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A B S T R A C T

Polyaniline-based cryogels were prepared by oxidative cryopolymerization in the presence of various concentrations of poly(N-vinylpyrrolidone) and phytic acid used as a polymer support and a dopant, respectively. Mechanical strength and handling stability of the resulting macroporous materials (pore size up to 70 pm) were significantly improved by the addition of poly(N-vinylpyrrolidone) into the polymerization system compared to the cryogels crosslinked only by phytic acid. Increase of poly(N-vinylpyrrolidone) concentration in the reaction medium above 5 wt%, while not noticeably changing mechanical properties, was found to lead to a decrease of conductivity and specific surface area. Introduction of optimal amount of phytic acid (0.2 M) as an additional codopant, in opposite, allowed enhancement of the material conductivity and specific surface area as well as increase of their tensile modulus. Polyaniline-poly(N-vinylpyrrolidone) cryogels containing phytic acid also showed better cytocompatibility due to lower cytotoxicity and improved cell adhesion and proliferation.

Keywords: Polyaniline Cryogel Conductivity Biocompatibility

Polyaniline (PANI)-based cryogels are composite materials which consist of the conducting polymer and a water-soluble polymer support forming a three-dimensional macroporous network [**1**]. They are designed to combine intrinsic physicochemical characteristics of PANI with mechanical and chemical properties of the supporting polymer to overcome inherent brittleness of the conducting polymer for improved handling properties and opening new potential application options. Being a subclass of conducting polymer hydrogels, cryogels can be used in similar ways, for example, as sensors [**2-4**], supercapacitors [**5,6**], antibacterial materials [**7,8**], in tissue engineering [**9,10**] and drug-delivery [**11,12**]. Polyaniline cryogels are prepared by oxidative polymerization of aniline in a frozen medium containing a water-soluble polymer **[1]**. The prefix "cryo" in the term cryogel, therefore, refers to the cryogelation process which is a crucial step in the preparation procedure. Freezing of the reaction medium and subsequent formation of ice crystals acting as pore-forming agents, around which a conducting polymer network is formed, are stages, which define macroporous morphology and properties of the resulting materials [**13**]. Influencing the balance between the polymerization rate and the rates of ice crystal formation and growth by variation of

the reaction medium composition (copolymerization approach) or freezing temperature allows tuning pore size, mechanical properties and specific surface area of the cryogels for a required application [**14,15**].

Choosing an appropriate polymer support for a conducting polymer gel preparation allows tailoring the material properties for the specific purpose. For instance, using poly(N-isopropylacrylamide) leads to thermoresponsive materials [**4**], poly(vinyl alcohol) is suitable for the preparation of the cryogels with superelasticity [**16**] or gelatin [**17**] can serve as a sacrificial template for the preparation of a cryogel derived macroporous conducting polymer. Poly(N-vinylpyrrolidone) (PVP) being an inert and biocompatible polymer, widely used for various biomedical purposes [**18**], can be a promising support for cryogels for bio-applications. It was shown [**19**] to be used in a PANI-based hydrogel. However, to the best of our knowledge, cryopolymerization of similar systems has not been reported yet.

Phytic acid is an organic acid, which is abundant in natural sources such as legumes, cereals and nuts, and represents up to 85% of phosphorus supply in plants [**20**]. Being a polybasic acid, which contains twelve acidic protons, phytic acid can be used as a dopant and crosslinker for PANI chains. The latter allows preparation of PANI hydrogels without additional polymer supports or matrices, where phytic acid serves as the only gelating agent **[21,22]**. The resulting macroporous materials show high surface area and specific capacitance, and can be used as supercapacitors [**21**], sensors [**21**] or adsorbents [**22**]. A combined approach involving cryogelation together with crosslinking by phytic acid for preparation of PANI-phytic acid cryogels was also reported [**23**]. Directed freezing and freeze-drying applied in the work [**23**] resulted in aerogels with anisotropic conductivity, high specific surface area and specific capacitance. Moreover, the cryogelation approach coupled with introduction of additional polymer support was also described [**3,24**]. In the mentioned works [**3,24**], however, freezing of polymerization mixture inducing gelation of poly(vinyl alcohol) was performed after PANI-phytic acid hydrogels had formed. This fact might affect morphology and mechanical properties of the materials compared to simultaneous formation of the hydrogel and the supporting polymer matrix.

It should be additionally noted that using phytic acid as the only dopant results in PANI of lower conductivity compared to the one doped by conventional hydrochloric acid [**25**]. Therefore, it was suggested [**25, 26**] to use chloride and phytate in a mixed dopant approach for achieving both high conductivity and desired morphology.

In the present work, we have prepared PANI cryogels supported by PVP by a previously unreported combination of cryopolymerization and a mixed dopant approach with chloride, sulfate and phytate ions as codopants and studied influence of polymerization mixture composition on the morphology, mechanical and physicochemical properties of resulting materials. In contrast to the published works, flash-freezing of the reaction mixture has been utilized to ensure simultaneous formation of PVP and PANI-phytic acid hydrogel networks to minimize potential interference of growing ice crystals on the formed conducting polymer phase.

2. Experimental

2.1. Chemicals

Aniline (Penta, Czech Republic), aniline hydrochloride (Penta, Czech Republic), ammonium peroxydisulfate (Lach-Ner, Czech Republic), poly (N-vinylpyrrolidone) (PVP) (molecular weight 360000, Sigma-Aldrich, China) and phytic acid (50 wt% solution, Sigma-Aldrich, Japan) were used as received.

2.2. Synthesis of cryogels

Polyaniline-poly(N-vinylpyrrolidone) cryogels were prepared by oxidative cryopolymerization of aniline hydrochloride (0.2 M) by ammonium peroxydisulfate (0.25 M) in aqueous solution of PVP (5-8 wt %) containing various concentrations of phytic acid (0.04 M, 0.2 M, 1 M). After mixing of precooled monomer and oxidant solutions, the polymerization mixture was quickly sucked into plastic syringes, frozen in dry ice/ethanol bath at — 78 °C and left to polymerize in a freezer at —24 °C for 7 days. After thawing, the cryogels were removed from the syringes, washed with excess of water and freeze-dried. Similar procedure was applied for preparation of reference cryogels in the absence of phytic acid (6 wt% PVP) or in the absence of PVP (0.2 M phytic acid). The latter material was prepared using aniline as a monomer instead of aniline hydrochloride.

2.3. Characterization

Morphology of freeze-dried PANI-PVP cryogels was studied by a scanning electron microscope (SEM) MAIA3 (Tescan, Czech Republic). Static mechanical properties of water-swollen PANI-PVP cryogels (cylindrical specimens - diameter 3 mm, length 60 mm) were investigated using electromechanical testing machine Instron 6025/5800R (Instron, USA) equipped with a 10 N load cell at room temperature in deionized water and with a cross-head speed of 10 mm min^{-1}. The values reported in the manuscript are averages of at least three measurements.

