# MACROPOROUS HYDROGELS BASED ON 2-HYDROXYETHYL METHACRYLATE. PART 1. COPOLYMERS OF 2-HYDROXYETHYL METHACRYLATE WITH METHACRYLIC ACID

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A series of macroporous crosslinked copolymers of 2-hydroxyethyl methacrylate (HEMA) with methacrylic acid (MA) was prepared in the presence of fractionated particles of sodium chloride. The hydrogels were characterized by the number of pores in unit volume, the pore size, the water content in pores and diffusion parameters. The structure of the hydrogels was followed by confocal and scanning electron microscopy.

**Keywords**: Diffusion; Hydrogels; 2-Hydroxyethyl methacrylate; Macroporous polymers; Morphology; Polyanions; Gels.

Macroporous hydrogels find application as biocompatible implants in tissues, such as in the central nervous system[1,2] or as substrates in cellular and tissue engineering[3–7]. Porosity is usually achieved by one of four basic methods:

*1*. Crosslinking polymerization in the presence of substances that are solvents for the monomers, but precipitants for the formed polymer[1–8].

*2*. Crosslinking polymerization in the presence of soluble substances (sugars, salts) which are washed out from the hydrogel after polymerisation[3–6].

*3*. By crosslinking polymerization in the presence of substances liberating gases which remain in the resulting hydrogel[9].

*4*. By frost sublimation of the hydrogel swollen in water[10,11].

Using any of these procedures, hydrogels with communicating or non-communicating pores can be obtained, *i.e.* the pores are or are not interconnected. In the present work we have chosen procedure No. *2*, which enables us to vary the pore size in a wide range. We wished to produce hydrogels with communicating pores, therefore we had to use a relatively large amount of sodium chloride in the polymerization mixture. At the same time we had to use a NaCl/monomer ratio leading to the polymers of suitable mechanical properties. The higher the content of sodium chloride, the more interconnected are the pores, but the poorer is the strength of the resulting hydrogel. The hydrogels described in this communication are to be used as implants in the central nervous system.

## EXPERIMENTAL

### Preparation of Hydrogels

Macroporous hydrogels were prepared in the pelleting apparatus shown in Fig. 1, using the following mixtures: 2-hydroxyethyl methacrylate (0.67 g); 2,2′-azobis(isobutyronitrile) (0.0067 g); sodium chloride (10.02 g); ethylene dimethacrylate (0.019 g); poly(ethylene glycol), $M_w$ = 400 (3.79 g); methacrylic acid, 0–17.9 wt. % relative to the total monomer weight (0–25 mole %); 2-hydroxyethyl methacrylate (Röhm) containing 0.11 wt.% of ethylene dimethacrylate as crosslinking agent.

The used sodium chloride was screen-fractionated into three fractions with grain sizes below 0.03 mm, 0.03–0.05 mm and 0.05–0.09 mm. Thorough mixing of the components produced a paste that was transfered into the polymerization chamber of the pelleting apparatus, the chamber was closed by a flange with fastening screws and the tightening screw was tightened in a standard way with a moment of force of 10 Nm. The whole pelleting apparatus was thermostatted to 80 °C for 8 h; after cooling the hard pellet was taken out and weighed. From the weight of the pellet the content of sodium chloride

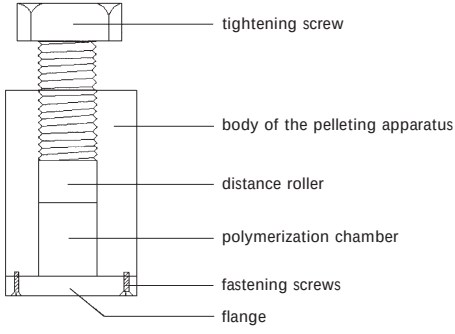


FIG. 1
Pelleting apparatus for the preparation of macroporous hydrogels

($m_{NaCl}$) and the content of polymer ($m_H$) were calculated. The pellet was then washed with a 1% solution of sodium hydroxide (5 × 8 h), thus transforming the carboxyl groups of methacrylic acid (MA) to carboxylate groups, and finally washed with a physiological solution (5 × 8 h). The volume of the swollen macroporous hydrogel ($V_H$) was calculated from its dimensions.

