Polish Journal of Chemical Technology, 17, **4**, 32 — 36, 10.1515/pjct-2015-0065

Encapsulation of L-ascorbic acid *via* polycaprolactone-polyethylene glycol-casein bioblends

Erhan Ozsagiroglu[1*], Yuksel Avcibasi Guvenilir[1]

[1]*Istanbul Technical University, Chemical and Metallurgical Engineering Faculty, Chemical Engineering Department, 34469, Maslak-Istanbul, Turkey*
[*]*Corresponding author: e-mail: ozsagiroglu@itu.edu.tr*

The aim of this study was to encapsulate, L-ascorbic acid, in biopolymers in order to obtain (i) enhancing its encapsulation efficiency (ii) increasing drug release ratio using different pH mediums. Microparticles based on polycaprolactone, polyethylene glycol and casein are prepared by spray drying technique. Microparticles are in vitro characterized in terms of yield of production, particle size, morphology, encapsulation efficiency, and drug release. In this manner, the importance of the study is producing of a stable and effective drug encapsulation system by PCL-PEG-CS polymer mixture by spray dryer. We achieved minimum 27.540±0.656 μm particle size with 0.512 m^2/g surface area, 84.05% maximum drug loading, and 68.92% drug release ratio at pH 9.6. Release profiles are fitted to previously developed kinetic models to differentiate possible release mechanisms. The Korsmeyer–Peppas model is the best described each release scenario, and the drug release is governed by non-Fickian diffusion at pH 9.6. Our study proposed as an alternative or adjuvants for controlling release of L-ascorbic acid.

Keywords: casein, drug encapsulation, L-ascorbic acid, polycaprolactone, polyethylene glycol, spray dryer.

INTRODUCTION

Drug delivery research aims to conveniently administer complex drugs to the target tissue in the biological system in a more stable and reproducible controlled way so that it would achieve higher activity at a minimal dose for prolonged period at the site devoid of side effects. Entrapment of a drug into a polymeric system may protect the drug from inactivation and help to retain its activity for prolonged durations, decrease its toxicity, dosing frequency and offers flexibility in administration[1].

Drug encapsulation in colloidal delivery systems is an efficient approach to improve the pharmacokinetics of hydrophilic drugs[2]. These carriers encompass a broad range of dispersion systems ranging from submicron emulsions to colloidal particles, such as bio-based polymeric (polycaprolactone (PCL), polyethylene glycol (PEG), chitosan, casein (CS), and starch) aiming to protect the drug against degradation, sustain drug release, increase patient comfort by avoiding repetitive bolus injections or the use of perfusion pumps and reduce side effects[3–5].

L-ascorbic acid, commonly called vitamin C, is a highly abundant and essential metabolite for plants and animals. It is an important dietary supplement for some animals, such as primates and humans, which lack the capacity to synthesize L-ascorbic acid. It has attracted considerable attention from the scientific community due to their numerous beneficial effects for human health, which include antioxidant and immune booster effects[6]. Unfortunately, while L-ascorbic acid provides beneficial therapeutic effects, its stability also easily effected temperature, sun-light and O_2. Thus, encapsulation of L-ascorbic acid would provide a stable and highly effective drug[7].

Spray-drying is known to be a convenient one-step process for the continuous conversion of liquid formulations into dry particulates, which is well-established in many relevant branches such as the chemical, food or pharmaceutical industry, due to the high flexibility to manipulate particle properties (e.g. size and morphology)[8]. It results in powders with good quality, low water activity, easier handling and storage[9, 10]. Equipment is readily available and production costs are lower than most other methods. Another advantage of spray drying is that it is possible to control particle size and morphology by varying process parameters and formulation. This is of great importance for the preparation of powders for drug encapsulation. Therefore, spray drying can be considered as an interesting alternative to any other methods for the preparation of amorphous solid dispersions, soluble complexes, encapsulated systems, solid self-emulsifying systems and nano-dispersions of poorly soluble drugs[11–13].