The DC electrical conductivity of freeze-dried cryogels was determined by a van der Pauw method on compressed pellets (diameter 13 mm, thickness 1.0 ± 0.3 mm) placed in sample holder fitted with gold plated spring loaded electrodes. A Keithley 230 Programmable Voltage Source in serial connection with a Keithley 196 System DMM served as a current source, the potential difference between the potential probes was measured with a Keithley 181 Nanovoltmeter (Keithley, USA). Measurements were carried out at constant ambient conditions at 24 ± 1 °C and relative humidity 35 ± 5%. The electrical conductivity σ was calculated from the applied current I and the measured potential difference U in the linear part of the current-voltage characteristics.

Specific surface area of cryogels was calculated from physisorption measurements on a volumetric sorption analyzer Autosorb-iQ (Anton Paar QuantaTec Inc., USA) using the Brunauer-Emmet-Teller (BET) method. 50 mg of the samples were outgassed for 2 h at 120 °C. The measurements were performed with nitrogen at 77 K at a relative pressure of 0.1-0.4.

Raman spectra of freeze-dried materials were measured with a InVia Reflex Raman microspectrometer (Renishaw, United Kingdom). The spectra were excited with a diode 785 nm laser. The scattered light was registered with a Peltier-cooled CCD detector (576 x 384 pixels) and analyzed by the spectrograph with holographic grating 1200 lines mm^{-1}. The spectra were obtained at several spots of the sample, and the samples were found to be inhomogeneous, average spectra are thus presented.

PANI-PVP cryogels (6 wt% PVP, 0.2 M and no phytic acid) were tested for cytotoxicity and proliferation of cells on the surface. The mouse embryonic fibroblast cell line (ATCC CRL-1658 NIH/3T3, USA) was used for all experiments. The cultivation medium consisted of Dulbecco's Modifed Eagle's Medium (PAA Laboratories GmbH, Austria) containing 10% bovine calf serum (BioSera, France) and 1% of Peni- cillin/Streptomycin (GE Healthcare HyClone, United Kingdom).

The cytotoxicity determination was performed according to ISO standard 10993-12; concretely the 0.1 g of PANI-PVP cryogel per 1 mL of media were used for extract preparation. The samples were disintegrated in culture medium and incubated for 24 h at 37 °C with stirring. The extracts were

subsequently filtered using Syringe filter 0.22 |im. The parent extracts (100%) were then diluted in fresh culture medium to obtain a series of dilutions with concentrations of 75, 50, 25, 10, and 5% of extracts. All extracts dilutions were used immediately for testing. The cytotoxicity testing was done as follows: cells were seeded to pre-cultivate in the 96 well microtitration test plates dishes (TPP, Switzerland) in seeding concentration 1 x 10^5 cells per mL. After 24 h of pre-cultivation, the extracts were added to pre-cultivated cells. All assays were performed in quadruplets. After another 24 h, when the cells were cultivated in the presence of diluted extracts, the extracts were sucked up, replaced by fresh medium, and cell viability was determined.

The cell viability was evaluated by MTT assay (MTT cell proliferation kit, Duchefa Biochemie, Netherlands). Infinite M200 Pro NanoQuant (Tecan, Switzerland) was used for measuring the absorbance at 570 nm. The reference wavelength was adjusted on 690 nm. The results are presented as reduction of cell viability in percentage when compared to those cultivated in medium without the extracts of tested materials (reference cell viability corresponds to 1).

Before cell proliferation testing, the samples were sterilized by 70% ethanol for 24 h and washed four times (after 12 h) with ultra-pure water to remove the residual ethanol.

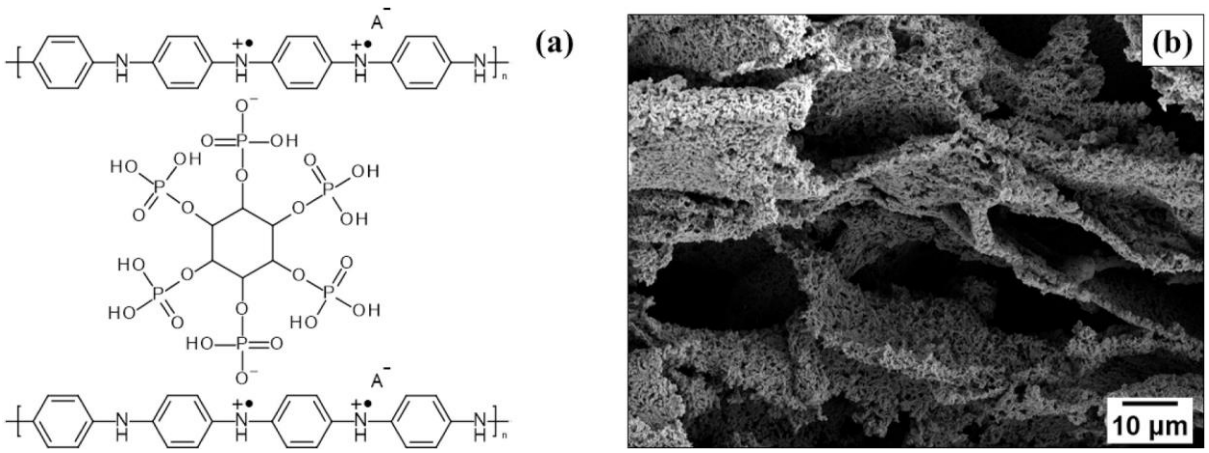

Fig. 1. (a) Scheme of PANI and phytic acid interaction; (b) SEM image of PANI-phytic acid cryogel prepared in aqueous solution of phytic acid (0.2 M) in the absence of PVP.

Then the samples were immersed into medium for next 24 h. The surface of PANI-PVP cryogels, approximately 0.79 cm^2, was overflowed with 0.5 mL of cell suspension in a concentration of 1 x 10^6 cells per mL. After 48 h of cultivation, staining of DNA and F-actin was used to determine the amount of cells and their cytoskeleton. Before staining, cells were fixed and permeabilized. Firstly, the cells were fixed using 4% formaldehyde (Penta, Czech Republic) for 15 min, then washed by phosphate buffered saline (PBS, Invitrogen, USA) and subsequently poured with 0.5% Triton X-100 (Sigma-Aldrich, USA) for 5 min to permeabilization. After this time cells were washed 3 times by PBS. Then Hoechst 33258 (Invitrogen, USA) at a concentration of 5 µg per mL was added to the new PBS to stain DNA. At the same time two drops of ActinRed™ 555 (Thermo Fisher Scientific, USA) per 1 mL of PBS were used for visualization of F-actin cytoskeleton and left to incubate for 30 min in the dark. The morphology of cells was observed using an inverted Olympus phase contrast microscope IX 81 (Olympus, Germany) and also confocal laser scanning microscope Olympus FV 3000.

3. Results and discussion

3.1. Polyaniline-phytic acid cryogel

Due to the fact that phytic acid can perform not only as a dopant for PANI but also as a gelator during formation of hydrogels (**Fig. 1**a) without additional supporting polymers [**21**], a reference synthesis was performed, in which oxidative cryopolymerization of aniline was carried out without PVP in an aqueous solution of phytic acid (0.2 M).

