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Molecular Crystals and Liquid Crystals

ISSN: 1542-1406 (Print) 1563-5287 (Online) Journal homepage: http://www.tandfonline.com/loi/gmcl20

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To cite this article: B. Kaczmarek, A. Sionkowska & E. Markiewicz (2016) L-ascorbic acid release from polymeric matrixes based on blends of chitosan, collagen and hyaluronic acid, Molecular Crystals and Liquid Crystals, 640:1, 46-53, DOI: 10.1080/15421406.2016.1255509

To link to this article: http://dx.doi.org/10.1080/15421406.2016.1255509

MOLECULAR CRYSTALS AND LIQUID CRYSTALS
2016, VOL. 640, 46–53
http://dx.doi.org/10.1080/15421406.2016.1255509

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L-ascorbic acid release from polymeric matrixes based on blends of chitosan, collagen and hyaluronic acid

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ABSTRACT

3D porous composites based on blends of chitosan, collagen and hyaluronic acid were obtained. L-ascorbic acid was incorporated into the composites. The structure of composites was studied by IR spectroscopy and SEM. Moreover, mechanical properties, porosity and density of the blends were studied. The release of L-ascorbic acid from biopolymeric matrixes was studied by UV-Vis spectroscopy.

The results showed that biopolymeric matrixes based on the blends of chitosan, collagen and hyaluronic acid can be used as 3D systems for L-ascorbic acid delivery. The addition of hyaluronic acid to chitosan/collagen blends leads to the decrease of amount of released agent.

KEYWORDS

collagen; chitosan; hyaluronic acid; drug release; L-ascorbic acid

1. Introduction

Natural polymers are widely used in biomedical sciences for preparation of several biomaterials. They can be shaped into films, 3D scaffolds and gels. Nowadays biopolymeric materials can be also applied as drug delivery systems [1-3]. The drug can be incorporated into the polymer solution, and during mixing for some time, the homogenous mixture can be obtained. Hydrophilic as well as hydrophobic active compounds can be used to be released from polymeric matrixes. The release of several active compounds from polymeric matrixes was studied, for example: theophylline [1,4,5], acetaminophen [4], p-nitroaniline [6,7] propranolol [1], diclofenac sodium, salicylic acid, sulfasalazine [8], acyclovir, chlorhexidine diacetate [9], ibuprofen, antipyrine [6], ofloxacin [1,5], raparnycin, emodin, curcumin, heparin [10], lidocaine [11], indomethacin [12], caffeine, dyphylline [13], saligenin [14]. L-asorbic acid, known as vitamin C, is widely used as pharmaceutical compound and active agent in cosmetic preparations. It is used for treatment of depression, dementia, Alzheimer's disease or to decrease the absorption of iron from food. The use of L-ascorbic acid includes also the positive effect in the heart, blood vessels or medical treatments of collagen disorders. One of the essential role of L-ascorbic acid in tissue engineering science is its role as cofactor for the hydroxylation of proline and lysine residues in collagen [15].

Biopolymeric matrixes as drug delivery systems are beneficial compared to conventional dosage forms. They improve efficacy and patient compliance as well as reduces the toxicity of drug in other places [16-18]. There are two cases in which controlled release is beneficial. First

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one is a distribution of drug molecules to encounter tissues and cause the side effects which can prohibit the treatment for example during the chemotherapy. The second one is when the natural distribution of the drug does not allow molecules to reach the place of action.

The aim of the study was to prepare the composite based on the blend of chitosan, collagen and hyaluronic acid with the addition of L-ascorbic acid. Vitamin C enhances the resistance of human body to viral and bacterial infections, what improves the efficiency of medical treatment. Such materials could be beneficial for the tissue regeneration due to the biological properties of vitamin C, where the system would be better protected against virus and bacteria after the implementation.

2. Materials and methods

2.1. Materials

Collagen (Coll) was isolated from rats tail tendons [19,20]. Chitosan (CTS) and hyaluronic acid (HA) were purchased from the Sigma Aldrich company (Poland). Collagen and chitosan ($M_v = 5.4 \times 10^5$ g/mol; DD = 77%) solutions were prepared in 0.1M acetic acid, both in 1% wt concentration [19]. Chitosan and collagen mixtures were prepared by mixing two solutions in the weight ratio 50/50. Hyaluronic acid as 1% solution in 0.1M acetic acid was added in the weight ratios 1% to chitosan/collagen blend (CTS/Coll/1HA), 2% (CTS/Coll/2HA), and 5% (CTS/Coll/1HA). To the 15 ml mixture of biopolymers 10 mg of L-asorbic acid (vitamin C) (Sigma Aldrich, Poland) was added. Solutions were mixed and put into the polystyrene container and then placed in a freezer at −80°C. Frozen mixtures were lyophilized at −55°C and 5Pa for 48h (ALPHA 1-2LD plus, CHRIST, Germany). 3D porous scaffolds of chitosan/collagen in ratio 50/50 as well as a pure chitosan and collagen scaffolds were left as a control.

