Novel macroporous amphoteric gels: Preparation and characterization

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Abstract. Macroporous amphoteric gels based on allylamine, methacrylic acid and acrylamide crosslinked by N,N′-methylenebisacrylamide were synthesized by radical copolymerization of monomers in cryoconditions. The composition of cryogels was determined by combination of potentiometric and conductimetric titrations. The morphology of cryogels was evaluated by scanning electron microscope (SEM). Cryogels exhibited sponge-like porous structure with pore size ranging from 50 to 200 μm. The values of the isoelectric points (IEPs) determined from the swelling experiments arranged between 3.5 and 4.3. The high adsorption-desorption capacity of amphoteric cryogels with respect to mM and trace concentrations of copper, nickel, and cobalt ions was demonstrated. It was shown that the macroporous amphoteric cryogels are able to adsorb up to 99.9% of copper, nickel, and cobalt ions from 10^{-3} mol·L^{-1} aqueous solution.

Keywords: polymer gels, smart polymers, macroporous amphoteric cryogels, morphology, swelling

1. Introduction

Hydrogels can be in the form of macroscopic networks or confined to smaller dimensions such as microgels, which are crosslinked polymeric particles [1]. When the size of microgels is in the submicron range, they are known as nanogels [2]. According to the IUPAC definition, materials having pores of larger than 50 nm are called macroporous [3]. However, recently much attention is paid to the materials with pore sizes between 1–100 microns, and beyond. Such polymers sometimes are called supermacroporous polymers. According to the cryopolymerization concept macroporous gels are formed in moderately frozen solutions of monomeric and/or polymeric precursors. Contrary to conventional gels in which solvent is retained within the volume, the cryogels are heterophase systems where solvent resides both inside the interconnected macropores and is bound to the polymer network. Comprehensive information on the structure, properties and application of cryogels based on natural and synthetic polymers can be found in a review article [4]. Recently published fundamental book [5] describes both the pioneering articles devoted to cryogels and gives an up-to-date compilation of modes of production of macroporous hydrogels, characterization of such materials, and applications with regard to both biotechnology and biomedicine.

Synthetic amphoteric gels belong to 'smart' materials due to their response to temperature, pH, ionic strength, water-organic solvent composition, electric field, etc. [6, 7]. In the last years the most attention was paid to amphoteric nanogels [8–15] and microgels [16–20] however, to our knowledge, there

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is no any available information on macroporous amphoteric gels of synthetic origin. Amphoteric cryogels as distinct from nonionic, anionic and cationic macroporous materials consist of both acidic and basic monomers.

In the present communication we report for the first time the preparation protocol and characterization of amphoteric cryogels that will have potential applications for the encapsulation of cells, immobilization of enzymes, purification and separation of proteins, recovery of metal ions and as catalysts.

2. Experimental part
2.1. Materials
Monomers and initiators – acrylamide (AAm, 99% purity, from Sigma-Aldrich Chemical Co., Milwaukee, WI, USA), allylamine (AA, 99% purity, from Sigma-Aldrich Chemical Co., Milwaukee, WI, USA), methacrylic acid (MAA, 99% purity, from Sigma-Aldrich Chemical Co., Milwaukee, WI, USA), N,N,N′,N′-tetramethylethylenediamine (TMED, 99% purity, from Sigma-Aldrich Chemical Co., Milwaukee, WI, USA), ammonium persulfate (APS, 99% purity), and crosslinking agent N,N′-methylenbisacrylamide (MBAA, 99% purity, from Sigma-Aldrich Chemical Co., Milwaukee, WI, USA) – were purchased from Aldrich and used without further purification.

