Acta Pharm. **66** (2016) 269–277
DOI: 10.1515/acph-2016-0024

Original research paper

Release of selected amino acids from zinc carriers

RENATA DYJA[1*]
BARBARA DOLIŃSKA[1,2]
FLORIAN RYSZKA[2]

[1] *Department of Applied Pharmacy and Drug Technology Medical University of Silesia 41-200 Sosnowiec, Poland*

[2] *Pharmaceutical Research and Product Plant »Biochefa« 41-200 Sosnowiec, Poland*

Accepted February 16, 2016
Published online May 19, 2016

The paper deals with the results of an investigation of the release of selected amino acids (histidine, tryptophan, tyrosine) from model suspensions prepared by co-precipitation with zinc chloride. It has been proven that the influence of the Zn(II)/amino acid molar ratio on dissolution profiles of the tested amino acids and dissolution half-life ($t_{1/2}$) of histidine or tryptophan is significant. The amount of amino acid in the dispersed phase (supporting dose) is a determinant of the amino acid release profile. There is a minimal supporting dose (30.0 μmol of histidine or 17.4 μmol of tryptophan) that provides release of similar amounts of amino acid (4.1–4.6 μmol of histidine or 8.7–9.9 μmol of tryptophan) after the same time intervals. The tyrosine release profiles follow first order kinetics since the supporting dose (0.9–11.2 μmol) is limited by the tyrosine low solubility in water.

Keywords: amino acids, zinc, suspensions, dissolution, kinetics

Zinc in conjunction with protein, peptides or low molecular weight substances in the form of suspension provides prolonged drug action (1–6). Co-precipitation with zinc salts is one of the methods for obtaining sustained release drug carriers (4, 5). Co-precipitation involves intercalation of a low-molecular weight drug molecule into layered zinc hydroxide, layered zinc hydroxide salt, or layered double hydroxide (4–7). Sparingly soluble zinc compounds form matrices for sustained release of p-coumaric acid, levodopa (4, 5). These carriers are biocompatible and are an attractive alternative to other sustained release drug formulations (5).

Amino acids (AAs) are used as model substances in studies on suspensions (6–9). AA bound in the dispersed phase is a model of the supporting dose that slowly releases the drug. Zn(II)/drug molar ratio determines the dissolution profile of a suspension prepared by the co-precipitation method (3, 8).

On the basis of preliminary experiments, optimal variants of suspensions were obtained by co-precipitation of selected amino acids with zinc chloride. The most probable compound formed under the conditions reported in the paper is layered zinc hydroxide

* Correspondence; e-mail: rdyja@sum.edu.pl

salt. AAs-histidine (His), tryptophan (Trp) and tyrosine (Tyr) served as model substances. Influence of the Zn(II)/AA molar ratio on the release of AA from suspensions was investigated. Due to their aromatic structure, the tested AA could be easily determined by the spectrophotometric method.

EXPERIMENTAL

Materials

L-Histidine (His, $C_6H_9N_3O_2$, ≥ 99 % pure; M_r = 155.15), L-tryptophan (Trp $C_{11}H_{12}N_2O_2$, ≥ 98 % pure; M_r = 204.23, p*I* = 5.89) and L-tyrosine (Tyr $C_9H_{11}NO_3$, ≥ 98 % pure; M_r = 181.19, p*I* = 5.66) were purchased from Sigma Chemical Corp., USA. All other reagents were supplied by Avantor Performance Materials Poland S.A., Poland (formerly POCH, Poland). The reagents satisfied the requirements of the standards.

