Acta Poloniae Pharmaceutica – Drug Research, Vol. 69 No. 4 pp. 739–749, 2012 ISSN 0001-6837
Polish Pharmaceutical Society

DEVELOPMENT AND OPTIMIZATION OF FORMULATION FOR TREATMENT OF COPPER DEFICIENCY IN HUMAN ORGANISM

IVANA M. SAVIC[1]*, GORAN S. NIKOLIC[1], IVAN M. SAVIC[1], KATARINA NIKOLIC[2] and DANICA AGBABA[2]

[1]Faculty of Technology, University of Nis, Bulevar oslobodjenja 124, 16000 Leskovac, Serbia
[2]Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, Belgrade, Serbia

Abstract: The aim of this study was to design and optimize a new tablet formulation for treatment of copper deficiency in human organism by using an experimental design. The new no-veneered tablets, prepared by a wet granulation technique, are containg active substance, a copper(II) complex with polysaccharide pullulan. The binder concentration, the disintegrant concentration and the resistance to crushing were used as independent variables in the formulation, while *in vitro* measured drug release characteristics of the tablets was response variable in a full factorial design 2^3 modeling. A cubic model for data fitted was used to examine the obtained results. They showed that the resistance to crushing has the most significant effect on copper(II) complex release from the formulation, while the disintegrant concentration has smaller influence on dissolution profile of copper(II) complex and the binder concentration had minor impact in this study. Lower value of resistance to crushing has influence on better dissolution profile. Furthermore, physical characteristics of the tablets were evaluated, *viz*., drug content, hardness, thickness, friability, disintegration time, mass variation, particle size and size distribution.

Keywords: copper, experimental design, dissolution, profile

Copper is one of essential elements in human organism (1). It is the primary element in melanin production (2). Melanin is responsible for pigmentation in the eyes, hair and skin. It is a powerful antioxidant, which removes free radicals and helps to prevent cell structure (3). Also, it has anticarcinogenic properties and helps to alleviate some arthritis pain (4). Copper has an important role in the metabolism and transition of iron in the body (1). Microcytic hypochromic anemia is one of the outcomes of copper deficiency (5). There are lots of preparations that are used for its treatment.

During years of research to develop products for the treatment of copper deficiency in the human body, the attention of researchers is mainly focused on simple organic or inorganic compounds and their salts. Based on the large number of literature data about the biological properties of these compounds (6, 7), currently dominated by products based on copper salts, primarily $CuSO_4$, $CuCl_2$ and $CuCO_3$, they are most suitable in the treatment of hipocupremical anemia. However, chlorides and carbonates are poorly soluble, furthermore, chlorides are hygroscopic, and carbonates are rapidly oxidized. Sulfates are salts resistant, easy purified and sulfate ions can be easily excreted from the body. However, in commercial OTC or parapharmaceutical products, these salts are present in combination with salts of other oligo- and micronutrients. It is believed that they have poor stability, partial toxicity and the inability to complete absorption of copper in the body (8).

In addition to inorganic forms of minerals, today are increasingly used so-called "chelated" forms, or organic bound microelements. Minerals associated with the amino acid or peptide are better protected during passage through the stomach and absorbed form of copper chelate is much greater than the absorption of sulfates. Synthesis of new organic-inorganic complexes based on copper ions with polysaccharides (dextran, pullulan) (9), was opened a wide field study of new formulations. Preliminary pharmacological studies of these bioactive complexes in animals showed lower toxicity compared with commercially used inorganic salts of copper (10). Positive results opened the way for the

* Corresponding author: e-mail: vana.savic@yahoo.com; phone: +381 16 242859; fax: +381 16 242859

development of new formulations for the treatment of copper deficiency in the human organism.

Therefore, the aim of this study was to develop and optimize new formulation with bioactive complex of copper(II) with pullulan (Cu(II)-RLMP), for treatment of copper deficiency in human organism. Development and optimization of the drug formulation was performed by use of the full factorial experimental design methodology. This method enables the desired formulation to be achieved as fast as possible (11–13). The binder concentration, the disintegrant concentration and the resistance to crushing were used as independent variables in the formulation, while *in vitro* measured drug release characteristics of the tablets was response variable in the study.

EXPERIMENTAL

Materials

The synthesis of Cu(II)-RLMP was described in detail by Nikolic et al. (19). The synthesis of the complex was performed at pH from 7 to 8. The reactant solution contained a reduced low-molar pullulan (RLMP, m. w. 6000 g/mol) and $CuCl_2$. This mixture was kept at a boiling point of the solution (9, 14–17). The complex is green, amorphous, almost odorless and freely soluble in water at 25°C.