2.2. Scaffolds characterization

2.2.1. Mechanical tests

Mechanical properties were measured by mechanical testing machine (Z.05, Zwick/Roell, Germany) for each kind of sample. Cylindrical samples with a diameter of 20 mm and a height of 13 mm were prepared for mechanical testing. Samples were put between two discs and compressed by the 0.1N force with the cross-head speed 5 mm/min. Measurements were carried out in room temperature and humidity. Analysis were made for five samples of each kind of composite. The average and standard deviation were calculated.

2.2.2. Scanning electron microscope

The morphology of the samples was studied using Scanning Electron Microscope (SEM) (LEO Electron Microscopy Ltd, England). Scaffolds were frozen in liquid nitrogen for 3 min. A freezing of sample allows a gentle cutting it with a razor scalpel to observe the interior structure. Samples were then covered by gold and SEM pictures were made with the resolution 500 μm.

2.2.3. Infrared spectroscopy

Spectra of the attenuated total reflected infrared spectroscopy were obtained. For 3D specimens IR spectra were obtained using a Nicolet iS10 spectrophotometer equipped with an ATR

device with diamond crystal [21]. All spectra were recorded in absorption mode at a 4 cm^{-1} interval and 64 scans.

2.2.4. Porosity and density

The porosity and density of obtained scaffolds were measured by the liquid displacement [21]. In this study the isopropanol was used in known volume (V_1) in which the scaffold of known weight (W) was immersed for 5 min. The total volume of isopropanol with impregnated scaffold was V_2. After the scaffold remove the volume of isopropanol was measured (V_3). The density (d) and porosity (Є) were calculated by using the equations:

$$d = \frac{W}{V_2 - V_3}$$

$$\varepsilon = \frac{V_1 - V_3}{V_2 - V_3}$$

2.3. L-ascorbic acid release

Scaffolds with the addition of L-ascorbic acid were immersed in PBS solution. The released rate depends of solution conditions. PBS has pH = 7.4 and it is relevant to the pH of blood. The use of such system is proper to examine the released rate of vitamin C after application in human body. After 5 min, 30min, 1h, 4h and 24h the amount of 2.5 ml of PBS solution with released L-ascorbic acid was taken out and it was replaced by the fresh portion of PBS. Released L-ascorbic acid was detected by UV-Vis spectroscopy with measurement of absorption at 302 nm. The concentration of L-ascorbic acid was calculated by the use of standard curve method.

3. Results and discussion

3.1. Structure of composites

SEM images show (examples of images are presented in Figure 1) that porous material with interconnected pores was obtained. The shape of pores is irregular with size between 10-100 μm. After addition of L-ascorbic acid the structure of scaffolds is only slightly altered.

3.2. Infrared spectroscopy

In IR spectra of composites based on the blends of chitosan, collagen and hyaluronic acid the characteristic peaks for all components of the blend were found. They are listed in Table 1. There is no additional peak from L-ascorbic acid in IR spectra after incorporation of this agent into the polymeric matrix and the significant shifts of existing bands are not observed. It suggests that strong bonds between polymers and vitamin C are not formed, however, it can be attached to the polymers by the weaker bonds, for example Van der Waals interactions.

3.3. Porosity and density

The porosity (ε) and density (d) for 3D composites were calculated and the results are shown in Table 2. The highest porosity was observed for pure collagen and pure chitosan scaffolds.