Preparation of AA-Zn(II) suspensions

AA (His, Trp or Tyr) was dissolved in 0.015 mol L^{-1} HCl solution. Amount of 0.75 mL of 0.1 mol L^{-1} $ZnCl_2$ in 0.003 mol L^{-1} HCl solution was mixed with 5 mL of 0.001, 0.005, 0.006, 0.01 or 0.02 mol L^{-1} AA solution yielding Zn(II)/AA molar ratios 15:1, 3:1, 2.5:1, 1.5:1 or 0.75:1, respectively. 0.75:1 Zn(II)/Tyr molar ratio was not obtained because of the limited solubility of Tyr in 0.015 mol L^{-1} HCl. Then, 1.05–1.35 mL of 0.2 mol L^{-1} NaOH solution was added. The volume of added 0.2 mol L^{-1} NaOH solution provided the optimal pH value for AA binding in the dispersed phase and was determined on the basis of preliminary experiments. Total volume was adjusted to 10.0 mL with distilled water. The suspensions were stirred using a magnetic stirrer at 500 rpm for 25 min. The suspensions were then left at room temperature for 1 h.

Optical microscopy of AA-Zn(II) suspensions

Optical microscope (MT 4000 Series Meiji Techno Co. LTP, Japan) was used to study the particle size of the suspension. A drop of undiluted formulation was viewed using total magnification of 100 or 400×.

Characterization of AA-Zn(II) suspensions

The suspensions (10 mL) were centrifuged for 10 minutes at 4000 rpm. The pH values, concentrations of AA and Zn(II) were then determined in the supernatant. The amounts of AA (supporting dose) and Zn(II) in the dispersed phase were calculated as the difference between the initial amount and those determined in the supernatant. The precipitate was intended for use in the *in vitro* release study of AA.

In vitro *release study of AA from suspensions*

The precipitate was re-suspended in 10 mL of 0.9 % NaCl solution and stirred with a magnetic stirrer at 500 rpm for 30 min. The suspension was subjected to centrifugation at 4000 rpm for 10 min and the amount of dissolved AA was determined in the supernatant.

The precipitate was re-suspended in 10 ml of fresh 0.9 % NaCl solution and the procedure was repeated. The study was carried out until dissolution of 95 % of the AA supporting dose or for 5 h.

Spectrophotometric determination of AA

Absorbance (x) was measured in 1 cm quartz cuvettes using a CE 3021 UV-Vis spectrometer (Cecil, UK). The photometric accuracy of the spectrophotometer was ± 0.005. AA concentration (y) was calculated on the basis of regression equations (His $y = 0.0387x + 0.0053$ $R^2 > 0.999$; Trp $y = 0.1758x + 0.0087$ $R^2 > 0.999$; Tyr $y = 0.0466x + 0.0256$ $R^2 > 0.999$). Absorbance was measured at wavelengths (λ_{max}) at which maximum absorbance was observed (λ_{max}(His) = 211.0 nm, λ_{max}(Trp) = 218.5 nm, λ_{max}(Tyr) = 222.5 nm).

Complexometric determination of Zn(II)

Zn(II) was determined by complexometric titration using 0.05 mol L^{-1} EDTA in the presence of Eriochrome Black T in pH 10 ammonia buffer until the initially violet solution turned blue (Polish Pharmacopeia VI).

Determination of pH values

pH values were measured using a Microcomputer pH-meter CP-315 (POCH, Poland). The electrode was calibrated using 0.05 mol L^{-1} sodium phosphate buffer solution purchased in Chempur, Poland.

Analysis of AA release data

The release kinetics of AA from the suspensions were elucidated by fitting the release data to kinetic models (first order or zero order) (Table I) and by comparison of the squared correlation coefficients R^2 values. AA dissolution half-lives ($t_{1/2}$, h) were calculated according to the kinetics with the higher value of R^2 (Table I).

Table I. First order kinetics, zero order kinetics and Korsmeyer-Peppas equations (10)

	First order kinetics	Zero order kinetics	Korsmeyer-Peppas model
Linear equation	$\ln (100–Q) = – k_1 t + \ln 100$	$Q = k_o t$	$\ln (M_t/M_\infty) = \ln k + n \ln t$
$t_{1/2}$	$t_{1/2} = 0.693/k_1$	$t_{1/2} = 50/k_o$	

Q – percent of AA released after time I (h); k_1 – first order release constant (1/h); k_o – zero order release constant (%/h), $t_{1/2}$ – dissolution half-life (h), M_t/M_∞ – fraction of AA released at time t (h); n – Korsmeyer-Peppas diffusion exponent.