The bioactive complex of copper(II) with pullulan (Cu(II)-RLMP) contained 13.04% of copper (18). This complex is very stable during prolonged storage (19) and non toxic. The structure of bioactive complex Cu(II)-RLMP was confirmed using physicochemical and spectroscopic methods (20).

Polyvinyl pyrrolidone K-30 (PVP K-30) was purchased from BASF (Germany) and corn starch from ISP Chemical (USA). All other chemicals used were of analytical grade.

Experimental design

The full factorial design was used to evaluate the effect of changes in mixture compositions on dependent variables. Also, the statistical optimization of the formulation, with the least number of experiments, was achieved by this design (21). The full factorial design is straight optimization on the basis of a chosen optimality criterion and model.

The number of experiments is the function of the number of dependent and independent variables. The response is measured for each trial. The model is fitted by carrying out multiple regression analysis and F-statistic parameters to identify statistically significant terms (22).

The reduced equation contains only statistically significant terms. After that, the response surface plots are drown in order to see the change of variables. The optimum point of formulation content is identified from the plot. Design-Expert software (version 7.1.6, Stat-Ease Inc., USA) was used for experimental design and statistical evaluation of the data.

The level of a single component cannot be changed independently in the design (23). The sum of the mixture components has to be equal to 100%. In the present case, tablets were prepared with a constant drug content (0.23%), lactose (75.11 %) and silica (0.66%). Therefore, the experimental range is between 0 and 24% (m/m).

Table 1. Experimental ranges for independent variables.

Factor	Experimental range	
	Low value	High value
X_1 (PVP K 30)	2.5%	2.7%
X_2 (corn starch)	20.0%	20.5%
X_3 (resistance to crushing)	50 *N*	70 *N*

Table 2. Ingredients of tablets.

Ingredient (mg / tablet)	F1	F2	F3	F4	F5	F6	F7	F8
Cu(II)-RLMP	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
PVP K-30	16.25	17.55	16.25	17.55	16.25	17.55	16.25	17.55
Corn starch	130.00	130.00	133.25	133.25	130.00	130.00	133.25	133.25
Lactose	488.25	488.25	488.25	488.25	488.25	488.25	488.25	488.25
Magnesium stearate	9.70	9.70	9.70	9.70	9.70	9.70	9.70	9.70
Silica	4.30	4.30	4.30	4.30	4.30	4.30	4.30	4.30

In Table 1 are shown the restrictions imposed on the mixture component proportions. Constraints were applied based on the applicable amounts of the components in pharmaceutical formulations (24). Percentage of Cu(II)-RLMP dissolved (Y_1) was considered as dependent variables (responses) in this study.

Development of tablets

The composition of formulations is shown in Table 2. The no-veneered tablets were prepared by the wet granulation method. Cu(II)-RLMP was used as an active substance, isopropyl alcohol as a binder solvent, lactose as a diluent, silica as an adsorption agent and a mixture of corn starch and magnesium stearate as a disintegrator and lubricant.

The ingredients were weighed accurately and passed through a sieve of 0.425 mm to get uniform size particles. Then, these were mixed geometrically for 5–10 min. Granulation was done with a solution of PVP K-30 in sufficient amount of isopropyl alcohol. The granules (40 meshes) were dried in conventional hot air oven at 45°C. Drying of the granules was stopped when the sample taken from the oven reached a loss on drying (LOD) value of 1 to 3%, as measured by a moisture balance at 105°C. The dried granules were passed through a sieve of 1.2 mm, lubricated with magnesium stearate (0.5% w/w) and then compressed on a single punch tablet machine (Erweka EK 0, Germany). The tablets were round and flat with an average diameter of 13.0 ± 0.1 mm and a thickness of 3.2 ± 0.2 mm.

Properties of granules

Angle of repose and flow rate

The flow properties of granules (before compression) were characterized in terms of flow rate and angle of repose (25). For determination of flow rate and angle of repose (θ), the granules were poured through the walls of a funnel. It was fixed at a position such that its lower tip was at a height of exactly 2.0 cm above hard surface. The granules were poured till the time when upper tip of the pile surface touched the lower tip of the funnel. The $\tan^{-1}$ of the (height of the pile / radius of its base) gave the angle of repose.

Particle size and distribution

The particle size and size distribution were estimated by the Crystalsizer (Retsch, USA). It is a compact laboratory instrument for particle size analysis of agglomerated and pourable solids in a size range from 0.7 to 2500 μm.