On the basis of these remarks, aim of this work was the encapsulation of L-ascorbic acid in microspheres based on bio-based polymers (PCL-PEG-CS) in order to obtain (i) enhancing of encapsulation and (ii) increasing of drug release ratio simulated with different pH mediums. We used ternary polymer mixture to obtain best performance from an encapsulation material because a combination of these materials can achieve superior characteristics than each component individually and spray dryer was our tool to obtain microspheres. The study includes three different steps: first step is to evaluate effects of spray drying conditions and composition of the microencapsulating formulation; second part is encapsulation of L-ascorbic acid; final part is controlled release potential of the prepared formulations employing plain and is followed by monitoring their *in vitro* drug release profile.

MATERIAL AND METHODS

Material

Polycaprolactone with M_n = 10.000 g/mol, M_w = 14.000 g/mol and casein from bovine milk are purchased from Sigma-Aldrich. Polyethylene glycol (PEG-6000) is a gift from Ashland Inc. L-ascorbic acid powder (Vitamin C) is also obtained from Sigma-Aldrich. Acetic acid (Merck) and formic acid (Merck) are used as solvent agents for polymer mixtures and used without any further purification. Oxalic acid and 2.6-Dichloroindophenol sodium

salt hydrate are used to determine L-ascorbic acid in UV spectrum.

Preparation of polymer mixture

PCL+PEG+CS polymer mixture solutions are prepared by acetic acid-formic acid solutions with 3:7 (v/v) ratios for ~24 h, followed by a filtration step (1.5 μm; 934-AH® Binderless Glass Microfiber Filter Media). The value of 3:7 (v/v) acetic acid:formic acid is determined by previous studies[14–16]. Polymer amounts are 10 wt% and mixtures are prepared by 1:1:1 (wt/wt) ratio.

Drying, drug loading and drug release studies

Although in industrial spray drying processes, dispersions with high solid concentration are recommended (>30%) to reduce costs and increasing drying efficiency, in this study dispersions with low concentration of total solids were selected in order to avoid obstruction of the pneumatic nozzle when using blends with the highest concentration of gum[17].

Polymer solutions are fed to spray dryer (Yamato ADL 310 lab scale spray dryer) by a peristaltic pump and first of all, best drying conditions are determined by changing drying temperature (120°C, 135°C, and 150°C) and feed flow rate (3 mL/min, 6 mL/min, and 9 mL/min). Atomizer pressure is constant at 1 barg.

Secondly, particle diameter and particle size distribution are determined and L-ascorbic acid in different weight ratios are loaded for microsphere which have the lowest particle diameter. L-ascorbic acid loading studies are done by indirect loading (pH 7.0) of drug to microspheres at 25°C and 200 rpm. L-ascorbic acid contents are 5 wt%, 10 wt%, and 15 wt%, respectively. Encapsulation efficiency is calculated by Eq. 1[18]:

$$Encapsulation\ efficiency\ \% = \frac{Final\ L-ascorbic\ acid\ amounts}{Initial\ L-ascorbic\ acid\ amounts} x100 \quad (1)$$

Liquid phase is analyzed by UV at 518 nm to calculate Eq. (1)[19–22]. Calculations in drug release step of the study are also done by UV. 0.5 mg blend/1 mL release medium with different pH saline solutions (pH 2.8, 7.4, and 9.6) ratio is used. Polymeric drug is dispersed in the release medium and incubation occurred at 25°C with shaking. Drug release is calculated by Eq. (2)[18].

$$Drug\ release\ \% = \frac{C(t)}{C(0)} x100 \quad (2)$$

Where;

C(t) refers to drug amount in any time and C(0) to drug amount at t = 0.

Methods

Particle Size Analyzer (Malvern Mastersizer 2000), Mastersizer is used to obtain particle size diameter with distribution. Scanning Electron Microscopy (SEM-Jeol, JSM-6390 LV) is used to obtain morphological structures of microspheres. Particle morphology is determined by this way. Thermogravimetric analysis or thermal gravimetric analysis (TGA, Perkin-Elmer Diamond TG/DTA) is used to analyze blend structure. Ultraviolet Analysis (UV-UV Mini 1240 SHIMADZU) is used to calculate drug encapsulation efficiency and drug release ratios.

