RIGA TECHNICAL UNIVERSITY
Faculty of Material Science and Applied Chemistry
Institute of Polymer Materials

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Doctoral study programme "Chemical Technology"

POLY(VINYL ALCOHOL) CRYO-HYDROGEL SYSTEMS FOR DEVELOPMENT OF BIOMATERIALS

Summary of Doctoral thesis

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Professor, Dr.habil.sc.ing.
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APPROVAL

With the following I approve that I have elaborated present doctoral dissertation, which is submitted to be reviewed in Riga Technical University to qualify for the doctoral degree in Chemical Engineering. The doctoral dissertation is not submitted anywhere else to qualify for a scientific degree.

Jolanta Staško ……

Date ……

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Jolanta Staško

CONTENTS

ABBREVIATIONS

CFU – colony forming units;

C_X - concentration of PVS water solution, where $_X$ – concentration of solution wt.%;

DSC – differential scanning calorimetry;

E – initial modulus of elasticity;

E_a – conditional tensile modulus of elasticity;

E_C - initial compression modulus of elasticity;

E_{Ca} – conditional compression modulus of elasticity;

$E_{C\varepsilon}$ – effective compression modulus of elasticity;

E_ε – effective tensile modulus of elasticity;

$\bar{E}_{C\varepsilon}$ - average compression modulus of elasticity calculated from $E_{C\varepsilon}(\varepsilon)$;

$\bar{E}_\varepsilon$ - average tensile modulus of elasticity calculated from $E_{C\varepsilon}(\varepsilon)$;

f-t – freezing-thawing;

M – molecular mass;

M_C – average molecular mass between crosslinks;

M_{CE} - average molecular mass between crosslinks, calculated from rubber elasticity theory;

$M_{C\varphi}$ - average molecular mass between crosslinks, calculated from polymer equilibrium swelling theory, *Flory-Rehne*r approach;

MOD – modifiers;

n_C – number of freezing-thawing cycles;

PVA – poly(vinyl alcohol);

R – universal gas constant;

S – cross-section area;

SEM – scanning electron microscopy;

T – absolute temperature, ^oK;

t – time;

$T^{\bullet}_A$ – average temperature changing rate of thawing;

$T^{\bullet}_S$ - average temperature changing rate of freezing;

T^b_{H20} – beginning temperature of water removal;

T^e_{H20} – end temperature of water removal;

TG – thermogravimetry;

T_{krit} – critical temperature;

t_{krit} – critical time;

T_{min} – minimal temperature of freezing;

TS – therapeutic substance;

t_S* - freezing time;

TS_N – natural occuring therapeutic substance;

TS_S – synthetic therapeutic substance;

V_1 – the molar volume of solvent;

ϑ – specific volume of the polymer;

ν_0 – initial rat of water uptake;

ρ – density;
σ – tensile/compression stress;
σ_B/ρ –specific strength;
ϕ – the functionality of crsslinks;
φ_P – volume fraction of pores;
φ_{POL} – volume fraction of polymer;
χ - *Flory-Huggins* interaction parameter between the polymer and swelling agent;
$\psi^{\infty D}_{H2O}$ – weight fraction of water in gel at the equilibrium state of sorption after desorption;
ψ_{H2O} – water weight fraction;
ΔH – melting enthalpy;
ΔH_{H20} – endothermic heat effect of water evaporation;
ε – relative deformation;
ε_B - relative deformation of fracture;
φ^{∞}_{POL} – volume fraction of polymer at the equilibrium state of sorption;
φ_{H2O} – volume fraction of water;
φ^{P}_{H20} – water volume fraction in the pores of gel;
φ_{POL} – volume fraction of polymer;
φ^{pol}_{H20} – water volume fraction in gel;
φ^*_{POL} – actual volume fraction of polymer;
$\rho_{x\varphi}$ - crosslinking density calculated from sorption data;
σ_B – tensile strength at break;
σ_{CB} – compression stress;
$\psi^{\infty *}_{H2O}$ – initial weight fraction of water;
ψ^{∞}_{H2O} – weight fraction of water at the equilibrium state of sorption;
ψ^{∞}_{POL} – weight fraction of polymer at the equilibrium state of sorption;
ψ_{GEL}- gel weight fraction;

A – St.-John`s-wort;
BR – *Viride nitens*;
C – starch;
HC – hemicellulose;
K – calendula;
KMC – sodum salt of carboxymethylcellulose;
MZ – methylene blue;
P – peppermint;
PRO – paste of pine extract.

OVERALL REVIEW OF THE THESIS

The state of the art

Wound dressings are one of the most important variety of medical material application. Nowadays, the range of materials used in wound dressings is extremely broad. Following materials are widely used: textile material dressings (medical gauze, elastic bandage), membranes, gels, various composite dressings etc.

The interest in specific (multi-purpose) dressings continues to grow in recent years. Multi-purpose dressings are especially necessary for severe wounds healing: burns, diabetic ulcers, etc., which needs special care. For that reason, nowadays there is sharp increase in the need for dressings, which are able to meet many of requirements at the same time, thus ensuring the wound healing process in particularly comfortable conditions and promoting the formation of new tissues, as well. Dressing change should be minimally traumatic and may not damage the newly formed epithelium cells. Dressing should be easy to use and as possible adaptable to individual needs. Considering these high expectations and nowadays determined individual approach for patient wounds healing, it is necessary to develop a dressing with a broader functionality and more comfortable use in practice.

The applied idea of the study is to create polymer material that can be used for the development of effective dressings, containing therapeutic substances. The number of important requirements is established for dressings. The dressing must effectively absorb wound exudates, provide optimal water, air and heat exchange between the wound and the environment, protect wound against infection from environment. Dressing must be with adequate mechanical strength and, finally, it should be in close contact with the wound surface and at the same time be readily detachable from it.

Evaluating the major properties of diverse polymers, including biocompatibility and toxicity, it is concluded that *polyvinyl alcohol (PVA) in hydrogel form* is particularly suitable for this purpose. Analysis of advantages and disadvantages of different PVA hydrogel synthesis methods leads to the conclusion, that the most appropriate method of obtaining PVA hydrogel is a *cryogenic* cross-linking of PVA in aqueous solutions, by formation of intermolecular crosslinks in successive freeze - thaw (f-t) cycles.

The most important parameter, which characterizes the structure of the gel, is the crosslinking density that determines the significant characteristics of the gel properties: swelling ability, strength characteristics and thermal resistance.

Large swelling capability, which is optional constraint for medical gel, requires low values of crosslinking density. At the same time, adequate strength and thermal resistance of swollen gel can be achieved for highly cross-linked gels. This means that must be found the degree of crosslinking value that provides a sufficient level of all these parameters, prioritizing the gel swelling ability.

Aim

Therefore the aim of the work is to study the formation process of poly(vinyl alcohol) gel, ongoing in freez-thaw cycles, in the area of low values of crosslinking density and to find the conditions under which develops gels with complex of properties suitable for the pharmaceutical polymer dressing material.

Tasks

Several tasks must be performed:

1. To develop the technology of successive freezing-thawing of PVA water solutions and to identify conditions those provide formation of gels with high water sorption capacity.
2. To find out the most important parameters, which characterize molecular structure and morphology of obtained hydrogels.
3. To assess the effect of temperature and cyclic water desorption-sorption cycles on the stability of obtained hydrogels.
4. To estimate strength-deformation characteristics obtained hydrogels.
5. To apprise the possibility to modify the structure and properties of PVA hydrogel by of use of gel-forming additives.
6. To evaluate the cytotoxicity and cell proliferation *in vitro* of synthesized gels.
7. To perform microbiological studies of synthesized gels by establishing colonization level and adhesion activity microorganisms.
8. To develop methods ensuring required content of medicine water solutions in the ready for use gel based drug delivery forms.
9. To develop production technology of cryo-hydrogel dressing prototype.

