Acta Poloniae Pharmaceutica – Drug Research, Vol. 75 No. 6 pp. 1369–1376, 2018
DOI: 10.32383/appdr/89799

ISSN 0001-6837
Polish Pharmaceutical Society

PHARMACEUTICAL TECHNOLOGY

PHYSICOCHEMICAL CHARACTERISTICS OF FUROSEMIDE INCORPORATED EUDRAGIT MICROSPHERES FORMULATED BY SPRAY DRYING

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Abstract: Microspheres are one of the multicompartment drug delivery systems. The active substance is incorporated into a polymeric matrix. The aim of the study was to determine physicochemical properties of furosemide microspheres prepared with Eudragit L30 D-55 matrix and to analyze their stability and drug release. A test formulation at the drug-polymer ratio of 1 : 2 was used in the study. Furosemide was incorporated into the enteric matrix because it reduces gastric acid secretion and impairs gastric secretory functions. The optimum spray drying parameters were determined on the basis of preliminary tests: aspirator efficiency: 80%, T_{in}: 140°C, pump capacity: 10%. The designated Hausner ratio and Carr index indicate good flow properties of the obtained product. The stability tests show that the microspheres are physically stable under the applied storage conditions. Based on the analysis of furosemide release from microspheres, it has been found that it is a two-step process. In the first step, 28.68% of the active substance was released into a pH 1.2 buffer solution. In the second step, the remaining part of the active substance was released into a pH 6.8 buffer within 30 min.

Keywords: furosemide, Eudragit L30 D-55, spray drying technique, microspheres

Microspheres are one of the multicompartment drug delivery systems, which are manufactured for sustained or controlled drug delivery. The active substance is incorporated into a polymeric matrix in this type of particles. Their size ranges from 1 to 500 μm. Microspheres have many advantages such as reduced drug fluctuation in the therapeutic range, lowered side effects and improved patient compliance (1). They are also promising particles for insoluble therapeutic drugs (2) and may be used for targeted delivery of the active substance to specific tissues, which is crucial in the therapy of, for example, inflammatory disorders (3). However, they have a short residence time at the site of absorption.

Microspheres can be prepared using various methods. However, one of the most common techniques is spray drying. Different types of polymers, both natural and synthetic, can also serve as matrices in the preparation of microspheres. Among synthetic polymers, the Eudragit series (acrylate and methacrylate polymers available in different ionic forms) are often used due to their inertness and solubility in relatively nontoxic solvents as well as lack of toxic effects (4). The nature of polymers influences the rate of diffusion of active substance molecules from a microsphere matrix. The final product obtained by spray drying should be smooth, monolithic and sphere-shaped (5).

We used furosemide as a model drug. Furosemide is an anthranilic acid derivative (4-chloro-2-[(2-furanylmethyl)-amino]-5-sulfamoylbenzoic acid) and serves as a potent loop diuretic mainly in the treatment of edematous states associated with cardiac, renal, and hepatic failure, and in the treatment of hypertension. It may exist in five forms, three polymorphs and two solvates, which was confirmed using different analytical methods (6).

The aim of the study was to determine physicochemical properties of furosemide microspheres

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prepared with Eudragit L30 D-55 matrix and to analyze their stability and drug release. A test formulation at the drug-polymer ratio of 1 : 2 was used in the study. Furosemide was incorporated into the enteric matrix because it reduces gastric acid secretion and impairs gastric secretory functions (7).

MATERIALS AND METHODS

Furosemide (Polfarmex S.A., Kutno, Poland), Eudragit® L30 D-55, 30% aqueous dispersion (Evonik Industries, Germany), triethyl citrate (98%, Sigma-Aldrich, USA). All the chemicals were of analytical grade.