RESULTS AND DISCUSSION

Yield and particle size distribution with standard deviation are shown for all studies in Figure 1 and Figure 2, respectively. Due to the Figure 2, Mastersizer analyses are done 3 times and particle diameter distribution is determined by standard deviation of the results. Final values demonstrate particle size [μm] with standard deviation of PCL-PEG-CS microspheres. Figure 1 determines that drying efficiency increase by decreasing of flow rates at 120°C, 135°C and 150°C. The cause of these results are that increasing drying temperature resulted in agglomeration of polymer mixture, so contact time with polymer mixture and drying temperature had adversely effect on diameter distribution[23]. Due to these results, 135°C and 3 ml/min are the best drying conditions because the lowest particle diameter and highest surface area are also obtained with 27.540±0.656 μm particle size with 0.512 m^2/g surface area in this study. The yields from spray drying have also been significantly decreased at higher flow rates due to the less contact time with hot drying air[24].

Figure 1 shows the tendency of the yield to be higher when the spray rate was low. Additionally, particle size diameters are increased at higher spray rates as seen in Figure 2. It is revealed that we have to avoid higher spray rates because of its reverse effect on encapsulation material.

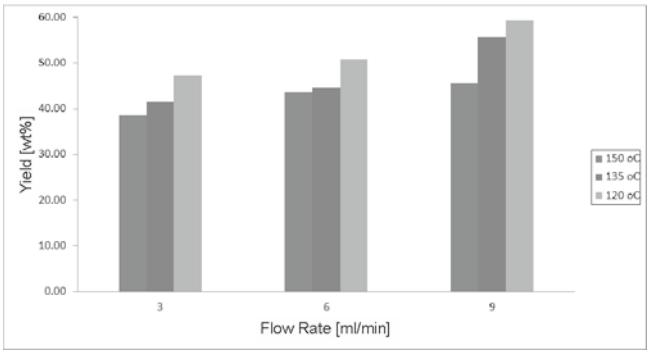

Figure 1. Yield [wt%] at different drying temperature [°C] and flow rates [ml/min]

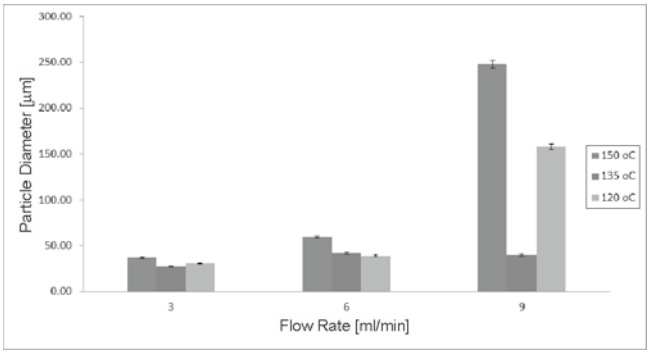

Figure 2. Particle diameter [μm] with standard deviations at different drying temperature [°C] and flow rates [ml/min]

SEM micrographs are shown in Figure 3 for PCL--PEH-CS microsphere obtained at 135°C and 3 ml/min.

Observing the external morphology, particles show a spherical shape and various sizes with no apparent cracks or fissures, which is an advantage, since it implies that capsules have lower permeability to gases, increas-

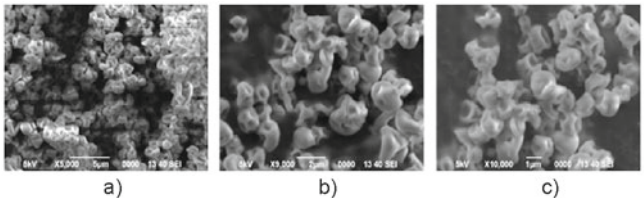

Figure 3. SEM micrographs of PCL-PEH-CS microsphere (a) 5,000X; (b) 9,000X; (c) 10,000X

ing protection and retention of the active ingredient (L-ascorbic acid). Moreover, the variety in size is a typical characteristic of particles produced by spray drying. The mixtures of different wall materials are influenced on microparticles morphology[25, 26]. As well, the picture shows that there are no agglomeration and adherence between microspheres during drying process.

TGA curve for PCL-PEG-CS bio-blend is shown in Figure 4. The sample is placed in furnace and it is burned in the N_2 atmosphere without O_2 with the ramp 30°C/min up to 800°C. TGA scan is obtained from the measurement to determine decomposition temperature and char yield.

When heating a ternary blend (PCL-PEG-CS), three well-separated peaks are obtained on the TGA thermogram as shown in Figure 4. Customarily, the peak temperatures are referred to the degradation temperatures of the blending components[27]. Figure 4 shows encapsulation material thermal degradation and it begins about 200°C with CS degradation, following at 350°C with PCL degradation and finalized at 400°C with PEG-6000 degradation.