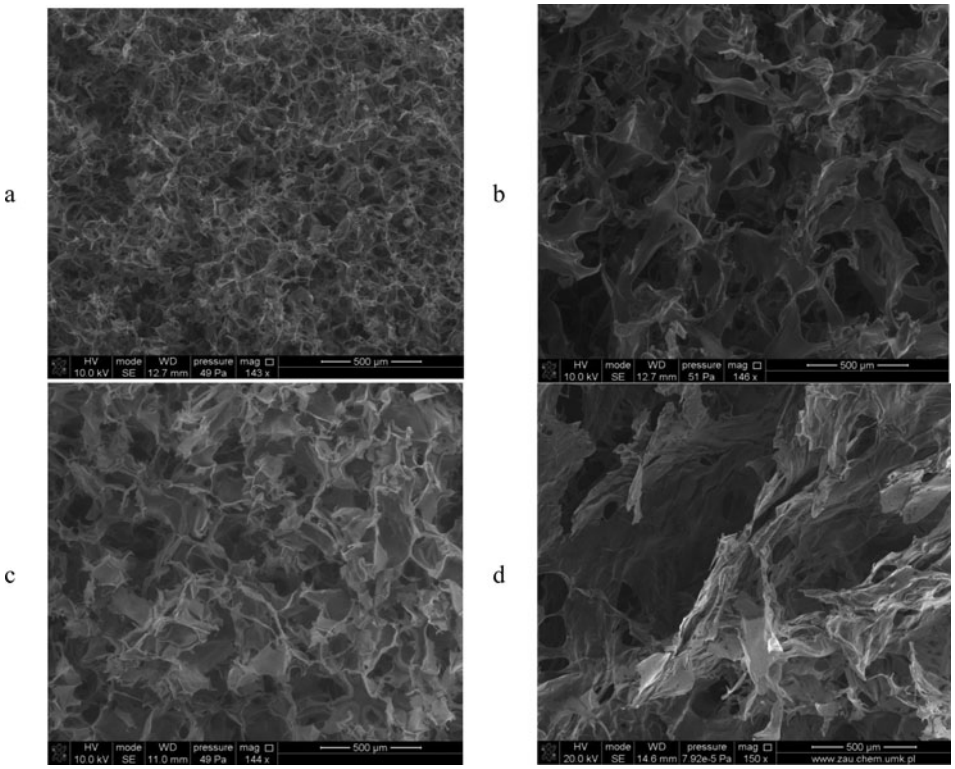

Figure 1. SEM images of a) chitosan b) chitosan/collagen in ratio 50/50 c) chitosan/collagen with 5% of hyaluronic acid d) chitosan/collagen with 5% of hyaluronic acid and vitamin C.

Table 1. Characteristic peaks for chitosan (CTS), collagen (Coll), their mixture (CTS/Coll) with 5% of hyaluronic acid (CTS/Coll/5HA) with and without addition of vitamin C.

	Wavenumber [cm^{-1}]		
Specimen	without	with vit. C	Characteristic group
CTS	3357	3353	O–H
	1645	1641	C=O
	1556	1550	N–H
	1081	1077	C–O–C
Coll	3323	3311	Amide A
	1642	1631	Amide I
	1552	1548	Amide II
	1242	1233	Amide III
CTS/Coll	3329	3328	Amide A
	1660	1657	Amide I
	1558	1550	Amide II
	1240	1240	Amide III
	1076	1072	C–O–C
CTS/Coll/5HA	3335	3329	Amide A
	1644	1635	Amide I
	1536	1541	Amide II
	1231	1238	Amide III
	1070	1070	C–O–C

Table 2. The porosity (ε) and density (d) of chitosan (CTS), collagen (Coll), their mixture (CTS/Coll) and with 1% addition of hyaluronic acid (CTS/Coll/1HA) etc. with and without addition of vitamin C.

	without		with vit. C	
Specimen	ε [%]	d [mg/cm^3]	ε [%]	d [mg/cm^3]
CTS	86	59	87	58
Coll	91	20	92	21
CTS/Coll	60	19	62	20
CTS/Coll/1HA	65	21	65	21
CTS/Coll/2HA	69	39	68	38
CTS/Coll/5HA	71	43	70	44

For the composite of chitosan and collagen in ratio 50/50 the decrease of porosity and density is observed. The addition of hyaluronic acid increases the porosity of scaffold as well as its density. The addition of L-ascorbic acid to the composite does not influence its porosity and density.

3.4. Mechanical tests

Mechanical properties are very important for any biomaterials to be applied in human body. For this reason the mechanical test was done in our experiment. Mechanical parameters as Young modulus (Figure 2) and maximum compressive force (Figure 3) were determinated for all kinds of samples with and without addition of vitamin C.

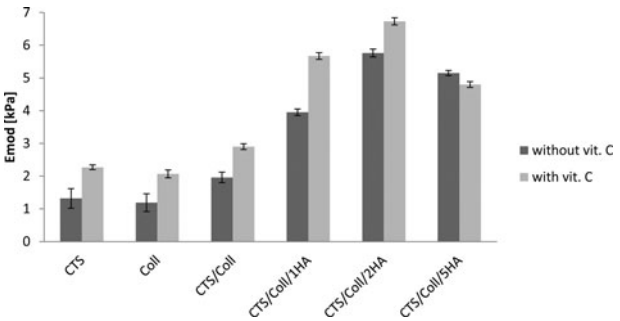

Figure 2. Young modulus (E_{mod}) for chitosan (CTS), collagen (Coll) and their mixture (CTS/Coll) with addition of 1% hyaluronic acid (CTS/Coll/1HA) etc. with and without addition of vitamin C.