Preparation of microspheres by spray drying

The study used a test formulation of furosemide with Eudragit L30 D-55, dispersed according to the manufacturer's guidelines at a 1 : 2 ratio. For this purpose, 250 mg of furosemide, 1666 mg of 30% Eudragit L 30 D-55 aqueous dispersion and 112.5 mg of triethyl citrate (plasticizer), i.e. 15% against the dry weight of the starting substances of furosemide and Eudragit L30 D-55 (749.8 mg), were used. The mixture was filled up to 100 mL with water, stirred with a magnetic stirrer for 6 h, and then spray dried using the Büchi Mini Spray Dryer B-191 (Büchi Labortechnik AG, Flawil, Switzerland). The type and amount of the plasticizer were selected on the basis of the results of Jankowski et al. (5). The authors have found that the addition of 10% to 15% of triethyl citrate improves the shape and morphology of microspheres as well as spray drying efficiency. Triethyl citrate also increases the elasticity of polymeric matrices (8). A standard nozzle with a diameter of 0.7 mm, a pressure of 3.5 bar, and an air flow rate of 600 L/h were used. The optimum spray drying parameters were determined on the basis of preliminary tests: aspirator efficiency of 80%, T_{in}: 140°C, pump capacity of 10%. The liquid formulation was fed to the atomizer by means of a peristaltic pump and sprayed into a hot air stream. After evaporation of the solvent, the dry product was separated in a cyclone and collected in a receiver. The resulting microspheres were stored in a sealed container at room temperature.

Physicochemical characteristics of microspheres

Analysis of microsphere production efficiency

Microsphere production efficiency (9) was calculated as % of the mass of obtained microparticles against the total amount of the applied furosemide, polymer and plasticizer – triethyl citrate (Formula 1):

$$\%\ yield = \frac{Practical\ mass\ (microspheres)}{Teoretical\ mass\ (polymer + drug + plasticizer)} \times 100$$

Drug loading

Microspheres (25 mg) were dissolved in 50 mL of a pH 7.4 phosphate buffer and stirred with a magnetic stirrer for 24 h. The solution was then filtered through a membrane filter with a 0.45 µm pore size. The furosemide content was determined using a spectrophotometric method at a wavelength λ = 277 nm. The percent drug loading (9) was determined according to Formula 2:

$$Percent\ loading = \frac{Weight\ of\ drugin\ microsphers}{Weight\ of\ microspheres} \times 100$$

Analysis of bulk density and tapped density

Bulk density [g/mL] was calculated as the quotient of the sample mass and its apparent volume (Formula 3). The test sample was weighed and placed in a measuring cylinder to read its volume.

$$Bulk\ density = \frac{Mass\ of\ microspheres}{Initial\ volume} \quad (3)$$

The jolting volumeter STAV II (Engelsman AG, Germany) was used to determine tapped density [g/mL]. It was calculated as the quotient of the sample mass and its apparent volume after compaction – 250 strokes/min (Formula 4).

$$Tapped\ density = \frac{Mass\ of\ microspheres}{Volume\ of\ microspheres\ after\ tapping} \quad (4)$$

The Hausner ratio (10) was determined according to the Formula (Formula 5):

$$Hausners\ ratio = \frac{Tapped\ density}{Bulk\ density} \quad (5)$$

Carr Index (10) (Formula 6):

$$Carr\ Index = \frac{Tapped\ density - bulk\ density}{Tapped\ density} \times 100$$

Analysis of absolute density

An automatic helium pycnometer (Accupyc 1340 Pycnometer, Micromeritics USA / Syl & Ant Instruments) was used to determine absolute density. It determines the helium volume displaced by the analyzed sample from the measuring chamber. The test samples were pre-desorbed. Helium purging was repeated 10 times.

Determination of product porosity

After calculating the bulk density, tapped density and absolute density, the porosity of the loose product (ε_B) and the porosity of the tapped product

(ε_T) were determined using the formulas 7 and 8, respectively (11-13):

$$\varepsilon_B = \frac{1 - Bulk\ density}{Absolute\ density} \times 100 \quad (7)$$

$$\varepsilon_T = \frac{1 - Tapped\ density}{Absolute\ density} \times 100 \quad (7)$$

Analysis of microsphere size and morphology using an optical microscope and SEM

The prepared microspheres were observed using microscopic methods. Microparticles were subjected to SEM analysis using the ultra-high Resolution Scanning Electron Microscope (Hitachi UHR FE-SEM SU 8010, Tokyo, Japan). The samples for SEM analysis were coated with gold using a vacuum sputter coater. The Meiji MT4300H microscope (Saitama, Japan) equipped with a Moticam camera was used. The microspheres were analyzed at x 100 magnification. Motic Images Plus 2.0 ML and Image J were used to determine the size of 100 randomly selected particles. A median filter was applied to improve the image quality. The analysis was performed according to USP < 776 > (14). The average particle size (D) was determined using the Edmondson's equation 9 (15):

$$D = \frac{\Sigma nd}{\Sigma n} \quad (9)$$

where: d – mean size range, n – number of observed microspheres

Microsphere stability analysis

Zeta potential analysis

The zeta potential of microsphere surfaces was determined using the NanoPlus HD analyzer (ParticulateSystems USA / Syl & Ant Instruments). The sample containing microparticles was diluted with deionized water and then sonicated in an ultrasonic scrubber for 60 sec. The zeta potential was determined by a method based on the combination of a heterodyne system and photon correlation, performing the Fourier transform (FFT) for the resulting correlation.