Scientific novelty

Crosslinking conditions that provide formation of slightly cross-linked hydrogels with high water sorption capacity together with sufficiently high characteristics of strength-deformation and thermal stability has been established. Relationship between hydrogel crosslinking degree and these characteristics has been investigated and confirmed.

It is revealed that synthesized hydrogels morphologically fit to a composite consisting of a water-swollen PVA gel and water inclusions with complicated pore hierarchy.

The practical value

Cryo-hydrogel dressing prototypes as well technological scheme of their production have been elaborated.

Cytotoxicity and cell proliferation *in vitro* of the synthesized gels has been evaluated.

Microbial colonization and adhesion intensity on the gel surface of has been established. Biocompatibility of PVA cryo-hydrogels has been demonstrated.

Methods ensuring required content of medicine water solutions in the ready for use gel based drug delivery forms have been developed.

Statements of defend

Gels with the crosslinking density, which provides large water swelling capacity and at the same time sufficient strength-deformation characteristics and heat resistance parameters can be obtained at the selected conditions of PVA gel cryosynthesis and at a small number of the freezing-thawing cycles (≤ 3)

Complex of properties of synthesized gels meets the requirements of drug containing dressing material.

Composition and volume of the thesis

The 130 pages of dissertation, including 83 pictures and 25 tables, comprise the introduction and 3 chapters: a review of literature, methodological part, experimental part, and overall conclusions, a list with 100 references, 2 annexes.

Approbation of the thesis

Results presented in the dissertation are approbated in 2 SCI journal articles, 4 full text articles in conference proceedings and have been protected by 1 patent of Latvia.

BRIEF CONTENT OF THESIS

In introduction actuality and the aim of the study are justified and working guidelines set out.

The first chapter contains a review of the literature. Information on the use of polymers in medicine field is summarized. Characteristics of structure and properties of the polymer gels, and especially polymer hydrogels, are discussed. The important role of gels in the field of development of medical materials, particularly dressing materials, is analyzed.

PVA as a potential polymeric material which can be used for drug containing dressings in the form of a hydrogel is described in details. The selection of cryogenic freezing-thawing crosslinking method from the range of potential methods of PVA hydrogels synthesis is justified.

Aim and tasks of the study are formulated in the end of the chapter.

The second chapter contains in detailed description of the studied materials.

The process of obtaining of cryogels (Fig. 1) and the cryogenic process time-temperature regime characteristics (Fig. 2) are described in this chapter.

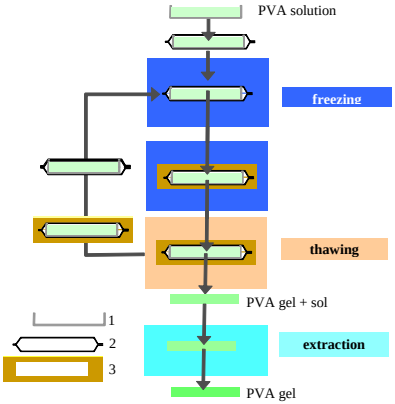

Fig. 1 Cryosynthesis process of PVA gel: 1 – aluminium mould, 2 – sealable plastic bag, 3 – foam capsule

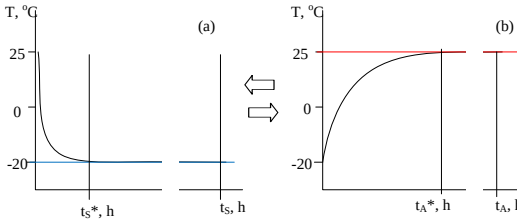

Fig. 2 Time-temperature regime scheme of freezing (a) - thawing (b) process.

The basic content of the chapter is the characterisation of research methods of synthesized hydrogels and determinable parameters:

- Sorption and extraction methods for determination of gel content and gel sorption capacity.
- Methods for determination of strength-deformation parameters of swollen gels in tensile and compression.
- Research technique of gel structure and ongoing processes by use of differential scanning calorimetry (DSC) and thermogravimetry (TG).
- Gel morphology studies by scanning electron microscopy (SEM).
- Gel sorption - desorption (drying) kinetic studies.
- Technique of cytotoxicity and cell proliferation evaluation on the gel surface.
- Microbiological studies of gels.
- Methodology of evaluation of adhesiveness of gels.

In the third chapter experimental results are summarised and discussed.

SUMMARY AND EVALUATION OF EXPERIMENTAL RESULTS

1. The option of the polymer and conditions of cryoprocess

The DSC analysis of freeze-thaw process shows (Fig. 3), that the crystallization of water in PVA aqueous solution begins below -8^{o}C and completely ends below -18^{o}C. At -20^{o}C water is completely frozen. Therefore, this temperature was chosen as the lowest freezing temperature in experiments.

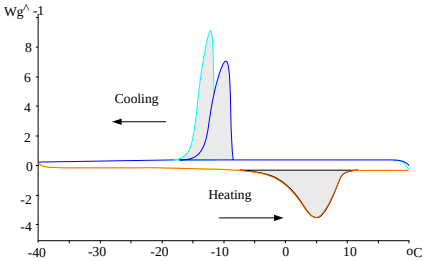

Fig. 3 DSC thermogram of freeze-thaw process of PVA aqueous solution (C_{15})

Studies of the effect of concentration C of PVA solutions and molecular mass M_{PVS} on gel formation, showed:

- at low concentration of solution (C_5, C_{10}) stable cryogels does not form irrespective of PVA molecular mass (Tabe 1).
- PVA (25) (in brackets polymer molecular weight · 10^{-3}) does not form cryogels at the range of all concentrations and number of freeze - thaw (f-t) cycles.

Table 1

PVA cryogel formation characteristics

Molecular mass of PVA M · 10^{-3}, g/mol	PVA solution concentration				n_C
	C_5	C_{10}	C_{15}	C_{20}	
25 (88 %)					1
					2
					3
					5
					10
88 (88 %)					1
					2
					3
					5
					10
130 (98 %)					**1**
					2
					3
					5
					10
145 (98 %)					1
					2
					3
					5
					10

	Gel does not form
	Heterogeneous gel forms
	Homogeneous gel forms

- PVA (88) forms homogeneous cryogels at high concentrations of solution (C_{15}, C_{20}) and at large number of cycles $n_C \geq 3$.
- PVA (130) and PVA (145) form a homogeneous cryogels at high concentration of solution (C_{15}, C_{20}) already at the first f-t cycle.
- PVA solutions with a concentration ≥ 25% is hard to use due to their high viscosity.

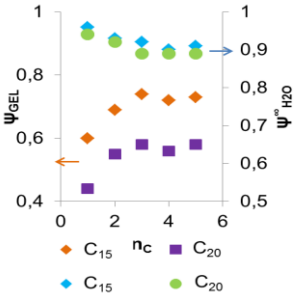

Fig.4. The dependence of hydrogel characteristics ψ_{GEL} and ψ^{∞}_{H2O} on the number of f-t cycles n_C

The dependence of the values of most important criteria of the efficiency of hydrogel synthesis process: gel content ψ_{GEL} (characterizing crosslinking process outcome and indicating what part of the polymer molecules has formed cross-linked structure) and gel water sorption capacity ψ^{∞}_{H2O} (characterizing quality of formed gel - the cross-links density) on the f-t number cycles n_C is reversal (Fig. 4): ψ_{GEL} value increases, but ψ^{∞}_{H2O} decreases with increasing n_C; $n_C \leq 3$ provides $\psi_{GEL} \geq 0,8$ and $\psi^{\infty}_{H2O} \leq 0,95$.