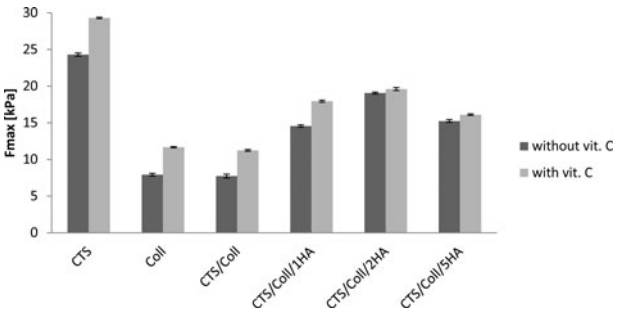

Figure 3. Maximum compressive force (F_{max}) for chitosan (CTS), collagen (Coll) and their mixture (CTS/Coll) with addition of 1% hyaluronic acid (CTS/Coll/1HA) etc.

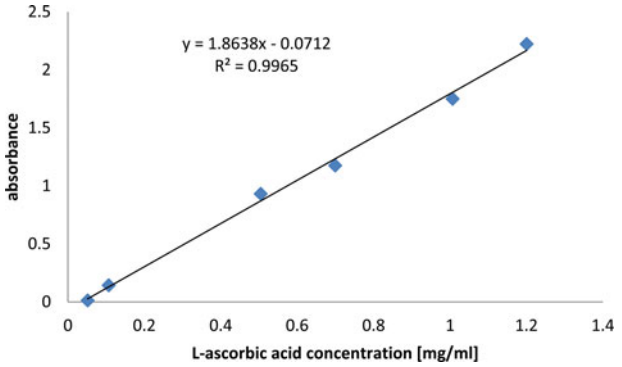

Figure 4. The standard curve of absorbance at 302 nm versus the concentration of L-ascorbic acid.

The results showed that the addition of hyaluronic acid improves the Young modulus. Composites based on the mixture of polymers have higher the mechanical parameter. The addition of vitamin C to the material improves its stiffness. The highest Young modulus was found for chitosan/collagen composite with 2% addition of hyaluronic acid in both case, with and without vitamin C addition.

The addition of hyaluronic acid improves the maximum compressive force for collagen and CTS/Coll composites, it increases with increasing amount of HA. The lowest maximum of compressive force was found for collagen and the addition of chitosan slightly improves it. The addition of L-ascorbic acid improves the maximum compressive force for each kind of sample.

3.5. L-ascorbic acid release

The standard curve showing absorbance of the L-ascorbic acid in PBS solution at 302 nm versus its concentration is presented in Figure 4.

The above standard curve was used to calculate the concentration of the released L-ascorbic acid from polymeric matrixes. The results are shown in Table 3 and Table 4.

Table 3. The concentration of the released L-ascorbic acid from chitosan (CTS), collagen (Coll) and their mixture (CTS/Coll) calculated by the use of standard curve method.

Specimen	Time of immersion [min]	Concentration [mg/ml]
CTS	5	0.0425
	30	0.0919
	60	0.1053
	240	0.1434
	1440	0.1285
	2880	0.1210
Coll	5	0.0867
	30	0.0863
	60	0.0616
	240	0.0429
	1440	0.0330
	2880	0.0386
CTS/Coll	5	0.0733
	30	0.0960
	60	0.0877
	240	0.1312
	1440	0.0917
	2880	0.0461

Table 4. The concentration of the released L-ascorbic acid from chitosan and collagen mixture with 1% (CTS/Coll/1HA), 2% (CTS/Coll/2HA) and 5% (CTS/Coll/5HA) addition of hyaluronic acid calculated by the use of standard curve method.