Data analysis

The results were presented as the mean value (x ± SD) of 5 experiments and were analyzed using Excel (Microsoft Office) and Statistica 10 (Statsoft) computer programs. The influence of Zn(II)/AA on selected parameters was analyzed using ANOVA and Tukey's

post-hoc test. The influence of the Zn(II)/AA molar ratio on dissolution profiles of AA was analyzed using ANOVA. The level of statistical significance was $p < 0.05$.

Determination and evaluation of theoretical AA release profiles

Theoretical AA release profiles were determined on the basis of the plot of the amount of AA released after the first 0.5 h against the amount of AA in the dispersed phase (AA supporting dose). Theoretical amount of AA released after the 0.5 h interval (y) corresponded to the remaining amount of AA (new supporting dose) (x). Similarity of theoretical and empirical AA release profiles was tested by means of relative error δ_x, which was calculated by the equation:

$$\delta_x = \frac{100 \times \sum_{j=1}^{n} \left| x_j - y_j \right|}{\sum_{j=1}^{n} x_j}, \text{ where}$$

j is a number of time points, n is a total number of time points, x_j is amount of AA released during 0.5 h at time point j (%) (curve 1) and y_j is amount of AA released during 0.5 h at time point j (%) (curve 2).

Dissolution profiles were also compared using a model independent method. Fit factors- f_1' (difference factor) and f_2 (similarity factor) were calculated by the equations:

$$f_1' = \frac{100 \times \sum_{j=1}^{n} \left| R_j - T_j \right|}{\sum_{j=1}^{n} \left(R_j + T_j \right) / 2},$$

$$f_2 = 50 \times \log \left\{ \left[1 + \left(1 / n \right) \sum_{j=1}^{n} \left| R_j - T_j \right|^2 \right]^{-0,5} \times 100 \right\}$$

where j is a number of time points, n is a total number of time points, R_j and T_j are cumulative percent of AA dissolved at each time point (curve 1 and curve 2, respectively).

According to the FDA guide for industry, f_1' values up to 15 (0–15) and f_2 values greater than 50 (50–100) ensure sameness or equivalence of the two curves (11).

RESULTS AND DISCUSSION

Properties of the obtained suspensions (pH value, AA and Zn(II) concentration in the dispersed phase and particle size) are summarized in Table II.

Parenteral suspensions with protein-peptide hormones should be in the pH range 7.10–7.50. For research purposes and in order to obtain suspensions with the optimal amount of AA bound in the dispersed phase, the extended pH range was applied. Percent of AA bound in the dispersed phase was 57.3–84.4 % (His), 48.4–84.4 % (Trp) and 17.0–36.1 % (Tyr). The pH values that provided optimal variants of suspensions were 8.24–9.90 (His),

Table II. Properties of the AA-Zn(II) suspensions

AA	Zn(II)/AA molar ratio	*c*(AA) (mmol L^{-1})[a]	*c*(Zn(II)) (mmol L^{-1})[a]	pH	AA_b (mmol L^{-1})[b]	$Zn(II)_b$ (mmol L^{-1})[b]	Particle size (μm)
His	15:1	0.5	7.5	8.24 ± 0.02	0.3 ± 0.0	7.1 ± 0.2	1–5
	3:1	2.5	7.5	9.82 ± 0.02	1.7 ± 0.0	6.2 ± 0.0	1–5
	1.5:1	5.0	7.5	9.33 ± 0.03	4.2 ± 0.0	7.1 ± 0.0	< 1
	0.75:1	10.0	7.5	9.90 ± 0.00	5.7 ± 0.1	5.1 ± 0.2	< 1
Trp	15:1	0.5	7.5	7.01 ± 0.00	0.2 ± 0.0	6.0 ± 0.0	1–5
	3:1	2.5	7.5	7.34 ± 0.03	1.2 ± 0.0	6.2 ± 0.0	1–5 (aggregates 1–5)
	2.5:1	3.0	7.5	7.43 ± 0.03	1.7 ± 0.0	6.5 ± 0.0	1–5 (aggregates 1–10)
	1.5:1	5.0	7.5	7.48 ± 0.02	4.2 ± 0.0	6.7 ± 0.0	1–10 (aggregates 1–10)
	0.75:1	10.0	7.5	7.79 ± 0.02	8.3 ± 0.1	7.0 ± 0.0	1–25 (aggregates 5–25)
Tyr	15:1	0.5	7.5	7.91 ± 0.02	0.1 ± 0.0	7.2 ± 0.0	1–5
	3:1	2.5	7.5	7.90 ± 0.03	0.9 ± 0.1	6.7 ± 0.2	1–5
	1.5:1	5.0	7.5	7.98 ± 0.05	1.2 ± 0.0	6.2 ± 0.0	15

