HIDROGELURI COMPOZITE SENSIBILE LA pH ȘI TEMPERATURĂ PENTRU TRATAMENTUL ARSURILOR
pH AND TEMPERATURE SENSITIVE COMPOSITE HYDROGELS FOR BURN TREATMENT

ȘTEFANIA MARIN[1], MĂDĂLINA GEORGIANA ALBU KAYA[1*], GEORGETA VOICU[2], GRAȚIELA TEODORA TIHAN[2]

[1]Institutul Național de Cercetare Dezvoltare Textile și Pielărie (INCDTP), Scucursala ICPI, Departamentul Colagen, str. Ion Minulescu, nr. 93, sector 3, București, România.
[2] Facultatea de Chimie Aplicată și Știința Materialelor, Universitatea Politehnica București, Strada Polizu, nr. 1-7, București, România

In this work the attention was focused on the superior properties of polyvinylalcohol – collagen hydrogels.Burns leads to exposure of the deeper layers of skin proportional to the size and depth of the wound. The pH level in the wound bed is a key parameter for assessing indicative of wound healing problems and temperature the same. The lower temperatures can stop the activity of the cells involved in the healing process.The aim of this study was to characterize hydrogels previously obtained in terms of pH and temperature sensitivity in order to choose the most appropriate formulation that could be used for burn healing. Hydrogels were made from collagen gel with initial concentration of 2.85% and polyvinyl alcohol having a molecular weight of 60 000 Da. The hydrogels obtained were lyophilized in order to be analyzed by microstructural appearance (SEM images) and also from the point of view of their sensitivity to different values of pH and temperature. Based on result obtained it was demonstrated that the sample with equal proportions of collagen and polyvinyl alcohol shows that it has the required properties in order to be used as a dressing in healing burns. This hydrogel will be basic support for new smart dressings with included nanoparticles, with targeted applications in medicine.

În această lucrare atenția s-a axat pe proprietățile superioare ale hidrogelurilor realizate din alcool polivinilic și colagen. Arsurile duc la expunerea straturilor pielii proporțional cu dimensiunea și adâncimea rănii. Nivelul pH-ului în patul rănii este un parametru cheie în ceea ce privește problemele de vindecare ale rănii la fel și temperatura.O temperatură scăzută poate stopa activitatea celulelor implicate în procesul de vindecare. Scopul acestui studiu a fost de a caracteriza hidrogelurile obținute din colagen și alcool polivinilic, din punct de vedere al sensibilități la pH și temperatură, astfel încât să se poată alege cea mai bună formulare care poate fi utilizată pentru tratamentul arsurilor. Hidrogelurile au fost realizate din colagen cu concentrația inițială de 2,85% și alcool polivinilic cu masa moleculară de 60 000 Da. Hidrogelurile obținute au fost liofilizate pentru a putea fi ulterior analizate microstructural (imagini SEM) și din punct de vedere al sensibilității acestora la diverse valori ale pH-ului si temperaturii. În urma analizelor s-a demonstrat că proba cu proporții egale de colagen și alcool polivinilic prezintă proprietățile necesare pentru utilizarea ca pansament în vindecarea arsurilor. Aceast hydrogel va fi suportul de bază pentru noi pansamente inteligente cu nanoparticule încorporate, cu aplicații țintite în medicină.

Keywords: *composite, collagen, polyvinyl alcohol, hydrogels, pH, temperature*

1. Introduction

The skin has an acid pH which is called 'acid mantle'. Acid mantle of the skin provides an impermeable barrier in order prevent bacterial infections. pH is maintained between 4 and 6 by the proton pump and by converting free fatty acids by secretory phospholipases [1]. Burns leads to exposure of the deeper layers of skin proportional to the size and depth of the wound and extracellular fluid is exudates. The pH level in the wound bed is a key parameter for assessing indicative of wound healing problems [2]. In contrast to the healthy skin or to the wounds in the process of healing that present a slightly acidic pH (5.5-6.5), chronic infected wounds often show pH values greater than 7.4 due to the alkaline secondary products appeared from the proliferation of bacterial colonies [3]. In many cases, irregular vascular structure of chronic wound infection causes a heterogeneous distribution in the wound bed resulting in a drastic variation in pH in the affected area [4].

The skin plays an important role in thermoregulation by intense vascularization. Skin temperature of the human body is the result of thermal balance between energy supplied and energy lost. Skin temperature is influenced by physiological and environmental factors and can be affected by variations in ambient temperature, humidity and blood flow [5]. The ideal temperature

* Autor corespondent/*Corresponding author*,
E-mail: albu_mada@yahoo.com.

of the wound was identified as being the body temperature, namely 37 ° C. The lower temperatures can stop the activity of the cells involved in the healing process.