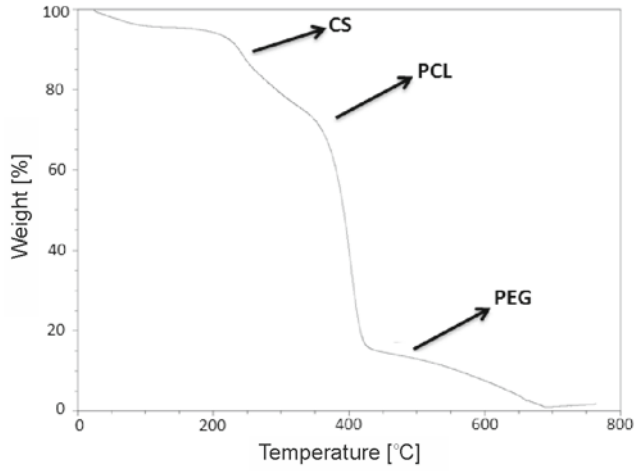

Figure 4. TGA curve of PCL-PEG-CS microsphere obtained at 135°C, 3 ml/min

Drug encapsulation studies are performed under three different L-ascorbic acid concentrations (5 wt%, 10 wt%, and 15 wt%) at pH 7.0 with different loading time and PCL-PEG-CS particle amounts. It is expected that encapsulation efficiency of a drug changes depending on structures of the microspheres[28]. Particle diameter also affects the encapsulation efficiency. Drug-encapsulated amount depends on surface area of the microspheres. Microsphere obtained at 135°C and 3 ml/min is used in drug loading studies because this microsphere had the lowest particle diameter and also best distributed particles due to the Mastersizer analyses (see also in Fig. 1 and 2). Figure 5 and Figure 6 indicate that how much L-ascorbic acid is encapsulated due to the time and particle amount, respectively. All measurements were carried in triplicate and values are presented as the mean ± standard deviation (SD).

Figure 5 shows drug absorbance is achieved peak value (84.05% loading efficiency) in one hour; thereafter it is smoothly decreased in all L-ascorbic acid solutions. SD values are calculated ±4.0, ±2.5 and ±1.5 for drug loading environment at 5 wt%, 10 wt% and 15 wt% L--ascorbic acid solutions, respectively.

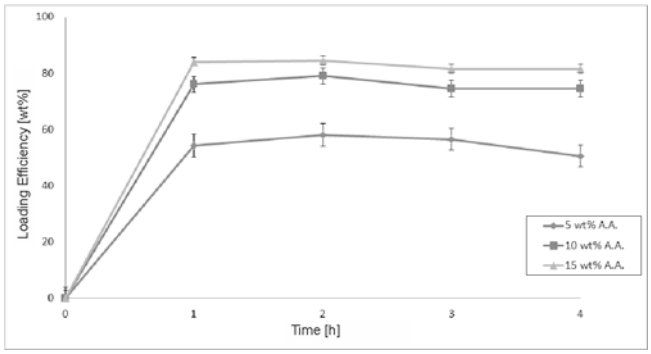

Figure 5. Effects of drug loading time on encapsulation of L-ascorbic acid

Although there is no significant difference, Figure 6 indicates that the encapsulation efficiency tended to increase from 0.5 to 2.0 mg particle/mL L-ascorbic acid solution. On the contrary, increasing weight percent of L-ascorbic acid in solution significantly increased loading rates. It changes from 49.93% to 84.05% by PCL-PEG--CS microsphere amounts from 0.5 to 2.0 mg particle/mL solution. It is also facts that low diameter and well distributed structure of microspheres are reasons to gain high L-ascorbic acid loading values[29]. Thus, when highly water-soluble drugs, such as L-ascorbic acid, are encapsulated using ternary polymer blends, % encapsulations achieved are expectably high. SD values are calculated ±5.0, ±2.9 and ±1.5 for drug loading environment at 5 wt%, 10 wt% and 15 wt% L-ascorbic acid solutions, respectively.

In-vitro release profiles of L-ascorbic acid from the microspheres produced by PCL-PEG-CS are shown in Figure 7. Experimental uncertainties in the % drug released, based on three replicates, are approximately changed from 3.4% to 5.7%.