Fig. 1b shows that PANI-phytic acid cryogel is a double-porous material which consists of porous (pore size up to 2 pm) twodimensional conducting polymer layers forming a 3D structure with much larger pores (up to 30 pm).

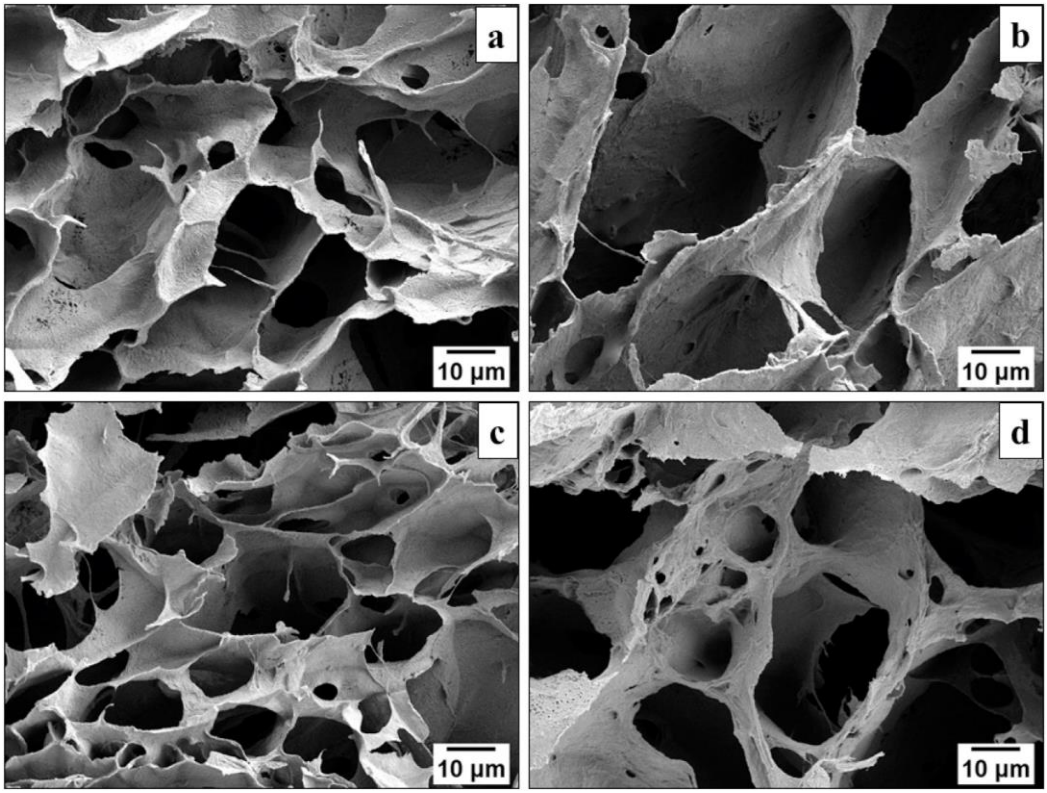

Fig. 2. SEM micrographs of PANI-PVP cryogels prepared in 0.2 M phytic acid solution containing following concentrations of PVP: (a) 5 wt%, (b) 6 wt%, (c) 7 wt%, (d) 8 wt%.

Table 1 Mechanical properties, conductivity and specific surface area of PANI-PVP cryogels prepared using various concentrations of PVP in polymerization mixture containing 0.2 M of phytic acid

PVP concentration, wt%	Tensile strain at break, %	Tensile stress at break, kPa	Tensile modulus, kPa	Conductivity, S cm^{-1}	BET surface area, m^2 g^{-1}
5%	41 ± 6	3.5 ± 0.4	96 ± 13	2×10^{-1}	38.7
6%	63 ± 7	4.2 ± 0.7	112 ± 25	1×10^{-1}	28.0
7%	59 ± 10	3.9 ± 0.3	108 ± 60	8×10^{-2}	22.2
8%	70 ± 19	4.0 ± 0.3	88 ± 14	7×10^{-2}	11.0

Double-porous morphology was previously described in the literature [**21**] for similar materials prepared without freezing the reaction medium. However, the largest reported pores were around units of micrometers. That value is similar to pore size in the individual polymer layers in our PANI-phytic acid cryogels but considerably smaller than the one for the pores between the layers. Thus, in the case of the cryogelated materials, significantly larger pores can be observed due to formation of ice crystals upon freezing of the reaction medium.

One of the main motivations for preparation of free-standing PANI-based hydrogels is enhancement of the conducting polymer processibility and applicability. Therefore, mechanical strength of the resulting materials is a crucial parameter for assessment. In the case of PANI-phytic acid cryogels mechanical integrity was found to be very low, the gels were difficult to handle and most of them were destroyed during the washing procedure. Thus, it was concluded that using of phytic acid as the only gelator is not enough for ensuring handling stability of the materials and all further experiments were performed with cryogels additionally containing PVP as a supporting polymer.

3.3. Polyaniline-poly(N-vinylpyrrolidone) cryogels: effect of phytic acid Concentration

Polyaniline-poly(*N*-vinylpyrrolidone) cryogels were prepared by oxidative cryopolymerization of aniline hydrochloride in the aqueous solution of PVP containing phytic acid. In order to study the influence of the reaction medium composition on the morphology, mechanical strength and conductivity of the cryogels, the synthesis was carried out using various concentrations of the supporting polymer and the dopant. In the first step, the cryopolymerization was performed varying a concentration of PVP from 5 wt% to 8 wt% with a constant concentration of phytic acid (0.2 M).
SEM images of the resulting cryogels (**Fig. 2**) prepared using all studied concentrations of PVP show similar macroporous structure with pore sizes 10-40 pm typical for conducting polymer hydrogels prepared by cryopolymerization technique in the presence of a polymer support [**1,14**]. The porous structure of cryogelated materials is determined by size and distribution of ice crystals, which are formed upon freezing of the reaction medium and serve as pore-forming agents [**13**]. It was recently shown [**15**] that it is possible to influence the pore size of PANI-based cryogels by shifting the balance between growth of ice crystals and formation of polymer network around them. In the present case, when PVP concentration is the only variable in the composition of the polymerization mixture there is no observed variation in the pore sizes in the cryogels with the change of the parameter. Therefore, we can assume that the mentioned shift of balance does not occur.

The main role of a water-soluble polymer, such as PVP, in the preparation of conducting polymer-based cryogels is enhancement of material's mechanical strength and overcoming inherent brittleness of conducting polymers. It can be expected that changing the ratio between a conducting polymer and a polymer support in the cryogel might influence mechanical characteristics of the material. However, according to assessment of mechanical properties of PANI-PVP prepared using different concentrations of PVP (**Table 1**), changing the strengthening agent concentration from 5 wt% to 8 wt% does not lead to noticeable change of tensile characteristics of the cryogels, while showing significant improvement compared to the reference PANI-phytic acid cryogels prepared in the absence of PVP, for which mechanical characteristics could not be measured due to the poor handling stability.