Accelerated aging test

Microsphere stability was tested by the accelerated aging test under conditions specified by ICH Q1A (R2) "Stability testing of new products": 40°C ± 2°C/75% RH ± 5% RH and 5°C ± 3°C. The test portions of microspheres (15 mg) were transferred to vials, capped and placed for one month in a thermostat and refrigerator. After 7, 14 and 30 days, the furosemide content in the samples was measured by a spectrophotometric method at wavelength λ = 277 nm, and the microsphere size and color were determined (16, 17).

Analysis of release of furosemide from microspheres

The furosemide content in the microspheres and the amount of released furosemide were determined by a spectrophotometric method at wavelength λ = 277 nm (The Cecil 302 UV-VIS spectrophotometer, Instrument Cheminst, Poland). The standard curve of absorbance as a function of the concentration of standard solutions described by the equation y = 0.066x was applied. The determination factor R^2 was 0.9999, and the linear regression error was 1.97×10^{-4}. The method precision was positively assessed based on the values of standard deviation, relative standard deviation, and coefficient of variation.

Table 1. Physical characteristics of microspheres.

Physical characteristics	
Bulk density [g/mL]*	0.2483 ± 0.01
Tapped density [g/mL]*	0.2759 ± 0.02
Hausner's coefficient*	1.11 ± 0.02
Carr's index [%]*	10.0 ± 0.02
Absolute density [g/mL] (mean ± SD, n = 10)	1.4536 ± 0.0016
Porosity of the loosely packed product (ε_B) [%]*	51.7 ± 0.02
Porosity of the tapped product (ε_T) [%]*	49.8 ± 0.02
Particle size [μm] (mean ± SD, n = 10)	24.1 ± 5.2
Percent loading [%]*	25.34 ± 1.0
Production yield [%]*	86.00 ± 1.5

*Data are expressed as the mean ± S.D, n = 3

The rate of the active substance release was measured in a rotary vane (DT 600, ERWEKA GmbH, Germany) at T = 37°C ± 0.5°C and 50 rpm in two steps, i.e. for the first 2 hours in 750 mL of 0.1 M HCl (pH = 1.2) and then for another hour in a pH = 6.8 ± 0.1 buffer solution. For this purpose, 250 mL of 0.2 M sodium phosphate solution was added and the pH value of 2 M NaOH was adjusted (18). The percentage of furosemide released per unit of time was determined and the release profile was plotted.

All measurements were repeated several times, depending on the analyzed parameter. A series of measurements were analyzed and the arithmetic mean of the values obtained and the mean error (standard deviation) were calculated. Microsoft Excel 2007 was used for these calculations. The kinetics of furosemide release was analyzed based on mathematical models. Experimental results were approximated with zero-order, first-order and Korsmeyer-Peppas models. KinetDS 3.0 Rev. 2010 was used for the analysis (19). The determination coefficient (R^2) was applied as the criterion of the correctness of models. The best model of active substance release kinetics is characterized by the highest R^2 (optimally ranging from 0.970 to 1) (20).

RESULTS

The product is white, odorless powder that was tested according to the recommendations of the European Pharmacopoeia and quality standards. The physical properties of the analyzed microspheres are shown in Table 1.

The bulk density of the loosely packed material is characterized by the degree of loose packing of the product and is dependent on the size and shape of particles. The tapped density after applying a standard amount of compactive effort determines the degree of packing after compaction. Smaller particles move into free spaces formed between larger particles, and, as a consequence, the volume occupied by the product is reduced. When these parameters are determined, it is possible to calculate the Hausner ratio (HR) and the Carr index (I_{Carr}). Their values indicate that the obtained product is characterized by low cohesiveness (HR < 1.2) and very good flowability (I_{Carr} < 15) (21). The porosity of the material is related to bulk density, tapped density, and true density. It includes the outer (a system of empty spaces between particles) and inner porosity (a system of pores and capillaries within a single particle). The results show significant porosity of microspheres.