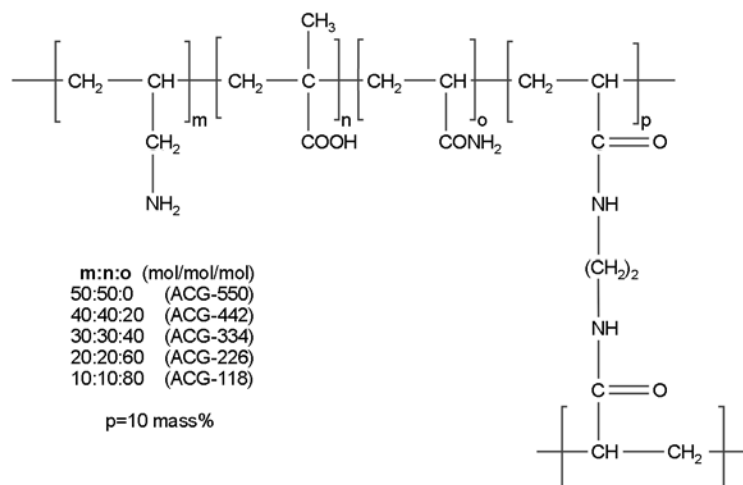### 2.2. Synthesis of amphoteric cryogels
A mixture of AA (66 mg or 1.15 mmol), MAA (99 mg or 1.15 mmol), AAm (660 mg or 9.3 mmol), MBAA (82 mg) corresponding to molar ratio of monomers AA:MAA:AAm = 10:10:80 mol%/mol%/mol% was dissolved in 9.7 mL of deionized water, flushed

by nitrogen for 10 min and degassed in vacuo for about 5 min to eliminate the dissolved oxygen. After addition of 10 µL of TMED the solution was cooled in an ice bath for 4–5 min. Then 0.1 mL of aqueous solution of APS (10 wt%) preliminary cooled in an ice bath for 4–5 min was added and the reaction mixture was stirred for 1 min. The total volume of reaction mixture was divided into 10 parts and each part containing 1 mL of reaction mixture was placed into the 10 plastic syringes with diameter 5 mm with closed outlet at the bottom. The solution in syringe was frozen within 10 min at –12°C and was kept frozen during 48 h. After completion of the reaction the sample was thawed at room temperature. The prepared cryogel sample was washed out thoroughly by distilled water every 2–3 hours during several days then successively washed out by 25, 50, 75 and 96% ethanol to dehydrate then dried in air and finally in vacuum oven to constant mass at room temperature. The amount of monomers in initial feed (in mg and mol%) used for synthesis of cryogels with different molar ratio of monomers at constant concentration of MBAA (82 mg or 10 wt%) is given in Table 1.

Thus a series of amphoteric cryogels (ACG) with initial molar ratios of AA:MAA:AAm = 10:10:80;

Table 1. Amount of AA, MAA and AAm in the feed used for synthesis of cryogels

Initial monomer feed					
[mg]			[mol%]		
AA	MAA	AAm	AA	MAA	AAm
131.3	197.8	460.6	20	20	60
197.0	296.7	327.0	30	30	40
262.7	395.6	163.5	40	40	20
328.3	494.5	0	50	50	0

Figure 1. Structural units of amphoteric cryogels derived from AA, MAA and AAm crosslinked by MBAA

20:20:60, 30:30:40, 40:40:20 and 50:50:0 mol%/mol%/mol% were synthesized and abbreviated as ACG-118, ACG-226, ACG-334, ACG-442 and ACG-550 (Figure 1).

2.3. Potentiometric and conductimetric titrations

The fine powdered cryogel samples (10 mg) were put to 10 mL of distilled water, stirred 1 h and titrated by 0.1M HCl or KOH. Potentiometric and conductimetric titrations were carried out on the pH/conductivity meter 'Mettler Toledo MFC 227' (Switzerland) at room temperature. For calculation of pK_b of allylamine groups the Henderson-Hasselbalch equation $pOH = pK_b + n \cdot \log(\beta/(1 - \beta))$ (where β is the degree of ionization of primary amine groups of cryogels, n is the specific parameter for polyelectrolytes) was used.