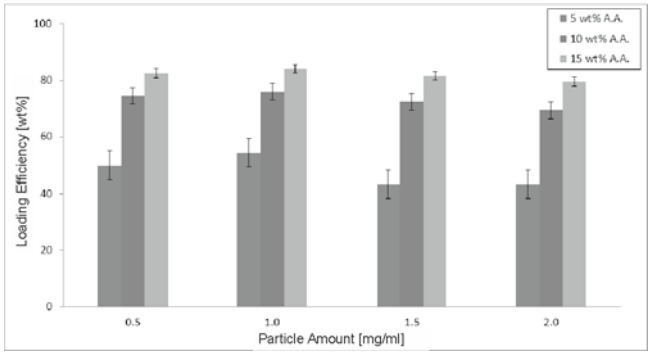

Figure 6. Effects of particle amount on encapsulation of L-ascorbic acid

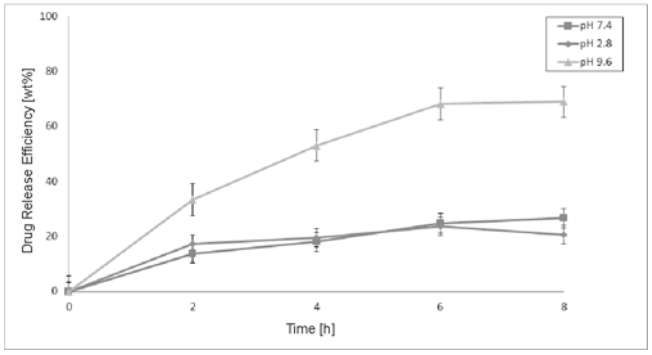

Figure 7. L-ascorbic acid release with different pH mediums in 8 hours

The % drug release initially in 2 h is 17.21 ±3.36 at pH 2.8, 13.84 ±3.50 at pH 7.4 and 33.39 ±5.7 at pH 9.6. Maximum drug release ratio achieved in 6 h is 68.21 ±5.7 at pH 9.6. The results indicate that L-ascorbic acid in the bioblend based microspheres are easily degraded higher pH values particularly at basic scale. Previous studies are showed that casein is soluble at basic pH values because of this reason L-ascorbic acid release are more effective at pH 9.6[30]. In additionally, since CS degraded L-ascorbic acid release is increased, it also indicates CS and L-ascorbic acid interaction is stronger than PCL and PEG. Due to the previous studies, our experiments showed that results are very encouraging particularly with high drug release ratio for a new L-ascorbic acid delivery drugs by PCL-PEG-CS based blends[6, 7, 17].

To describe drug release mechanism more precisely, there is a more comprehensive but still very simple semi-empirical formulations, called zero order kinetics, first order kinetics, Higuchi, Hixson-Cromwell, and the Korsmeyer-Peppas power law[31, 32, 33]. So the drug release data were fitted to these kinetic models to analyze the release kinetics and the mechanism from the polymeric drugs. Based on the best correlation coefficient values, the most appropriate model was selected to explain the release behavior of the drug. The values of the release exponent (n), kinetic rate constant (k) and the correlation coefficient (R^2) are tabulated in the Table 1. L-ascorbic acid release from microparticles exhibits high correlation with the Korsmeyer–Peppas semi empirical model, with $R^2 > 0.94$. The values of "n" determined by the Korsmeyer–Peppas semi empirical model, ranged from 0.259 to 0.553 as tabulated in the Table 1. The results indicate that the formulations at pH 2.8 and pH 7.4 exhibit Fick diffusion mechanism ($n<0.5$), so the drug release is governed by diffusion. The formulation at pH 9.6 exhibits anomalous transport (i.e non-Fickian diffusion mechanism), so the drug release is governed by both diffusion of the drug and dissolution of the polymeric network[32].