The particle size significantly influences the behavior of the product during storage and further processing. The mean size of microspheres (24.1 ± 5.2 μm), measured by means of a microscopic method, determines cohesiveness and influences their dosing (11-13). In the microscopic image, most microparticles are spherical in shape, sometimes with pits on the surface (Figs. 1, 2).

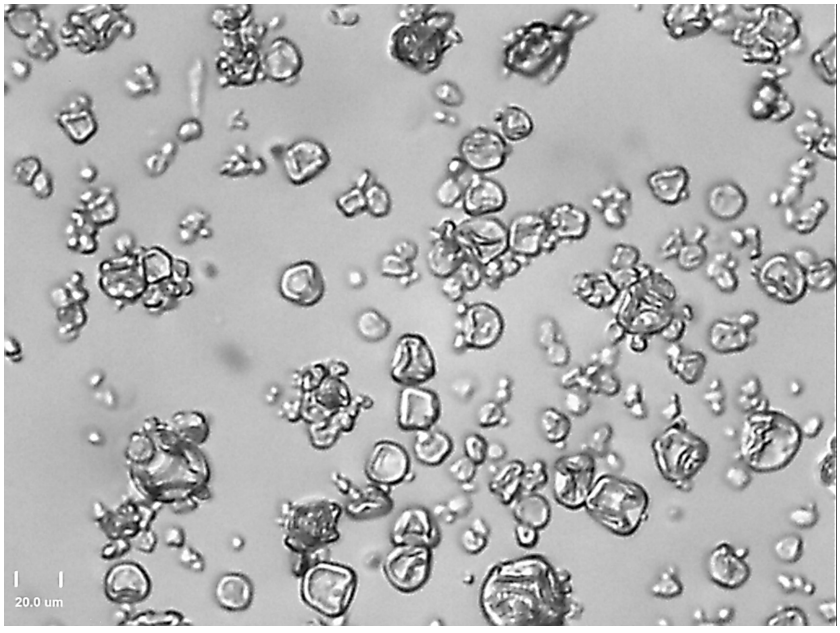

Figure 1. Optical microscopic image of spray-dried furosemide microspheres (100 x magnification)

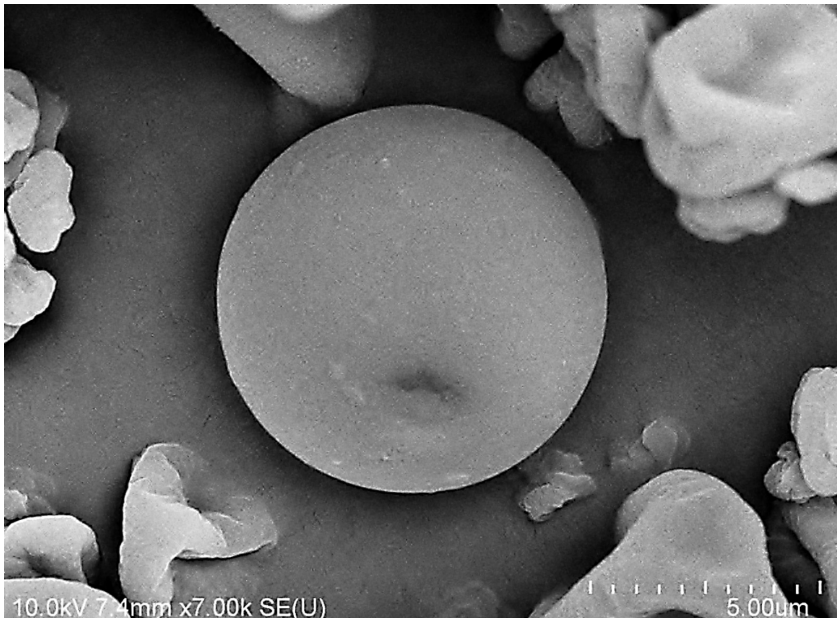

Figure 2. SEM microphotographs of spray-dried furosemide microspheres (10000 × magnification)