Characterization of tablets

The prepared tablets (20 tablets) were evaluated for mass uniformity (Eur. Ph.) (26). Hardness (6 tablets) was measured by a hardness tester (Erweka tester, Germany), thickness (10 tablets) was measured by a Erweka Multicheck tester (Germany), friability (10 tablets) was determined using a Erweka Friability tester TDR 100 (Germany). Disintegration test was performed using Disintegration test apparatus (Erweka ZT 301, Germany) by placing each tablet in each basket with the disc. The process was carried out using water maintained at 37°C.

The drug content in each formulation was determined by triturating 20 tablets. The powder equivalent to average weight was added in 100 mL of water, followed by stirring for 30 min. The solution was filtered through a 0.45 μm membrane filter. The dilution suitability and the absorbance of resultant solution was measured spectrophotometrically at 640 nm using water as blank.

Homogeneity of tablets was estimated by ATR-FTIR instrument. FTIR spectra were obtained by using a FTIR microspectroscopy system. ATR-FTIR spectrometer Bruker Tensor-27 was in conjunction with a FTIR Bruker Hyperion-1000/2000 microscope attachment. It was equipped with the 4× viewing objective (objective magnification 4×, visible magnification 57×) and 15× IR Schwarzschild objective (objective magnification 15×, visible magnification 215×). The standard detector, a 250 μm liquid nitrogen cooled, mid-band mercury-cadmium-telluride (MCT) detector (ATR objective GmbH, Germany) with preamplifier, with the range of the IR spectrum from 7000 to 600 cm^{-1} was used in this work. The spectra were measured with 4 cm^{-1} resolution and 200 scans co-addition. The spectrometer was linked to a PC equipped with Bruker OPUS software to allow the automated collection of IR spectra. The measurements were conducted in the reflection mode. In the region from 4000–600 cm^{-1} all spectra were interactive polynomials baseline corrected and area normalized. All experiments were performed at ambient temperature, 25 ± 2°C. Thus, various tests could be performed by the Bruker Hyperion microscope.

The microscopy images of tablets are also taken by Stereo zoom microscope KRÜSS (MSZ 5600) with 45° inclined optical head. The flat-field eyepieces was 20× and dioptric adjustable interocular distance was 51–75 mm. This microscope was equipped with zoom lenses for magnification from 0.7× to 4.5×. Nikon-Coolpix 4500 digital camera was connected to the microscope. Nikon digital camera offers two kinds of zoom. An optical zoom,

in which the cameras telescoping lens can be used to magnify the subject up to 4× and a digital zoom, in which digital processing is used to further magnify the image up to 4×. Together they gave a total magnification of up to 16×.

In vitro drug release studies

The *in vitro* drug release studies were conducted by using the USP 28 type II (10) (paddle) dissolution apparatus (Erweka). The hydrochloric acid (1000 mL, pH 1.2) and phosphate buffer (pH 7.56) were used as a medium. The study was conducted at 37 ± 0.5°C and at paddle rotation of 50 rpm. The samples of 5 mL were collected at predetermined time intervals and replaced with fresh hydrochloric acid and phosphate buffer. The samples were filtered and diluted. The drug content in the samples was estimated at 640 nm by using a validated UV-VIS spectrophotometric method (27). Cumulative percentage of drug release was calculated by using an equation obtained from a standard curve. Mathematical models (zero-order, first-order and Korsmayer-Peppas) were applied to analyze the release mechanism and pattern (28).

Stability studies

Optimized formulation tablets were packed in suitable primary packaging. Then, the accelerated stability test (29) was performed, keeping them at 45°C and 75% relative humidity (RH) for 6 months. At the end of 3 months, tablet properties including hardness, friability and desintegration time as well as drug content and dissolution were evaluated.

RESULTS AND DISCUSSION

Experimental design

To simultaneous and rapid evaluation of the effect of independent variables (the binder concentration PVP K-30 (X_1), the disintegrant corn starch concentration (X_2) and the resistance to crushing (X_3)) on the appropriate response (the percentage of Cu (II)-RLMP dissolved in 60 min (Y_1)), the methodology of experimental design and statistical analysis were applied. By applying experimental design, the interaction effect between variables can be estimated and prediction of their levels can be made. Critical independent variable can be identified based on the statistical analysis.

The full factorial experimental design was applied to the following data: the binder concentration PVP K30 (X_1), the disintegrant corn starch concentration (X_2) and the resistance to crushing (X_3) as independent variables and the percentage of Cu (II)-RLMP dissolved in 60 min (Y_1) as response variable. The concentration range of ingredients for preparing the eight formulations and the observed responses are given in Table 3.