CONCLUSIONS

The current study investigates the fabrication of L-ascorbic acid drugs by bioblends for advanced spray-drying applications. In comparison the spray dryer method to others, we obtained much more uniform microspheres and lower particle diameters. Significantly, the experimental results indicate that to obtain particle diameter 27.540 ±0.656 μm with 0.512 m^2/g surface area does not need to any additive effects during drying process. Furthermore, the microspheres can be straightforwardly transformed into the L-ascorbic acid loaded drugs. The dissolution rates of the drugs from PCL-PEG-CS microspheres are clearly improved at higher pH as compared to neutral and acidic pH values. The shift of the mechanism from diffusion controlled to an anomalous transport changing the pH of the medium from acidic to basic conditions as seen drug release kinetic studies. It is also indicated that drug release ratio can be adjustable by this way, thus preventing the drug release until the target has been achieved. Towards the aim of the study, improving the vitamin C drugs, our method provides easily procurable and effective solution.

LITERATURE CITED

1. Chakkarapani, P., Subbiah, L., Palanisamy, S., Bibiana, A., Ahrentorp, F., Jonasson, C. & Johansson, C. (2015). Encapsulation of methotrexate loaded magnetic microcapsules for magnetic drug targeting and controlled drug release. *J. Magn. Magn. Mater*. 380, 285–294. DOI: 10.1016/j.jmmm.2014.11.006.

2. Zhou, G., Zhao, Y., Hu, J., Shen, L., Liu, W. & Yang, X. (2013). A new drug-loading technique with high efficiency and sustained-releasing ability via the Pickering emulsion interfacial assembly of temperature/pH-sensitive nanogels. *React. Funct. Polym*. 73, 1537–1543. DOI: 10.1016/j.reactfunctpolym.2013.02.011.

3. Ozsagiroglu, E., Iyisan, B. & Avcibasi Guvenilir, Y. (2013). Comparing the In-Vitro Biodegradation Kinetics of Commercial and Synthesized Polycaprolactone Films in Different Enzyme Solutions. *Ekoloji*. 86, 90–96. DOI: 10.5053/ekoloji.2013.8611.

4. Xu, X. Khan, M.A. & Burgess, D.J. (2012). A quality by design (QbD) case study on liposomes containing hydrophilic API: II. Screening of critical variables, and establishment of design space at laboratory scale. *Int. J. Pharm*. 423, 543–553. DOI: 10.1016/j.ijpharm.2011.11.036.

5. Patel, A.R. & Velikov, K.P. (2011). Colloidal delivery systems in foods: A general comparison with oral drug delivery. *LWT-Food Sci. Technol*. 44, 1958–1964. DOI: 10.1016/j.lwt.2011.04.005.

6. Huang, M., Xu, Q. & Deng, X.X. (2014). L-Ascorbic acid metabolism during fruit development in an ascorbate-rich fruit crop chestnut rose (Rosa roxburghii Tratt). *J. Plant Physiol*. 171, 1205–1216. DOI: 10.1016/j.jplph.2014.03.010.

7. Khalid, N., Kobayashi, I., Neves, MA., Uemura, K., Nakajima, M. & Nabetani, H. (2014). Monodisperse W/O/W emulsions encapsulating l-ascorbic acid: Insights on their formulation using microchannel emulsification and stability studies. *Colloid Surface A.* 458, 69–77. DOI: 10.1016/j.colsurfa.2014.04.019.

Table 1. Kinetic parameters of L-ascorbic acid release from the PCL-PEG-CS tablets

pH	Zero-order		First-order		Higuchi		Hixson-Crowell		Korsmeyer-Peppas		
	k_0 [min^{-1}]	R^2	k_1 [min^{-1}]	R^2	k_H [$min^{-1/2}$]	R^2	k_{HC} [min^{-1}]	R^2	n	R^2	k_{HP} [min^{-n}]
2.8	0.0204	0.9209	0.0003	0.9210	0.6767	0.9396	0.0004	0.9210	0.2588	0.9471	0.0493
7.4	0.0376	0.9588	0.0005	0.9607	1.2403	0.9682	0.0007	0.9601	0.4960	0.9733	0.0127
9.6	0.1014	0.8866	0.0022	0.9107	3.4266	0.9371	0.0026	0.9044	0.5526	0.9586	0.0246

8. Beck-Broichsitter, M., Paulus, I.E., Greiner, A. & Kissel T. (2015). Modified vibrating-mesh nozzles for advanced spray--drying applications. *Eur. J. Pharm. Biopharm*. 92, 96–101. DOI: 10.1016/j.ejpb.2015.03.001.