For further studies following f-t parameters were used (Table 2):

Table 2

Summary of freez-thaw parameters

Lowest freezing temperature	T_{min}	-20°C
Average rate of temperature change at freezing	$T^{\bullet}_S$	~ 2°C/min
Retention time at T_{min}	t_S*	12 h
Average rate of temperature change at thawing	$T^{\bullet}_A$	0,27°C/min
Number of freezing-thawing cycles	n_C	≤ 3

15 and 20 % (C_{15} and C_{20}) PVA (130) water solution was used.

2. Morphology of hydrogel

Information on the morphology of synthesized hydrogels can be obtained by the analysis of the SEM microphotographs.

For preparing SEM samples, gels were slowly frozen to -20°C at the thermostat, then frozen in liquid nitrogen (- 196°C) and broken. Fractures were dried at a low temperature (-25°C) to constant weight and analyzed by SEM without defrosting.

Framework configuration of the gel remains in the cryogenic drying process (Fig. 5).

SEM microphotographs analysis suggests that the synthesized cryogels morphologically corresponds to a composite, consisting of a water-swollen PVA gel and water inclusions (pores) with rather complex pore hierarchy. Such structure is schematically shown in Fig. 6.

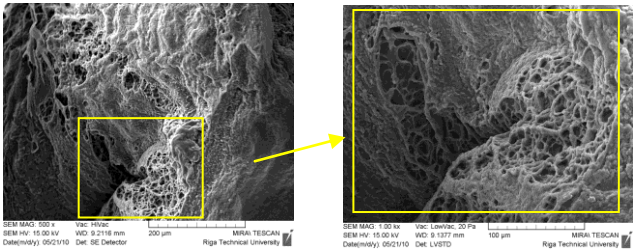

Fig. 5 PVA hydrogel SEM microphoto (C_{15}, PVS III, $n_C = 3$)

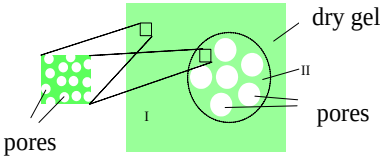

Fig. 6 Schematic structure of porous of dried gel

Using image processing program Vega TC, cross-sectional area of each pore (approximated by an oval) on the SEM microphotographs was determined. The distribution of the pore area and the total pore area fraction were calculated.

The gel base is fine-pored structure with a relatively small pore sizes (the dominant pore cross-sectional area is 0,3 – 0,5 μm^2). In this structure areas with larger pores (10 – 50 μm^2) is distributed. The walls of the pore are fine-pored itself. The total pore volume fraction φ_P does not exceed 0,3.

3. Evaluation of thermal stability of PVA gels in the water environment

There was the reason to believe that cross-links, which form in PVA aqueous solution in freezing-thawing cycles, no differ by nature from intermolecular bonds linking the macromolecules in cryogenically unprocessed PVA samples. For that reason in addition unfrozen swollen PVA samples ($n_C = 0$) were also included in the thermal stability tests of PVA cryogels.

Studied samples were placed in water at 20°C and heated at a rate of 2°C/min. Values of several "critical" temperatures T_{krit} where recorded, at which substantial changes in the samples where observed:

T^{I}_{krit} - samples become more transparent (sample swelling degree increases, the existing associates dissipate less light).

T^{II}_{krit} – samples lose the original regular form (sample swelling degree further increases, weaker intermolecular bonds breaks, the degree of swelling in the sample volume becomes unequal).

T^{III}_{krit} – samples either dissolves or splits (disintegrate in small fragments of swollen gel).

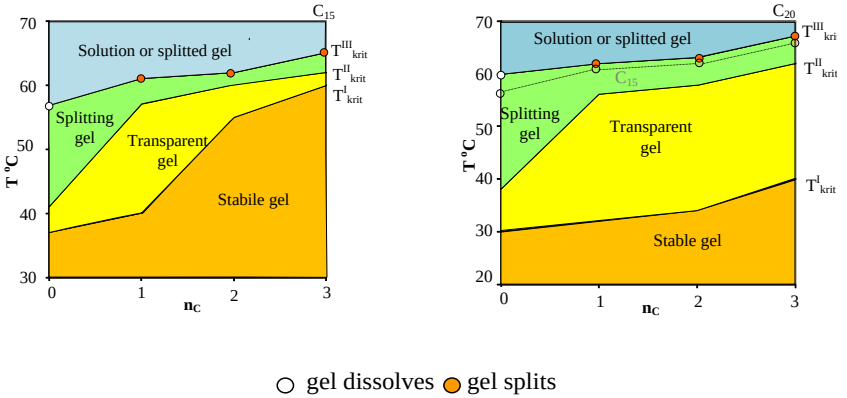

Fig. 7 The dependence of critical temperature from the number of f-t cycle

Figure 7 shows the critical temperature values and the corresponding gel state areas. As can be seen, the values of the critical temperature increase with the number of f-t cycles. The highest increase was observed in the case of T^{II}_{krit} (for 17°C, C_{15} and 24°C, C_{20}).

T^{III}_{krit} value increases less (for 4°C, C_{15}; and 6°C, C_{20}). Though, if the not frozen PVA gels dissolve, the cryogels splits, maintaining most durable cross-links in gel fragments also over the T^{III}_{krit}.

For another group of samples durability of cross-links was determined, namely the time period t_{krit}, in which samples dissolves/splits in water at different temperatures: 25°C, 40°C, 60°C. There is a large difference between the value of t_{krit} for unfrozen and frozen-thawed samples (Fig. 8). As might be expected, durability of unfrozen samples is negligible. They dissolve in a rather short period of time even at the lowest temperature. Increasing of the cycle number considerably raise the gel durability. Gel samples ($n_C = 3$) withstand with no visible changes even 20 days at a temperature of 60°C (just 5°C below the T^{III}_{krit}).

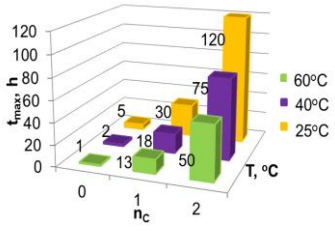

Fig. 8 dependence of t_{krit} on exposing temperature and number of f-t cycles (C_{15})

All findings clearly show both: a significant increase of the total energy of crosslinks as well growth of proportion of more resistant crosslinks by increase of number of f-t cycles.

The results suggest that the thermal resistance of synthesized cryogels is sufficient for medical use. However, it imposes certain restrictions on sterilization conditions of gel products.

4. DSC and TG studies of PVA gels

DSC and TG studies were performed to determine the role of water in formation of crosslinked structures of swollen PVA hydrogels.

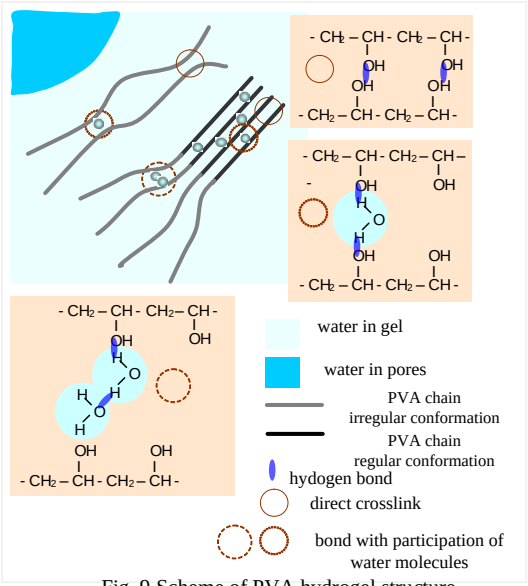

Fig. 9 Scheme of PVA hydrogel structure

There is a reason to believe that cross-linked gel structure consists of both direct intermolecular bonds between the hydroxyl groups of PVA and the intermediate links with the participation of water molecules (Fig. 9). The hydrogen bonds in these structures may play a major role. The higher is gel crosslinking degree, the more stable cross-links can be formed.

It was interesting to find out how the temperature affects the water leaving from the swollen gel. DSC and TG seemed appropriate methods for this purpose.