Heat is a marker that helps of establishment of infections in wounds and could be used as an early indicator regarding the severity of the wound before any visible changes in terms of appearance wound could be observed [6]. When the temperature of the wound falls below body temperature, healing may be delayed due to low re-epithalization, lack of deposits of collagen, reduced epithelial cells and fibroblasts during the inflammatory phase and a high percentage of wound infections [7].

In vitro studies have shown that a temperature of 33 ° C is the critical level at which neutrophils, fibroblasts and epithelial cells shows minimal activity. Meanwhile, studies have shown an improvement in wound healing when the temperature is higher. Studies have shown that the most effective temperature of wound healing is the normal body temperature and healing is delayed when the temperature drops below normal body temperature or when rises above 42 ° C [8].

Hydrogels are functional polymeric materials with moderate degree of crosslinking and three-dimensional structure. These are insoluble in water, but have a very high absorption capacity [9].

Polyvinyl alcohol (PVA) is one of the most commonly used synthetic polymers for hydrogels obtaining because of biocompatibility. This property has made possible its use in medical applications such as artificial organs, drug delivery systems, contact lenses and wound dressings [10]. Collagen (COLL) is the most common protein in the human body and has the ability to take over the function and shape of biological tissue. Due to its biocompatibility is the mainly component in biomaterials development with applications such as dressings, devices for tissue engineering scaffold type or controlled delivery systems [11]. Indomethacin (IND) is an anti-steroidal and anti-inflammatory drug. It is used to relieve pain and inflammation in rheumatic diseases, sprains, backache, gout or menstrual pain [12] and it can be successfully used in bioactive wound dressing development.

The aim of this study was to characterized the hydrogels obtained previously [13] in terms of sensitivity to temperature and pH in order to chose the most appropriate formulation that could be use for burns healing.

2. Materials and methods

The type I fibrillar collagen gel having a concentration of 2.85% (w/w) was extracted from calf hide using technology developed at the Research-Development Textile Leather National Institute Division Leather and Footwear Research Institute – Collagen Department [14]. Polyvinyl alcohol, with molecular weight of 60 000 Da was purchase from Sigma-Aldrich and glutaraldehyde from Merck (Germany). The indomethacin was obtained from Fluka.

The collagen gel with the initial concentration of 2.85% and acid pH was adjusted using 1M sodium hydroxide at pH 7.3 in order to increase biocompatibility. The final concentration of used collagen gel was 1% (w/v). Thereafter PVA with a concentration of 0.5% and 0.2% indomethacin were dispersed in the collagen gels in different proportions, according to the compositions shown in Table 1. For cross-linking 0.025% glutaraldehyde solution was used and the ratio between COLL:GA was 9:1.

Table 1

Composition of collagen hydrogels

Compoziția hidrogelurilor de colagen [13]

	COLL, %	PVA, %	IND, %	GA, %
S1	0	100	0.2	0.025
S2	100	0		
S3	50	50		
S4	75	25		
S5	25	75		

The collagen gels were freeze-dried using Delta 2-24 LSC (Martin Christ, Germany) lyophilizer, in order to be analyzed in terms of sensitivity to temperature and pH.

2.1. Scanning Electron Microscopy

Microstructural characterization was made using scanning electron microscope (SEM) Hitachi S-2600N. Obtained collagen matrices were analyzed in section.

2.2. pH and Temperature Sensitivity

To determine the influence of pH on the obtained hydrogels, was watched over their behavior in time, at the immersion in solutions with different pHs. Thus, solutions with pH of 3.5, 7.4 and 9 were prepared, in which were immersed samples of hydrogels from lyophilized matrices. They were placed in an oven at three different temperatures: 37 °C, 39 °C and 41 °C. All samples were tested in triplicate for a better acuity. The samples behavior was followed for five days, in terms of their tendency of gradual degradation processes that can be correlated *in vivo* with the gradual degradation of the hydrogel to eliminate indomethacin and also to release collagen.

Thus, after immersion in solutions with various pHs, the samples were removed from solution and were weighed. The percentage of degradation of the tested matrices was determined using the following equation (eq. 1):

$$\text{Weight loss [\%]} = \frac{Wi - Wt}{Wt} * 100 \qquad (1)$$

where Wi is the initial weight and the weight Wt is recorded at time t.

