Journal of Applied Polymer Science

The use of liposomes in the modification of polycaprolactone fibers

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ABSTRACT: Polycaprolactone is fully bioresorbable and biocompatible material. Liposomes containing nanocopper, nanosilver, and nanogold are known to have antifungal and antibacterial properties and to further aid in the synthesis of collagen and elastin in the skin. It is possible to combine the properties of polycaprolactone fibers and liposomes in new approaches to deliver active substances through cosmetics and medicines. The aim of the research was to examine the possibility of simple modification of PCL fibers with use of nanocopper, nanogold, and nanosilver incorporated liposomes. The size and the type of the liposomes were examined using optical microscopy and DLS techniques. The fibres modified with liposomes were investigated using SEM and FTIR techniques. Additionally the contact angle measurements were performed. The study shows an innovative method of modifying polycaprolactone nonwoven textiles. This combination of PCL fibers and liposomes allows easy and efficient preparation and delivery of active substances to a particular location. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 43299.

KEYWORDS: electrospinning; fibers; functionalization of polymers; properties and characterization

Received 26 September 2015; accepted 1 December 2015
DOI: 10.1002/app.43299

INTRODUCTION

Polycaprolactone (PCL) is fully bioresorbable and biocompatible material approved by The European Union and the American Food and Drug Administration.[1] It is a hydrophobic, semi-crystalline polymer with a low melting point (60°C), whose high biocompatibility made it one of the earliest commercialized polymers. PCL has a range of applications in Medicine, as medical implants and surgical sutures, Cosmetology and Aesthetic Medicine, as a dermal filler, and the pharmaceutical industry, as dressings.[1–6] The flexibility and extensibility of PCL and its copolymers and composites are also valuable in tissue engineering, especially when incorporated in electrospun fibers with hierarchically arranged structures,[2] sometimes also when used with other active encapsulated substances to form a system for the controlled release of drugs.[7] Polymers with a wide range of physicochemical properties have a strong influence on the release profile of a drug. Polymeric drug delivery systems have shown to improve the bioavailability and therapeutic efficacy of drugs, and reduce their associated toxicity,[6,8] which facilitates the sustained delivery of drugs and also helps in providing targeted therapy.[6,9] In recent years, ultrafine, micro, and nanofibers synthesized by such techniques as electrospinning, phase separation, template synthesis, and self-assembly have attracted much attention due to their biomedical applications.[7]

Electrospun nanofibers in drug delivery systems comprise a plurality of modes of delivery namely, transcutaneous,[10] oral,[11] with sustained release,[12] targeted delivery,[13] and biomedical implants.[14] Tunable nanometer size nanofibers with surface functionality and mechanical properties are widely used in drug delivery systems[15–17] and cosmetic applications,[18,19] in the form of dressing materials,[20–22] drug-eluting stents,[14] transdermal patches for drug delivery[23–25] and blood vessels.[26]

Liposomes, in turn, are extremely good carriers of active substances. The use of liposomes as carriers increases the durability of the products, protects them against harmful external factors

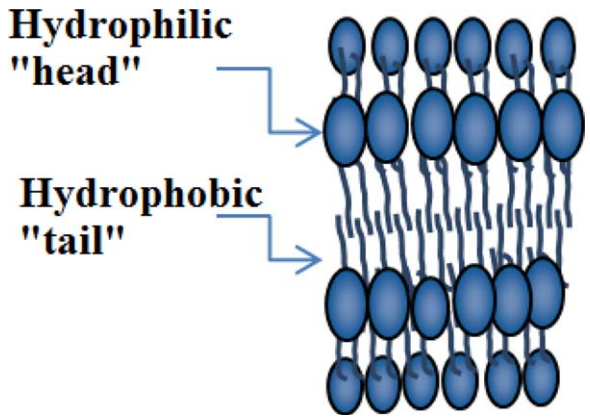

Figure 1. Construction of the lipid bilayer. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

and allows them to be delivered to a specific location (targeted therapy).[27] Liposomes are lipid vesicle structures ranging in size from 0.02 to 10 μm which are composed of phospholipids and surrounded by a lipid bilayer. They are formed by hydratation of phospholipids and can be constructed of several layers separated by phospholipid bilayers (Figure 1).[28] Depending on the method of preparation, liposomes can be divided into small unilamellar vesicles SUV (0.02–0.05 μm), large unilamellar vesicles LUV (0.05–1 μm), giant unilamellar vesicles GUV (>1 μm) and multilamellar vesicles MLV (0.4–10 μm). Besides the size of the liposomes, a key distinction between them is based on the number of lipid bilayers present.[28,29]

The liposomes are used as carriers of active substances in medicines. Hydrophilic compounds are incorporated in the aqueous core of the liposome, while hydrophobic compounds remain in the lipid bilayer.[30] Liposomes can be used as drug transporters with local or systemic effect. Lipid vesicles are currently used in drug delivery systems for substances with analgesic (brucine), cytostatic, antihistamine, antiviral (acyclovir), and even hormonal (estradiol, melatonin) effects.[31,32] Liposome suffer a much more efficient mode of drug delivery than the traditional system, and allows the dose of the drug to be reduced, as well as its toxicity.[30] This approach also allows damage to healthy tissue to be minimized in cancer treatments.