Gels dried up to equilibrium weight at 25, 60 and 105°C were studied.

Flat endothermic peak on the DSC thermograms (Fig. 10) corresponds to the water removal process from the gel. It is possible to determine temperature limits of water removal process (the beginning temperature T^b_{H2O} and the end temperature T^e_{H2O}) as well weight fraction of removed water, from endothermic heat effect of water evaporation ΔH_{H2O}.

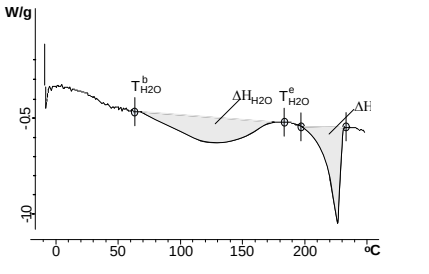

Fig. 10 DSC thermogram of C_{15} PVA unfrozen sample dried of 25°C (n_C = 0)

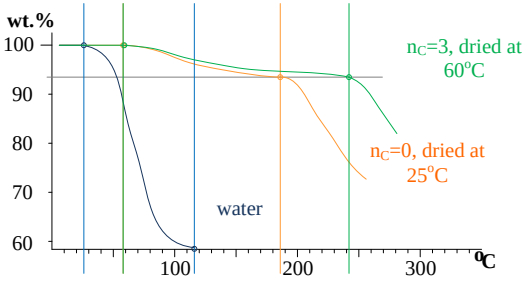

Fig. 11 TG thermogram C_{15}: unfrozen dried sample 25°C (n_C = 0), 60°C dried PVA gel (n_C = 3) and pure water

The values of water removal temperature interval for unfrozen samples determined from DSC thermogram: 62 - 182°C and TG thermogram: 58 - 186°C (Fig. 11) are rather close. Compared with pure water (26 - 117°C), this interval is

significantly shifted towards higher temperatures, suggesting a strong enough interaction of water and PVA macromolecules also in the unfrozen system.

There is gradual shift of the temperature interval of water removal towards higher temperatures with increase of the number of f-t cycles suggesting the raise of the resistance of PVA - water links.

Drying of gels at low temperatures (25 and 60°C) the system leaves more than 90% of the total water content. This is considered as free water.

Removable water (less than 8%), determined by TG analysis, is bonded water. The amount of bonded water slightly increases (Fig. 12 a) by increase of the number of f-t cycle.

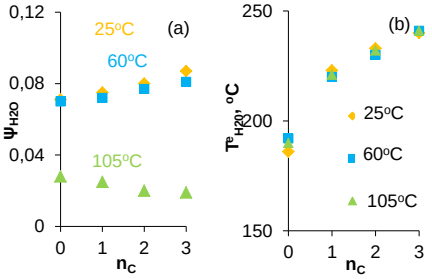

Fig. 12 Water weight fraction ψ_{H2O} in gels (a) and T^e_{H2O} values (b)

Increase of n_C, leads to expanding of the temperature range of water removing and raise the end temperature of water removal T^e_{H2O} (Fig. 12 b).

This means that in the consecutive f-t cycles the ability of water molecules more efficiently associate with hydroxyl groups of PVA macromolecules increases: temperatures necessary to remove bonded water becomes higher (from 186 to 241°C).

Drying at 105°C does not release system from bonded water. About 20 to 40% of bonded water remains in the system. This amount decreases with increase of number of f-t cycle.

Both unfrozen sample and cryogel DSC termogram show pronounced endothermic peak with a maximum at 225 - 228°C (Fig. 10). This peak obviously corresponds to the melting to PVA crystalline phase and coincides with melting peak of solid PVA crystalline phase: 228°C. Apparently, heating the amorphous gel in DSC analysis over the temperature range 70 - 180°C, the gel-forming intermolecular bonds gradually degrade and at the same time mutual adjustment of PVA macromolecules takes place. Crystalline phase forms in the result. Exothermic effect is this process “spread” over a wide temperature range and is masked by the endothermic effect of water removal process. The water content in the gel and the degree of crosslinking of PVA (the number of f-t cycles) has negligible effect on formation of crystalline phases in the heating process.

5. Behavior of gels in desorption-sorption cycles

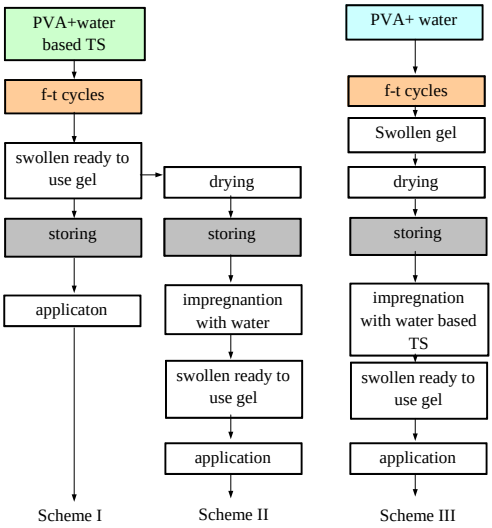

Fig. 13 Obtaining of PVA gels with therapeutic substances

Thinking about the possibility of use of synthesized hydrogels in medicine, is necessary to know in what particular form the gel containing water solutions of therapeutic substances (TS), can be stored prior to utilization. Large content of TS water solutions in the gel can make awkward safe storage of gels in swollen state.

There may be also other medical gel obtaining schemes and gel storage forms (Fig. 13).

Schemes II and III envisage drying of obtained gels, storage in the dry state, and subsequent impregnation with aqueous solution of TS.

It was important to find out whether the relatively weak cross-linked network still remains during the drying process and is able to provide subsequent efficient gel swelling.

Effect of drying on the gel structure and the sorption capacity was studied as follows.

PVA gels were subjected to desorption (drying) – water absorption cycles. Samples were dried (at 25°C, 60°C and 105°C, respectively) until the constant weight. Water content in the gel ψ_{H2O} was determined by periodic weighing. After each drying, samples are placed in distilled water at 20°C. Absorbed water content in the gel ψ_{H2O} was fixed up to the equilibrium sample weight.

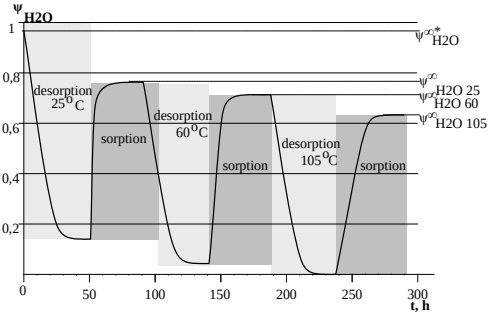

Fig. 14 Scheme of change of water content ψ_{H2O} in desorption - sorption cycles

Schematic representation of the kinetic curves of water content $\psi_{H2O}(t)$ is given in Fig. 14.

From the sorption curves $\psi_{H2O}(t)$ a number of kinetic parameters were identified: weight fraction of water at the equilibrium state of sorption ψ^{∞}_{H2O}, weight fraction of water at the equilibrium state of sorption after desorption $\psi^{\infty D}_{H2O}$, initial rate of water uptake $v_0 = \lim(d\ \psi_{H2O}/dt)|_{t\to 0}$, weight

fraction of water after 15 min and 1 h of sorption ($\psi_{H2O(15)}$, $\psi_{H2O(60)}$), as well as the time required to reach 80% of ψ^{∞}_{H2O}.

After drying at 25°C, gels still contain 12-15% of the initial water content, after drying at 60°C - only 5-10%.

Irrespective of drying conditions, after drying gels are able to absorb water intensively and to reach high equilibrium water content, maintaining cross-linked structure (Table 3).