9. Rassu, G., Nieddu, M., Bosi, P., Trevisi, P., Colombo, M., Priori, D., Manconi, P., Giunchedi, P. & Gavini, E. Boatto, G. (2014). Encapsulation and modified-release of thymol from oral microparticles as adjuvant or substitute to current medications. *Phytomedicine*. 21, 1627–1632. DOI: 10.1016/j.phymed.2014.07.017.

10. Carneiro, H.C.F., Tonon, R.V., Grosso, C.R.F. & Hubinger, MD (2013). Encapsulation efficiency and oxidative stability of flaxseed oil microencapsulated by spray drying using different combinations of wall materials. *J. Food Eng*. 115, 443–451. DOI: 10.1016/j.jfoodeng.2012.03.033.

11. Bürki, K. Jeon, I., Arpagaus, C. & Betz, G. (2011). New insights into respirable protein powder preparation using a nano spray dryer. *Int. J. Pharm*. 408, 248–256. DOI: 10.1016/j.ijpharm.2011.02.012.

12. Erbay, Z. & Koca, N. (2014). Exergoeconomic performance assessment of a pilot-scale spray dryer using the specific exergy costing method. *Biosyst. Eng*. 122, 127–138. DOI: 10.1016/j.ijpharm.2011.02.012.

13. Paudel, A., Worku, ZA., Meeus, J., Guns, S. & Van den Mooter, G. (2013). Manufacturing of solid dispersions of poorly water soluble drugs by spray drying: Formulation and process considerations. *Int. J. Pharmaceut*. 453, 253–284. DOI: 10.1016/j.ijpharm.2011.02.012.

14. Van der Schueren, L., Steyaert, I., De Schoenmaker, B. & De Clerck, K. (2012). Polycaprolactone/chitosan blend nanofibres electrospun from an acetic acid/formic acid solvent system. *Carbohyd. Polym*. 88, 1221–1226. DOI: 10.1016/j.carbpol.2012.01.085.

15. Van der Schueren, L., De Meyer, T., Steyaert, I., Ceylan, Ö., Hemelsoet, K., Van Speybroeck, V. & De Clerck, K. (2013). Polycaprolactone and polycaprolactone/chitosan nanofibres functionalised with the pH-sensitive dye Nitrazine Yellow. *Carbohyd. Polym*. 91, 284–293. DOI: 10.1016/j.carbpol.2012.08.003.

16. Horton, D.C., Derveer, D.V., Krzystek, J., Telser, J., Pittman, T., Crans, D.C. & Holder, A.A. (2014). Spectroscopic Characterization of L-ascorbic Acid-induced Reduction of Vanadium(V) Dipicolinates: Formation of Vanadium(III) and Vanadium(IV) Complexes from Vanadium(V) Dipicolinate Derivatives. *Inorg. Chim. Acta*. 420, 112–119. DOI: 10.1016/j.ica.2013.12.001.

17. Porras-Saavedra, J., Palacios-González, E., Lartundo-Rojas, L., Garibay-Febles, V., Yáñez-Fernández, J., Hernández-Sánchez, H., Gutiérrez-López, G. & Alamilla-Beltrán, L. (2015). Microstructural properties and distribution of components in microparticles obtained by spray-drying. *J. Food Eng*. 152,105–112. DOI: 10.1016/j.jfoodeng.2014.11.014.

18. Grund, J., Koerber, M., Walther, M. & Bodmeier, R. (2014). The effect of polymer properties on direct compression and drug release from water-insoluble controlled release matrix tablets. *Int. J. Pharmaceut*. 469, 94–101. DOI: 10.1016/j.ijpharm.2014.04.033.

19. Kadajji, V.G. & Betageri, G.V. (2011). Water Soluble Polymers for Pharmaceutical Applications. *Polymers*. 3(4), 1972–2009. DOI: 10.3390/polym3041972.

20. Li, F., Li, X. & Li, B. (2011). Preparation of magnetic polylactic acid microspheres and investigation of its releasing property for loading curcumin. *J. Magn. Magn. Mater*. 323, 2770–2775. DOI: 10.1016/j.jmmm.2011.05.045.

21. Orellana, B.R. & Puleo, D.A. (2014). Cyanide induced self-assembly and copper recognition in human blood serum by a new carbazole AIEE active material. *Mater. Sci. Eng. C* 43, 418–423. DOI: 10.1016/j.msec.2014.07.041.