The exceptional properties of liposomes are also useful in Dermatology and Cosmetology. They can be used in the delivery of biologically active antiaging substances, and can act as carriers of active compounds in transdermal therapy. The skin is the best site of application for active compounds, because liposomes are able to penetrate the deeper layers which are inaccessible to traditional preparations.[33] The lipophilic nature of the stratum corneum allows the penetration of nonpolar hydrophobic compounds with low molecular weight. However, as the penetration of active compounds is sometimes not sufficient to achieve any therapeutic effect, this rate has to be increased.

The most common methods of increasing penetration are associated with modifications to the drug molecule and its incorporation in the liposome carrier.[33] The incorporation of drugs into lipid vesicles improves the solubility and stability of the active compounds. Depending on the structure and properties of the drug it can be incorporated into the lipid shell or inside the liposome itself, with the distribution of the active substance affecting the rate of drug release. Currently, although the potential use of lipid vesicles for the transdermal application of such drugs as isotretinoin, ketoprofen, and retinol is under investigation, the use of liposomes in cosmetics, especially creams with filters, appears to offer the greatest chance of success.[34,35] In addition, other active compounds such as vitamins A, E, F (incorporated in lipid bilayer)[36,37] and C (encapsulated inside the liposome),[36] retinol,[38] and collagen[39] have also been encapsulated.

The use of liposomes in cosmetics has many advantages over conventional carriers. Previous studies indicate that liposomes offer non-sensitizing (non-allergenic) properties, easier skin penetration, easier healing and treatment of skin diseases, and do not require the use of preservatives.[27,40,41] Fortunately, it is possible to combine the properties of polycaprolactone fibers (PCL) and liposomes in new approaches to delivering active substances through cosmetics and medicines. Hence, the present study was conducted to determine the simple and efficient way of modification of PCL fibers to achieve this aim.

EXPERIMENTAL

Materials

Polycaprolactone and Electrospinning. Polycaprolactone for electrospinning (mol. wt 45.000, Sigma–Aldrich, UK) was prepared by dissolving PCL in chloroform and ethanol (ratio 9:1) at a concentration of 14 wt %.

The fibers were electrospun from a free surface of a thin polymeric layer using Nanospider™ needleless technology. This technology was developed at the Technical University of Liberec in cooperation with Elmarco.[42,43] The spinning electrode was connected to the positive pole of a high voltage source (Spellman Sl100) positioned 130 mm beneath a negatively charged collector (Spellman Sl100). A voltage of +50/−10 kV was applied. A nonwoven Spunbond (Pegas, CZ) was located under the collector to collect the electrospun material. The width of the Spunbondline was 300 mm. Electrospinning was performed at ambient temperature (23°C ± 2°C) and relative humidity (23% ± 3%).

Liposomes. Three types of commercially available liposomes were used for the surface modification of the polycaprolactone fibers, each one containing 50 ppm of the active substance: type one was nanocopper, which is known to have antifungal and antibacterial proprties and to further aid in the synthesis of collagen and elastin in the skin;[44] the second type liposomes with nanosilver, which show bactericidal and fungicidal properties;[44–46] the third was liposomes with nanogold, which are believed to purify the body through the skin and stimulate collagen synthesis.[46] The liposomes were immersed in a preparation comprising: *Aqua, Lecithin, D-panthenol, Glucose, Trilaureth-4-phosphate, Citric acid, Phenoxyethanol, Ethylhexylglycerin* (INCI terminology). It is a homogeneous, opaque, beige liquid with a pH of 5.30–6.80 and density 1.0 g mL^{-1}.