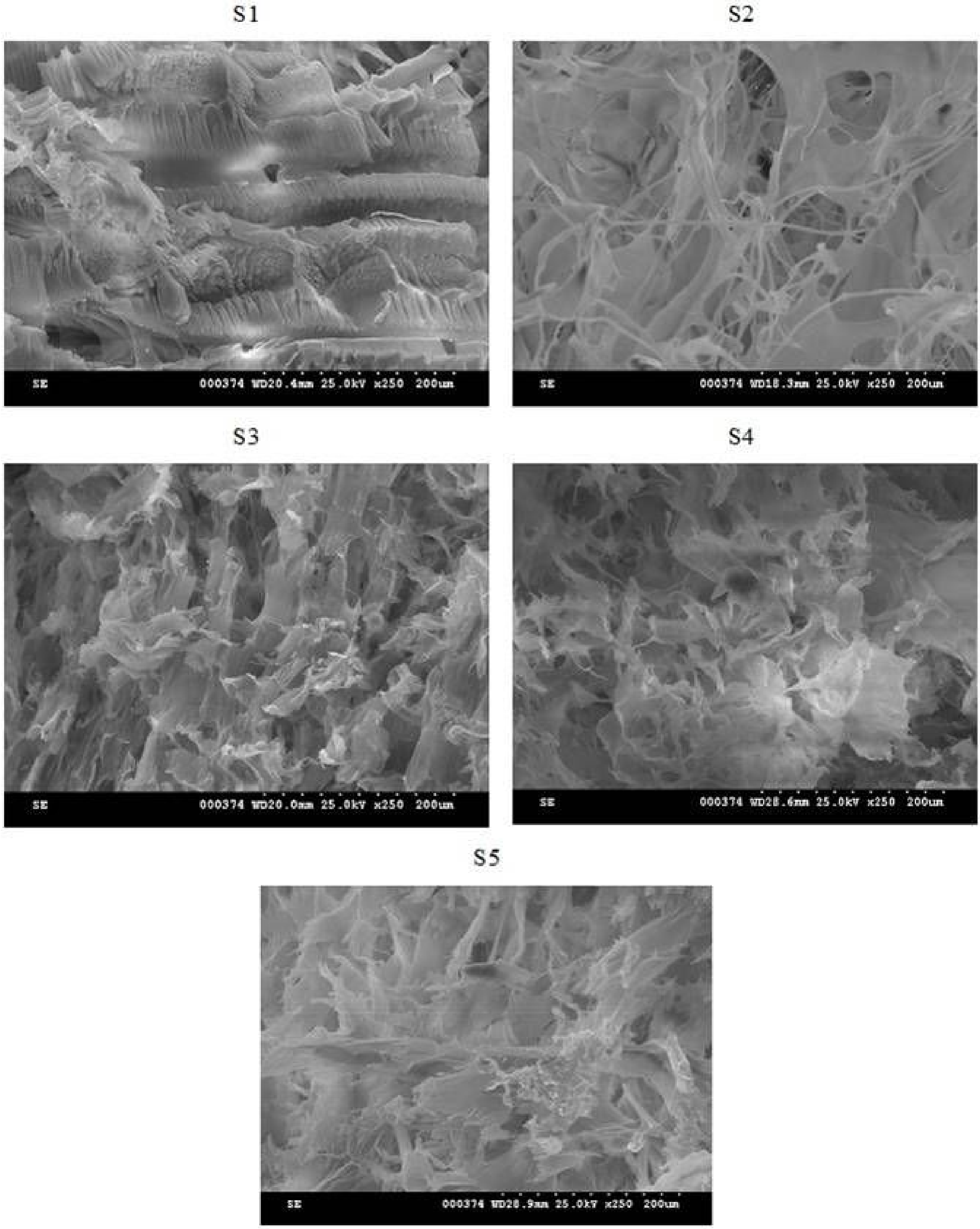

Fig. 1 - SEM images of lyophilized hydrogels, 250X magnification./ *Imagini SEM ale hidrogelurilor liofilizate, magnificație 250X.*

3. Result and discussion

Microstructural properties were investigated on lyophilized hydrogels. Obtained images are presented in Figure 1.

The sample homogeneity was the first aspect observed in microscopic analysis. For sample S1, PVA structure can be easily seen, namely laminated formation with well-defined structure. Collagen sample S2 shows the specific structure of collagen: interconnected pores formed by fibrils. Composite sample S3 and S4 are defined by homogeneity between collagen and PVA structure and high porosity. Considering the high amount of PVA from sample S5, namely 75%, SEM images shows its predominate structure.