2.4. Swelling experiments

The swelling capacity of cryogel samples as a function of pH was evaluated from the height measurements. For this dry gel sample with diameter 5 mm and height 10 mm was placed into a glass tube with diameter 10 mm the bottom of which was closed by Shott filter ended by valve. After passing of 100 mL of aqueous solutions through a gel sample with desired pH, that was adjusted by addition of 0.1 N HCl or KOH to distilled water to avoid the influence of buffer on swelling behavior, its height was accurately measured [16, 17]. Swelling experiments were repeated three times with experimental error not exceeding ±5%. The ratio of L_s/L_0 (where L_s is the height of swollen gel, L_0 is the height of dry gel) on pH was plotted. The flow-rate of water passing through the cryogel samples was measured at a constant hydrostatic pressure equal to 100 cm of water column corresponding to a pressure of ca. 0.01 MPa as described in [21]. Measurements were repeated three times and the values averaged.

2.5. Sorption of metal ions

Sorption of Cu^{2+}, Ni^{2+}, and Co^{2+} ions by ACG-334 was performed as follows: a piece of dry sample with diameter 5 mm and height 10 mm was placed inside of the glass tube with diameter 5 mm and height 50 mm the bottom of which was closed by Shott filter ended by valve. Then 1000 mL of aqueous solution of metal salts or their mixture with concentrations 10^{-3} or 10^{-5} mol/L was passed through the sample during 1 day. Desorption of metal ions was carried out by passing through gel sample 25 mL of 0.1 N HCl several times.

2.6. Methods

For SEM measurements cryogel samples were preliminary dehydrated by ethanol and dried at room temperature then in vacuum oven. The cylindrical sample with diameter 5 mm and height 10 mm was carefully cut by knife blade in parallel or perpendicular to the long axes and the longitudinal and cross sectional parts coated and uncoated with aluminum were examined on scanning electron microscope JSM5800 (Jeol, Japan). Probably due to the presence of electrically conducting amine and carboxylic groups we were able to observe the fine structure and longitudinal and cross sectional morphology of uncoated with aluminum cryogels samples. The concentration of adsorbed and desorbed metal ions was determined with the help of ion-plasma coupled emission spectrometer Optima 5100 DV (Perkin Elmer, USA). FTIR spectra were recorded on an Alpha-P (Bruker, Germany) in KBr pellets.

3. Results and discussion

Figure 2 shows the potentiometric and conductimetric titration curves of amphoteric cryogels. Gradual shifting of the inflection points to left side confirms overall decreasing of the content of carboxylic and amine groups in cryogels in the following order: ACG-550>ACG-442>ACG-334>ACG-226>ACG-118 and it is in good agreement with the variation of acid and base monomers in the feed. Compositions of amphoteric cryogels found from potentiometric and conductimetric titration curves together with ionization constant of allylamine groups (pK_b) and isoelectric pH (pH_{IEP}) are shown in Table 2.

For different samples the average molar composition of amphoteric cryogels found from the potentiometric titration curves is equal to 55.5±2.0 mol% of COOH group and to 44.5±2.0 mol% of NH_2 group while acid-base content found from conductometric titration curves corresponds to 63±4 mol% of COOH group and 37±4 mol% NH_2 group. In spite of inconsistency of potentiometric and conductimetric titration data, they indicate that the composition of amphoteric cryogels is lightly enriched by
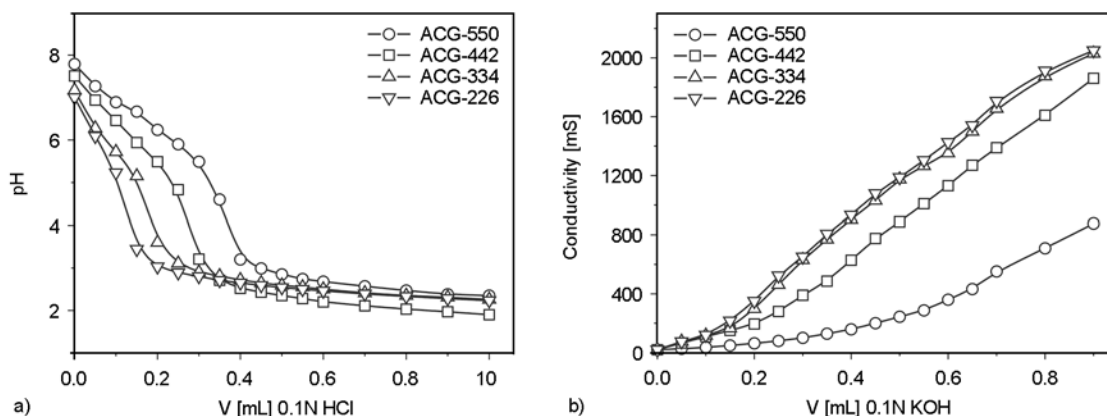
Figure 2. Potentiometric (a) and conductimetric (b) titration curves of amphoteric cryogels by 0.1M HCl (a) and KOH (b)