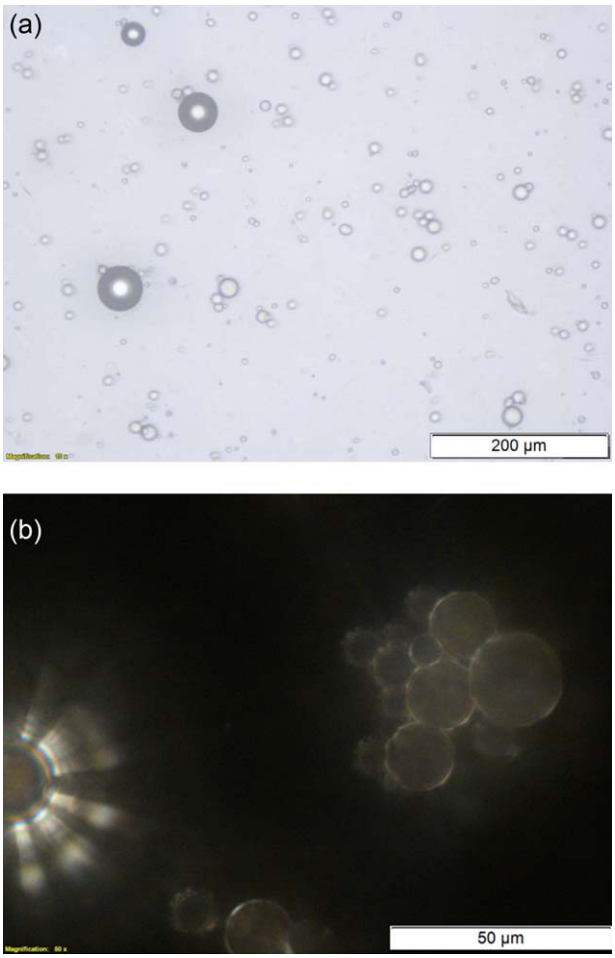

Figure 2. Reflected light optical microscopy image of liposomes with nanocopper: (a) bright field and (b) dark field. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

METHODS

Modification of Polycaprolactone Fibers Using Liposomes

The PCL samples were placed on the laboratory glass. First, 200 μL of liposome containing formulation was applied on both sides of the surface of the polycaprolactone samples using an automatic pipette. A size of the modified area was 10 × 10 mm^2. Each of the three formulations was applied on the separate PCL sample. The prepared samples were then analyzed immediately after application and 96 h after drying.

Optical Microscopy. Preparations containing all liposome types were analyzed using an Olympus GX-71 optical fluorescent microscope with reversed optics and digital camera. Image acquisition was performed using Stream software. The sample of the suspension was applied to a glass slide with a thickness of 0.1 mm and covered with a slip. Observations were carried out both using transmitted light and reflected light. In the latter, bright and dark filed method were used.

Dynamic Light Scattering DLS. Dynamic light scattering (DLS) was studied using a Zeta Sizer Nano Series potential analyzer (Malvern). The measurements were conducted in disposable polystyrene cuvettes. In each cuvette, 1-mL liposome solution was analyzed. For each sample, three series of measurements were taken and the results were averaged.