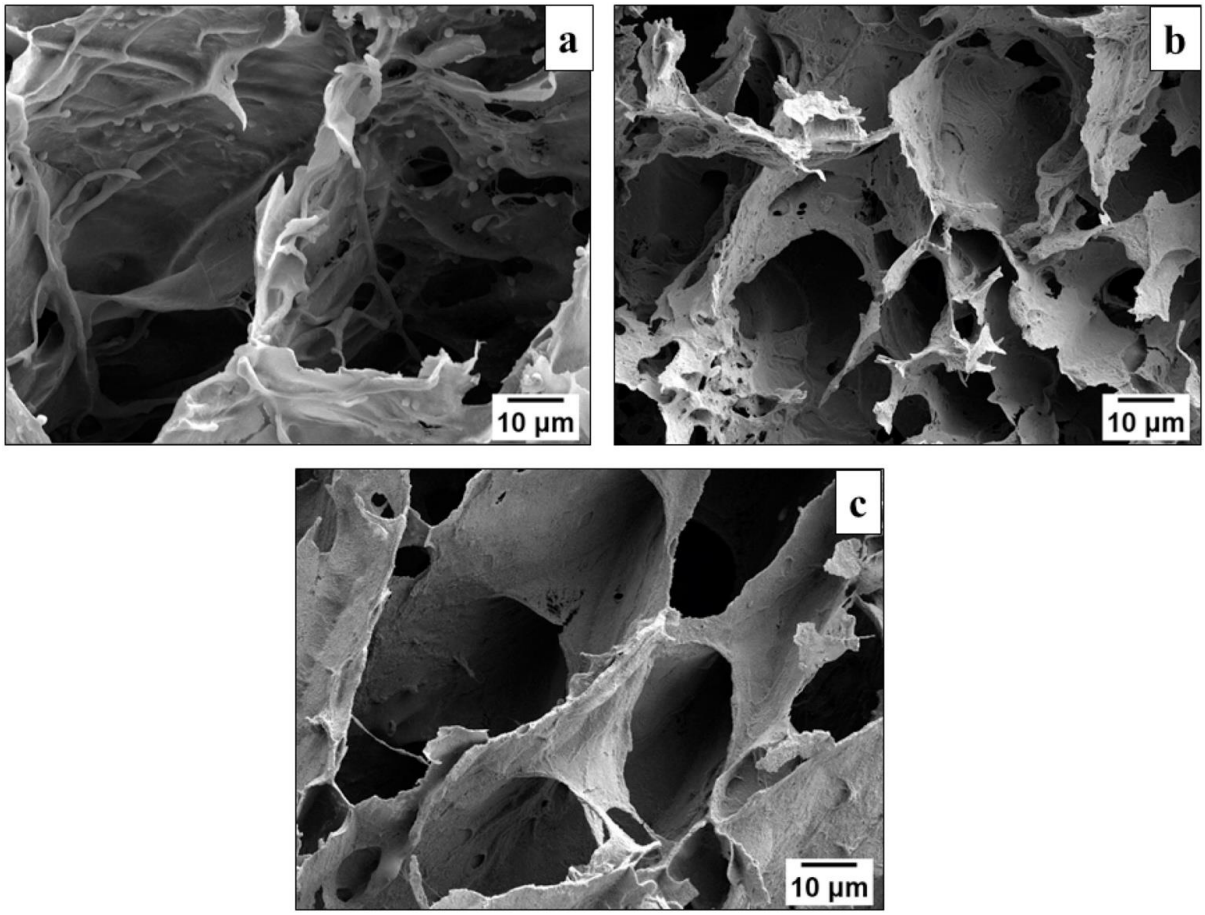

Fig. 3. SEM images of PANI-PVP cryogels prepared in 6 wt% solution of PVP in (a) the absence of phytic acid or containing following concentrations of phytic acid: (b) 0.04 M, (c) 0.2 M.

Table 2 Mechanical properties, conductivity and specific surface area of PANI-PVP cryogels prepared in 6 wt% solution of PVP containing various concentrations of phytic acid or in the absence of phytic acid.

Phytic acid concentration, M	Tensile strain at break, %	Tensile stress at break, kPa	Tensile modulus, kPa	Conductivity, S cm^{-1}	BET surface area, m^2 g^{-1}
0.2	63 ± 7	4.2 ± 0.7	112 ± 25	1×10^{-1}	28.0
0.04	55 ± 28	0.3 ± 0.2	16 ± 4	2×10^{-2}	5.8
0	196 ± 52	3 ± 1	15 ± 3	1×10^{-2}	9.3

The strengthening effect of addition of PVP into the polymerization system might be connected with the absence of pores in the PANI-PVP cryogel walls (**Fig. 2**), which were present in the structure of the material containing phytic acid as the only gelating agent (**Fig. 1**b) and could act as microdefects. Influence of PVP content in the initial polymerization solution on conductivity of PANI-PVP cryogels is shown in **Table 1**. Increasing PVP concentration from 5 wt% to 8 wt% leads to a slight conductivity

decrease from 2 x $1CT^1$ S cm^{-1} to 7 x $1CT^2$ S cm^{-1} which can possibly be explained by higher fraction of a non-conducting polymer component (PVP) in the resulting cryogels.

Specific surface area of PANI-PVP cryogels was found to decrease from 38.7 m^2 g^{-1} to 11.0 m^2 g^{-1} with increasing of PVP concentration from 5 wt% to 8 wt%. This is probably concerned with PVP interference with formation of pores, which can be assumed to take place based on the difference between porous structure of a reference cryogel prepared in the absence of PVP (**Fig. 1**b) and morphology of PVP-containing materials (**Fig. 2**).

3.3. Polyaniline-poly(N-vinylpyrrolidone) cryogels: effect of phytic acid concentration

The next step for optimization of the PANI-PVP cryogel preparation procedure was studying the influence of phytic acid concentration on the morphology, mechanical properties and conductivity of the materials. The cryogels were synthesized by the cryopolymerization technique in aqueous solution of PVP (6 wt%) in the presence of various concentrations of phytic acid (0.04 M, 0.2 M, 1 M) or in the absence of phytic acid. It should be noted that PANI-PVP cryogels prepared using 1 M phytic acid showed low mechanical strength and poor handling stability and were destroyed upon removal from plastic syringes immediately after preparation. Therefore, they were excluded from further studies.

Fig. 3 shows that all studied PANI-PVP cryogels prepared using various concentrations of phytic acid or in the absence of phytic acid have similar macroporous morphology typical for the cryogelation approach. However, the material obtained in the absence of phytic acid has notably larger pores with pore sizes reaching 70 pm while for the composites synthesized in the phytic acid containing solutions these values are lower (up to 40 pm). This effect might be attributed to the presence of additional type of ions (phytic acid) and difference in ionic strength in the system which can influence ice crystal formation [**27**] leading to the change in pore sizes of the resulting composites.
Influence of phytic acid concentration in the initial reaction mixture on mechanical properties of PANI-PVP cryogels is shown in **Table 2**. It can be seen that introduction of 0.2 M phytic acid into the polymerization medium results in higher tensile modulus and lower tensile strain can possibly be explained by the fact that phytic acid can act as a crosslinker in the system and its presence leads to obtaining materials with lower elasticity.