For interpretation of the results obtained during development and optimization of new formulation, several models of experimental designs were applied, such as linear, quadratic and special cubic model. Based on the statistical results, obtained after fitting the experimental data to these models, it was shown that a special cubic model was the most suitable for interpretation of results.

Using ED, the coefficients of proposed model were calculated, which represent the relationship between dependent and independent variables.

The obtained mathematical model is presented in the form of equation:

$$Y_1 = 92.793 - 1.307X_1 - 1.332X_2 - 3.403X_3 - 0.257X_1X_2 - 4.627X_1X_2 + 3.067X_2X_3 + 0.325X_1X_2X_3$$

The results of regression analysis for the simple model are presented in Table 4.

The main effects (X_1, X_2 and X_3) represent the average result of changing one factor at a time from its low to high value. The interactions (X_1X_2, X_1X_3, X_2X_3 and $X_1X_2X_3$) show how the Cu(II)-RLMP dissolved value changes when two or more factors are simultaneously changed.

The Y_1 values for the eight formulations show a wide variation of the response ranges from 87.21 to 98.66% (pH 1.2) and from 86.26 to 93.35% (pH 7.56) in 60 min. The data clearly indicate that the Cu(II)-RLMP dissolved is strongly dependant on the three factors.

Referring to the ANOVA (Table 4) X_1X_2, X_1X_3, X_2X_3 and $X_1X_2X_3$ were significant model terms ($p < 0.05$). The obtained results showed that the compression force (X_3) is the most significant factor for the Cu(II)-RLMP release parameter. The lower hardness of tablets gives better dissolution profile. The disintegrant concentration (X_2) has smaller influence on dissolution profile of Cu(II)-RLMP, while the binding concentration PVP K-30 (X_1) has minor impact in this study.

Evaluation of the model

The 3D response-surface plot represents the response as a function of the two factors (Figs. 1, 2). From Figures 1 and 2, one can see a few differently shaded zones. The respective zones indicate an optimal content of the formulation and the highest percentage of Cu(II) dissolved (Y_1 high) as well as not adequate content of the formulation where the percentage of Cu(II) dissolved is small (Y_1 low).

Table 3. Experimental plan for the design and the results.

Formulation	Variable factor (%)			Response % of Cu(II)-RLMP dissolved (Y_1)	
	% PVP K-30 (X_1)	% Corn starch (X_2)	Hardness (N)	pH 1.2	pH 7.56
1	2.5	20.0	50.0	94.73	88.75
2	2.7	20.0	50.0	98.66	93.35
3	2.5	20.5	50.0	90.94	90.08
4	2.7	20.5	50.0	93.65	91.05
5	2.5	20.0	70.0	93.24	89.64
6	2.7	20.0	70.0	87.21	90.13
7	2.5	20.5	70.0	94.88	79.44
8	2.7	20.5	70.0	89.04	86.26

Table 4. Experimental design results.

Factor	Degree of freedom	Sum of squares	Mean square	F-ratio	p value
X_1	1	3.419	3.419	8.41	0.0298
X_2	1	3.551	3.551	9.73	0.0324
X_3	1	23.154	23.154	26.90	0.0260
X_1X_2	1	0.133	0.133	24.09	0.0184
X_1X_3	1	42.827	42.827	30.21	0.0027
X_2X_3	1	18.819	18.819	11.34	0.0031
$X_1X_2X_3$	1	0.248	0.248	33.11	0.0045
Residual	7	92.152	92.152	–	–

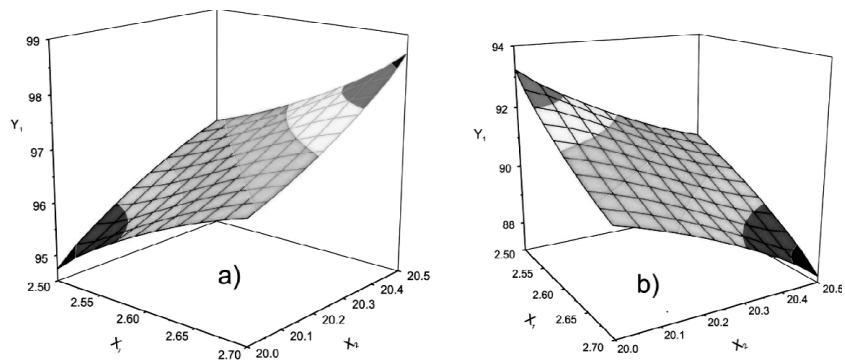

Figure 1. 3D response-surface plot of the response Cu(II)-RLMP dissolved in hydrochloric acid (pH 1.2) at hardness: a) 50 N and b) 70 N.