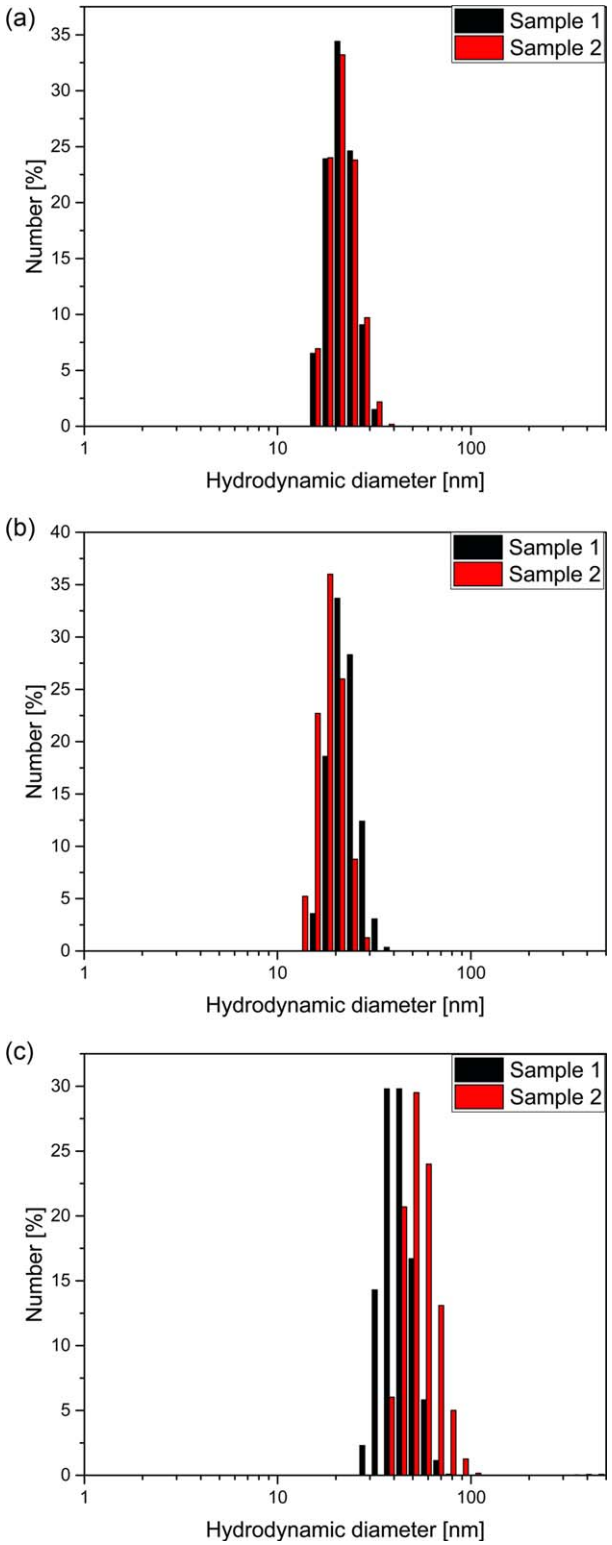

Figure 3. DLS size distribution of liposomes containing: (a) copper; (b) silver; and (c) gold nanoparticles. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

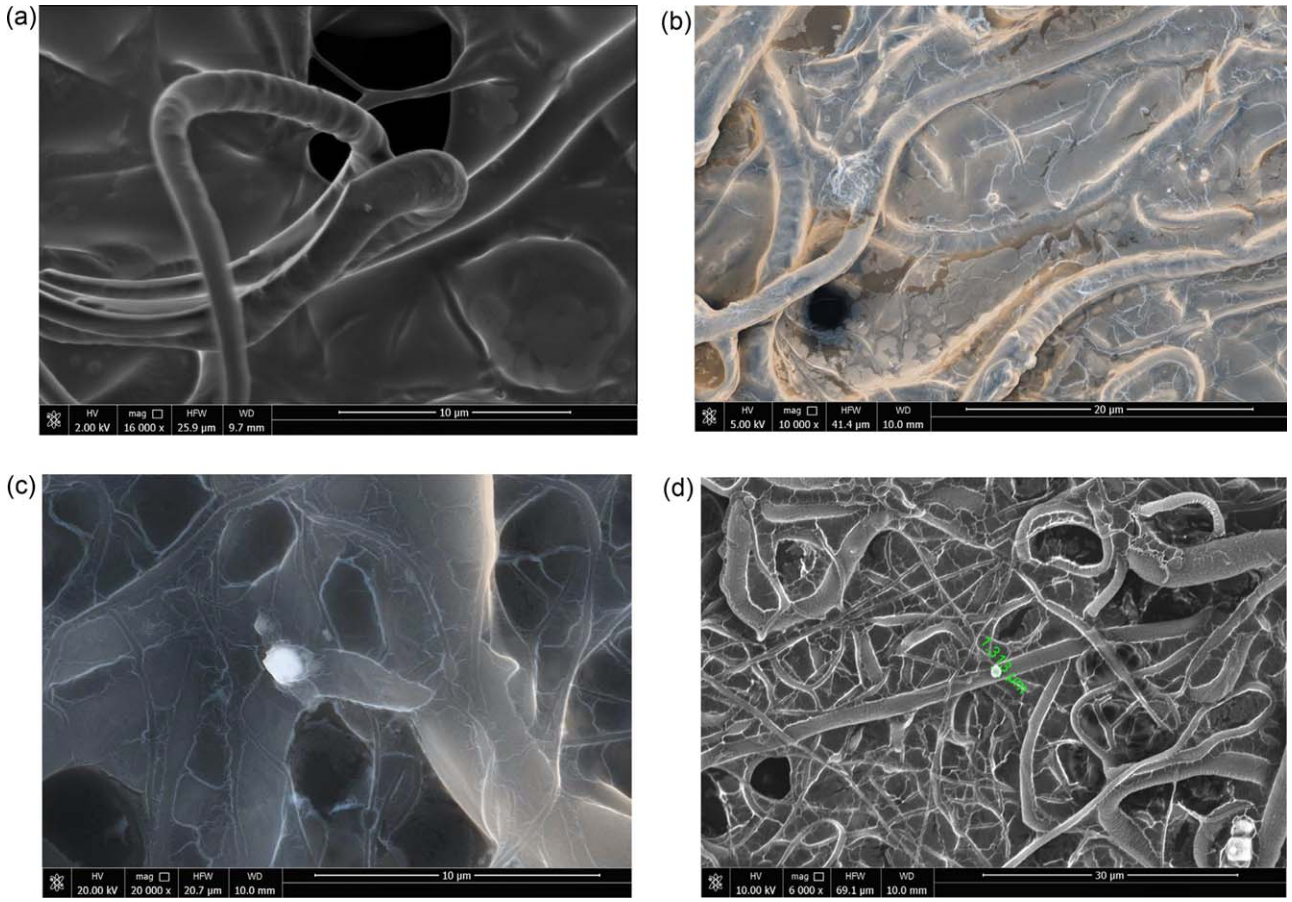

Figure 4. (a) Polycaprolactone fibers, (b–d) The PCL fibers modified with nanogold liposomes. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Scanning Electron Microscopy SEM. The PCL fibers were investigated before and after the modification using a QUANTA FEG 250 scanning electron microscope (SEM) (FEI Company) working in a high vacuum in secondary and backscattered electron detection mode. A sample was mounted on a sample holder using a conductive carbon tape and coated with a 4 nm layer of gold (Leica AM 600).