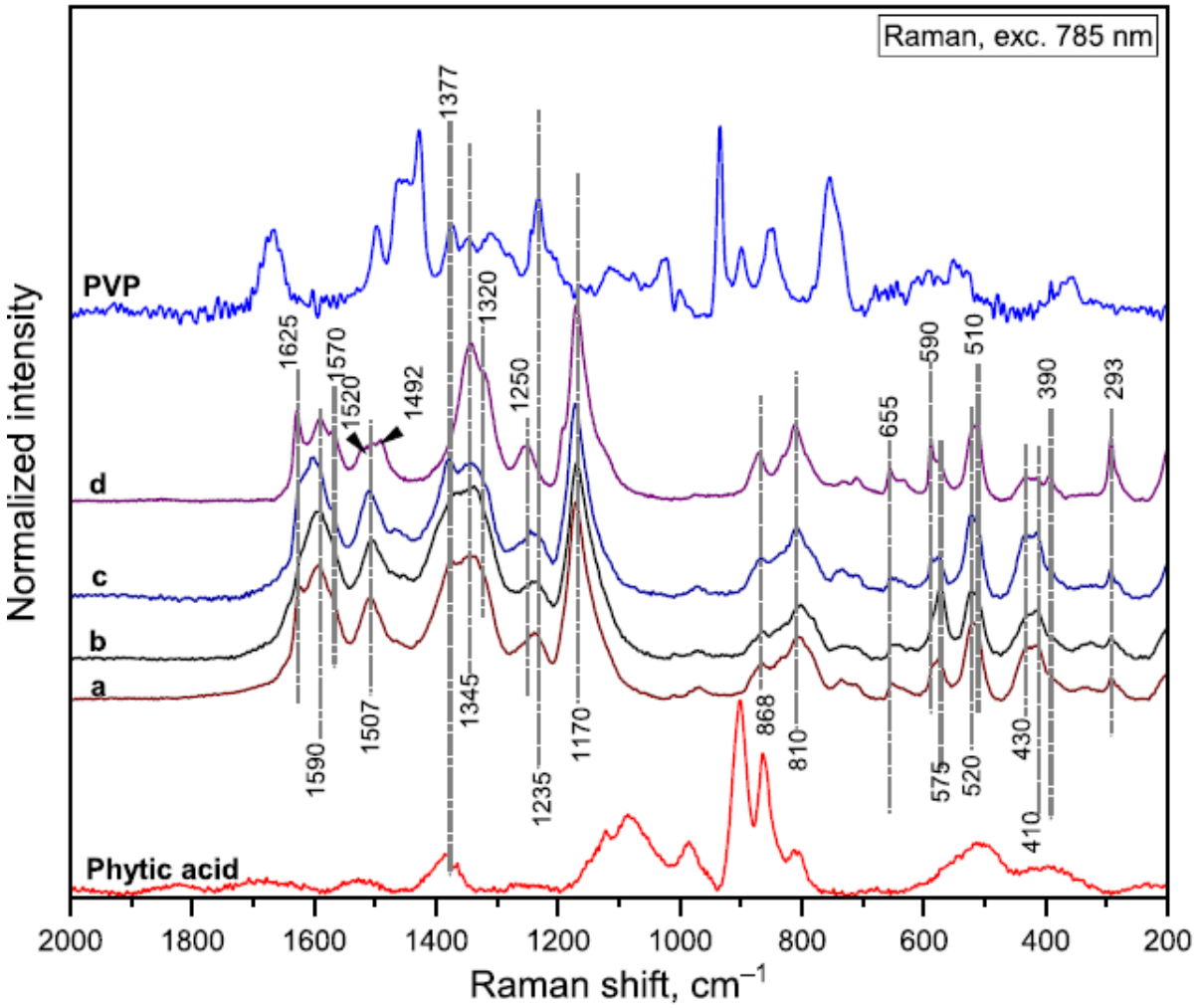

Fig. 4. Average Raman spectra of (a) PANI-phytic acid (0.2 **M**) cryogel synthesized without PVP; PANI-PVP (6 wt%) cryogel prepared (b) in the presence of 0.2 **M** phytic acid and (c) in the absence of phytic acid; (d) PANI-PVP (5 wt %) cryogel prepared in the presence of 0.2 **M** phytic acid (a typical spectrum of an inhomogeneity) and reference spectra of PVP and phytic acid. The spectra were excited with 785 nm laser line, normalized and shifted for clarity.

Increasing the phytic acid concentration further (to 1 M), as was mentioned before, led to mechanically weak cryogels, which were impossible to handle.

Conductivity of PANI-PVP cryogels (**Table 2**) was found to be in the same order of magnitude as observed for PANI-based cryogels containing dopants other than phytic acid (sulfate and chloride ions) [**14**]. However, addition of 0.2 M phytic acid into the polymerization medium resulted in a slight increase of the measured values: 1 x 10^{-2} S cm^{-1} for the material obtained in the absence of phytic acid, 1 x 10^{-1} S cm^{-1} for the cryogel prepared in 0.2 M solution of phytic acid. This can be attributed to higher resistance to deprotonation [**28**] of a phytic acid doped PANI which occurs during washing of the resulting cryogels with water.

Addition of 0.2 M phytic acid into the polymerization mixture was also found to increase specific surface area of resulting PANI-PVP cry- ogels up to 28.0 m^2 g^{-1} compared to the reference cryogel prepared in the absence of phytic acid (9.3 m^2 g^{-1}) (**Table 2**). This effect might be related to the morphology changes induced by the presence of phytic acid, which can be seen in **Fig. 3**. To summarize, 0.2 M is the optimal concentration of phytic acid in the polymerization mixture which leads to the noticeable improvement of mechanical strength, conductivity and specific surface area of PANI-PVP cryogels. Using 0.04 M phytic acid does not affect tensile modulus, conductivity and BET

surface area compared to the reference cryogel without phytic acid and 1 M phytic acid leads to the dramatic reduction of the cryogel mechanical strength.

3.4. Spectroscopic stud

To analyze chemical structure of PANI in the cryogels, Raman spectra excited with 785 nm laser line were obtained (**Fig. 4**). This excitation resonantly enhances various polaronic structures, allowing to study the protonation and oxidation states of the conducting polymer.

The samples were quite heterogeneous, polaron delocalization changed from spot to spot. Raman spectra were obtained at several spots of the sample and average spectra are presented (**Fig. 4**). Raman spectra display the features of protonated PANI **[29-34]**: semiquinonoid ring stretching of emeraldine salt structure at 1590 cm^{-1}, N-H deformation vibration at 1507 cm^{-1}, C-$N^{\bullet+}$ stretching vibration of delocalized 1340 cm^{-1} and localized 1377 cm^{-1} polaronic structures, C-N stretching vibration at 1250 and 1235 cm^{-1}, C-H deformation vibrations at 1170 cm^{-1}, and various skeletal deformation vibrations below 1000 cm^{-1}.