Increasing of PVP K30, in the range of 2.5 to 2.7%, increase the percentage of Cu(II)-RLMP dissolved. Also, the percentage of Cu(II)-RLMP dissolved grows with increasing percentage of disintegrant in the range of 20 to 20.5%. From 3D diagram it can be seen that the formulation with concentration of PVP K-30 from 2.65 to 2.7 and concentration of corn starch from 20.4 to 20.5% is the optimal content.

It can be concluded that the percentage of Cu(II)-RLMP dissolved in 60 min is significantly higher for tablets with hardness from 50 N than 70 N (Table 3).

Table 5. Properties of granules.

Formulation	Angle of repose (θ) [°]	Flow rate [s/100 g sample]	Particle size [μm]
F1	42.81	21.9	183
F2	44.30	22.3	180
F3	41.00	22.1	182
F4	41.81	22.4	180
F5	46.10	22.0	186
F6	44.18	22.1	184
F7	43.56	22.3	185
F8	45.32	22.4	183

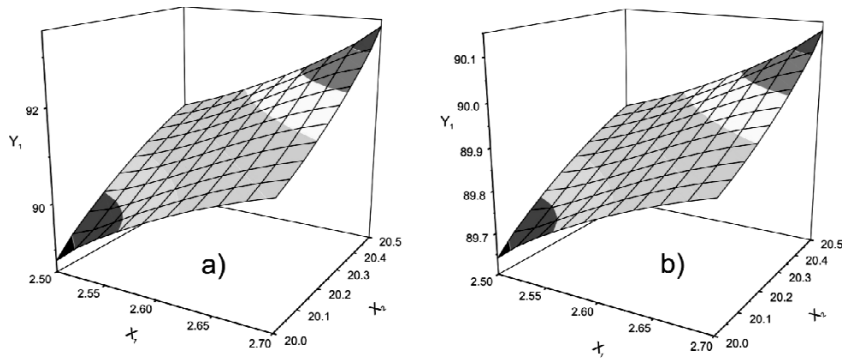

Figure 2. 3D response-surface plot of the response Cu(II)-RLMP dissolved in hydrochloric acid (pH 7.56) at hardness: a) 50 N and b) 70 N.

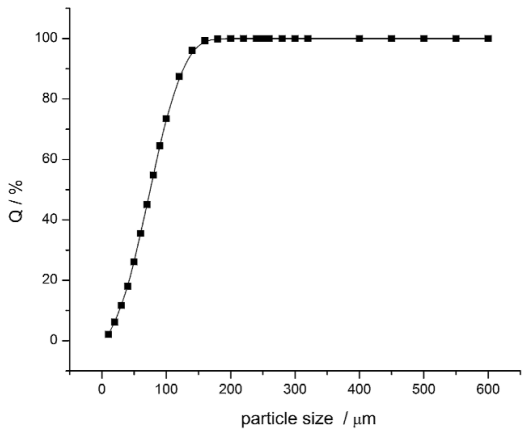

Figure 3. The size distribution of formulation F2 granules

Properties of granules

The granules prepared for compression of no-veneered tablets were evaluated for their flow properties (Table 5). Angle of repose was in the range of 41.0 to 46.1° for granules of different formulations. Flow rate was in the range of 21.9 to 22.4 s/100 g sample for all granules. These values indicate that the prepared granules had good flow properties. The particle size was from 180 to 186 mm (Table 5). Size distribution was shown in Figure 3 for formulation F2. It can be seen that in the granules prevailed particles with size larger than 180 μm.

Evaluation of tablets

The no-veneered tablets of Cu(II)-RLMP were prepared by the wet granulation technique using lactose, silica and PVP K-30. The magnesium stearate and corn starch were used as lubricant and disintegrant, respectively. The results of the physicochemical characterization are shown in Table 6.

The weight of tablets was varied between 650.3 and 652.0 mg for different formulations. The low values of standard deviation indicate weight uniformity. The variation in weight was within the range of ± 5% complying with pharmacopoeia specifications. The hardness for different formulations was found to be between 48.7 and 71.7 N. It indicates a satisfactory mechanical strength. The friability was below 1% for all the formulations,

Table 6. Physicochemical characterization of tablets.