Fourier Transform Infrared Spectroscopy FTIR. The Fourier Transform Infrared Spectroscopy (FTIR) analysis was performed with the use of a Nicolet iS50 FTIR spectrometer (Thermo Scientific). The readings were taken within a spectral range of 4000–600 cm^{-1} with a resolution of 0.5 cm^{-1} and using Deuterated Triglycine Sulfate (DTGS) KBr beam splitter. The measurements were conducted using a Harric Diffuse Reflectance Infra-red Fourier Transform DRIFT reflectance unit working at a beam incidence angle of 70° in an absorbance mode. A single measurement was composed of 120 scanning cycles.

Contact Angle. Contact angles were measured with a KRUSS Contact Angle Measuring Instrument, using the sessile drop method. A standard volume of 3 mL deionized water (specific conductivity: 0.1e1 mS cm^{-1}) was applied to the sample surface for each measurement. Each measurement was repeated three times and the results were averaged.

RESULTS AND DISCUSSION

Optical Microscopy

The appearance of the liposomes under the optical microscope is presented in Figure 2. Some of the liposomes occur in the form of single vesicles of similar size; however, most of the liposomes were arranged in aggregates. Although the lipid vesicles arranged in aggregates were larger than individual liposomes, they were of similar size to each other. Hence these appear to be large and giant LUV and GUV unilamellar liposomes, the presence of LUV was also confirmed by dynamic light scattering.

Dynamic Light Scattering DLS

The sizes of the nanocopper, nanosilver, and nanogold liposomes were characterized by DLS. The particle size distributions of all analyzed samples are presented in Figure 3. In all cases, the size distribution curves are approximately Gaussian, and the particle size varies between 10–100 nm. This observed difference in the size may be related to the increased tendency of the liposomes to agglomerate. DLS also indicates the presence of large particles that are visible in the optical microscope and SEM images. Nevertheless the optical microscopy examination indicates that all sizes of liposomes occur mostly in the form of conglomerates.

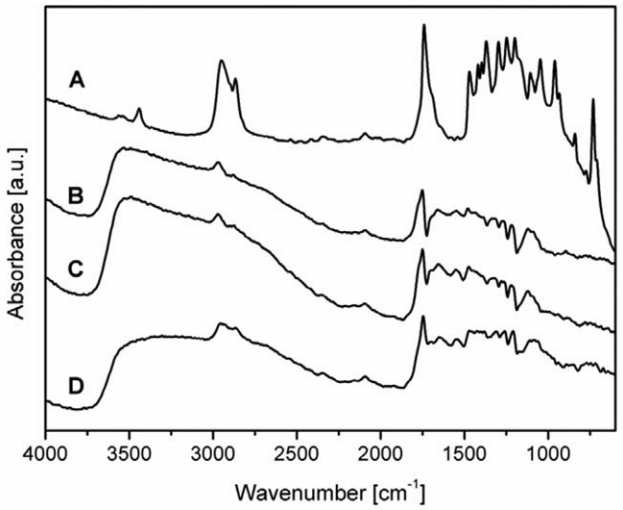

Figure 5. FTIR spectra of: (a) unmodified PCL, (b) PCL modified by Cu-containing liposomes, (c) PCL modified by Ag-containing liposomes, (d) PCL modified by Au-containing liposomes.

The findings indicate that the tested liposomes are small and large SUV- and LUV-type unilamellar liposomes, which are not all visible in the optical microscope image. Liposomes of this type are ideal for use in Cosmetology and Pharmacology as carriers of drugs and active ingredients.

Scanning Electron Microscopy SEM

SEM was used to determine the efficiency of the liposome modification of PCL fibers. The findings indicate such modification is possible through the direct application of the liposome formulation to the surface of the final PCL product. The SEM analysis of PCL fibers before and after the modification is presented in Figure 4. The liposomes are incorporated into the spaces between the individual fibers and attach to their surface. They mostly appear in the form of aggregates with a size of ~1 μm, due to the specificity of the commercial liposome preparation.

Fourier Transform Infrared Spectroscopy FTIR

The FTIR spectrum of the polycaprolactone (PCL) nonwoven fabric is presented in Figure 5(a). PCL is a semicrystalline polyester characterized by the presence of one polar ester group and five nonpolar ethylene groups in a single monomer unit. These groups dominate the FTIR spectrum.