In order to determine the hydrogels behavior, different pHs and temperature has been used. Therefore in Figure 2 is presented the influence of temperature and pH for S1 sample.

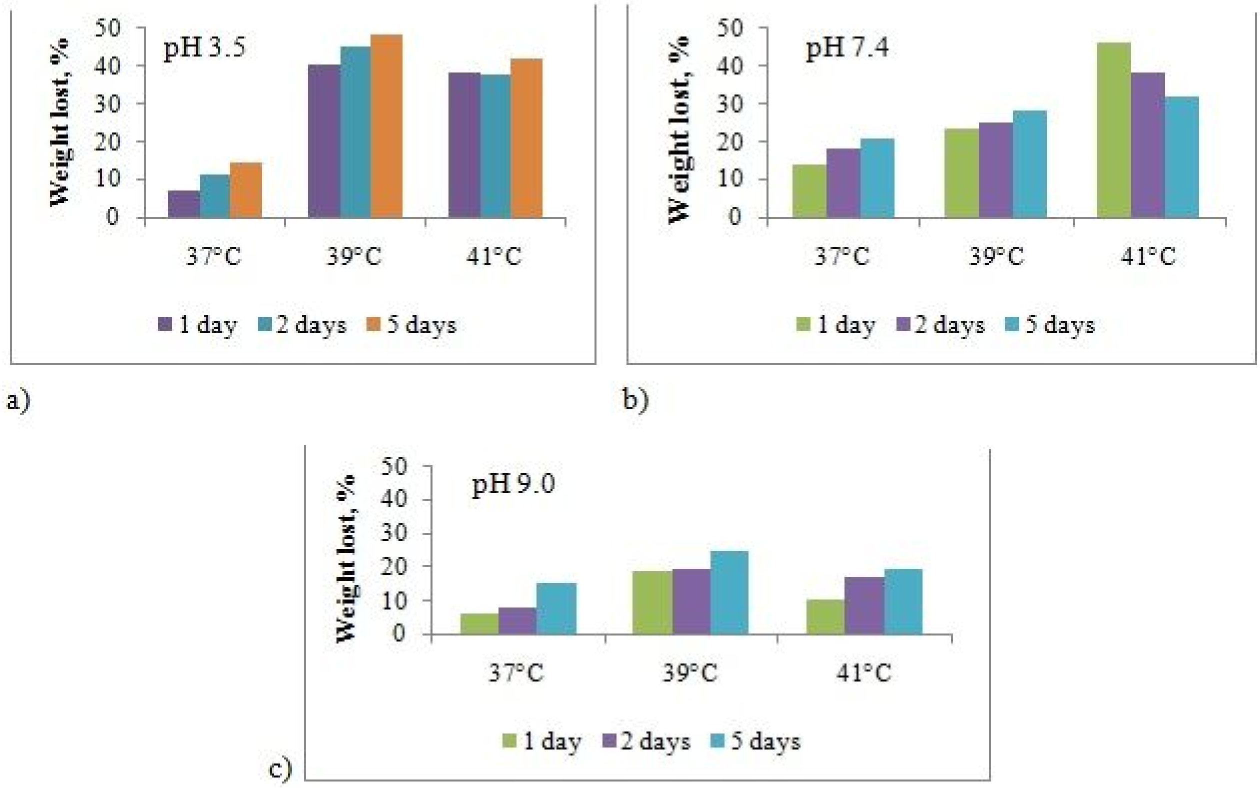

Fig. 2 -Temperature and pH influence for S1 sample./ *Influența pH-ului și a temperaturii asupra probei S1.*