Formulation	Uniformity of weight	Hardness[a] (mg)	Friability (*N*) (%)	Disintegration time (min)[a]	Drug content (mg)[a]
F1	652.0	48.7 ± 1.21	0.10	4.5 ± 0.77	1.513 ± 0.76
F2	650.3	50.0 ± 0.77	0.13	3.2 ± 1.13	1.492 ± 0.34
F3	651.9	51.7 ± 0.45	0.12	3.7 ± 1.02	1.512 ± 0.87
F4	651.4	52.1 ± 0.31	0.10	3.8 ± 0.88	1.518 ± 1.11
F5	650.9	70.8 ± 0.94	0.05	8.1 ± 1.61	1.519 ± 0.81
F6	650.8	69.1 ± 0.36	0.08	6.9 ± 1.05	1.488 ± 0.98
F7	650.7	71.7 ± 1.32	0.07	7.0 ± 0.85	1.494 ± 0.62
F8	650.9	68.4 ± 1.48	0.03	7.5 ± 0.28	1.515 ± 0.83

[a] Mean ± SD.

Table 7. Kinetics of *in vitro* copper (II) complex release from tablets (pH 1.2).

Formulation	Zero order		First order		Korsmeyer model	
	k_0 (mg/min)	r^2	k_1 (min^{-1})	r^2	n	r^2
F1	1.418	0.863	0.048	0.984	0.452	0.998
F2	1.439	0.840	0.070	0.941	0.390	0.974
F3	1.341	0.841	0.038	0.986	0.404	0.958
F4	1.395	0.874	0.044	0.974	0.441	0.970
F5	1.368	0.848	0.045	0.990	0.400	0.964
F6	1.240	0.810	0.032	0.964	0.336	0.896
F7	1.397	0.843	0.048	0.978	0.460	0.988
F8	1.339	0.876	0.036	0.992	0.406	0.978

Table 8. Kinetics of *in vitro* copper (II) complex release from tablets (pH 7.56).

Formulation	Zero order		First order		Korsmeyer model	
	k_0 (mg/min)	r^2	k_1 (min^{-1})	r^2	n	r^2
F1	1.214	0.740	0.033	0.937	0.271	0.874
F2	1.270	0.727	0.414	0.943	0.261	0.866
F3	1.219	0.712	0.035	0.935	0.248	0.876
F4	1.252	0.729	0.037	0.948	0.277	0.889
F5	1.272	0.803	0.035	0.964	0.342	0.944
F6	1.278	0.792	0.036	0.966	0.336	0.925
F7	1.129	0.806	0.024	0.954	0.340	0.954
F8	1.215	0.771	0.030	0.962	0.314	0.966

which is an indication of good mechanical resistance of the tablets. The disintegration time was found to be in the range of 3.24–8.1 min for all the formulations.

The drug content varied between 99.2 and 101.26%, in different formulations. The low coefficient of variation indicates a content uniformity in the prepared batches.

Table 9. Physicochemical characteristics of the optimized formulation (F2) after accelerated stability studies (45°C, 75% RH).

Dependent variable	Time (month)			
	0	1	2	3
Uniformity of weight (mg)	650.3	651.8	648.9	649.2
Hardness (*N*) (n = 10)[a]	50 ± 0.77	49.3 ± 1.01	48.7 ± 0.48	48.1 ± 0.57
Friability (%)	0.13	0.18	0.24	0.33
Disintegration time (min) (n = 6)[a]	3.2 ± 1.13	4.8 ± 0.81	5.3 ± 0.56	5.7 ± 0.73
Drug content (%)	99.46 ± 0.68	98.14 ± 0.34	97.41 ± 1.02	96.72 ± 0.98
Q_{60}[b] (%) (n = 3)[a] (pH 1.2)	98.6 ± 1.21	97.9 ± 0.96	97.1 ± 1.51	95.4 ± 1.71
Q_{60}[b] (%) (n = 3)[a] (pH 7.56)	93.3 ± 1.15	92.7 ± 0.65	91.2 ± 1.89	89.2 ± 1.66

[a] the mean ± SD. [b] drug dissolved in 60 min.

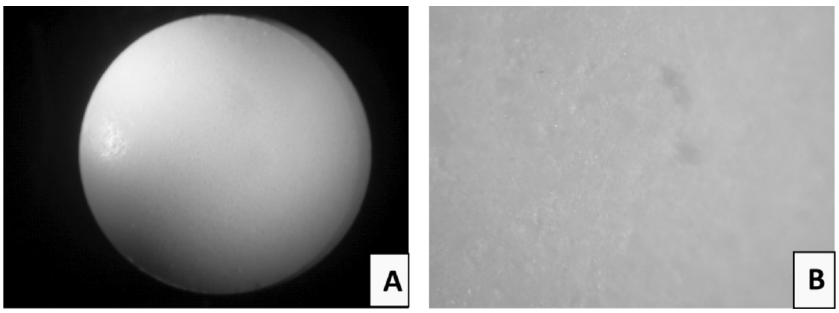

Figure 4. Stereo zoom microscope images of unbroken tablet with: **A** 28× and **B** 180× magnification