In the spectral range between 3000 and 2800 cm^{-1}, a broad band corresponding to stretching C—H vibrations of PCL methylene groups can be seen, with their symmetric and asymmetric modes corresponding to the 2921 and 2860 cm^{-1} maxima, respectively. In addition, the presence of aliphatic carbon chains is confirmed by the presence of absorption bands corresponding to different bending vibrations of C—H bonds culminating at the wave numbers of 1420, 1390, and 1368 cm^{-1}.[47]

All the remaining absorption maxima found in the spectrum are characteristic of PCL. A strong absorption band corresponding to the stretching vibration of a carbonyl group is observed at 1741 cm^{-1}. Within the range 1295–1170 cm^{-1}, four distinct maxima are present. The first, at 1293 cm^{-1}, is due to the stretching vibrations of C—O and C—C bonds. The bands at 1240 and 1190 cm^{-1} can be attributed to the asymmetric vibrations of the C—O—C atomic system. Finally, the 1170 cm^{-1} maximum situated at the slope corresponds to symmetrical C—O—C vibrations.[47,48] According to Coleman and Zarian,[49] such a combination of absorption maxima indicates the presence of high crystallinity of the PCL sample. In addition, the lack of a 1157 cm^{-1} band, which is associated with the C—O and C—C stretching vibrations in an amorphous structure, confirms the high degree of polymer crystallinity present in the sample.

Figure 5(b–d) presents the spectra of the nanocopper, nanosilver, and nanogold liposomes modified PCL fibers. The spectra appear quite similar, with a minor difference present in the spectral range of 3000–2800 cm^{-1} associated with the stretching vibrations of the C—H bonds. The spectrum of PCL modified using nanogold liposomes is comparable to that of pure PCL and it slightly differs from the other two spectra. In these spectra [Figure 5(b,c)], two additional bands appear at 2964 and 2872 cm^{-1}, corresponding to the symmetric and asymmetric stretching vibrations of the C—H bonds in the methyl groups.[47] In addition, maxima corresponding to the stretching vibrations of these bonds in methylene groups are also present, although their intensity is less than those seen in the spectrum of pure PCL. The presence of these bands may be either due to the PCL substrate, or they may be ascribed to the aliphatic chains of fatty acids constituting the liposome tails. In addition, a band corresponding to scissoring vibrations of CH_2 groups is present at 1470 cm^{-1}. The 3500–3000 cm^{-1} spectral range is covered by a very broad band, attributed to the vibrations of hydroxyl groups and originating from the (adsorbed) water molecules, used as a solvent in the liposome solution. This band is broad enough to be able to hide the vibrations of the N—H, C—N or, =CH bonds present in this absorption range.[47,48]

A high intensity maximum at 1751 cm^{-1} is present in the spectra of all the modified liposome samples. This may either originate from the polymer substrate, or may be associated with the fatty acid carbonyl group bound to glycerol. In all three spectra, this band is shifted slightly further toward higher wave numbers than those observed in the pure PCL spectrum, and has a slightly different form.

The most pronounced differences between the spectrum of the plain PCL substrate and those of its three modifications are found in the spectral range of 1650–1000 cm^{-1}. A broad band, ending at 1650 cm^{-1} in the spectra shown in Figure 5(b–d), corresponds to the stretching vibrations of C=O and N—H groups present in the liposome head.[50] In addition, a 1100 cm^{-1} maximum characteristic for stretching vibrations of an amine bond in aliphatic amines is present. An intense absorption band at 1212 cm^{-1}, on the other hand, indicates an interaction of amine bond with a metal-oxide (Me-O) connection.[51] The presence of this band suggests that a fraction of metal introduced to the liposome may be bound to its head instead of being trapped in its interior in metallic form.

The vibrations of a phosphate group present in the liposome head corresponds to infrared characteristic absorption bands in the spectral range 1300–1050 cm^{-1}. The two relatively distinct maxima visible at 1263 cm^{-1}, corresponding to the asymmetric

stretching vibrations of a PO_2^- connection, and 1230 cm^{-1}, indicating the presence of water molecules bound to phosphate groups. A shift of this 1230 cm^{-1} band toward higher wavelengths may indicate partial dehydratation of the phosphate groups. If so, it would suggest that the lowest degree of dehydratation was exhibited by copper modified liposomes, while the highest was shown by a modification with silver. The maximum observed at 1080 cm^{-1} can also be attributed to a phosphate group. However, this maximum corresponds to the symmetric stretching vibrations of phosphate groups, and its intensity is far lower than that of bands bound to asymmetric vibrations.[50]

Contact Angle

Previous studies indicate that polycaprolactone is hydrophobic.[2,4] However, non-woven PCL is hydrophilic. As both pure PCL and liposome-modified PCL were found to be hydrophilic, it may be concluded that liposome modification does not significantly affect the contact angle. The typical nonwoven construction causes spreading of droplets, which is probably caused by capillary forces. This distinctive design allows a solution to be absorbed between the individual fibers, enabling easier and more efficient surface modification.

