5).

In Table 5 they are listed the maximum concentration of ions which are not mask the colorimetric visualization of Fe^{2+}, demonstrating that materials based on hydrogels copolymerized with AMPS and BPhen incorporate to matrix have excellent sensitivity for Fe^{+2} in water. It seems that even that other ions are absorbed inside the hydrogel and could complex with BPhen, either no visible color is formed or the intensity is low enough to mask the color due to the $[(BPhen)_3Fe]^{2+}$ complex.

Table 5
Maximum concentration of common ions which do not interfere with the detection of 0.1 ppm of Fe^{2+}, using PAAm-50%AMPS/BPhen as indicator.

Ion	*Concentration (ppm)*	*Added as*
Na^+	20000	NaCl
K^+	20000	KCl
SO_4^{-2}	3000	Na_2SO_4
Ca^{+2}	50	$CaCl_2{\cdot}2H_2O$
Mg^{+2}	50	$MgCl_2{\cdot}6H_2O$
Cl^-	20000	NaCl
F^-	50	NaF
Mn^{+2}	50	$MnSO_4{\cdot}H_2O$
Zn^{+2}	50	$Zn(NO_3)_2{\cdot}6H_2O$
Ni^{+2}	50	$Ni(NO_3)_2{\cdot}6H_2O$
Cr^{+3}	50	$Cr(NO_3)_3{\cdot}9H_2O$
Cd^{+2}	50	$Cd(NO_3)_2{\cdot}4H_2O$
Co^{+2}	20	$CoCl_2{\cdot}6H_2O$
Cu^{+2}	20	$Cu(NO_3)_2{\cdot}5H_2O$

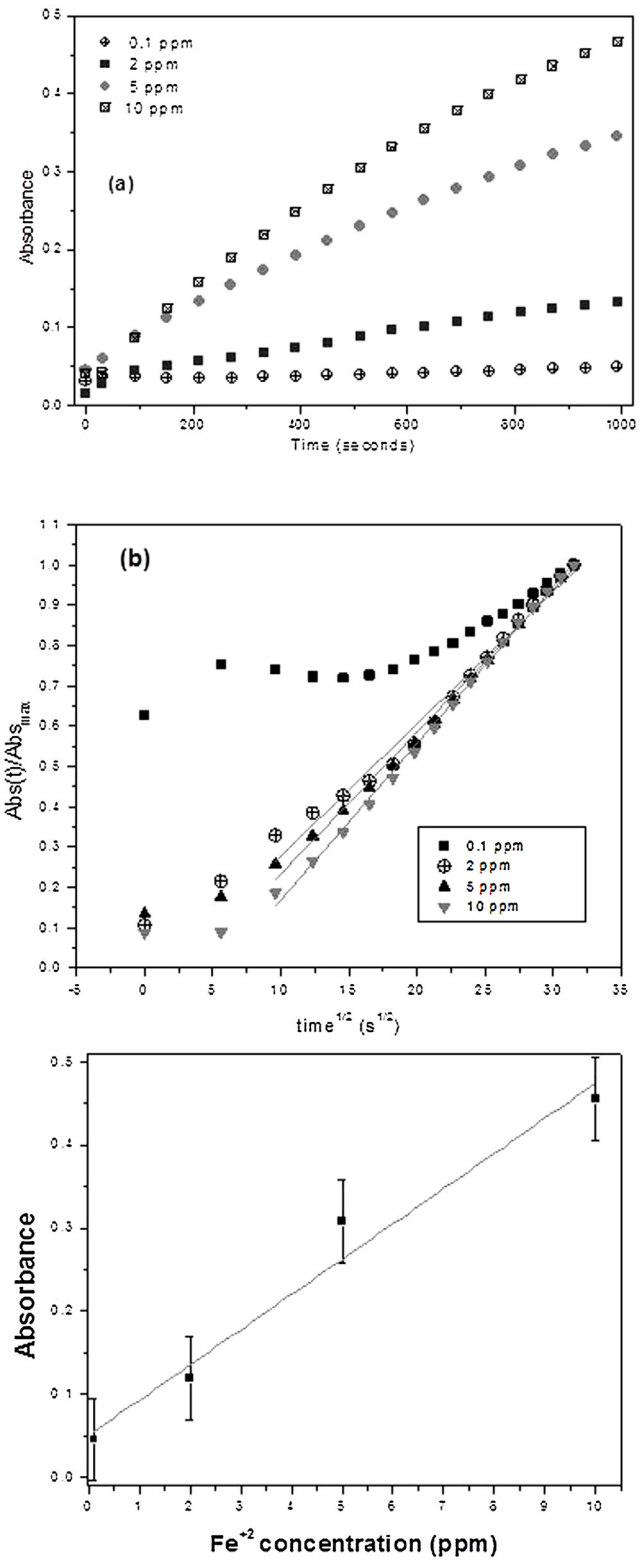


Fig. 6. a) Absorbance at 540 nm as a function of time after cell filling for a PAAm-co-50%AMPS/BPhen in presence of [Fe^{2+}] (10 ppm; 5 ppm; 2 ppm and 0.1 ppm). b) Plot of Abs (t)/Abs $_{max}$ ratio at 540 nm as a function of square root of time (time$^{1/2}$). **c)** Relationship between maximum absorbance of $[(BPhen)_3Fe]^{2+}$ inside the hydrogel and the Fe^{2+} concentration in the solution.