Samples of homogeneous hydrogels with the same concentrations of MA as in the macroporous hydrogels, but in the absence of sodium chloride, were further prepared. After washing with a solution of sodium hydroxide and physiological solution (the same procedure as with macroporous hydrogels), the volume fraction of dry polymer in equilibrium-swollen hydrogel ($Z_V$) was determined.

Characterization of Hydrogels

Macroporous hydrogels were characterized by the following quantities:

*1*. The number of pores in the macroporous hydrogel in 1 $cm^3$ of sample, *i.e.* the number of sodium chloride particles ($n$):

$n = m_{NaCl}/[\rho_{NaCl}(4/3)\pi(d/2)^3 V_H]$, where $\rho_{NaCl}$ is the density of sodium chloride (2.16 g $cm^3$) and $d$ is the average size of the fractionated sodium chloride particles (0.015, 0.04 and 0.07 mm).

*2*. Total volume of all pores in 1 $cm^3$ of the hydrogel, *i.e.* the volume of water in all pores ($V_V$):

$V_V = [V_H - m_H/(\rho_p Z_V)]/V_H$, where $\rho_p$ is the density of dry polymer (1.2 g $cm^3$).

*3*. Average volume of one pore:

$V_1 = V_V/n$

*4*. Diameter of one pore in macroporous hydrogel:

$d_H = 2[3V_1/(4\pi)]^{1/3}$

*5*. Total surface area of all pores in 1 $cm^3$ of macroporous hydrogel:

$S = 4\pi(d_H/2)^2 n$

All calculations were made under a simplifying assumption of spherical shape of sodium chloride particles, *i.e.* the irregular shape of NaCl crystals was approximated by spheres of average diameter given by the mesh size of the screen used for fractionation. The diameters were 0.015, 0.04 and 0.07 mm. Another assumption is 100% conversion of the polymerization, *i.e.* the weight of the dry matter of the hydrogel phase is equal to the weight $m_H$.

*6*. Tetramethylammonium diffusion measurements:

We studied the diffusion characteristics of the hydrogels by the real-time tetramethylammonium ($TMA^+$) iontophoretic method[12], which is used to study the diffusion parameters of extracellular space in the neural tissue and of hydrogels[13]. $TMA^+$ ions were introduced into the tissue with an iontophoretic micropipette, and their concentration was measured at a known distance with a $TMA^+$-selective microelectrode. The micropipette and microelectrode were glued together to stabilize the intertip distance. The acquired diffusion curves were fitted using a nonlinear curve-fitting algorithm to determine three ECS diffusion parameters: volume fraction ($\alpha$), tortuosity ($\lambda$) and non-specific $TMA^+$ uptake ($k'$). The volume fraction is the restricted volume of the tissue or hydrogel which is available for diffusion; tortuosity is the calculated factor defined as $\lambda = (D/ADC)^{1/2}$ reflecting the increased path length for the diffusion of $TMA^+$ ions between two points in the tissue or hydrogel due to various barriers which slow down their diffusion. Before measuring in hydrogel or tissue, several diffusion curves were recorded in agar where, by definition, $\alpha = 1$, $\lambda = 1$ and $k' = 0$, thus enabling the transport number of the electrode array to be determined.

Morphology of Hydrogels

The morphology of the prepared hydrogels was checked with a low-vacuum scanning electron microscope Aquasem (Tescan, Brno, Czech Republic). The pressure in the Aquasem microscope chamber can be as high as 500–1100 Pa, which makes it possible to observe samples containing frozen water. The samples were prepared by cutting the hydrogel in physiological solution with a razor blade: from the middle of hydrogel a thin cross-section (*ca* 0.5 mm thick) was cut off, removed from physiological solution and instantly frozen in liquid nitrogen. The frozen sample was placed on the cooled sample stage (–20 °C) of an Aquasem microscope, where it was fixed with a tiny amount of water (the water freezes immediately after touching the cooled sample stage). The samples were observed using two backscattered-electron detectors in combination with an ionization detector.