CONCLUSION

The study presents an innovative method of modifying polycaprolactone nonwoven textiles. The application of the described liposome formulation to the surface of PCL fibers expands their application spectra, especially the use of targeted therapy for individual patients. Liposomes, being extremely good carriers which increase the stability of the formulation, can be used to protect the incorporated compounds against the harmful effects of external factors. They can be combined with electrospun biocompatible nonwoven PCL textiles, whose fiber size, surface functionality, and mechanical properties can be tuned to individual needs. This combination of PCL fibers and liposomes allows easy and efficient preparation and delivery of active substances to a particular location. The use of liposome-modified polycaprolactone fibers in dermocosmetic masks, dressing materials, and transdermal patches represents a unique application of active substances in cosmetics and medicines.

ACKNOWLEDGMENTS

The study was supported by the Medical University of Lodz, Poland grant no.: 502-03/3-066-01/502-34-045 and statutory research activity no.: 503/3-066-01/503-31-001, 503/3-066-02/503-31-001.

REFERENCES

1. Figueiredo, V. M. *J. Cosmet. Dermatol.* **2013**, *12*, 73.
2. Ko, Y. I.; Lee, Y.; Devarayan, K.; Kim, B. S.; Hayashi, T.; Kim, I. S. *Mater. Lett.* **2014**, *131*, 128.
3. Lendlein, A.; Langer, R. *Science* **2002**, *296*, 1673.
4. Woodruff, M. A.; Hutmacher, D. W. *Prog. Polym. Sci.* **2010**, *35*, 1217.
5. Nicolau, P. J.; Marijnissen-Hofsté, J. *Eur. J. Aesth. Med. Dermatol.* **2013**, *3*, 19.
6. Kim, H. J.; Kim, T. H.; Kang, K. C.; Pyo, H. B.; Jeong, H. H. *Int. J. Cosmetic. Sci.* **2010**, *32*, 185.
7. Madhaiyan, K.; Sridharb, R.; Sundarrajanb, S.; Venugopala, J. R.; Ramakrishna, S. *Int. J. Pharm.* **2013**, *444*, 70.
8. Singh, S. *J. Nanosci. Nanotech.* **2010**, *10*, 7906.
9. Chakraborty, S.; Liao, I. C.; Adler, A.; Leong, K. W. *Adv. Drug Deliv. Rev.* **2009**, *61*, 1043.
10. Mendoza-Palomares, C.; Ferrand, A.; Facca, S.; Fioretti, F.; Ladam, G.; Kuchler-Bopp, S.; Regnier, T.; Mainard, D.; Benkirane-Jessel, N. *ACS Nano* **2012**, *6*, 483.
11. Ignatious, F.; Sun, L.; Lee, C. P. *J. Pharm. Res.* **2010**, *27*, 576.
12. Yu, D. G.; Branford-White, C.; Li, L.; Wu, X. M.; Zhu, L. M. *J. Appl. Polym. Sci.* **2010**, *117*, 1509.
13. Shen, X.; Yu, D.; Zhu, L.; Branford-White, C.; White, K.; Chatterton, N. P. *Int. J. Pharm.* **2011**, *408*, 200.
14. Yoo, J. J.; Kim, C.; Chung, C. W.; Jeong, Y. I.; Kang, D. H. *Int. J. Nanomed.* **2012**, *7*, 1997.
15. Azarbayjani, A. F.; Venugopal, J. R.; Ramakrishna, S.; Lim, P. F.; Chan, Y. W.; Chan, S. Y. *J. Pharm. Pharm. Sci.* **2010**, *13*, 400.
16. Yan, N.; Zhang, X.; Cai, Q.; Yang, X.; Zhou, X.; Wang, B.; Deng, X. *J. Biomater. Sci. Polym.* **2012**, *23*, 1005.
17. Yan, S.; Xiaoqiang, L.; Shuiping, L.; Xiumei, M.; Ramakrishna, S. *Colloids Surf. B* **2009**, *73*, 376.
18. Fathi-Azarbayjani, A.; Qun, L.; Chan, Y. W.; Chan, S. Y. *AAPS Pharm. Sci. Tech.* **2010**, *11*, 1164.
19. Opanasopit, P.; Ruktanonchai, U.; Suwantong, O.; Panomsuk, S.; Ngawhirunpat, T.; Sittisombut, C.; Suksamran, T.; Supaphol, P. *J. Cosmet. Sci.* **2008**, *59*, 233.
20. Chen, D. W.; Liao, J. Y.; Liu, S. J.; Chan, E. C. *Int. J. Nanomed.* **2012**, *7*, 763.
21. Grewal, H.; Dhakate, S. R.; Goyal, A. K.; Markandeywar, T. S.; Malik, B.; Rath, G. *Artif. Cells Blood Substit. Immobil. Biotechnol.* **2012**, *40*, 146.
22. Jannesari, M.; Varshosaz, J.; Morshed, M.; Zamani, M. *Int. J. Nanomed.* **2011**, *6*, 993.
23. Taepaiboon, P.; Rungsardthong, U.; Supaphol, P. *Eur. J. Pharm. Biopharm.* **2007**, *67*, 387.
24. Ngawhirunpat, T.; Opanasopit, P.; Rojanarata, T.; Akkaramongkolporn, P.; Ruktanonchai, U.; Supaphol, P. *Pharm. Dev. Technol.* **2009**, *14*, 70.
25. Wu, X. M.; Branford-White, C. J.; Zhu, L. M.; Chatterton, N. P.; Yu, D. G. *J. Mater. Sci. Mater. Med.* **2010**, *21*, 2403.
26. Janairo, R. R.; Henry, J. J.; Lee, B. L.; Hashi, C. K.; Derugin, N.; Lee, R.; Li, S. *IEEE Trans. Nanobiosci.* **2012**, *11*, 22.
27. Sarecka-Hujar, B.; Jankowski, A.; Wysocka, J. *Ann. Acad. Med. Siles.* **2011**, *65*, 45.
28. Bartosiewicz, D.; Kozubek, A. *Drug Poland* **2008**, *18*, 85.
29. Shaheen, S. M.; Ahed, F. R.; Hossen, N. *Pak. J. Biol. Sci.* **2006**, *9*, 1181.
30. Morrow, D. I. J.; McCarron, P. A.; Woolfson, A. D. *Open Drug Deliv. J.* **2007**, *1*, 36.
31. Qin, X. Q.; Yuan, Y.; Liu, C. *S. Acta Pharmacol. Sin.* **2007**, *28*, 1851.