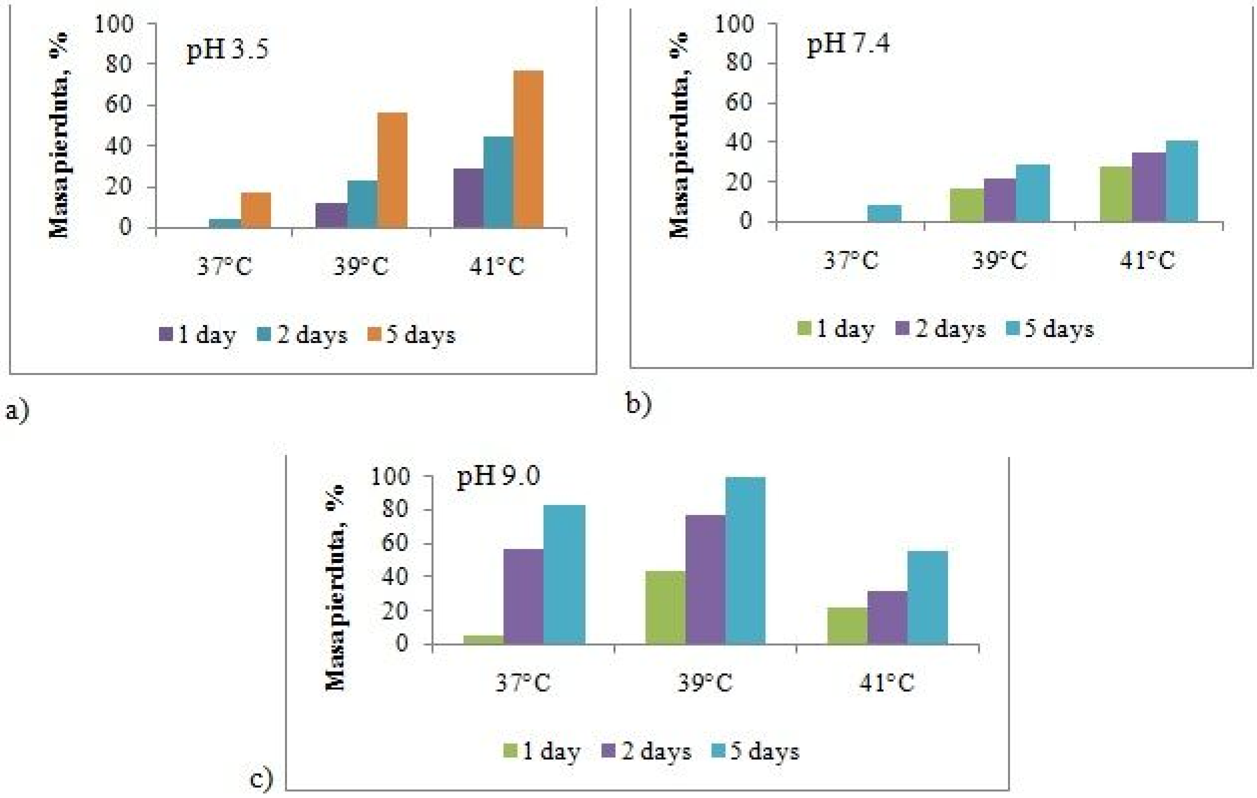

Fig. 3 -Temperature and pH influence for S2 sample./ *Influența pH-ului și a temperaturii asupra probei S2.*

It can be seen that the high degradation percentage, mostly 50%, is recorded at 39°C and pH 3.5. The pH increasing leads to the degradation inhibition so this type of hydrogel can notbe used for infected plague.

The temperature and pH influence for blank collagen sample S2 can be observed in Figure 3.

The image shows that at temperature of 39°C and pH 9 the collagen matrix is completely degraded, in comparison with the others temperatures. This behavior made this type of matrix properly for infected plague treatment