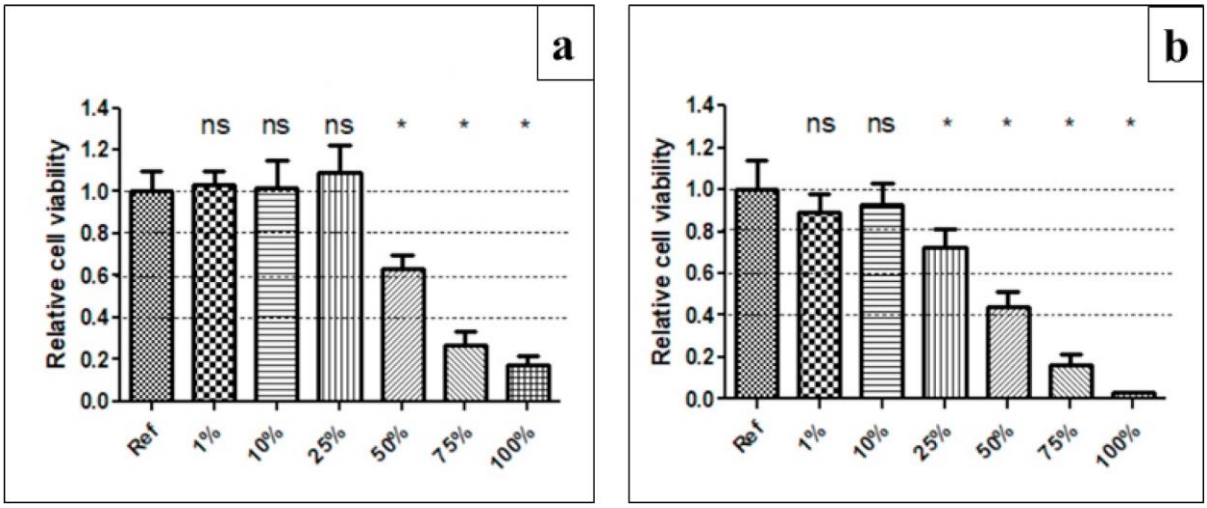

Fig. 5. Cell viability of extracts of PANI-PVP cryogels synthesized using 6 wt% of PVP in the presence of (a) 0.2 M phytic acid and (b) without it. Statistical significance was determined by ANOVA with post hoc Tukey's Multiple Comparison test; *P < 0.0001. (*) mark statistically significant difference compared to reference.

A group of additional bands connected with overoxidation [**29**] appear in the spectra of some areas in the cryogels (**Fig. 4**d): ring stretching of the quinonoid ring in a pernigraniline-like or benzoquinone-like structure at 1625 cm^{-1}, benzenoid ring stretching at 1590 and 1575 cm^{-1}, N^{+}—H stretching in quinonoid structure at 1520 cm^{-1}, C-N stretching at 1492 cm^{-1}, bipolaron C-$N^{\bullet+}$ stretching at 1320 cm^{-1}, and skeletal deformation peaks at 590, 510, 390 and 293

3.5. Biological properties

Influence of phytic acid presence on biocompatibility of PANI-PVP cryogels was studied by cytotoxicity and cell proliferation tests. The cryogels prepared at 6 wt% of PVP in the presence of 0.2 M phytic acid and without it were compared. The results of cytotoxicity evaluation are presented in **Fig. 5**. To determine any statistical differences between the reference and samples extracts, the ANOVA with post hoc Tukey's multiple comparison test was applied. The P values of < 0.0001 were

considered statistically significant. Extracts from both type of cryogels proved no cytotoxicity for concentration 1% and 10% in cultivation medium. The first difference occurs in 25% of extracts, when the sample with phytic acid shows statistically non-significant difference from the reference, while the sample without phytic acid shows a mild cytotoxicity. Statistically significant differences were noted for both types of extracts with 50% and over. Overall, the cytotoxicity effect for PAN- I-PVP cryogel containing phytic acid was slightly lower than without phytic acid. Previously cryogels of PANI with poly(vinyl alcohol) were studied in context of cytotoxicity by Humpolicek et al. [**35**] Compared to the material reported in the present manuscript, the PANI-poly(vinyl alcohol) cryogel has slightly lower cytotoxicity as only 100% extracts show mild cytotoxicity while all other concentrations were non-cytotoxic. The results of these two studies are, however, not fully comparable as different cell lines were used in there. On the other hand, the cytotoxicity of the presented PANI-PVP material is comparable to the one determined for polypyrrole-poly(vinyl alcohol) cryogel which was published in the study of Bober et al. [36], and where the same cell line was used. It is, therefore, obvious that cytotoxicity of PANI-PVP cryogels is common to those type of materials.

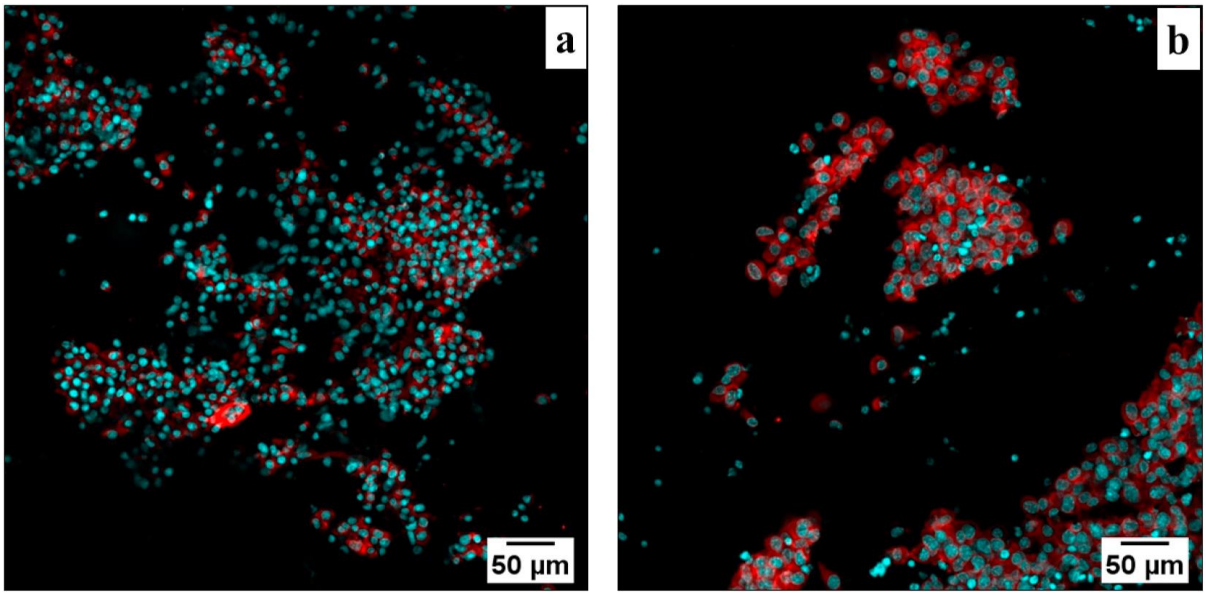

Fig. 6. Cell proliferation on surface of PANI-PVP cryogels synthesized using 6 wt% of PVP in the presence of (a) 0.2 M phytic acid and (b) without it.