[a] initial concentration
[b] concentration of amino acid or Zn(II) bound in the dispersed phase

7.01–7.79 (Trp) and 7.90–7.98 (Tyr) (Table II). The amount of Zn(II) bound (%) as the matrix of the dispersed phase was higher than 79 % (except for Zn(II)/His 0.75:1 suspension) (Table II).

The particle size of the dispersed phase of Zn(II)/His 15:1, 3:1, Zn(II)/Tyr 15:1, 3:1 and 1.5:1 and Zn(II)/Trp 15:1 suspensions was 1–5 μm and was smaller than 1 μm in the case of Zn(II)/His 1.5:1 and Zn(II)/His 0.75:1 suspensions. Zn(II)/Trp 3:1, 2.5:1, 1.5:1, 0.75:1 suspensions included, besides amorphous particles, ordered spherical aggregates with particle sizes 1–25 μm (Table II).

Fig. 1 depicts the influence of the amount of AA in the dispersed phase (supporting dose) on the amount of AA released from suspensions after 0.5 h. Theoretical AA release profiles were calculated on the basis of Fig. 1.

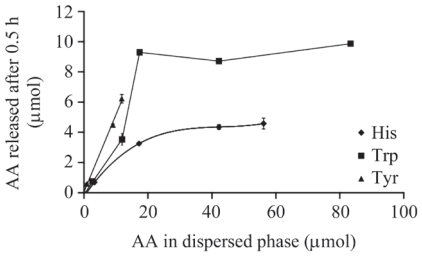

Fig. 1. The amount of AA released from suspensions after 0.5 h against the amount of AA in the dispersed phase (supporting dose). Theoretical AA release profiles were calculated on the basis of the graph.

Suspensions with 30–57 μmol His and 17–83 μmol Trp in the dispersed phase released similar amounts of AA after 0.5 h (4.1–4.6 μmol His and 8.7–9.9 μmol Trp). Suspensions with smaller amounts of AA in the dispersed phase released smaller amounts of AA after 0.5 h. However, the amount of Tyr released after 0.5 h was directly proportional to the amount of Tyr in the dispersed phase (Fig. 1).

Fig. 2 shows AA release profiles and Fig. 3 shows cumulative AA release profiles. Theoretical AA release profiles (calculated from Fig. 1) are also marked (Figs. 2 and 3). Relative error values $\delta_x < 20$ % and fit factor values ($f_1' < 15$ and $f_2 > 50$) confirmed the similarity of theoretical and empirical AA release profiles. This indicates that the amount of AA in the dispersed phase (maintenance dose) is the factor determining the AA release profile.