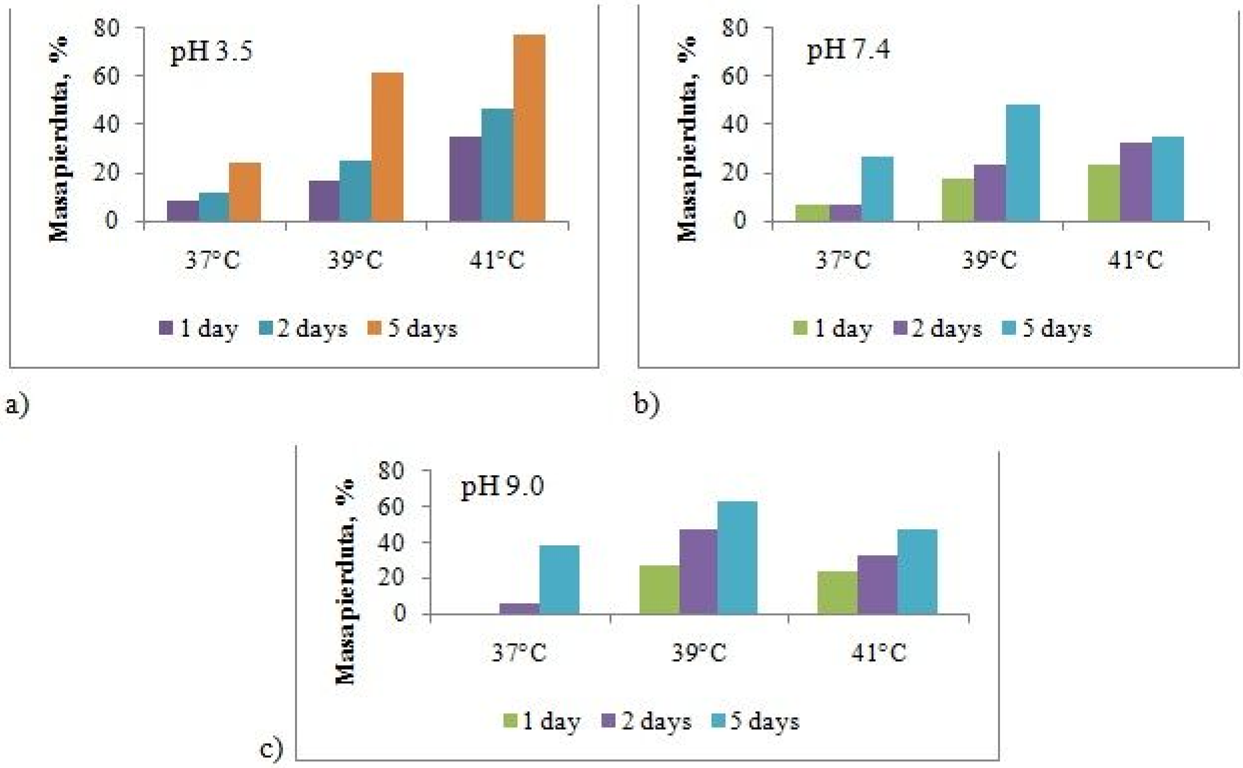

Fig. 4-Temperature and pH influence for S3 sample./ *Influența pH-ului și a temperaturii asupra probei S3.*

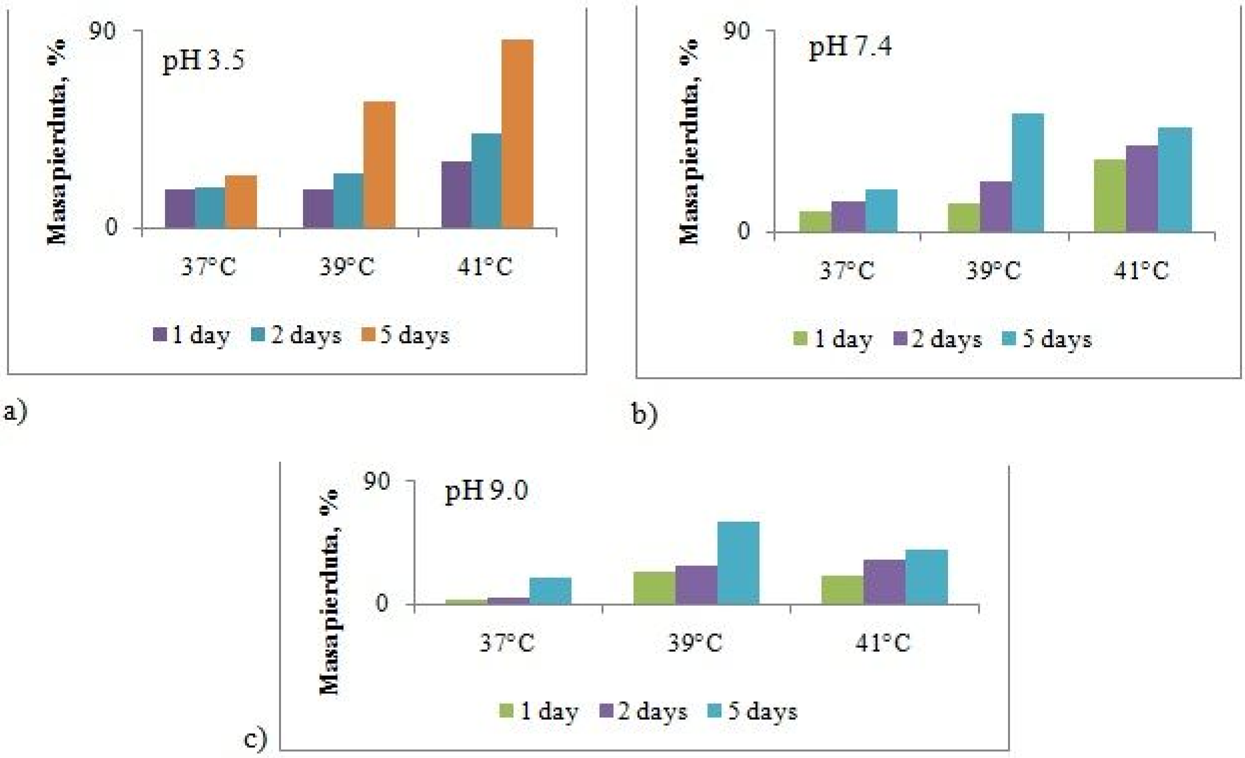

Fig. 5 -Temperature and pH influence for S4 sample./ *Influența pH-ului și a temperaturii asupra probei S4.*