Table 2. Composition, ionization constant of allylamine groups (pK_b) and the isoelectric pH (pH_{IEP}) of cryogels

Cryogels	–NH₂ groups [mol%]		–COOH groups [mol%]		pK_b	pH_{IEP}
	Potentiometric titration	Conductimetric titration	Potentiometric titration	Conductimetric titration		
ACG-550	46.7	41.0	53.3	59.0	5.44	4.0
ACG-442	42.4	37.0	57.6	63.0	5.25	4.1
ACG-334	43.3	33.3	56.7	66.6	5.78	4.2
ACG-226	–	41.4	–	58.6	5.62	4.3
ACG-118	–	38.5	–	61.5	–	3.5

acidic monomer probably due to relatively low activity of AA monomer in the copolymerization reaction [22].
The values of pK_b for ACG-550, ACG-442 ACG-334 and ACG-226 determined from Henderson-Hasselbalch equation are equal to 5.44; 5.25; 5.78 and 5.62 respectively. Such values of pK_b suggest effective complexation of allylamine groups with transition metal ions and formation of coordination bonds.
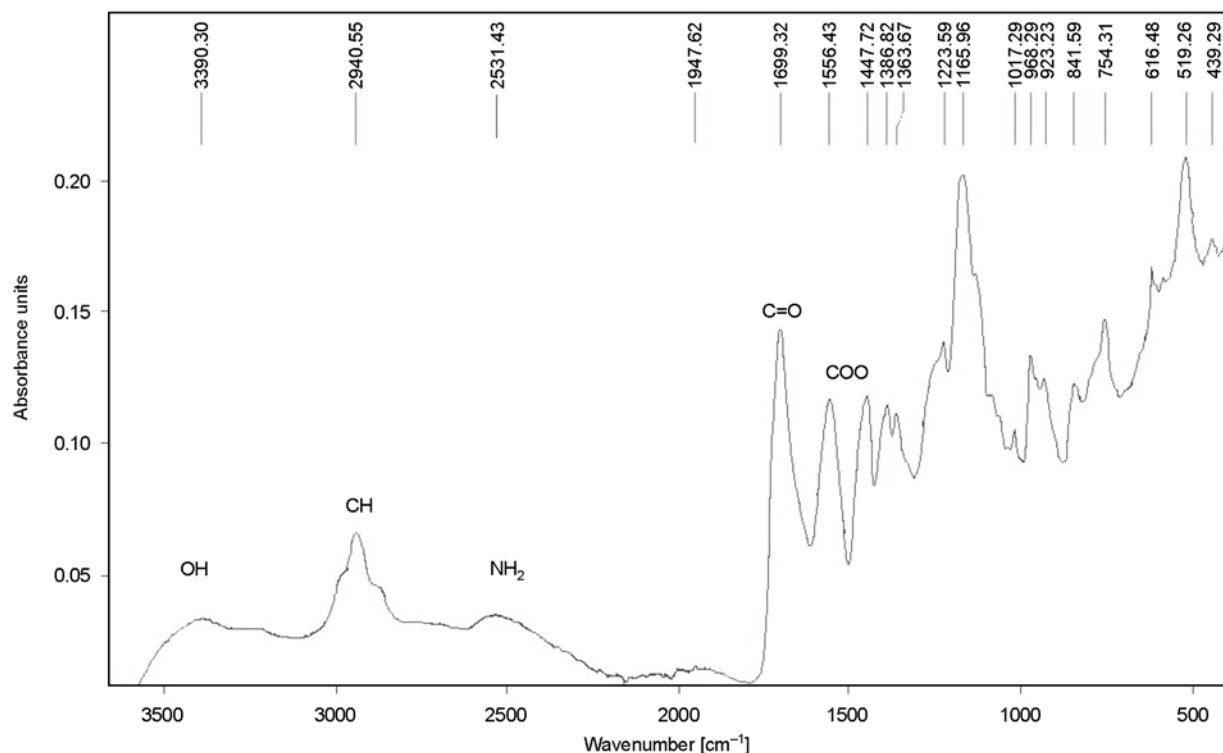
Figure 3. FTIR spectra of dry ACG-334