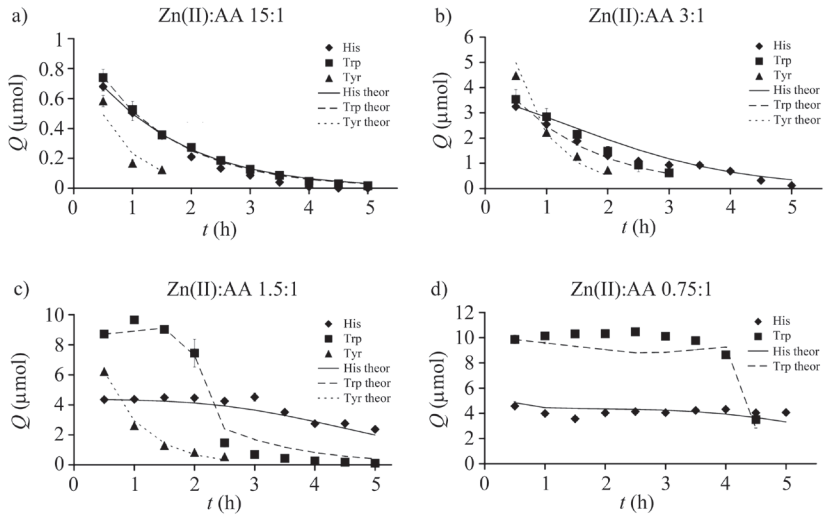

Fig. 2. The amount of AA (Q (μmol)) released over 0.5 h at specific time points. Suspensions with different Zn(II)/AA molar ratios: a) Zn(II)/AA 15:1, b) Zn(II)/AA 3:1, c) Zn(II)/AA 1.5:1 and d) Zn(II)/AA 0.75:1. Lines mark theoretical AA release profiles.

ANOVA showed a significant effect of the Zn(II)/AA molar ratio on the cumulative AA release (%) ($p < 0.05$).

Kinetic analysis of the AA release data (first order and zero order R^2 squared coefficients, $t_{1/2}$ (h) and Korsmeyer-Peppas diffusion exponent n values) is given in Table III. The data calculated from the theoretical dissolution profiles are also summarized in Table III (in parentheses).

Release kinetics (zero order or first order) was determined on the basis of the R^2 correlation coefficients and Korsmeyer-Peppas diffusion exponent n values (Table III). Korsmeyer-Peppas diffusion exponent n value equal to 1.0 is indicative of zero order kinetics (10). Release of AA from the Zn(II)/His 0.75:1 ($R^2 = 0.9995$, $n = 0.96$), Zn(II)/His 1.5:1 (R^2 =

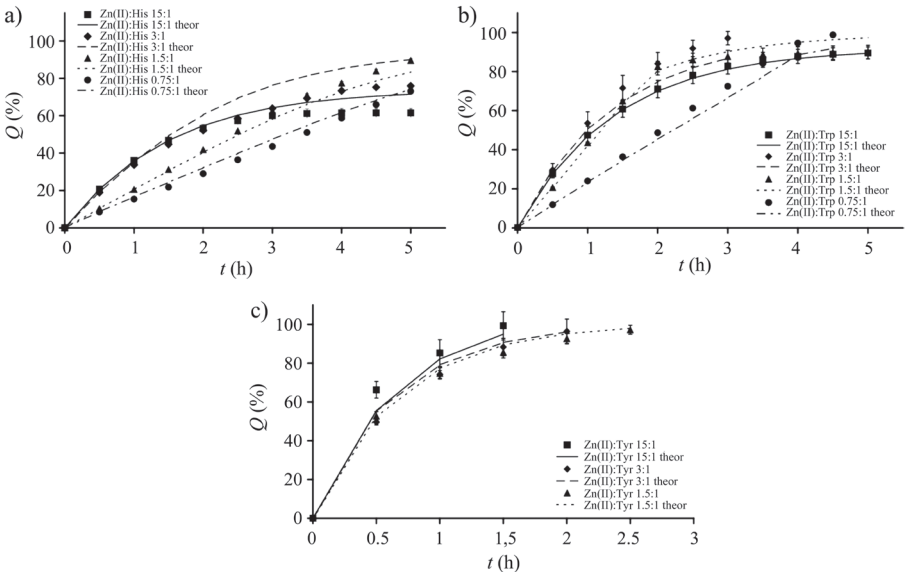

Fig. 3. Cumulative AA release profiles *Q* (%) *vs. t*(h); suspensions with different Zn(II)/AA molar ratios for: a) His, b) Trp and c) Tyr. Lines mark theoretical AA release profiles.