For sample S3, containing 50% COLL and 50% PVA, the expression under temperature and pH influence is display in Figure 4.

A considerable degradation percentage was recorded at 41°C and acid pH, the degradation being of approximately 80% compared to the initial weight of the sample. A significant weight loss, over 60%, was also registered at basic pH and a higher temperature then the body temperature, namely 39°C. Thus sample S3 can be successfully used both for acute wounds, under the healing process, and infected wounds, due to the favorable reaction in both cases. S3 sample reacts as expected favoring healing.

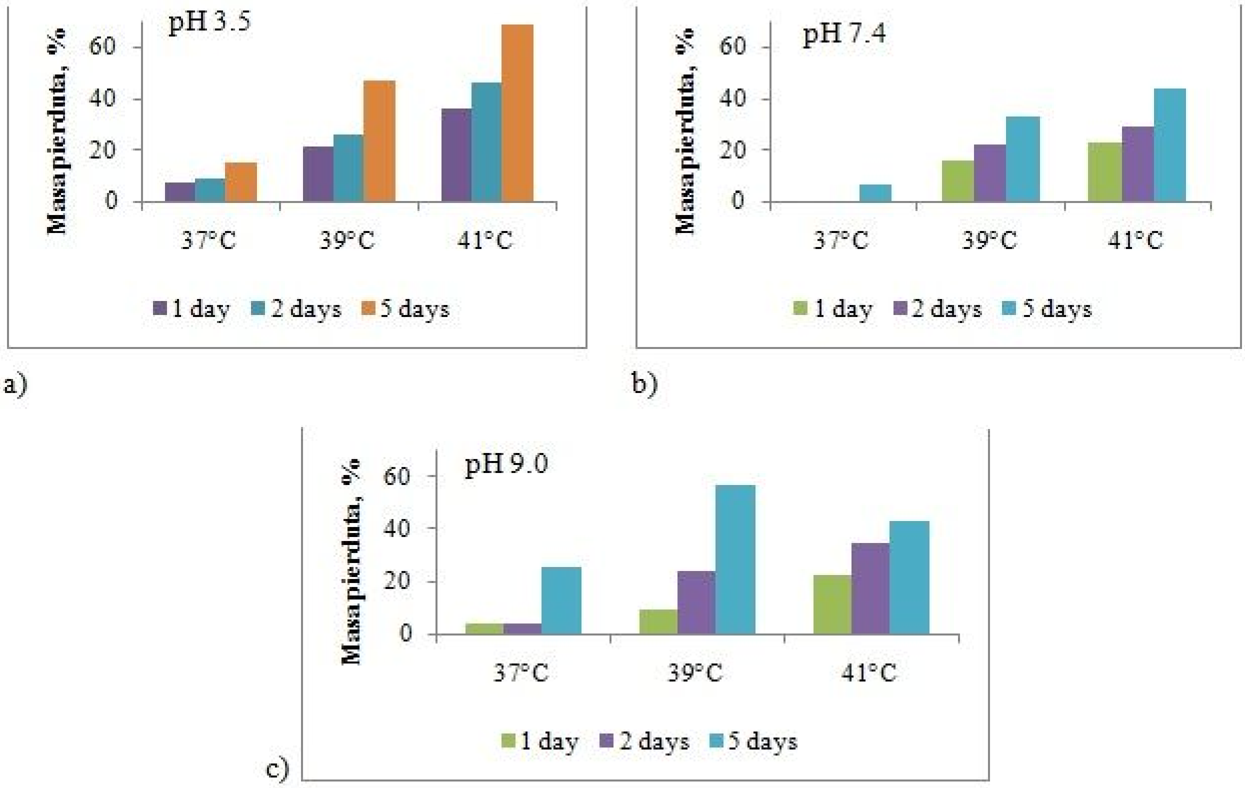

Fig. 6 -Temperature and pH influence for S5 sample./ *Influența pH-ului și a temperaturii asupra probei S5.*

Figure 5 brings out the reaction of sample S4 at various temperatures and pHs.