The microstructure of the samples was also characterized using a confocal microscope (Leica). Prior to observation in the confocal microscope the hydrogel samples were immersed in a solution of Lucipher Yellow (Sigma–Aldrich) for 1 min and washed with physiological solution for 10 min. The sample was then scanned using an objective with water immersion. In the pictures taken with the confocal microscope, individual pores were marked, and their average area in $mm^2$, $S_K$, was calculated using the Image analysis toolbox in Matlab 5.3 (The Mathworks, Inc.). Then for every sample, by approximation with a circle of the same area, the average diameter of the pore $d_K$ was calculated.

$$d_K = 2(S_K/\pi)^{1/2}$$

Similarly as above, also here the irregular shape of NaCl crystals was approximated by spheres of diameter $d_K$.

## RESULTS AND DISCUSSION

The dependence of the volume fraction of the dry polymer in the equilibrium-swollen homogeneous, *i.e.* nonporous hydrogel on the MA content in the copolymer (*x*, mole %) is shown in Fig. 2. As the macroporous hydrogels are envisaged to be used as implants in nervous tissue, all their properties were followed in physiological solution.

From Fig. 2 it follows that in the crosslinked copolymer with 2-hydroxyethyl methacrylate (HEMA), the dependence of $Z_V$ is steep up to about 9 mole % of MA and does not much change further, reaching the value of about 0.05. The contents of methacrylic acid higher than 25 mole % could not be measured because the hydrogel spontaneously decomposed during swelling. The values of $Z_V$ had to be measured for further calculations of the characteristics of macroporous hydrogels.

Figure 3 shows the dependence of hydrogel porosity on the methacrylic acid content in the polymer for three different fractions of the used sodium chloride. Porosity was characterized as the number of pores in 1 $cm^3$ of the hydrogel, *i.e.* as the density of all (communicating and noncommunicating) pores. The measured dependences correspond to the relation of $Z_V$

*vs* % MA according to Fig. 2; at a constant content of sodium chloride in the polymerization mixture, the density of pores is given by the volume of the hydrogel, *i.e.* by the degree of its swelling. As the degree of swelling changes but little above *ca* 9 mole % of MA, so the effect of the MA content

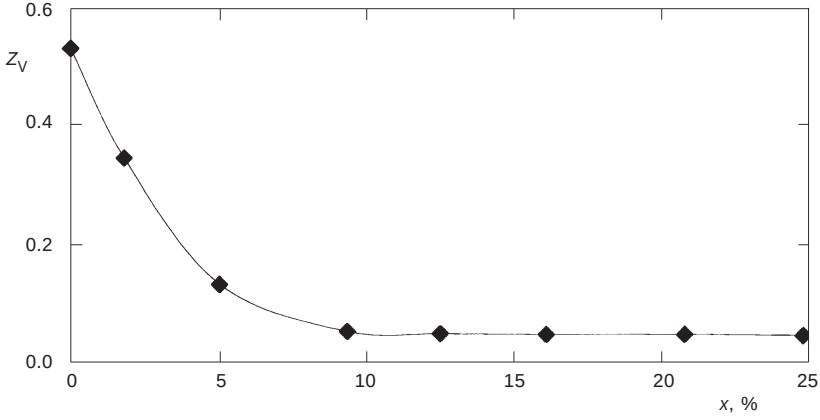


FIG. 2
Dependence of polymer volume fraction ($Z_V$) in an equilibrium-swollen homogeneous hydrogel on the MA content in the copolymer (*x*, mole %)