32. Jain, S. K.; Gupta, Y.; Jain, A. *Drug Deliv.* **2008**, *15*, 141.
33. Benson, H. *Curr. Drug Deliv.* **2005**, *2*, 23.
34. Raskovic, D.; Piazza, P. *J. Liposome Res.* **1993**, *3*, 737.
35. Rahimpour, Y.; Hamishehkar, H. *Expert Opin. Drug Deliv.* **2012**, *99*, 443.
36. Niki, E.; Kawakami, A.; Yamamoto, Y.; Kamiya, Y. *Chem. Soc. Japan* **1985**, *5858*, 1971.
37. Arsić, I.; Vuleta, G. *Int. J. Cosmet. Sci.* **2001**, *21*, 219.
38. Oh, Y. K.; Kim, M. Y.; Shin, J. Y. *J. Pharm. Pharmacol.* **2006**, *58*, 161.
39. Pajean, M.; Herbage, D. *Int. J. Pharm.* **1993**, *91*, 209.
40. Brown, M. B.; Martin, G. P.; Jones, S. A. *Drug Deliv.* **2006**, *13*, 175.
41. Puglia, C.; Bonina, F. *Expert Opin. Drug Deliv.* **2012**, *9*, 429.
42. Available at: http://www.elmarco.com/electrospinning/electrospinning-technology/
43. Jirsak, O.; Sanetrnik, F.; Lukas, D.; Kotek, V.; Martinova, L.; Chaloupek, J. Patent WO2005024101 (**2005**).
44. Kartal, S. N.; Green, F.; Clausen, C. A. *Int. Biodeterior. Biodegrad.* **2009**, *63*, 490.
45. Batory, D.; Gorzedowski, J.; Rajchel, B.; Szymanski, W.; Kolodziejczyk, L.; Vacuum **2013**, *110*, 78.
46. Bhattacharya, R.; Mukherjee, P. *Adv. Drug Deliv. Rev.* **2008**, *60*, 1289.
47. Silverstein, R. M.; Bassler, G. C.; Morrill, T. C. Spectrometric Identification of Organic Compounds; Wiley: Chichester, **1991**, Chapter 3, p 72.
48. Lambert, J. B.; Shurvell, H. F.; Lightner, D. A.; Cooks, R. G. Introduction to Organic Spectroscopy; MacMillan: New York, **1987**, Chapter 10, p 174.
49. Coleman, M. M.; Zarian, J. *J. Polym. Sci. Polym. Phys. Ed.* **1979**, *17*, 837.
50. Nzai, J. M.; Proctor, A. *J. Am. Oil. Chem. Soc.* **1998**, *70*, 1281.
51. Barbinta-Patrascu, M. E.; Ungureanu, C.; Iordache, S. M.; Iordache, A. M.; Bunghez, I. R.; Ghiurea, M.; Badea, N.; Fierascu, R. C.; Stamatin, I. *Mater. Sci. Eng. C* **2014**, *39*, 177.