22. Alexa, I.F., Ignat, M., Popovici, R.F., Timpu, D. & Popovici, E. (2012). *In vitro* controlled release of antihypertensive drugs intercalated into unmodified SBA-15 and MgO modified SBA-15 matrices. *Int. J. Pharmaceut*. 436, 111–119. DOI: 10.1016/j.ijpharm.2012.06.036.

23. Moribe, K. Makishima, T., Higashi, K., Liu, N., Limwikrant, W., Ding, W., Masuda, M., Shimizu, T. & Yamamoto, K. (2014). Encapsulation of poorly water-soluble drugs into organic nanotubes for improving drug dissolution. *Int. J. Pharmaceut*. 469, 190–196. DOI: 10.1016/j.ijpharm.2014.04.005.

24. Jensen, D.M., Cun, D., Maltesen, M.J., Frokjaer, S., Nielsen, H.M. & Foged, C. (2010). Spray drying of siRNA-containing PLGA nanoparticles intended for inhalation. *J. Control Release* 142, 138–145. DOI: 10.1016/j.jconrel.2009.10.010.

25. Wu, Y., Zou, L., Mao, J., Huang, J. & Liu, S. (2014). Stability and encapsulation efficiency of sulforaphane microencapsulated by spray drying. *Carbohyd. Polym*. 102, 497–503. DOI: 10.1016/j.carbpol.2013.11.057.

26. Yuzbasi, N.S. & Selçuk, N. (2011). Air and oxy-fuel combustion characteristics of biomass/lignite blends in TGA-FTIR. *Fuel Process. Technol*. 92, 1101–1108. DOI: 10.1016/j.fuproc.2011.01.005.

27. Wang, H., Helwa, Y. & Rempel, G.L. (2013). Preparation of polyacrylamide based microgels with different charges for drug encapsulation. *Eur. Polym. J.* 49(6), 1479–1486. DOI: 10.1016/j.eurpolymj.2013.04.003.

28. Kalhapure, R.S., Mocktar, C., Sikwal, D.R., Sonawane, S.J., Kathiravan, M.K., Skelton, A. & Govender, T. (2014). Ion pairing with linoleic acid simultaneously enhances encapsulation efficiency and antibacterial activity of vancomycin in solid lipid nanoparticle. *Colloid. Surface B*. 117, 303–311. DOI: 10.1016/j.colsurfb.2014.02.045.

29. Post, A.E., Arnold, B., Weiss, J. & Hinrichs, J. (2012). Effect of temperature and pH on the solubility of caseins: Environmental influences on the dissociation of αS- and β-casein. *J. Dairy. Sci*. 95, 1603–1616. DOI: 10.3168/jds.2011-4641.

30. Zhou, Y., Song, F., Li, Y.S., Liu, J.Q., Lu, S.Y., Ren, H.L., Liu, Z.S., Zhang, Y.Y., Yang, L., Li, Z.H., Zhang, J.H. & Wang, X.R. (2013). Double-antibody based immunoassay for the detection of β-casein in bovine milk samples. *Food Chem*. 141(1), 167–173. DOI: 10.1016/j.foodchem.2013.03.010.

31. de Almeida, R.R., Magalhães, H.S., de Souza, J.R.R., Trevisan, M.T.S., Vieira, I.G.P., Feitosa, J.P.A., Araújo, T.G. & Ricardo, N.M.P.S. (2015). Exploring the potential of *Dimorphandra gardneriana* galactomannans as drug delivery systems. *Ind. Crop. Prod*. 69, 284–289. DOI: 10.1016/j.indcrop.2015.02.041.

32. England, C.G., Miller, M.C., Kuttan, A., Trent, J.O. & Frieboes, H.B. (2015). Release kinetics of paclitaxel and cisplatin from two and three layered gold nanoparticles. *Eur. J. Pharm. Biopharm*. 92, 120–129. DOI: 10.1016/j.ejpb.2015.02.017.

33. Ozsagiroglu, E., Hayta, E.N. & Avcibasi Guvenilir, Y. (2015). Preparation of bioblends using spray dryer and their applications in drug delivery systems. Asian J. Chem. 27(10), 3734–3738. DOI: 10.14233/ajchem.2015.18958.