Regarding the sample S4, it also reacts well to both the temperature of 41°C, pH acid and at 39°C, basic pH, as is recorded significant degradation percentages over 80% and over 60%.

In Figure 6 is presented the degradation kinetic for sample S5 made of 75% PVA and 25% COLL.

S5 sample shows a considerable degradation percentage up to 70% when the sample is subjected at acid pH and temperature of 41°C. This behavior recommends the sample for treatment of acute plague.

4. Conclusions

After carried out studies it can be said that the sample S3 has the ability to lose about 70% from its initial weight, after 5 days of exposure to chosen environmental conditions. The matrix has the desired behavior because the wound pH, basic or acid, indicates the bad stage of the wound so the need for a faster degradation is preferred in order to achieved faster regeneration. Taking into account all of the favorable results for sample S3, made of 50% COLL and 50% PVA, it can be said that it can be used with a lot of success rate in acute wound healing.

Acknowledgment
This work was financially supported by NUCLEU project no. PN 16 34 02 03.

REFERENCES

1. A. Chan, T. Mauro, Acidification in the epidermis and the role of secretory phospholipases, Dermatoendocrinol, 2011, **3**, 84.
2. J.R. Sharpe, S. Booth, K. Jubin, N.R. Jordan, D.J. Lawrence-Watt, B.S. Dheansa,Progression of wound pH during the course of healing in burns, Journal of Burn Care and Research, 2013, **34**(3), 201.
3. R.Rahimi, M. Ochoa, T.Parupudi, X. Zhao,I.Yazdi, M. R.Dokmeci, A.Tamayol, A.Khademhosseini, B.Ziaie, Alow-cost flexible pH sensor array for wound assessment, Sensors and Actuators B:Chemical, 2016, **229**, 609.
4. S. Schreml, R.J. Meier, O.S. Wolfbeis, M. Landthaler, R.-M. Szeimies, P. Babilas, 2Dluminescence imaging of pH in vivo, Proceedings of the National Academy of Science, 2011, **108**(6), 2432.
5. G. F. Clough, M. K. Church, Vascular responses in the skin: an accessible model of inflammation. News in Physiological Science, 2002;**17**, 170.
6. G. Nakagami, H.Sanada, S.Iizaka, T.Kadono, T.Higashino, H.Koyanagi, N.Haga, Predicting delayed pressure ulcer healing using thermography: a prospective cohort study, Journal of Wound Care,2010, **19**(11), 465.
7. W. McGuiness, E. Vella, D. Harrison, Influence of dressing changes on wound temperature, Journal of Wound Care, 2004.**13**(9), 383.
8. J. McCulloch, C. A. Knight, Nanocontactnormiothermic wound therapy and offloading in the treatment of neurophatic foot ulcers in patients with diabetes. Ostomy Wound Management, 2002, **48**(3), 38.
9. L. L.Gan and L. P. Nong. Study on swelling performance of chitosan/gelatin/PVA complex hydrogel. Chemistry and Bioengineering, 2010, **27** (9), 33.
10. E. Kenawy, E. A. Kamoun, M. S.MohyEldin, M. A. El-Meligy, Physically crosslinked poly(vinyl alcohol)-hydroxyethyl starch blend hydrogel membranes: synthesis and characterization for biomedical applications, Arabian Journal of Chemistry, 2014, **7**, 372
11. R.Parenteau-Bareil, R. Gauvin, F.Berthod, Collagen-Based Biomaterials for Tissue Engineering Applications,Materials, 2010,**3**, 1863.
12. M. Rasekh, C.Karavasili, Y. L. Soong, N.Bouropoulos, M. Morris, D. Armitage, X. Li, D.Fatoutous,Z. Ahmad, Electrospun PVP–indomethacin constituents for transdermal dressings and drug delivery devices, International Journal of Pharmaceutics, 2014,**473**, 95.
13. S. Marin, M. V. Ghica, I. Titorencu, M. G. Albu Kaya, M. Ferdes, C. Dinu Pirvu, V. Pruna, Development and Characterization of Indomethacin Loaded Polyvinil Alcohol Collagen Smart Hydrogels for Burns Injuries, Proceedings of ICAMS 2016 – 6th International Conference on Advanced Materials and Systems, 2016, 281-286.
14. M.G. Albu, Collagen Gels and Matrices for Biomedical Applications, Lambert Academic Publishing, Saarbrücken, 2011, 23-24.