FTIR spectra of amphoteric cryogels show the characteristic bands of COO^- (1556 cm^{-1}), $C=O$ (1699 cm^{-1}), NH_3^+ (2532 cm^{-1}), CH (2940 cm^{-1}), and OH (3390 cm^{-1}) groups (Figure 3). The appearance of the band at 3390 cm^{-1} can be attributed to OH groups of hydrated water tightly bonded to functional groups of cryogels. Coexistence of COO^- and NH_3^+ reveals the zwitterionic structure of cryogels due to the fact that partly transition of protons from carboxylic groups to amine one may take place.

According to a commonly adopted concept [4, 5] cryogels can contain closed-, open-, or through pores that permit liquid to fill them and ensure unhindered convectional flow of solutes within interconnected pores. Cross- and longitudinal sections of dry ACG-118 and ACG-442 show sponge-like porous structure with pore size ranging from 50 to 200 μm (Figure 4a–4d) and the interconnected channels (Figure 4b).

Dynamics of water flux through the cryogel samples with a diameter 5 mm and height 10 mm were evaluated (Table 2). Increasing of the content of acidic and basic monomers in a series of ACGs leads to progressive decreasing of water flux. This phenomenon can probably be explained by different hydration degrees of ionic groups leading to 'apparent' decreasing of pore size of cryogels (Table 3).

One of the specific features of linear and crosslinked polyampholytes is the existence of so-called isoelectric points (IEPs) where intra- and intermolecular attractions of opposite fixed charges lead to pseudoneutral behavior and compact structure of amphoteric macromolecules [6, 7]. Figure 5 shows the swelling and deswelling of amphoteric cryogels as a function of pH. It is seen that the deswelling of amphoteric cryogels is minimal at the IEPs. The iso-