Table III. Kinetic analysis of the in vitro release data of His, Trp and Tyr from suspensions; values of R^2 *correlation coefficients,* $t_{1/2}$ *(h) and Korsmeyer-Peppas diffusion exponent n calculated from the theoretical dissolution profiles (in parentheses)*

AA	Zn(II)/AA molar ratio	R^2		$t_{1/2}$ (h)	Korsmeyer-Peppas diffusion exponent
		first order kinetics	zero order kinetics		*n*
His	15:1	0.324 (0.795)	0.000 (0.216)	2.77 ± 0.17 (2.31)	0.45 (0.52)
	3:1	0.950 (0.998)	0.575 (0.699)	2.15 ± 0.02 (1.48)	0.59 (0.68)
	1.5:1	0.926 (0.956)	0.982 (0.987)	2.59 ± 0.02 (2.86)	0.96 (0.97)
	0.75:1	0.937 (0.950)	> 0.999 (> 0.999)	3.43 ± 0.04 (3.65)	0.96 (1.01)
Trp	15:1	0.924 (0.934)	0.094 (0.050)	1.33 ± 0.18 (1.37)	0.51 (0.49)
	3:1	0.944 (0.999)	0.703 (0.613)	0.71 ± 0.12 (1.01)	0.65 (0.57)
	1.5:1	0.758 (0.987)	0.092 (0.387)	1.21 ± 0.16 (0.93)	0.61 (0.65)
	0.75:1	0.735 (0.873)	0.993 (0.992)	2.14 ± 0.02 (2.30)	0.99 (0.95)
Tyr	15:1	0.986 (0.972)	0.000 (0.190)	0.35 ± 0.07 (0.37)	0.37 (0.29)
	3:1	0.971 (0.998)	0.161 (0.000)	0.48 ± 0.09 (0.43)	0.48 (0.41)
	1.5:1	0.993 (0.999)	0.000 (0.000)	0.52 ± 0.06 (0.45)	0.41 (0.40)

0.982, n = 0.96) and Zn(II)/Trp 0.75:1 (R^2 = 0.993, n = 0.99) suspensions followed zero order kinetics, since the suspensions with higher AA supporting dose (> 30.0 μmol of His or > 17.4 μmol of Trp in the dispersed phase) released similar amounts of AA after 0.5 h (4.1–4.6 μmol of His or 8.7–9.9 μmol of Trp) (Fig. 1). Release of AA from the Zn(II)/His 15:1 or Zn(II)/Trp 1.5:1 suspensions followed neither zero order nor first order kinetics (Table III). The release of His from Zn(II)/His 15:1 was incomplete (61.5 %) (Fig. 3a). Release of Trp from the Zn(II)/Trp 1.5:1 suspension followed initially zero order kinetics (64.85 % of Trp supporting dose) as the amount of Trp in the dispersed phase was greater than 17.4 μmol during the first 2 h. Trp release was then reduced as the amount of Trp in the dispersed phase dropped below 17.4 μmol (Fig. 2c). In other cases, the release of AA from His-Zn(II), Trp-Zn(II) or Tyr-Zn(II) suspensions followed first order kinetics (R^2 > 0.900 (Table III). No release of Tyr following zero order kinetics was obtained because of the small amount of Tyr in the dispersed phase (0.9–11.2 μmol). Obtaining Tyr-Zn(II) suspensions with higher Tyr supporting dose was limited due to the low solubility of Tyr in water.

Release kinetics (zero order or first order) could be also determined on the basis of the theoretical AA release profiles; calculated R^2 values are summarized in Table III.

ANOVA showed a significant effect of the Zn(II)/AA molar ratio on the Korsmeyer-Peppas diffusion exponent n value in the case of all three AA (p < 0.05). However, Zn(II)/His 1.5:1 and Zn(II)/His 0.75:1 suspensions (p = 0.93) or Zn(II)/Trp 3:1 and Zn(II)/Trp 1.5:1 suspensions (p = 0.11) had the same n value. The n values were close to 0.5 in case of all tested Tyr-Zn(II) suspensions (n = 0.37–0.48), Zn(II)/His 15:1 suspension (n = 0.45) and Zn(II)/Trp 15:1 suspension (n = 0.51) indicating a Fickian diffusion mechanism (10). The n values less than 0.5 can be explained by the release of AA removably adsorbed on the zinc hydroxide surface.