The cytotoxicity is just the first prerequisite for application of any biomaterial. Here another parameter, the cell adhesion and their subsequent growth and proliferation, was determined. For the cell proliferation testing, the cells were seeded on PANI-PVP cryogel surface and allowed to grow for 2 days. It is clear **(Fig. 6)**, that cells were able to adhere on the surface. On the other hand, the cells form clusters and, thus, the surface was not covered homogeneously. Moreover, the morphology of cells shows limited spreading of cells on the material surface which can be connected to porous structure of the cryogel. Generally, there were more adhered cells on PANI-PVP cryogel prepared in the presence of phytic acid, than on the cryogel without one. This is an important improvement of cryogel properties in context of its practical application. To summarize, in terms of

toxicity and proliferation, addition of phytic acid into PANI-PVP cryogels allows higher biocompatibility of PANI-PVP based cryogels.

4. Conclusions

Polyaniline-based macroporous conducting cryogels supported with PVP were synthesized by *in situ* one-step procedure in the presence of phytic acid as a codopant and a crosslinker for PANI. The presence of PVP was shown to be crucial for mechanical integrity of the materials, despite having phytic acid in the system as a crosslinker. The introduction of optimal amount of phytic acid, in addition to improvement of mechanical strength of the composites, enhanced conductivity, specific surface area and biocompatibility of the cryogels, shown by cytotoxicity and cell proliferation tests.

References

1. J. Stejskal, P. Bober, M. Trchova, A. Kovalcik, J. Hodan, J. Hromadkova, J. Prokes, Polyaniline cryogels supported with poly(vinyl alcohol): soft and conducting, Macromolecules 50 (2017) 972-978, **https://doi.org/10.1021/acs. macromol.6b02526**.
2. H. Al-Sagur, K.S. Sundaram, E.N. Kaya, M. Durmus, T.V. Basova, A. Hassan, Amperometric glucose biosensing performance of a novel graphene nanoplatelets- iron phthalocyanine incorporated conducting hydrogel, Biosens. Bioelectron. 139 (2019) 111323, **https://doi.org/10.1016/j.bios.2019.111323**.
3. C.X. Hu, Y.L. Zhang, X.D. Wang, L. Xing, L.Y. Shi, R. Ran, Stable, strain-sensitive conductive hydrogel with antifreezing capability, remoldability, and reusability, ACS Appl. Mater. Interfaces 10 (2018) 44000-44010, **https://doi.org/10.1021/ acsami.8b15287**.
4. Z.X. Deng, Y. Guo, P.X. Ma, B.L. Guo, Rapid thermal responsive conductive hybrid cryogels with shape memory properties, photothermal properties and pressure dependent conductivity, J. Colloid Interface Sci. 526 (2018) 281-294, **https://doi. org/10.1016/j.jcis.2018.04.093**.
5. H.B. Huang, R.P. Chen, S.Y. Yang, L. Li, Y.L. Liu, J. Huang, Facile fabrication of MnO2-embedded 3-D porous polyaniline composite hydrogel for supercapacitor electrode with high loading, High Perform. Polym. 32 (2019) 286-295, **https:// doi.org/10.1177/0954008319860893**.
6. Z.Q. Niu, W.Y. Zhou, X.D. Chen, J. Chen, S.S. Xie, Highly compressible and allsolid-state supercapacitors based on nanostructured composite sponge, Adv. Mater. 27 (2015) 6002-6008, **https://doi.org/10.1002/adma.201502263**.
7. Y. Zhao, Z.H. Li, S.L. Song, K.R. Yang, H. Liu, Z. Yang, J.C. Wang, B. Yang, Q. Lin, Skin-inspired antibacterial conductive hydrogels for epidermal sensors and diabetic foot wound dressings, Adv. Funct. Mater. 29 (2019) 1901474, **https://doi.org/ 10.1002/adfm.201901474**.
8. H. Hosseinzadeh, A. Barghi, Synthesis of poly(AN)/poly(AA-co-AM) hydrogel nanocomposite with electrical conductivity and antibacterial properties, Polym. Compos. 40 (2019) 2724-2733, **https://doi.org/10.1002/pc.25080**.
9. M. Talikowska, X. Fu, G. Lisak, Application of conducting polymers to wound care and skin tissue engineering: a review, Biosens. Bioelectron. 135 (2019) 50-63, **https://doi.org/10.1016/j.bios.2019.04.001**.
10. N. Alegret, A. Dominguez-Alfaro, D. Mecerreyes, 3D scaffolds based on conductive polymers for biomedical applications, Biomacromolecules 20 (2019) 73-89, **https://doi.org/10.1021/acs.biomac.8b01382**.
11. K. Sharma, V. Kumar, B. Chaudhary, B.S. Kaith, S. Kalia, Application of biodegradable superabsorbent hydrogel composite based on gum ghatti-co-poly (acrylic acid-aniline) for controlled drug delivery, Polym. Degrad. Stab. 124 (2016) 101-111, **https://doi.org/10.1016/j.polymdegradstab.2015.12.021**.
12. J. Qu, X. Zhao, P.X. Ma, B.L. Guo, Injectable antibacterial conductive hydrogels with dual response to an electric field and pH for localized "smart" drug release, Acta Biomater. 72 (2018) 55-69, **https://doi.org/10.1016Zj.actbio.2018.03.018**.
13. V.I. Lozinsky, I.Y. Galaev, F.M. Plieva, I.N. Savinal, H. Jungvid, B. Mattiasson, Polymeric cryogels as promising materials of biotechnological interest, Trends Biotechnol. 21 (2003) 445-451, **https://doi.org/10.1016/j.tibtech.2003.08.002**.
14. K.A. Milakin, M. Trchova, U. Acharya, J. Hodan, J. Hromadkova, J. Pfleger, B. A. Zasoanska, J. Stejskal, P. Bober, Conducting composite cryogels based on poly (aniline-co-p-phenylenediamine) supported by poly(vinyl alcohol), Synth. Met. 246 (2018) 144-149, **https://doi.org/10.1016/j.synthmet.2018.10.008.**
15. K.A. Milakin, M. Trchovaa, U. Acharya, S. Breitenbach, C. Unterweger, J. Hodan, J. Hromadkova, J. Pfleger, J. Stejskal, P. Bober, Effect of initial freezing temperature and

comonomer concentration on the properties of poly(aniline-co-m- phenylenediamine) cryogels supported by poly(vinyl alcohol), Colloid Polym. Sci. 298 (2020) 293-301, **https://doi.org/10.1007/s00396-020-04608-5**.