Table 3. Dynamics of water flow-rate through amphoteric cryogels

Amphoteric cryogels	Water flux [mL/min]
ACG-118	1.20±0.10
ACG-226	0.35±0.05
ACG-334	0.18±0.05
ACG-442	0.05±0.01
ACG-550	0.02±0.01
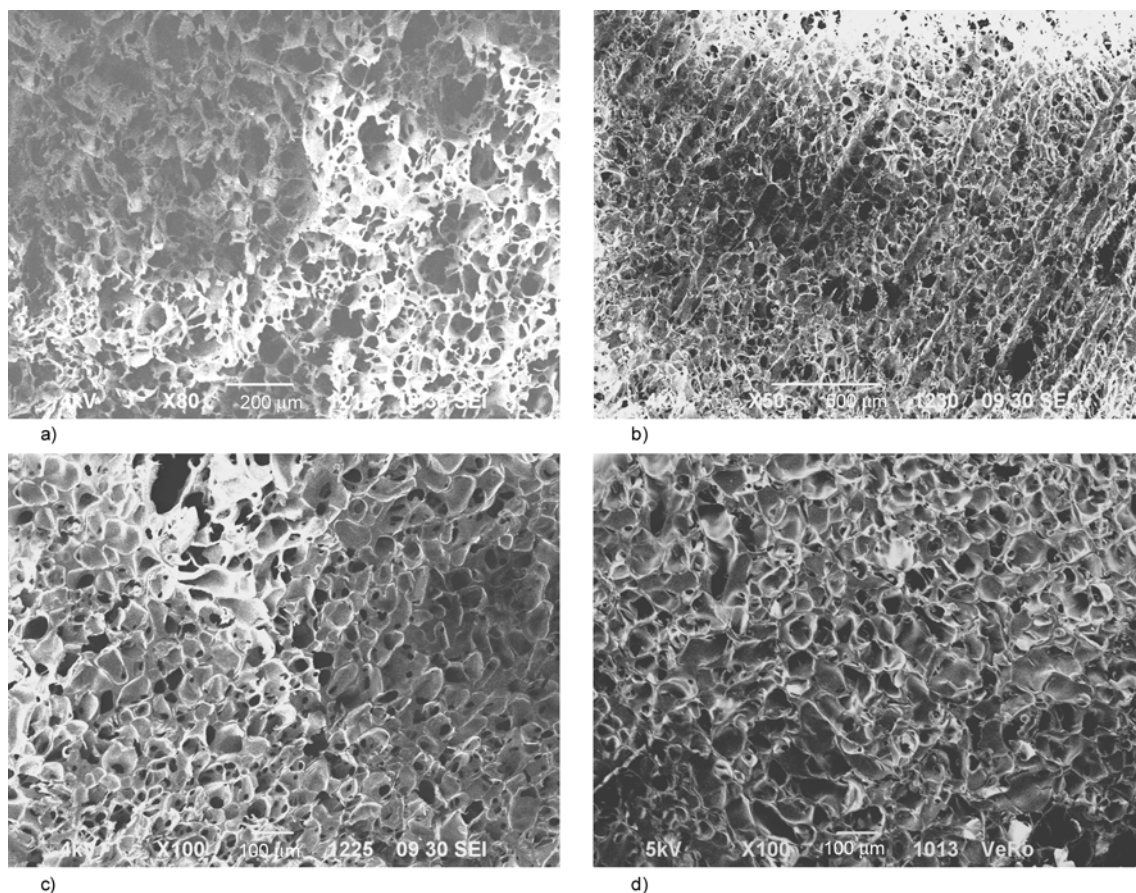
Figure 4. SEM images of cross (a, c, d) and longitudinal (b) sections of ACG-118 (a, b) and ACG-442 (c, d) uncoated (a, b, c) and coated (d) with aluminum
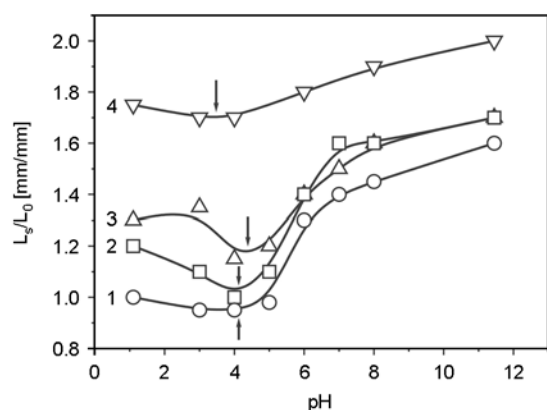
Figure 5. Swelling-deswelling curves of cryogels ACG-550 (curve 1), ACG-334 (curve 2), ACG-226 (curve 3), and ACG-118 (curve 4) on pH. Arrows show the IEPs

Table 4. Adsorbed and desorbed amounts of metal ions by ACG-334

Type of metal ions	Concentration of metal ions passed through ACG-334 [mol/L]	Adsorbed [%]	Desorbed [%]
Cu^{2+}		99.9	51.4
Ni^{2+}	10^{-3}	99.9	67.2
Co^{2+}		99,9	62.0
Cu^{2+}		65.0	98.8
Ni^{2+}	10^{-5}	73.0	95.2
Co^{2+}		72.3	91.1

electric pH (pH_{IEP}) of amphoteric cryogels found from the swelling-deswelling measurements arranges between 3.5 and 4.3 (see Table 2).

The ability of cryogels to bind metal ions was also studied. Sorption of metal ions is accompanied by colourization of samples. This may be due both existing of free (uncomplexed) metal ions and metal ions coordinated with amine and/or carboxylic groups within cryogels (Figure 6). Involvement of allylamine groups into complexation reaction with transition metals ions and formation of chelate structure was studied earlier [23].