Table 3

Weight fraction of water at the equilibrium state ψ^{∞}_{H2O} in dried gels after sorption

Equilibrium water content ψ^{∞}_{H2O}, weight fraction, **in bold** percentage from water content in the initial gel	n_c	Sorption after drying at 25° C		Sorption after drying at 60° C		Sorption after drying at 105° C	
		C_{15}	C_{20}	C_{15}	C_{20}	C_{15}	C_{20}
	1	0,76 **79**	0,78 **83**	0,73 **76**	0,73 **78**	0,62 **65**	0,59 **63**
	2	0,77 **83**	0,76 **83**	0,71 **76**	0,71 **77**	0,59 **63**	0,56 **61**
	3	0,77 **84**	0,74 **83**	0,69 **71**	0,71 **80**	0,56 **61**	0,57 **64**

Equilibrium water content ψ^{∞}_{H2O}, which dried gel reaches by sorption, decreases with the raise of drying temperature, but is still large enough (Table 3). After drying at 25°C, value ψ^{∞}_{H2O} exceeds 80% of the initial water content in the gel, after drying at 60°C it is 75-80%, and after 105°C - no less than 60%.

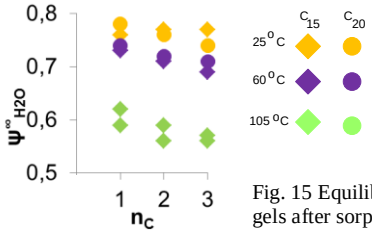

Fig. 15 Equilibrium water content in dried gels after sorption

Equilibrium water content is less for gels synthesized at higher number of f-t cycles (Fig. 15). The results show that in the drying process the cross-linked structure of gel is not destroyed. Moreover, in each of the drying cycles additional cross-links forms.

The increase of resistance of gel for water sorption also indicates the raise of crosslinking degree too: the initial water sorption rate v_0 decreases with number of f-t cycles (Fig. 16). The time required to achieve a certain amount of water in the gel, increases as well, see Figure 17.

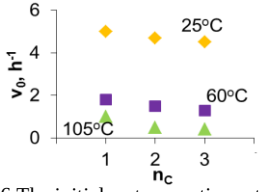

Fig. 16 The initial water sorption rate v_0 of PVA gels dried at different temperatures and obtained with different number of f-t cycles

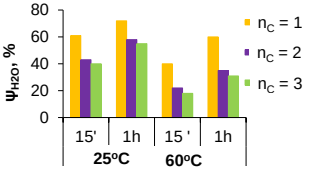

Fig. 17 Water content in gels after a sorption period (15 'and 1h), depending on the drying temperature (25 and 60°C) and the number of f-t cycles

After drying at 60°C gel contains only 5-10% water (Table 4). Dried gel is capable to absorb water intensively (80% of equilibrium water content can be reached within 6-10 h). This means that if necessary it is acceptable to store gels in dried form and saturate them with water solution of particular therapeutic substances just before use.

Table 4

Initial equilibrium water weight fraction $\psi^{\infty *}_{H2O}$ in gels and equilibrium water weight fraction ψ^{∞}_{H2O} afer drying (**in bold** percentage from $\psi^{\infty *}_{H2O}$)

n_C	$\psi^{\infty *}_{H2O}$		ψ^{∞}_{H2O} after drying at			
	C_{15}	C_{20}	25°C		60°C	
			C_{15}	C_{20}	C_{15}	C_{20}
1	0,96	0,94	0,13 **13,5**	0,11 **11,7**	0,04 **4,2**	0,07 **7,5**
2	0,93	0,92	0,13 **13,9**	0,11 **11,9**	0,05 **5,4**	0,09 **9,8**
3	0,92	0,89	0,14 **15,2**	0,11 **12,4**	0,05 **5,4**	0,09 **10,1**

6. Evaluation of crosslinking degree of PVA hydrogels

Crosslinked gel structure was quantitatively described by a statistical average molecular mass between closer crosslinks - M_C by use of the expression, postulated within polymer equilibrium swelling theory: *Flory-Reihner* approach (1) [1, 2]:

$$M_{C\varphi} = -\frac{\rho(1-2/\phi)V_1\left(\left(\varphi^{\infty}{}_{POL}\right)^{1/3} - 0{,}5\varphi^{\infty}{}_{POL}\right)}{\ln(1-\varphi^{\infty}{}_{POL}) + \chi\left(\varphi^{\infty}{}_{POL}\right)^2 + \varphi^{\infty}{}_{POL}} \qquad (1)$$

where:

$M_{C\varphi}$ – average molecular mass between crosslinks, g/mol,

ρ – density of unswollen polymer,

ϕ – functionality of the crosslinks, $\phi = 3$ [3],

$\varphi^{\infty}{}_{POL}$ – volume fraction of polymer at the equilibrium state of sorption,

V_1 – the molar volume of solvent (water),

χ – *Flory-Huggins* interaction parameter between swelling agent (water) and the polymer, $\chi = 0{,}49$ [4].

Values of crosslinking density $\rho_{x\varphi}$, were calculated as follows (2) [1, 4-5]:

$$\rho_{x\varphi} = \frac{1}{\vartheta M_C} \qquad (2)$$

ϑ- specific volume of the polymer.

For calculation of $M_{C\varphi}$ and $\rho_{x\varphi}$ the experimentally determined values of φ_{POL} can not be used because they were calculated assuming that all the water in the gel is evenly distributed in swollen gel structure. However, as can be seen from the SEM microphotographs, synthesized gels have a composite structure. Sufficiently large volume fraction of the water $\varphi^{P}{}_{H20}$ (see Fig. 5) is located in the gel pores. So water volume fraction directly in the swollen gel is (3):

$$\varphi^{pol}_{H20} = \varphi_{H20} - \varphi^{P}_{H20} \qquad (3)$$

where:

φ^{pol}_{H20} – water volume fraction in gel,

φ_{H20} – total volume fraction of water,

φ^{P}_{H20} – water volume fraction in the gel pores

The real polymer volume fraction φ^*_{POL} in swollen gel can be calculated as follows (4):

$$\varphi^{*}_{POL} = \frac{\varphi_{POL}}{\varphi_{POL} + \varphi^{pol}_{H2O}} \qquad (4)$$

where:

φ_{POL} – total volume fraction of polymer in composite gel.

We have calculated the pore volume fraction for one of the gel (n_C = 3, C_{15}). SEM image visual assessment suggests that φ_P value for other gels differs little. So we thought that it is possible to use the value φ_P = 0,29 for calculation of real volume fraction of polymer φ^*_{POL} also for other gels.

Crosslinking characteristics of synthesized gel are summarized in Table 5.

Table 5

Parameters characterizing crosslinking degree of gels

n_C	ψ^{∞}_{H2O}	φ^{∞}_{POL}	$\varphi^{\infty *}_{POL}$	$M_{C\varphi} \cdot 10^{-3}$ (g/mol)	$\rho_{x\varphi} \cdot 10$ (mol/l)
C_{15}					
1	0,96	0,032	0,046	47	0,27
2	0,93	0,056	0,080	12	1,05
3	0,92	0,064	0,092	8,6	1,49
C_{20}					
1	0,94	0,047	0,067	19	0,67
2	0,92	0,064	0,092	8,6	1,49
3	0,89	0,088	0,128	3,6	3,55

$M_{C\varphi}$ values decrease, while respective $\rho_{x\varphi}$ values significantly increase with raise of number of f-t cycles - more than 5 times. For gels obtained from concentrated PVA solution $\rho_{x\varphi}$ values are about 2-3 times higher (Table 5). Calculated $M_{C\varphi}$ and $\rho_{x\varphi}$ values indicate that the crosslinking degree of synthesized gels is relatively small and gels can be qualified as slightly cross-linked.