The $t_{1/2}$ values calculated according to the kinetics with higher R^2 value are summarized in Table III. ANOVA showed a significant effect (p < 0.05) of the Zn(II)/AA molar ratio on the $t_{1/2}$ value (His-Zn(II) or Trp-Zn(II) suspensions). However, Zn(II)/AA 1.5:1 and 15:1 Zn(II)/AA suspensions had the same $t_{1/2}$ value (His, p = 0.31; Trp, p = 0.72). Zn(II)/Tyr effect on the $t_{1/2}$ value was not significant (p = 0.08).

CONCLUSIONS

The amount of amino acid in the dispersed phase (supporting dose) is the determining factor of the release profile. There is a minimal supporting dose that provides the release of similar amounts of amino acid after the same time intervals (zero order kinetics).

Amino acid solubility in water is a limiting factor for obtaining suspensions with a supporting dose high enough to provide a release profile following zero order kinetics and sustained release.

REFERENCES

1. U. Gietz, T. Arvinte, M. Häner, U. Aebi and H. P. Merkle, Formulation of sustained release aqueous Zn-hirudin suspensions, *Eur. J. Pharm. Sci.* **11** (2000) 33–41; DOI: 10.1016/S0928-0987(00)00072-5.
2. K. Shi, F. Cui, H. Bi, Y. Jang, H. Shi and T. Song, Metal ions guided self-assembly of therapeutic proteins for controllable release: from random to ordered aggregation, *Pharm. Res.* **30** (2013) 269–279; DOI: 10.1007/s11095-012-0871-9.
3. B. Dolińska and F. Ryszka, Pulsatile and moderated release of dalareline from Zn(II) complexes in the form of suspension, *Boll. Chim. Farmac.* **142** (2003) 10–13.
4. T. Biswick, D. H. Park, Y. G. Shul, J. H. Choy, *p*-Coumaric acid-zinc basic salt nanohybrid for controlled release and sustained antioxidant activity, *J. Phys. Chem. Solid.* **71** (2010) 647–649; DOI: 10.1016/j.jpcs.2009.12.058.
5. A. U. Kura, S. H. Hussein Al-Ali, M. Z. Hussein, S. Fakurazi and P. Arulselvan, Development of a controlled release anti-parkinsonian nanodelivery system using levodopa as the active agent, *Int. J. Nanomed.* **8** (2013) 1103–1110; DOI: 10.2147/IJN.S39740.
6. C. G. Carbajal Arízaga, Intercalation studies of zinc hydroxide chloride: ammonia and amino acids, *J. Solid State Chem.* **185** (2012) 150–155; DOI: 10.1016/j.jssc.2011.11.016.
7. Á. Fudala, I. Pálinkó and I. Kiricsi, Preparation and characterization of hybrid organic-inorganic composite materials using the amphoteric property of amino acids: amino acid intercalated layered double hydroxide and montmorillonite, *Inorg. Chem.* **38** (1999) 4653–4658; DOI: 10.1021/ic981176t.
8. B. Dolińska and F. Ryszka, The influence of physicochemical properties of amino acids on their release from the Zn(II)-amino acid complexes in suspension, *Boll. Chim. Farm.* **141** (2002) 218–222.
9. B. Dolińska, The properties of solid Zn(II)-amino acid complexes in the form of suspensions, *Il Farmaco* **56** (2001) 737–740.
10. S. Jose, J. F. Fangueiro, J. Smitha, T. A. Cinu, A. J. Chacko, K. Premaletha and E. B. Souto, Predictive modeling of insulin release profile from cross-linked chitosan microspheres, *Eur. J. Med. Chem.* **60** (2013) 249–253; DOI: 10.1016/j.ejmech.2012.12.011.
11. P. Costa and J. M. Sousa Lobo, Modeling and comparison of dissolution profiles, *Eur. J. Pharm. Sci.* **13** (2001) 123–133; DOI: 10.1016/S0928-0987(01)00095-1.