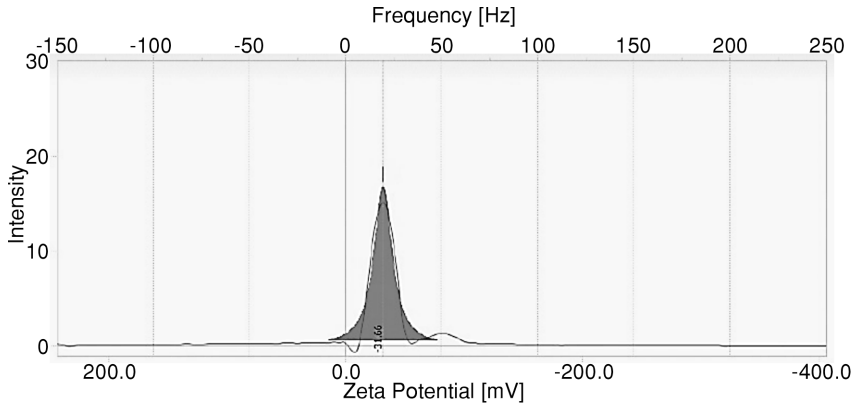

Figure 3. Zeta potential distribution curve of furosemide microspheres

The Zeta potential analysis and accelerated aging test were used to determine the stability of the obtained microspheres. The Zeta potential allows for the numerical determination of stability of the analyzed dispersion. The absolute value of Zeta > +30 mV or Zeta < -30 mV determines the stability of dispersion and can be used to assess its stability during storage. A sample of microspheres containing furosemide incorporated into the Eudragit L 30 D-55 matrix has a Zeta potential of -31.66 mV, and therefore their surface charge is sufficient to counter aggregation processes. The Zeta potential value also indicates the negatively charged surface of microspheres. This may suggest the presence of both furosemide and Eudragit L30 D-55 in the outer portion of microspheres. Eudragit L30 D-55 is a copolymer of methacrylic acid and ethyl acrylate at a 1 : 1 ratio (monomer pKa = 4.6) (22). Furosemide, on the other hand, is a weak acid (pKa = 4.25) and is ionized in aqueous environment to the negatively charged carboxylate (23). The Zeta potential distribution curve of the microsphere formulation is shown in Figure 3.

The smallest decrease in furosemide content in the obtained microspheres was observed in the sam-

ples stored at 5 ± 3°C. The furosemide content was found to be 99.98 ± 0.1% after 7 days, 99.97 ± 0.2% after 14 days and 99.87 ± 0.4% after 30 days of storage. At a temperature of 40 ± 2°C, the content of furosemide was the following: after 7 days – up to 99.86 ± 0.3%, after 14 days – 99.78 ± 0.3% and after 30 days – 99.50 ± 0.4%. The physical parameters of the microspheres (size, color) have not changed. The percentage of the active substance content indicates that the preparation is stable and the applied temperature and humidity parameters do not affect its physical properties.

Furosemide release from the prepared microspheres was examined using simulated gastric juice and simulated intestinal juice. Simulations of microspheres in the gastrointestinal tract were performed *in vitro*. When analyzing the profile of furosemide release from microspheres (Fig. 4), it was found that after one hour almost 1/3 of the active substance content was released into 0.1 M HCl. This amount did not change during the next hour of testing in this fluid. The amount of furosemide remaining in the microspheres was released into a pH 6.8 buffer within the next 30 min.

The parameters of matching mathematical models to the data of furosemide release from microspheres are shown in Table 2. In the first step, furosemide was released more slowly in relation to the second step.

Analysis of the kinetics of furosemide release from microspheres showed the best fit to the Korsmeyer-Peppas model. The exponents "n" defin-

Table 2. Parameters of matching mathematical models to the data of furosemide release from microspheres.