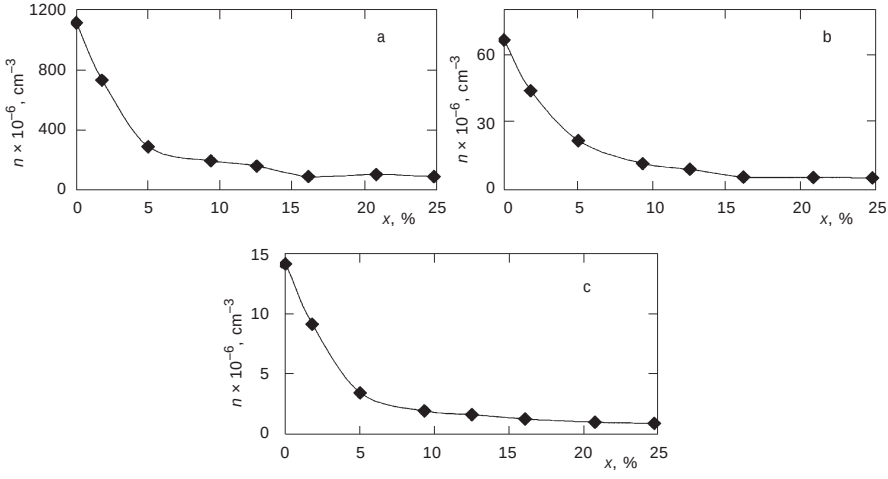


FIG. 3
Dependence of pore density (*n*, cm$^{-3}$) on the MA content (*x*, mole %) in a macroporous hydrogel for three average sizes of the fractionated sodium chloride particles (*d*, mm): 0.015 (a), 0.04 (b), 0.07 (c)

is also small in this region. Simultaneously the porosity of hydrogels grows sharply with decreasing particle diameter of the used sodium chloride, as at a constant NaCl weight in the polymerization mixture the number of its particles increases.

Interesting is the dependence of the specific pore volume (*i.e.*, the volume of pores in 1 $cm^3$ of macroporous hydrogel) on the content of methacrylic acid in the copolymer, as shown in Fig. 4.

Contrary to the dependence of $n$ on mole % MA, $V_V$ is only little affected by the size of NaCl particles; for individual fractions the measured curves differ but little, and for the MA content of *ca* 10 mole %, all three curves show a pronounced minimum. This can be explained in the following way: with increasing swelling ability of the hydrogel, the thickness of the walls between the pores increases, and thus also the overall volume of the hydrogel that has a negative influence on the specific pore volume. However, the surface area of the walls increases together with the pore volume, which leads to an increase in $V_V$. Both these effects are almost compensated in the ranges 0–5 mole % MA and 16–25 mole % MA, where $V_V$ is almost independent of the concentration of methacrylic acid and has a value of about 0.5 (in the macroporous hydrogel the volumes of polymer and pores are practically equal). On the contrary, at 9 mole % MA, the effect of wall thickness strongly predominates, $V_V$ shows a pronounced minimum and the pore volume is the lowest relative to the volume of the hydrogel.