$M_{C\varphi}$ and $\rho_{x\varphi}$ values were calculated also for dried gels, assuming that φ_P = 0,29. As can be seen (Table 6, Fig. 18) for gels, exposed to successive desorption - sorption cycles, $M_{C\varphi}$ values substantially decreases, while the corresponding $\rho_{x\varphi}$ values increases.

Crosslinking density of the gel should be related to resistance of gel to water sorption. We can assume that M_C value characterizes the cross-section of a "canal", through which water transfer in the gel occurs in sorption process. In accordance with scheme (Fig. 19), water transfer rate must be proportional to the canal "cross-section", consequently the value of M_C^2.

Table 6

Crosslinking degree characteristics of the dried gel

n_C	$M_{C\varphi} \cdot 10^{-3}$ (g/mol) and $\rho_{x\varphi} \cdot 10$ (mol/l), in brackets							
	Initial gels		Gels dried at					
			25°C		60°C		105°C	
	C_{15}	C_{20}	C_{15}	C_{20}	C_{15}	C_{20}	C_{15}	C_{20}
1	47 (0,27)	19 (0,67)	0,33 (38,8)	0,44 (29,2)	0,22 (57,0)	0,22 (58,2)	0,06 (209,8)	0,045 (284,4)
2	12 (1,05)	8,6 (1,49)	0,38 (33,8)	0,37 (34,8)	0,17 (74,8)	0,17 (74,8)	0,045 (284,4)	0,03 (387,9)
3	8,6 (1,49)	3,6 (3,55)	0,38 (33,8)	0,28 (45,1)	0,14 (94,8)	0,17 (74,8)	0,03 (387,9)	0,04 (355,6)

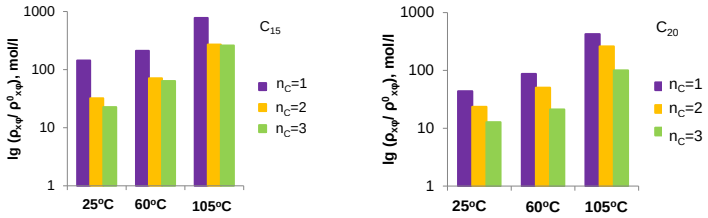

Fig. 18 The dependence of relative values crosslinking density $\rho_{x\varphi} / \rho^0_{x\varphi}$ of gels on drying temperature and number of f-t cycles ($\rho^0_{x\varphi}$ – the initial value of the gel crosslinking density)

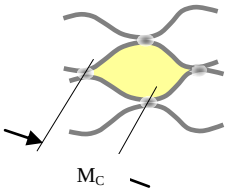

Fig. 19 Schematic representation of the"canal" in macromolecular structure of gel

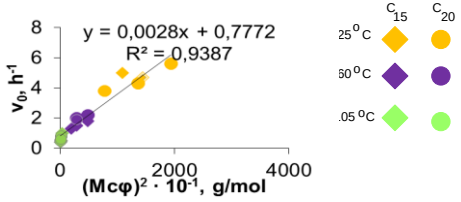

Fig. 20 Correlation of ν_0 and $M_{C\varphi}$ values

As can be seen from Figure 20, values of the initial rate of water sorption $\nu_0 = \lim(d\psi_{H2O}/dt)|_{t\to 0}$ decreases by decreasing of M_C the values. The correlation between ν_0 un M_C^2 values is close to linear.

7. Strength-deformation characteristics of hydrogel

Strength-deformation characteristics first of all are important constituent of the complex of hydrogel exploitation properties. On the other hand stress (σ) and deformation (ε) relationships gives additional information concerning the gel structure.

Relationships σ(ε) in tension and compression modes were experimentally obtained. A number of parameters were determined from the tension relationships: tensile strength at break σ_B, relative elongation at break ε_B and several types of tensile elasticity modulus: conditional modulus $E_a = \sigma_B/\varepsilon_B$, initial modulus E =

$\lim(d\sigma/d\varepsilon)|_{\varepsilon\to 0}$, effective modulus $E_\varepsilon = d\sigma/d\varepsilon$, as well as module $\bar{E}_\varepsilon$ - average value from the $E_\varepsilon(\varepsilon)$ relationship (Fig. 21, Table 7).

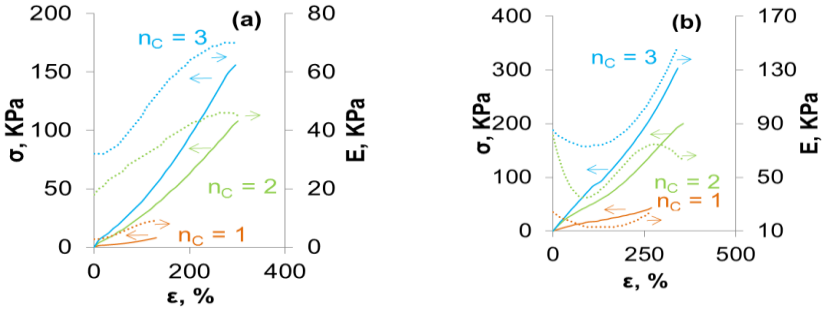

Fig. 21 σ(ε) and E(ε) relationships in tensile mode of PVA hydrogel: C_{15} (a) and C_{20} (b)

Table 7

Strength-deformation characteristics of gels in tensile and compressive mode

	C_{15}			C_{20}		
	n_C					
	1	**2**	**3**	**1**	**2**	**3**
E_a (KPa)	7	36	53	16	56	89
E_{Ca} (KPa)	7	29	38	-	-	-
$\bar{E}_\varepsilon$ (KPa)	6	35	53	17	58	93
$\bar{E}_{C\varepsilon}$ (KPa)	7	27	39	-	-	-
E (KPa)	3	18	32	26	80	85
σ_B (KPa)	9	108	156	43	200	303
ε_B	1,3	3,0	3,0	2,7	3,6	3,4
σ_B/ρ (m)	86	1036	1497	407	1893	2869
σ_{CB} (KPa)	0,7	2,64	0,004	-	-	-

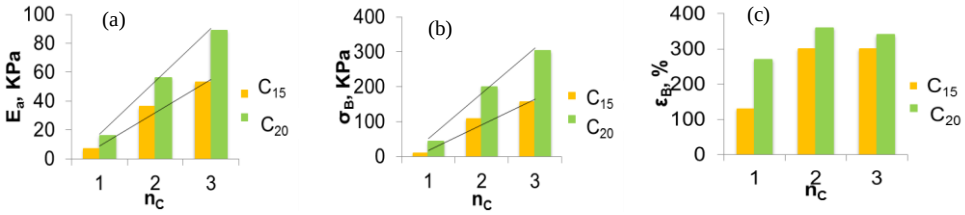

Fig. 22 Conditional tensile modulus E_a (a), tensile strength at break σ_B (b) and relative deformation at break ε_B (c) for PVA gels depending on n_C

E_a and σ_B values increase significantly (almost an order of magnitude) by increasing the number of f-t cycles. E_a, σ_B, ε_B values are higher for gels synthesized from more concentrated PVA solutions (C_{20}) (Fig. 22).

Considering that the degree of crosslinking of gel, calculated from the sorption data, increases by increasing the value of (Table 5), the growth E_a and σ_B values with raise of n_C is logical.

An attempt was made to calculate the statistical average molecular weight between crosslinks M_C by use an expression based on high-elasticity theory of crosslinked polymers [6] (5):

$$M_{CE} = \frac{3\vartheta RT}{E_a} \qquad (5)$$

where:

M_{CE} – average molecular weight between crosslinks, g/mol,

ϑ - specific volume of the polymer,

R – universal gas constant

T – absolute temperature

Calculated value of M_{CE} compared with the corresponding values calculated from the sorption data (Table 5) turned out to be incredibly high: $10 \cdot 10^{-4}$ to $130 \cdot 10^{-4}$ g/mol.