Zero - order kinetics	First - order kinetics	Korsmeyer - Peppas model		
R^2	R^2	R^2	k	n
pH = 1.2				
0.7086	0.7368	0.9983	0.44	0.963
pH = 6.8				
0.6298	0.7679	0.9949	1.846	1.021

R^2 - correlation coefficient; k - release rate constant; n - release exponent

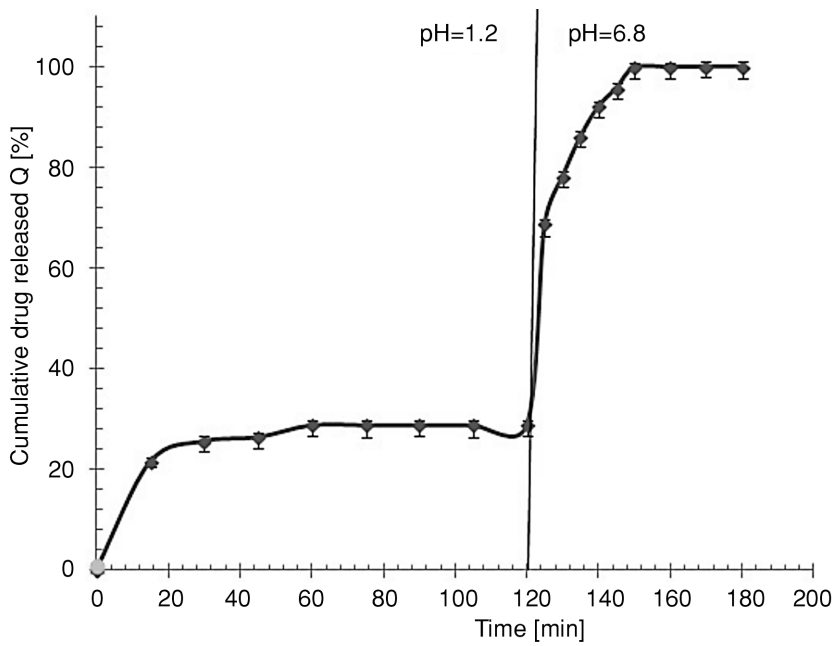

Figure 4. Cumulative percentage of furosemide release from microspheres (mean ± SD, n = 6)

ing the release mechanism for both model fluids are greater than 0.89, indicating the type referred to as "Super Case II transport". It combines polymer chain erosion and abnormal transport. In turn, abnormal transport refers to the combination of diffusion and erosion (24, 25).

DISCUSSION

Microspheres obtained by spray drying are characterized by spherical shape, which is typical of particles obtained by this method (5, 26). No cracked/damaged particles were observed in the microscopic images. The resulting bulk density and tapped density are within the acceptable range. The designated Hausner (HR < 1.2) and Carr (I_{Carr} < 15) parameters indicate good flow properties of the obtained product (27). The stability tests show that the microspheres are physically stable under the applied storage conditions. At lower temperatures, their stability is higher. A similar relationship was observed by Anupam et al., who tested the stability of duloxetine microspheres for 30 days at 4^{o}C and 25^{o}C. At lower temperatures, microsphere stability was higher (28).

Based on the analysis of furosemide release from microspheres, it has been found that it is released in two steps. In the first step, 28.68% of the active substance was released into a pH 1.2 buffer solution. In the second step, the remaining part of the furosemide was released into a pH 6.8 buffer within 30 min. The resulting release profiles suggest that in the first phase furosemide located close to or on the surface of microspheres was probably dissolved and diffused, whereas in the second phase the drug was released by diffusion and degradation/erosion of the polymer matrix. This is due to the presence of furosemide on the surface of microspheres (Zeta potential of -31.66 mV) and significant porosity of the polymer matrix (ε_B = 51.7%; ε_T = 49.8%), which facilitated penetration of the acceptor fluid and elution of furosemide. The resulting release profile is similar to the kinetics of the Korsmeyer-Peppas model, which suggests that the mechanism of drug release was controlled by diffusion and erosion. The release of the active substance from the Eudragit L30 D-55 matrix was also observed by other authors, despite the fact that the manufacturer determined its solubility at pH = 5.5. Bhadra et al. incorporated erythromycin stearate into the Eudragit L100 D-55 (powder), and found that 5-10% was released in pH = 1.2 (29). In the study of Balwierz et al., from ~ 29 to ~ 50% of potassium losartan was released into a pH 1.2 acceptor fluid depending on the amount of Eudragit L30 D-55 used. The authors suggested that the release of an increased amount of the active substance resulted from a possible interaction between the analyzed drug and polymer (26).

Some of the authors suggest also that obtaining furosemide-containing microspheres may increase its bioavailability (30, 31).