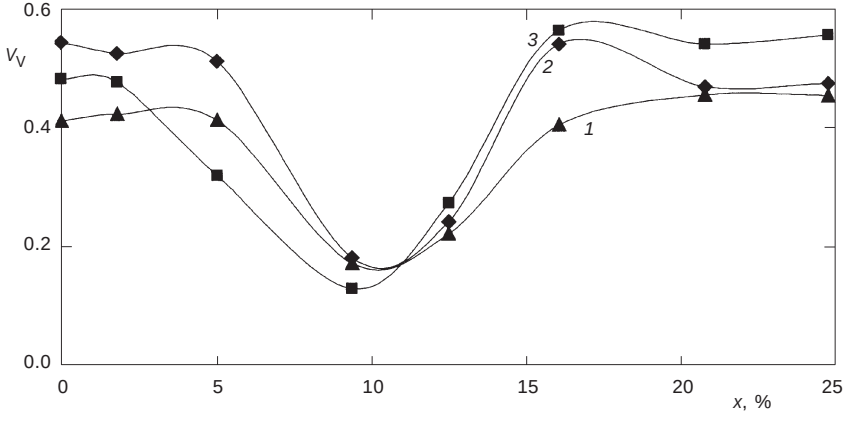


FIG. 4
Dependence of specific pore volume ($V_V$) in a macroporous hydrogels on the MA content ($x$, mole %) for three average sizes of the fractionated sodium chloride particles ($d$, mm): 0.015 (*1*), 0.04 (*2*), 0.07 (*3*)

Figure 5 shows the dependences of the average values of the volume of one pore in swollen macroporous hydrogel for all three studied fractions of sodium chloride. From Fig. 5 it follows that $V_1$ grows monotonical by increasing MA content in the measured range, but the growth is somewhat sharper at a higher amount of the ionogenic component in the copolymer. A similar trend is observed in the dependence of pore diameter on the MA content in the copolymer, as shown in Fig. 6. At low fractions of the ionogenic component in the copolymer, the calculated pore diameter is smaller than the particle size ($d$) of the used sodium chloride, at higher fractions $d_H > d$. The values of $d$ and $d_H$ agree in the range 9.3–12.7 mole % MA,

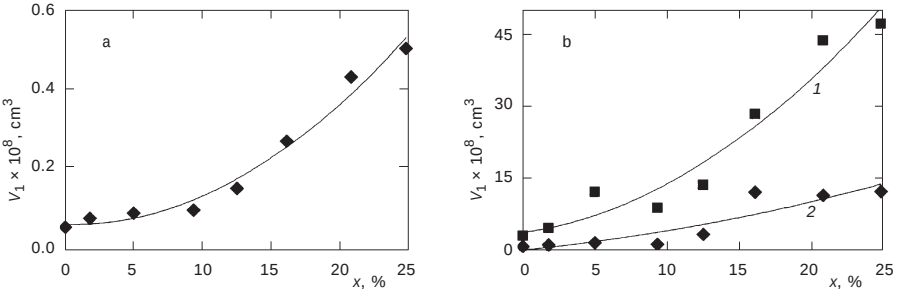


FIG. 5
Dependence of the single-pore volume ($V_1$) on the MA content ($x$, mole %) for three average sizes of the fractionated sodium chloride particles ($d$, mm): a 0.015; b 0.04 (*1*), 0.07 (*2*)

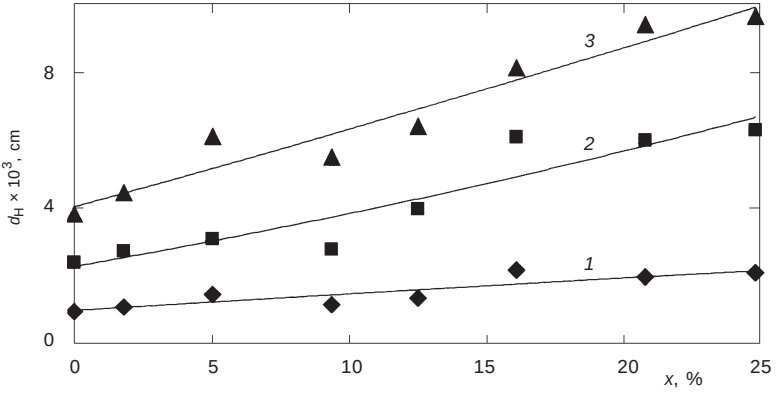


FIG. 6
Dependence of pore diameter ($d_H$) on the MA content ($x$, mole %) for three average sizes of the fractionated sodium chloride particles ($d$, mm): 0.015 (*1*), 0.04 (*2*), 0.07 (*3*)

which almost coincides with the range of pronounced minima on the curve of $V_V$ *vs* mole % MA (Fig. 4). The stated dependence of pore diameter on the amount of methacrylic acid can be well approximated in the given range by straight lines, which are the steeper, the larger are particles of the used sodium chloride. With increasing *d*, the amount of the ionogenic component in the copolymer, where $d = d_H$ increases (9.26, 10.65, 12.73 mole % MA).