3.9. Determination of iron in a real sample: bovine milk

One of the advantages of analytical methods involving two phases, like liquid/liquid extraction [45] or solid phase extraction [46], is that the chemical substance of interest could be extracted into the other phase removing the interferents present in the matrix and/or conditioning the sample for direct measurement. In the case of milk, the dispersion of fat and proteins in food makes it opaque and precludes the direct colorimetric determination of substances like iron requiring complex workout to remove dispersed phases, such as drying and ashing the milk [47]. Such procedure not only makes the method more complex and prone to errors but could also remove part of the analyte. The determination of iron content in milk is important on nutritional grounds since can vary widely being the main source of the mineral for infants [48]. Indeed, commercially milk is sometimes fortified by addition of iron. The hydrogel based detection system was used directly by immersion of a PAAm-co-50%AMPS/BPhen cylinder in milk, followed by gentle washing with distilled water and visual detection of the coloration (Fig. 7). The one shot detection was not possible because the milk masked the color but when the sample was diluted (1/2) the color is observed easily, which it allows only knowing if the iron concentration is above the detection limit (0.1 ppm). Such data could be enough for nutritional purposes. However, if a quantitative determination is required, a strategy is required. One possibility is to set the gel in decreasing quantitative dilutions of the original milk sample to find at which dilution the 0.1 ppm detection limit is reached. Another approach is based in the kinetics of diffusion of the ion inside the hydrogel in way similar to enzymatic kinetics [49]. Since the time to detect color varies with concentration, a calibration curve can be produced. Moreover, it was found (Section 2.6) that the detection limit depends on the nature of the hydrogel matrix. Therefore, using a set of different gels, the iron content could be quantitatively measured.

Of those approaches, we chose to quantitatively dilute the milk and try to detect iron at 0.1 ppm. The amount of Fe^{+2} in a commercial milk sample was 1.25 ppm determined by colorimetric method ashing. Fig. 7b shows the colors observed to dilution different of milk. To 1/10 dilution was obtained a similar color regarded to 0.1 ppm of Fe^{2+} in water (Fig. 7a).

4. Conclusions

Incorporation of organic molecules poorly soluble in water, such as ligands, inside hydrophobic matrix (hydrogel) is possible by swelling of the hydrogel in a solvent (e.g. ethanol), or mixture of solvents (e.g. ethanol/water), which are also able to swell the hydrogel in a large degree and dissolve the ligand at the same time. If the ligand and/or complex are insoluble in water, the material could be used for ion detection without loss to the external solution in a way similar to liquid/liquid extraction.

Using combinatorial synthesis to produce a hydrogel library and a high-throughput visual, it was possible to find hydrogels materials which allow the colorimetric detection of Fe^{2+} using Phen as ligand. Then, the partition coefficients are measured. According to the results, hydrogels with $-SO_3$- groups were selected because of their better behavior. In order to improve the material performance, it was studied with other more hydrophobic ligand as BPhen, which is insoluble in water.

In function of capacity of complex adsorption inside hydrogel, we could select complex/hydrogel matrixes (HML) to different applications. PAA/Phen matrix present the better performer as retainer of complex Phen-Fe^{+2} from aqueous medium, because the complex is strongly retained inside matrix. While PAAm-*co*-50%AMPS/BPhen proved to be the better visual sensor with lower detection limit (0.1 ppm) of Fe^{2+} at 15 min of exposition in water. In addition, this system showed very high selectivity for Fe^{+2} ions with low interference of other ions.

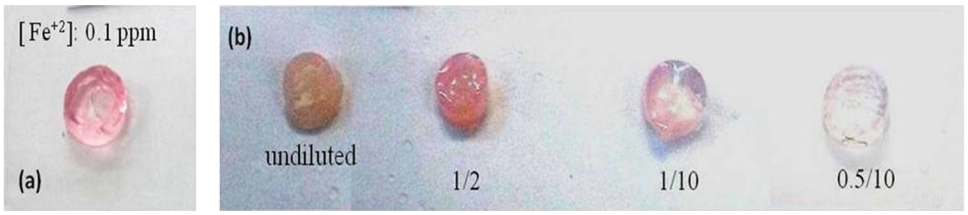


Fig. 7. a) Photograph of PAAm-co-50%AMPS/BPhen immersed in Fe^{2+} solution in water at 0.1 ppm b) Photographs of PAAm-co-50%AMPS/BPhen which have been immersed in different dilutions of milk (0.5/10, 1/10, 1/2 and undiluted).

Using a specially designed set-up, the absorption of the ion into the hydrogel was studied in-situ by spectrophotometry and it was found that at concentrations larger than 0.1 ppm, the mass transport is diffusion controlled. The sensor system is used to detect iron in real samples of milk, showing a simplified procedure compared with conventional determination. Because the fabrication process of these systems is very easy and it is possible change the ligand used, this method could be extended to detect other metallic ions. Given the analogous behavior of polymeric hydrogel matrixes with organic solvents, literature data on the extraction of metallic complexes [44] could be used as guidance.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.snb.2016.10.013.

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