CONCLUSION

Spray drying provides microspheres with furosemide and Eudragit L30 D-55 at a 1:2 ratio, in which the breakdown of the active substance is varied throughout the whole mass. The study of drug release has confirmed its release from both the outer and inner microsphere structure. This creates the basis for the development of a furosemide formulation with a two-step release mechanism.

Conflict of interest

The authors confirm that this article content has no conflicts.

Acknowledgements

The authors would like to thank PhD Jagna Karcz from Laboratory of Scanning Electron Microscopy, Faculty of Biology and Environmental Protection, the University of Silesia in Katowice for SEM measurement.

REFERENCES

1. Haznedar S., Dortunç B.: Int. J. Pharm. 269, 1 (2004).
2. Wang Z., Chen B., Quan G., Li F., Wu Q. et al.: Int. J. Nanomedicine 7, 5807 (2012).
3. Lautenschläger C., Schmidt C., Fischer D., Stallmach A.: Adv. Drug Deliv. Rev. 5, 71 (2014).
4. Dewan I., Islam M.M., Al-Hasan M., Nath J., Sultana S. et al.: J. Pharm. (Cairo) 2015, 254930 (2015).
5. Jankowski A., Balwierz R., Marciniak D., Łukowiec D., Pluta J.: Acta Pol. Pharm. 71, 5 (2014).
6. Babu N.J., Cherukuvada S., Thakuria R., Nangia A.: Cryst. Growth Des. 10, 1979 (2010).
7. Ayalon A., Corcia A., Caplan S.R.: Isr. J. Med. Sci. 16, 678 (1980).
8. Sengel-Turk C.T., Hascicek C., Gonul N.: AAPS PharmSciTech. 12, 1127 (2011).

9. Majeed A., Ranjha N.M., Hanif M., Abbas G., Khan M.A.: 74, 565 (2017).
10. Mullaicharam A.R., Al-qartoobi M.S.S.: WJPPS 2, 3984 (2013).
11. Domian E.: ŻNTJ 4, 45 (2005).
12. Peleg M.: Food Proc. Eng. 1, 303 (1978).
13. Schubert H.: J. Food Eng. 6, 1 (1987).
14. The United States Pharmacopoeia (General Chapter <776>). Inc., Rockville 2000.
15. Swamy N.G., Abbas Z.: Indian J. Pharm. Sci. 73, 608 (2011).
16. Li D., Gong L.: Drug Des. Devel. Ther. 10, 2815 (2016).
17. ICH, Q1A(R2), Stability Testing of New Drug Substances and Products. International Conference on Harmonization, IFPMA, Geneva (2003).
18. Ching A.L., Liew C.V., Heng P.W., Chan LW.: Int. J. Pharm. 355, 259 (2008).
19. Mendyk A., Jachowicz R.: Expert Syst. Appl. 32, 1124 (2007).
20. Saifullah M., Yusof Y.A., Chin N.L., Aziz M.G., Mohammed M.A.P. et al.: J. Food Eng. 178, 60 (2016).
21. Urooj S., Ali A., Khan H., Shah S.H., Asad M.H.H. et al.: Acta Pol. Pharm. 75, 107 (2018).
22. Varum F.J., Merchant H.A., Goyanes A., Assi P., Zboranová V. et al.: Int. J. Pharm. 468, 1-2 (2014).
23. Nikolakakis I., Panagopoulou A., Salis A., Malamataris S.: AAPS PharmSciTech. 16, 1 (2015).
24. Costa P., Sousa Lobo J.M.: Eur. J. Pharm. Sci. 13, 123 (2001).
25. Siepmann J., Peppas N.A.: Adv. Drug Deliv. Rev. 48, 139 (2001).
26. Balwierz R., Jankowski A., Jasińska A., Marciniak D., Pluta J.: 73, 1275 (2016).
27. European Pharmacopoeia, 9th edn., Council of Europe EDQM, Strasbourg (2017).
28. Setia A., Kansal S., Goyal N.: Int. J. Pharm. Investig. 3, 141 (2013).
29. Bhadra S., Prajapati A.B., Bhadra D.: J. Pharm. Bioallied Sci. 8, 135 (2016).
30. Derakhshandeh K., Karimi M., Azandaryani A.H., Bahrami G., Ghanbari K.: Iran J. Basic Med. Sci. 19, 1049 (2016).
31. Kuchekar B.S., Boldhane S.P.: JPR 2, 110 (2009).

Received: 19. 11. 2017