As seen from Table 4 the ACG-334 adsorbs up to 99.9% of metal ions from aqueous solution containing 10^{-3} mol/L of copper, nickel, and cobalt ions. In the course of passing of 1000 mL of metal ions with concentration of 10^{-5} and 10^{-3} mol/L through amphoteric cryogel ACG-334 gradually colorization of cryogel samples (especially for copper ions) was observed that is in favour of complexation reaction. The sorption capacity of ACG-334 with respect to

10^{-3} mol/L of copper, nickel, and cobalt ions is equal to 634.9; 586.4; 588.7 mg respectively per 1 g of dry cryogel while the same parameter for 10^{-5} mol/L of copper, nickel, and cobalt ions is correspondingly equal to 41.3; 42.8 and 42.7 mg per 1 g of dry sample.

Desorption of metal ions by 0.1 N HCl is in the range from 51 to 67%. In spite of the fact that the adsorbed amount of metal ions from aqueous solution containing 10^{-5} mol/L is low, the desorbed amount of copper, nickel, and cobalt ions is high and equal to 98.8; 95.2 and 91.1% respectively. Preferentially adsorption of Cu^{2+} ions (79%) in comparison with Ni^{2+} (38%) and Co^{2+} ions (32%) from their mixture was also observed from aqueous solution containing 10^{-5} mol/L of metal ions indicating on the specific binding of copper ions. High adsorption capacity of amphoteric macroporous gels with respect to mM and trace metal ions may be perspective for purification of the wastewaters and analytical purposes. The reduction of cryogel-metal complexes by $NaBH_4$ leads to formation of nano- and microsized particles of metals in reduced and oxidized forms [24, 25] immobilized on the inner and surface parts of amphoteric cryogels. The chemical composition of the Ni containing sample by energy dispersive X-ray
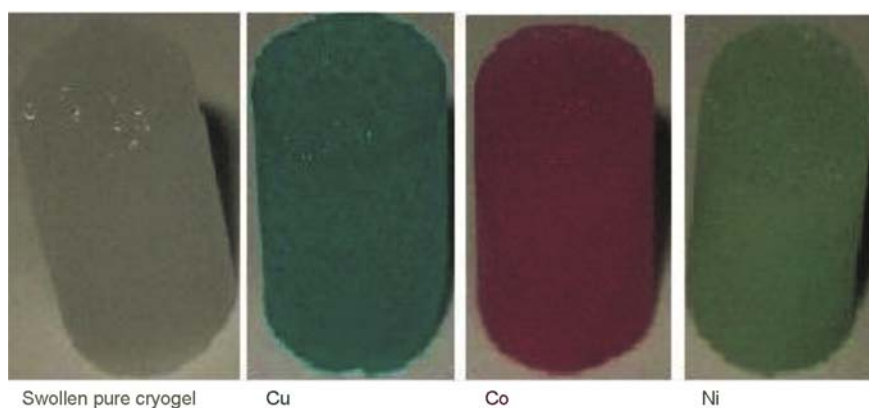
Figure 6. Adsorption of metal ions by macroporous amphoteric cryogel ACG-334

attached to SEM revealed that up to 34% mass % of Ni particles are formed. Thus amphoteric cryogels can serve as efficient heterogeneous supports for metal nanoparticles and as flow microreactors in catalysis.

4. Conclusions

SEM images of cross- and longitudinal sections of amphoteric cryogels show sponge-like porous structure and the interconnected channels. The content of acid-base groups determined by combination of potentiometric and conductimetric titrations is in favour of acidic groups. The isoelectric points of amphoteric cryogels found from the swelling measurements are arranged between 3.5 and 4.3. It has been shown that macroporous amphoteric cryogels are able to adsorb up to 99.9% of copper, nickel, and cobalt ions from 10^{-3} mol/L aqueous solution while to desorb from 91 to 98% metal ions from 10^{-5} mol/L aqueous solution. Desorption of metal ions by 0.1 N HCl ranges from 51 to 69%. Specific sorption of copper ions by amphoteric cryogel from the mixture of copper, nickel, and cobalt ions with concentration of 10^{-5} mol/L is also observed.

Acknowledgements

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