It means that the experimental E_a values used for calculation are not correct. E_a characterizes the gel as a composite, consisting of water swollen gel matrix with isolated water inclusions, which volume fraction φ_P we have evaluated in the second chapter. Tensile stresses do not actually distribute throughout the whole composite cross section area S, but just on the part of it: $S^* = S (1 - \varphi_P)$.

Using for M_{CE} calculation values of modulus E_a^*, corresponding to the stress distribution in the cross section area S^* ($E_a^* = E_a [1/(1- \varphi_P)]$, $E_a^* > E_a$) only slightly smaller M_{CE} values were obtained. For example, if the E_a = 53 KPa , E_a^* = 75 kPa, $M_{CE} \cdot 10^{-4}$ = 18 g/mol, $M_{CE}^* \cdot 10^{-4}$ = 12,5 g/mol.

Apparently the stress distribution in the real gel samples is rather complex. Actual values stresses in the gel, which are necessary for calculation of the modulus of elasticity of the gel, failed to identify.

Nevertheless, the composite modulus E_a value can be used as a relative parameter, which characterizes crosslinking degree of the gel.

As shown in (Fig. 23) $M_{C\varphi}$ and $1/E_a$ values correlate almost linearly.

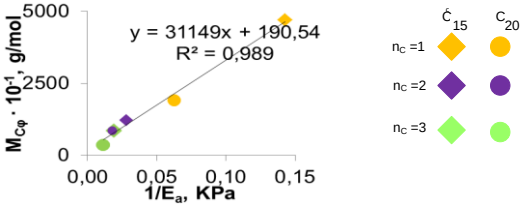

Fig. 23 Correlation of $M_{C\varphi}$ and E_a values

Values of tensile strength σ_B and relative deformation at break ε_B of synthesized gels are entirely determined by gel crosslinking degree (Fig. 24).

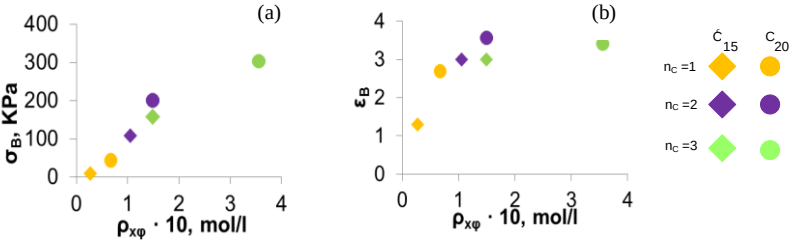

Fig. 24 Values of σ_B (a) and ε_B (b) depending on the crosslinking density $\rho_{x\varphi}$

σ_B values of swollen gels seems rather small. However, the specific strength of gels σ_B/ρ (where ρ – density of gel), expressed as self support length (Table 7), shows, that strength of gels is sufficient to ensure reliable handling of swollen gel articles in practice.

8. PVA gel`s composites with polysaccharides

In order to improve the deformation characteristics of the hydrogels, PVA composites with several polysaccharides were made. All used additives are characterized by a high content of hydroxyl groups in macromolecules and, consequently, by large intermolecular interaction energy. So they are basically insoluble in water, but effectively swell. It seemed possible that the swollen polysaccharide particles (the initial size 15-30 μm), will create an self phase in PVA gel and interact with PVA macromolecules. It could increase resistance of composite to deformation.

Following polysaccharides were used: carboxymethylcellulose sodium salt (KMC), hemicellulose (water soluble fraction) (HC) and Alojas potato starch (C). Swollen polysaccharide particle suspension (water content 10%) was put into PVA solution before freeze-thaw process.

Polysaccharide additives promote the formation of PVA cryogels. In the presence of additives gels can be obtained also from 10% PVA aqueous solutions. The highest ψ_{GEL} values show PVA C_{10} gels with starch.

Compression strength-deformation characteristics (stress σ_{CB}, corresponding to the relative compression deformation $\varepsilon = 0{,}1$ and several types of compression modulus values: conditional compression modulus $E_{Ca} = \sigma_{CB}/0{,}1$, initial modulus $E_C = \lim(d\sigma/d\varepsilon)|_{\varepsilon\to 0}$, effective modulus $E_{C\varepsilon} = d\sigma/d\varepsilon$ as well as module $\bar{E}_{C\varepsilon}$ as average value from the $E_\varepsilon(\varepsilon)$ relationship) for gels with additives were determined (Table 8).

There is a significant increase of modulus E_{Ca} (Fig. 25) and σ_{CB} values by adding polysaccharides in PVA gels especially at a small number of f-t cycles. This shows that dispersed polysaccharide microgels effectively participate in the structure of PVA gel composite.

Table 8

Strength-deformation characteristics in compression of hydrogel and hydrogel composites (C_{15})

				Composites (content of additive 5%)								
	Pure PVA gel			C			KMC			HC		
	n_C											
	1	2	3	1	2	3	1	2	3	1	2	3
$E_C a$, KPa	7	29	38	28,5	53,8	74,2	9,1	35,5	42,9	15,8	35,3	59,5
$\bar{E}_C \varepsilon$, KPa	7	27	37	30,5	53,6	80,4	9,4	36,2	42,3	14,7	35,1	58,4
σ_{CB}, KPa	0,07	3	4	3	5	7	1	4	5	2	3,5	5,5
				Composite, content of additive, 10 %								
$E_C a$, KPa	-	-	-	12,6	44,8	87,6	20,5	35,3	36,8	13,7	46,1	47,9
$\bar{E}_C \varepsilon$, KPa	-	-	-	12,4	44,4	87,2	20,1	36,5	38,0	13,5	44,8	47,6
σ_{CB}, KPa	-	-	-	1	5	9	2	4	4	1,5	5	5

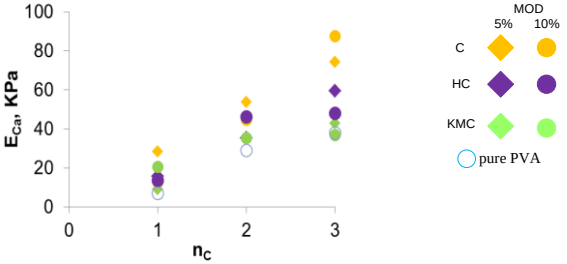

Fig. 25 Values of elastic modulus E_{Ca} of PVA hydrogel - a polysaccharide composites depending on the number of f-t cycles

9. Modification of PVA hydrogel with therapeutic substances

It has been found, that wound dressings, in which therapeutic substances are incorporated, shows positive results in wound treatment. Use of herbal therapeutic substances in traditional medicine has been particularly increased in recent years [7-9]. This is because highly potent synthetic therapeutic substances in some cases may prevent the development of new cells and hence hinder the successful healing of wounds.

Ethanol extracts of natural therapeutic substances (TS_N) and synthetic therapeutic substances solutions (TS_S) were added in PVA aqueous solution prior freezing-thawing. Following TS_N extracts: the calendula (K), peppermint (P), St.-John's Worth (A) [7-9] and provitamin paste (PRO) as well as some simple synthetic antibacterial TS_S solutions: *Viride Nitens* (BR) and methylene blue (MZ) were selected [10-11].

The effect of presence of these TS on the gel formation process was tested. The concentration of TS solution not hindering the formation of gel was determined. It is found that in presence of 0,5 and 2,5% solutions of K, A, P gel is forming, gel is forming also at all concentrations of PRO (0,5%, 2,5% and 5%). 5% TS_N extracts

hinder gel formation. Stable gels are forming by adding TS_S - BR, MZ at concentrations 0,15 and 0,3%.

Almost all TS slightly reduce water sorption capacity of synthesized gels.

The colonization level and the adhesion activity of the microorganisms *Pseudomona aeruginosa* and *Staphylococcus epidermidis* on PVA gels i*n vitro* were studied.