Specimen	Time of immersion [min]	Concentration [mg/ml]
CTS/Coll/1HA	5	0.0457
	30	0.0665
	60	0.0777
	240	0.1165
	1440	0.1337
	2880	0.1279
CTS/Coll/2HA	5	0.0430
	30	0.0736
	60	0.0709
	240	0.1049
	1440	0.1473
	2880	0.1346
CTS/Coll/5HA	5	0.0390
	30	0.0594
	60	0.0747
	240	0.1206
	1440	0.1473
	2880	0.1346

The amount of L- ascorbic acid released from the scaffold depends on the composition of 3D scaffolds where the active agent was incorporated. The highest concentration of active compound for composites based on chitosan and collagen blend (however without hyaluronic acid) was released after 4h of immersion in PBS. The highest release of L-ascorbic acid from scaffolds with hyaluronic acid was observed after 24h. The addition of hyaluronic acid to chitosan/collagen blends leads to the decrease of amount of released agent. Nevertheless from each kind of matrix L-ascorbic acid was released.

4. Conclusion

In 3D porous composites based on the blends of chitosan, collagen and hyaluronic acid the active agent, L-ascorbic acid can be incorporated. The amount of L- ascorbic acid released from the scaffold depends on the composition of 3D scaffolds. The highest concentration of active compound for composites based on chitosan and collagen blend was observed after 4h of immersion in PBS. The highest release of L-ascorbic acid from chitosan/collagen scaffolds with hyaluronic acid was observed after 24h. The addition of hyaluronic acid to chitosan/collagen blends leads to the decrease of amount of released agent. One can conclude that the composites of chitosan, collagen and hyaluronic acid can be used as matrixes for L-ascorbic acid release.

Acknowledgment

Financial support from the National Science Centre (NCN, Poland) Grant No UMO-2013/11/B/ST8/04444 is gratefully acknowledged.

References

[1] Yang, L., & Fassihi, R. (1997). *Int. J. Pharm.*, *155*, 219.
[2] Mathiowitz, E., Jacob, J.S., Jong, Y.S., Carino, G.P., Chickering, D.E., Chaturvedi, P., Santos, C.A., Vijayaraghavan, K., Montgomery, S., Bassett, M., & Morrell, C. (1997). *Nature*, *386*, 410.

[3] Peppas, N., & Langer, R. (1994). *Science*, *263* 1715.
[4] Streubela, A., Siepmannb, J., Peppasc, N.A., & Bodmeiera, R. (2000). *J. Cont. Rel.*, *69*, 455.
[5] Iwanaga, K., Kawai, M., Miyazaki, M., & Kakemi, M. (2012). *Int. J. Pharm.*, *436*, 869.
[6] Berkland, C., Kipper, M.J., Narasimhan, B., Kim, K.K., & Pack, D.W. (2004). *J. Cont. Rel.*, *94*, 129.
[7] Shen, E., Kipper, M. J., Dziadul, B., Lim, M.K., & Narasimhan, B. (2002). *J. Cont. Rel. 82*, 115.
[8] Proikakis, C.S., Tarantili, P.A., & Andreopoulos, A.G. (2006). *European. Polym. J.*, *42*, 3269.
[9] Tallury, P., Alimohammadi, N., & Kalachandra, S. (2007). *Dental Materials*, *23*, 404.
[10] Pan, C. J., Tang, J. J., Weng, Y. J., Wang, J., & Huang, N. (2009). *Coll. Surf. B: Biointerfaces*, *73*, 199.
[11] Polakovica, M., Gornerb, T., Grefb, R., & Dellacherieb, E. (1999). *J. Cont. Rel.*, *60*, 169.
[12] Karatas, A., & Baykara, T. (2001). *Farmaco.*, *56*, 197.
[13] Neau, S. H.,. Howard, M. A., Claudius, J. S, & Howard, D. R. (1999). *Int. J. Pharm.*, *179*, 97.
[14] Gopferich, A. (1996). *Biomaterials*, *17*, 103.
[15] Hata, R., & Senoo, H. (1989). *J. Cell. Physiol.*, *138*, 8.
[16] Borgquist, P., Körner, A., Piculell, L., Larsson, A., & Axelsson, A. (2006). *J. Cont. Rel. 113*, 216.
[17] Huang, X., & Brazel, C. S. (2001). *J. Cont. Rel.*, *73*, 121.
[18] Uhrich, K. E., Cannizzaro, S. M., Langer, R. S., & Shakesheff, K. M. (1999). *Chem. Rev.* 99, 3181.
[19] Sionkowska, A., Kaczmarek, B., & Lewandowska, K. (2014). *J. Mol. Liq.*, *199*, 318.
[20] Sionkowska, A, Kaczmarek, B., Stalinska, J., & Osyczka, A. M. (2014). *Key Eng. Mat.*, *587*, 205.
[21] Kozlowska, J., & Sionkowska, A. (2015). *Int. J. Biol. Macromol. 74*, 397.