The poorly pronounced minima indicate a dependence of the total surface area (*S*) of the pore surface (per 1 $cm^3$ of hydrogel) on the content of the ionogenic component in the polymer, as shown in Fig. 7, while the values of *S* grow in the whole measured range with decreasing size of sodium chloride particles.

The diffusion parameters were measured in the hydrogels with 5–21 mole % MA. The values of volume fraction $\alpha$ in the prepared hydrogels were lower than in the dilute agar gel, where diffusion proceeds freely in the whole volume and[12] $\alpha = \lambda = 1$. The values of $\alpha$ measured in the prepared hydrogels lie between 0.75 and 0.96, while their dependence on the methacrylic acid content was not proved. On the assumption that the prepared hydrogels contain only communicating pores and diffusion proceeds only through these pores, the values of $V_V$ and $\alpha$ should be identical. As this identity has not been proved and $\alpha > V_V$, it has to be concluded that a part of the pores in the hydrogels is noncommunicating and diffusion of $TMA^+$ proceeds partly also through the walls between the pores.

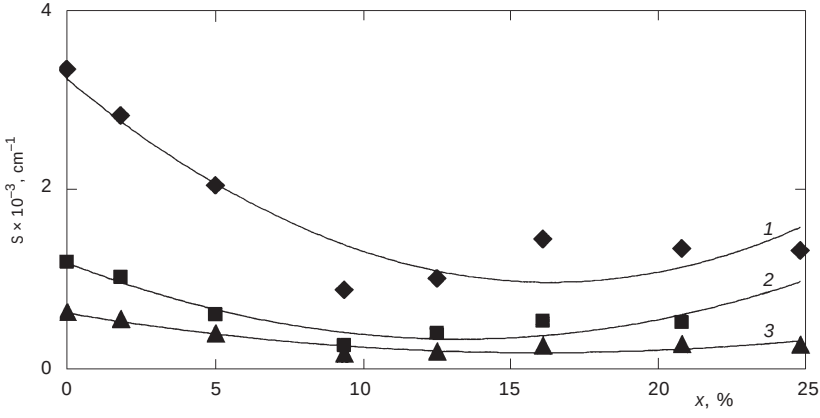


FIG. 7
Dependence of total pore surface area (*S*) on the MA content (*x*, mole %) for three average sizes of the fractionated sodium chloride particles (*d*, mm): 0.015 (*1*), 0.04 (*2*), 0.07 (*3*)

The values of tortuosity λ in the prepared hydrogels lie in the range 1.04–1.09. From this it follows that the diffusion of $TMA^+$ ions in the hydrogels is somewhat more hindered than in dilute agar gel, where[12] λ = 1. The values of non-specific uptake $k'$ corresponding to loss of diffusible $TMA^+$ ions, lie at *ca* $1 \times 10^{-7}$ $cm^2$ $s^{-1}$; the value is almost the lowest measurable and comparable with the value for agar. Therefore we do not assume that in the described macroporous hydrogels the binding of $TMA^+$ to functional groups of the polymer occurs.

A standard method for the observation of hydrogel morphology in aqueous medium is the environmental scanning electron microscopy (ESEM). An example of the hydrogel with 16.1 mole % MA and the NaCl fraction 0.05–0.09 mm using an Aquasem microscope is shown in Fig. 8, from which it is clearly evident that the pores are mostly communicating. A small drawback of this method is the need to place the sample in water vapour in vacuum, where changes in sample morphology at reduced pressure cannot be completely excluded.