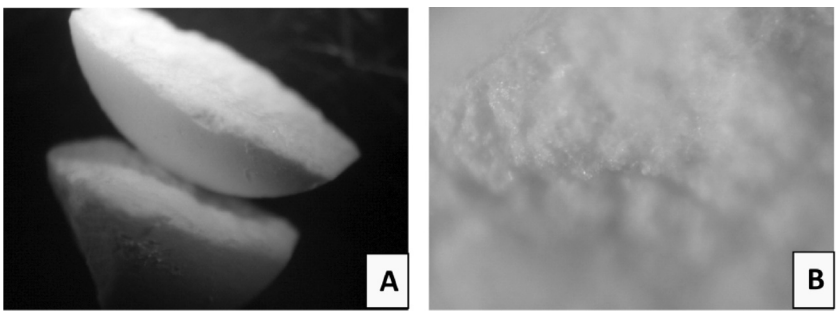

Figure 5. Stereo zoom microscope images of broken tablet with: **A** 28× and **B** 180× magnification

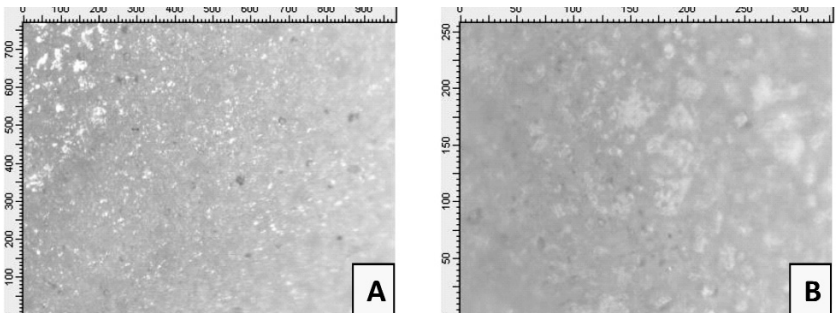

Figure 6. ATR-FTIR microspectroscopic images of unbroken tablet with **A** 57× and **B** 215× magnification

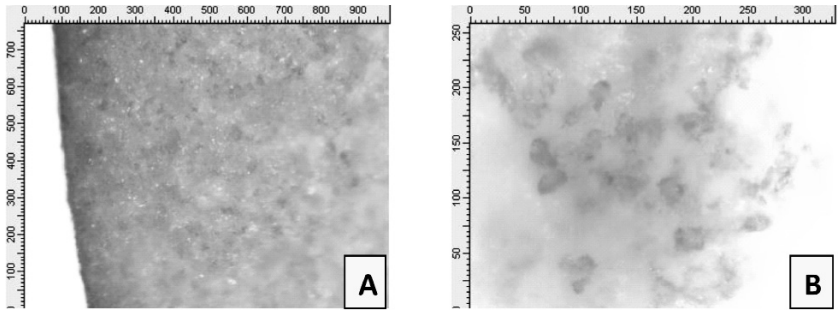

Figure 7. ATR-FTIR microspectroscopic images of broken tablet with **A** 57× and **B** 215× magnification

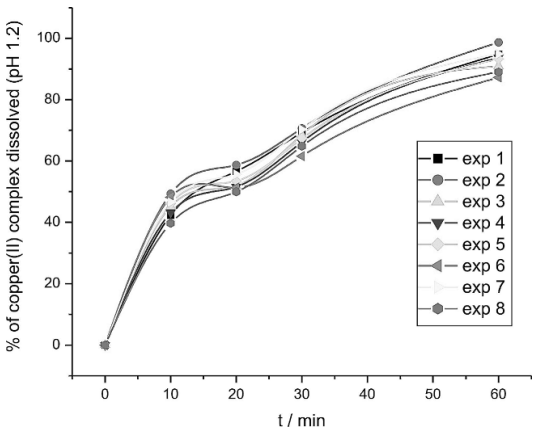

Figure 8. *In vitro* dissolution profile of tablets in hydrochloric acid (pH 1.2)