Adhesion intensity on pure PVA gels is rather small. With both bacteria adhesion starts at concentration of 10 CFU/ml. Somewhat higher adhesion was observed with *Ps.aeruginosa*. PVA gels with 0.3% BR shows lowest observed intensity of adhesion *S.epidermidis* at all bacteria concentrations.

The lowest intensity of colonization of *S.epidermidis* shows PVA gels with TS_S (Br, MZ 0,15 and 0,3%). TS_S do not hinder *Ps.aeruginosa* colonization. On the PVA gels with TS_N and composites with polysaccharides very intensive colonization was observed.

The intensity of colonization of the bacteria and the possible biofilm formation were confirmed visually by use of SEM. It was observed that *Ps.aeruginosa* formes the biofilm on the PVA gels with TS_S (higher concentration) after 24 h incubation.

Less pronounced biofilm was observed on the gels with the TS_N and on composites with polysaccharides (after 24 h incubation in 10^4 CFU/ml). In the sample with K, microcolonies of *S.epidermidis* partially covered with glycocalyx were observed, while *Ps.aeruginosa* was colonizing the surface as an even layer of separate cells.

Studies of PVA gel biocompatibility and cytotoxicity *In vitro* also were performed. It is shown that pure PVA gel is biocompatible and can be used as biomaterial for the development of wound dressing.

10. Determination of gel adhesion

Dressing gel layer should be in close contact with the wound surface but at the same time it must be easily detachable from it. The adhesion ability of gel dressing to different surfaces: glass, various polymers, skin etc. was assessed. Maximum peeling load does not exceed 25 g/cm. This level of adhesion of the gel is considered to be the appropriate for wound dressing.

11. Development of PVA cryo-hydrogel dressing prototypes

Prepared dressing containing PVA cryo-hydrogel is a composite material consisting of water swollen PVA hydrogel layer and support layer.

One of criteria of dressing efficiency is certain water and air permeability. Dressing must also ensure optimal heat exchange between the wound and the environment. Therefore support layer itself must be sufficiently permeable. At present work dressing prototypes were made on several support layers: the paper (kraft paper) and a variety of textiles (cotton medical gauze, cotton textile, elastic bandage).

The process of obtaining of dressing at the laboratory scale is simple.

Layer of PVA solution of certain thickness (3 - 5 mm) is applied to the support layer, by use of squeegee blade applicator.

Examples of PVA hydrogel dressings are shown in the Figure 26.

Fig. 26 Dressing with a cotton textile support layer (a) and gauze support layer (b)

Use of gauze support makes dressing semi-transparent, allowing visual assessment of the wound (Fig. 26 b).

Laboratory studies suggest the following technological scheme of production of PVA cryo-hydrogel dressing on an industrial scale (Fig. 27).

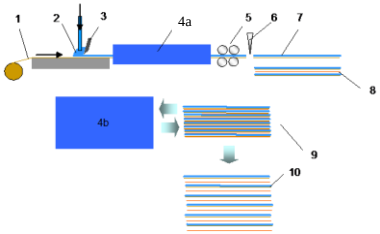

Fig. 27 Technological scheme of industrial-scale production of PVA cryo-hydrogel dressing: 1 - support layer 2 - dosing of PVA solution, 3 – equipment of squeegee-type PVA layer application and calibration, 4a, b - cryo-thermostat, 5 - pulling equipment, 6 - cutting machine, 7 - PVA solution sheet with support layer, 8 - dividing polymer film, 9 - sheet pack, 10 - PVA cryo-hydrogel dressing sheet

Fixed-width band of support layer 1 with a constant speed continuously moves (arrow) on the horizontal base. The PVA aqueous solution is continuously dosed on the support layer (equipment 2), the PVA solution thickness is calibrated (equipment 3). Coated material further passes through the cryo-thermostat 4a, the PVA coating freezes. The pulling equipment 5 provides the necessary tension and moving of the band. Frozen band is cut into fixed length sheets 7 with equipment 6. Using dividing polymer films 8 sheets are arranged into packages 9. Packages are exposed to cyclic freezing-thawing. Freezing is carried out in cryotermostat 4b, thawing can be made either in a special thermostat or directly in factory premises.

After thawing the sheets 10 are released from dividing films and cut into dressing articles of necessary size and configuration.

Articles are packaged in an appropriate package and sterilized (γ-radiation is preferred).

CONCLUSIONS

1. Cryogenic synthesis technology of poly(vinyl alcohol) (PVS) hydrogels by use of PVA aqueous solutions is developed. Temperature-time regime of cryoprocess, the range of polymer molecular mass and solution concentration, that provides formation of gel with a small degree of crosslinking, is based.
2. The dependences of most important efficiency criteria of hydrogel synthesis process: gel content ψ_{GEL} and water sorption capacity ψ^{∞}_{H2O} on the number of

freezing - thawing (f-t) cycles n_C are reversal: ψ_{GEL} value increases, but ψ^{∞}_{H2O} value decreases with increasing of n_C; $n_C \leq 3$ provides $\psi_{GEL} \geq 0,8$ and $\psi^{\infty}_{H2O} \leq 0,95$.

3. Analysis of obtained SEM microphotographs suggests that the morphology of synthesized cryo-hydrogel corresponds to a composite, consisting of a water-swollen PVA gel and water inclusions (pores) with complex pore hierarchy and shape. The gel base has fine-pored structure with relatively small pore sizes (the dominant pore cross-sectional area is 0,3 – 0,5 μm^2). Areas with larger pores (10 – 50 μm^2) are distributed in fine-pored gel structure. The total pore volume fraction φ_P does not exceed 0,3.
4. The critical temperature, above which the PVA cryogels loses integrity and splits, as well as the durability of the gel at different temperatures in water medium increases with the number of f-t cycles. This is a result of increase of total energy of crosslinks and growth of proportion of resistant crosslinks.
5. Most of the water in swollen cryogel (> 90%) is weakly tied in cryogel structure and leave the gel at drying temperature $\leq 60^oC$. It is considered as free water. The rest firmly bonded water leaves the gel at a much higher temperature. Amount of bonded water, temperature interval of water removal and end temperature of this interval increases with the number of f-t cycles.
6. After drying gels are able to absorb water intensively and to reach high equilibrium water content. Drying does not destroy the cross-linked structure of gel. Moreover, in each drying cycle additional cross-links forms. As a result initial gel water sorption rate and sorption capacity decreases by raise of the drying temperature.
7. The average molecular mass between crosslinks M_C and corresponding crosslinking density, calculated from equilibrium gel swelling degree, allows to consider synthesized cryogels as slightly cross-linked.
8. Strength-deformation characteristics (tensile and compression modulus of elasticity, tensile strength at break, relative deformation at break) of cryogels are completely determined by crosslinking density.
9. Polysaccharide additives promote the formation of PVA cryogels. PVA cryorogel composites with polysaccharides show significantly higher resistance to deformation.
10. Methods ensuring necessary content of water solution of therapeutic substances in ready for use cryo-hydrogel drug delivery forms are developed.
11. Cytotoxicity and cell proliferation *in vitro* of the synthesized gel is evaluated. Microbial colonization level and adhesion activity of gels is established. Synthesized gels are biocompatible.
12. Manufacturing technology of cryo-hydrogel dressing prototypes is developed. Prototypes with a porous and dense support layer are prepared and tested. Version of technological scheme for industrial production of dressings is worked out.

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APPROBATION OF THE THESIS

Results presented in the dissertation are approbated in 2 SCI journal articles, 4 conference full-text articles in proceedings and have been protected by 1 patent of Latvia.

Articles in journals and full-text articles in scientific proceedings

1. J.Greidāne, M.Kalnins, A.Dzene, V.Tupureina. On the preparation and characteristics of polymer systems for wound dressings, *Scientific Papers of the Institute of Environment Protection Engineering of the Wroclow University of Technology* (Poland, Wroclaw, 2006), 2006, 81, 199-203.
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