16. L. Li, Y. Zhang, H.Y. Lu, Y.F. Wang, J.S. Xu, J.X. Zhu, C. Zhang, T.X. Liu, Cryopolymerization enables anisotropic polyaniline hybrid hydrogels with superelasticity and highly deformation-tolerant electrochemical energy storage, Nat. Commun. 11 (2020) 62, **https://doi.org/10.1038/s41467-019-13959-9.**
17. K.A. Milakin, U. Acharya, M. Trchovaa, B.A. Zasonaska, J. Stejskal, Polypyrrole/ gelatin cryogel as a precursor for a macroporous conducting polymer, React. Funct. Polym. 157 (2020) 104751**, https://doi.org/10.1016/j. reactfunctpolym.2020.104751**.
18. M. Kurakula, G.S.N.K. Rao, Pharmaceutical assessment of polyvinylpyrrolidone (PVP): as excipient from conventional to controlled delivery systems with a spotlight on COVID-19 inhibition, J. Drug Deliv. Sci. Technol. 60 (2020) 102046, **https://doi.org/10.1016/jjddst.2020.102046**.
19. C. Dispenza, C. Lo Presti, C. Belfiore, G. Spadaro, S. Piazza, Electrically conductive hydrogel composites made of polyaniline nanoparticles and poly(N-vinyl-2- pyrrolidone), Polymer 47 (2006) 961-971, **https://doi.org/10.1016/j. polymer.2005.12.071**.
20. R.K. Gupta, S.S. Gangoliya, N.K. Singh, Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains, J. Food Sci. Technol. 52 (2015) 676-684, **https://doi.org/10.1007/s13197-013-0978-y**.
21. L.J. Pan, G.H. Yu, D.Y. Zhai, H.R. Lee, W.T. Zhao, N. Liu, H.L. Wang, B.C.K. Tee, Y. Shi, Y. Cui, Z.N. Bao, Hierarchical nanostructured conducting polymer hydrogel with high electrochemical activity, Proc. Natl. Acad. Sci. U.S.A. 109 (2012) 9287-9292, **https://doi.org/10.1073/pnas.1202636109**.
22. H.J. Kim, S. Im, J.C. Kim, W.G. Hong, K. Shin, H.Y. Jeong, Y.J. Hong, Phytic acid doped polyaniline nanofibers for enhanced aqueous copper(II) adsorption capability, ACS Sustain. Chem. Eng. 5 (2017) 6654-6664, **https://doi.org/ 10.1021/acssuschemeng.7b00898**.
23. R. Yao, Z.J. Yao, J.T. Zhou, Novel multi-configuration aniline/phytic acid based aerogel with directed higher performance, Mater. Lett. 198 (2017) 206-209, **https://doi.org/10.1016/j.matlet.2017.03.032**.
24. J. Ye, D.J. Shi, Z.K. Yang, M.Q. Chen, Interpenetrating network hydrogels based on nanostructured conductive polymers for flexible supercapacitor, Polym. Sci. 60 (2018) 647-654, **https://doi.org/10.1134/S0965545X18050164**.
25. Y. Gawli, A. Banerjee, D. Dhakras, M. Deo, D. Bulani, P. Wadgaonkar, M. Shelke, S. Ogale, 3D polyaniline architecture by concurrent inorganic and organic acid doping for superior and robust high rate supercapacitor performance, Sci. Rep. 6 (2016) 21002, **https://doi.org/10.1038/srep21002**.
26. H.H. Wu, C.W. Chang, D.L. Lu, K. Maeda, C.C. Hu, Synergistic effect of hydrochloric acid and phytic acid doping on polyaniline-coupled g-C3N4 nanosheets for photocatalytic Cr(VI) reduction and dye degradation, ACS Appl. Mater. Interfaces 11 (2019) 35702-35712, **https://doi.org/10.1021/ acsami.9b10555**.
27. S.W. Wu, C.Q. Zhu, Z.Y. He, H. Xue, Q.R. Fan, Y.L. Song, J.S. Francisco, X.C. Zeng, J.J. Wang, Ion-specific ice recrystallization provides a facile approach for the fabrication of porous materials, Nat. Commun. 8 (2017) 15154**, https://doi.org/ 10.1038/ncomms15154**.
28. D. Mawad, C. Mansfield, A. Lauto, F. Perbellini, G.W. Nelson, J. Tonkin, S.O. Bello, D.J. Carrad, A.P. Micolich, M.M. Mahat, J. Furman, D.J. Payne, A.R. Lyon, J.
    J. Gooding, S.E. Harding, C.M. Terracciano, M.M. Stevens, A conducting polymer with enhanced electronic stability applied in cardiac models, Sci. Adv. 2 (2016), e1601007, **https://doi.org/10.1126/sciadv.1601007**.

29. Z. Moravkova, E. Dmitrieva, Structural changes in polyaniline near the middle oxidation peak studied by in situ Raman spectroelectrochemistry, J. Raman Spectrosc. 48 (2017) 1229-1234, **https://doi.org/10.1002/jrs.5197**.
30. S. Quillard, G. Louarn, J.P. Buisson, M. Boyer, M. Lapkowski, A. Pron, S. Lefrant, Vibrational spectroscopic studies of the isotope effects in polyaniline, Synth. Met. 84 (1997) 805-806, **https://doi.org/10.1016/S0379-6779(96)04155-0**.
31. M.I. Boyer, S. Quillard, E. Rebourt, G. Louarn, J.P. Buisson, A. Monkman, S. Lefrant, Vibrational analysis of polyaniline: a model compound approach, J. Phys. Chem. B 102 (1998) 7382-7392, **https://doi.org/10.1021/jp972652o**.
32. M. Cochet, G. Louarn, S. Quillard, J.P. Buisson, S.J. Lefrant, Theoretical and experimental vibrational study of emeraldine in salt form. Part II, Raman Spectrosc 31 (2000) 1041-1049, **https://doi.org/10.1002/1097-4555(200012)31:12<1041:: AID-JRS641>3.0.CO;2-R.**
33. M.I. Boyer, S. Quillard, G. Louarn, G. Froyer, S. Lefrant, Vibrational study of the FeCl3-doped dimer of polyaniline; A good model compound of emeraldine salt, J. Phys. Chem. B 104 (2000) 8952-8961, **https://doi.org/10.1021/jp000946v**.
34. G. Cirie-Marjanovie, M. Trchova, J. Stejskal, The oxidative polymerization of aniline in water: Raman spectroscopy, J. Raman Spectrosc. 39 (2008) 1375-1387, **https://doi.org/10.1002/jrs.2007.**
35. P. Humpolmek, K.A. Radaszkiewicz, Z. Capakova, J. Pachermk, P. Bober, V. Kasparkova, P. Rejmontova, M. Lehocky, P. Pomzil, J. Stejskal, Polyaniline cryogels: biocompatibility of novel conducting macroporous material, Sci. Rep. 8 (2018) 135, **https://doi.org/10.1038/s41598-017-18290-1**.
36. P. Bober, Z. Capakova, U. Acharya, B.A. Zasonska, P. Humpolmek, J. Hodan, J. Hromaadkovaa, J. Stejskal, Highly conducting and biocompatible polypyrrole/poly (vinyl alcohol) cryogels, Synth. Met. 252 (2019) 122-126, **https://doi.org/ 10.1016/j.synthmet.2019.04.015**.