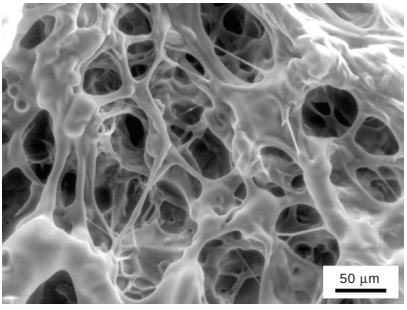


FIG. 8
An Aquasem microscope picture of a hydrogel with 11.3 mole % of MA and NaCl fraction 0.05–0.09 mm

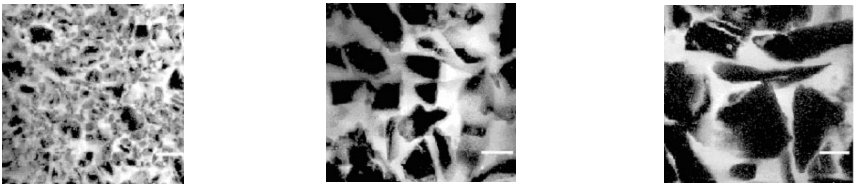


FIG. 9
Reconstruction of hydrogel with 8 mole % MA and NaCl fraction 0–0.03 mm (a), 0.03–0.05 mm (b), 0.05–0.09 mm (c) based on confocal microscope pictures. Scale 40 µm

On the contrary, the confocal microscope enables us to observe the microstructure of the prepared hydrogels in a medium where no deformation of their structure occurs, *i.e.* directly in physiological solution. Reconstructions of hydrogels with 9.35 mole % MA and different fractions of sodium chloride are shown in Fig. 9a–9c, while hydrogels with the same fraction of sodium chloride and a different content of methacrylic acid are compared in Fig. 10a and 10b. The pore volume of the hydrogel in Fig. 10a is almost double compared with the hydrogel in Fig. 10b. Although the figures obtained with the confocal microscope have a poorer resolution and do not show the spatial structure of the hydrogels, they complement the picture obtained with the electron microscope.

A series of sections obtained with the confocal microscope could be easily treated with methods of picture analysis and used for verification of the assumed morphology of the hydrogel. In Fig. 11 we see a correlation beteen

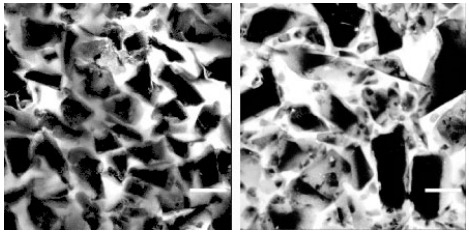


FIG. 10
Reconstruction of hydrogel with NaCl fraction 0.03–0.05 mm and MA content 12.5 mole % (a), 24.8 mole % (b) based on confocal microscope pictures. Scale 40 μm

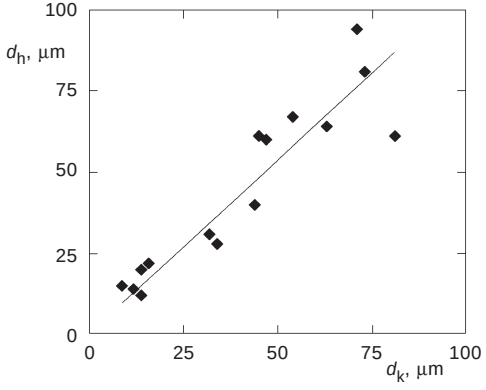


FIG. 11
Correlation of calculated pore diameters ($d_H$) and averages obtained by picture analysis ($d_K$); $d_H = 1.076 d_K$, $R^2 = 0.8417$

the value of $d_H$ calculated from the average volume of pores in the hydrogel, and the value $d_K$ obtained by calculation of the average pore volume from sections displayed using the confocal microscope. The slope of the line relating the values of $d_H$ *vs* $d_K$ (with a correlation coefficient of 0.84) is 1.08, *i.e.* $d_H = 1.08 d_K$. The deviation of 1.08 (theoretical value is 1) between the discussed values can be interpreted as a small difference between the assumed and observed pore size.

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