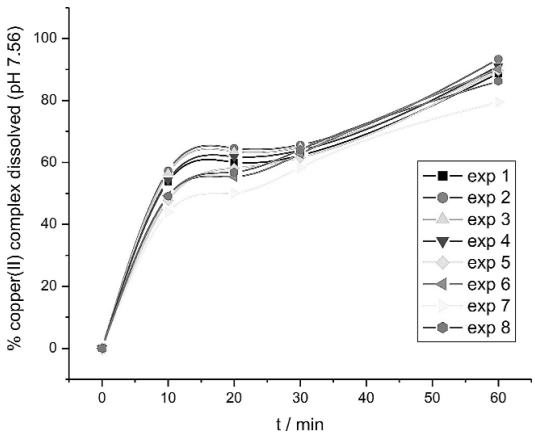

Figure 9. *In vitro* dissolution profile of tablets in phosphate buffer (pH 7.56)

Stereo zoom microscope images of unbroken and broken tablets showed high homogeneity of tablets (Figs. 4, 5), and equal distribution of active pharmaceutical substance Cu(II)-RLMP in excipients. The size or scale, within which the homogeneity was maintained, was crucial in defining a phase. In general, the composition and physical state become more and more heterogeneous as the scale went smaller.

This statement was also confirmed by ATR-FTIR microspectroscopic imaging and spectroscopy, which were used in analyzing pharmaceutical material (Figs. 6, 7).

In vitro dissolution studies

The pharmacokinetic parameters of Cu(II)-RLMP were used to calculate a theoretical drug release profile for the eight dosage forms (29). Percentage of complex dissolved was determined by a VIS spectrophotometric method at 640 nm, after 10, 20, 30 and 60 min. The *in vitro* drug release profile of the Cu(II)-RLMP complex for all the formulations is shown in Fig. 8 (in hydrochloric acid) and Fig. 9 (in phosphate buffer).

Percentage of the drug released in 60 min for the F1–F4 formulations, (resistance to crushing of 50 N) were 94.73, 98.66, 90.94 and 93.65% in hydrochloric acid; and 88.75, 93.35, 90.08 and 91.05% in phosphate buffer, respectively.

The obtained values have indicated that the percentage of released Cu(II)-RLMP complex is the highest for the F2 formulation in hydrochloric acid and phosphate buffer. Percentage of the Cu(II)-RLMP complex released for the F2 formulation is negligibly higher in hydrochloric acid.

Percentage of the drug released in 60 min for the F5–F8 formulations (resistance to crushing of 70 N) were 93.24; 87.12; 94.88 and 89.04% in hydrochloric acid; and 89.64; 90.13; 79.44 and 86.26% in phosphate buffer, respectively.

For the tablets with hardness of 70 N the percentage of the complex released in 60 min is the

highest for formulation F7 in hydrochloric acid, and for formulation F6 in phosphate buffer.

From the experimental results it can be concluded that the tablets with smaller hardness have a better drug release profile than tablets with higher hardness in both investigated media. This finding is in very good agreement with the results of the previously performed experimental design (see Table 4).

The formulation F2, with the best dissolution profile (see Figure 8 and 9) and the best mechanical characteristics, was selected as optimal formulation for producing tablets with the Cu(II)-RLMP complex.

Drug release kinetics

The data obtained from *in vitro* dissolution studies were fitted in different models viz., zero order, first order and Korsmeyer-Peppas equation (Tables 7 and 8). The zero order plots were not found to be fairly linear ($r^2 < 0.900$).

In order to confirm the exact mechanism of drug release from these tablets, the data were fitted according to the first order equation and the regression analysis was performed. The coefficients of regression, r^2, were spanned from 0.941 to 0.992 in hydrochloric acid (Table 7) and from 0.935 to 0.966 in phosphate buffer (Table 8) for the examined formulations.

Hardness of the tablets was stable in the stability study (around 50 N). Although disintegration time and friability of tablets increased from 3.2 to 5.7 s and 0.13 to 0.33% respectively, drug content of the tablets was relatively stable, from 99.46 to 96.72%, in the stability study. More than 80% of Cu(II)-RLMP complex was dissolved from all tablets in 60 min of the test (Q60). An increase of the disintegration time of tablets exposed to the stability testing conditions has influenced on slight decrease of the Q60 values for the tablets. However, according to the statistical analysis (ANOVA), no significant difference was observed for the drug dissolved from tablets during the stability test. In conclusion, the optimized formulation F2 could be considered stable even after 3 months of being kept under accelerated stability conditions.

CONCLUSION

In a phase of developing a new drug solid dosage form it is important to identify rapidly the best component composition. The complex of polysaccharide pullulan with copper(II) ion was used for preparing the tablets for treatment a copper deficiency in human organism. The no-veneered tablets with PVP K-30 and corn starch as variable components was prepared and optimized using experimental design. The quantitative effect of these factors on the percentage of Cu(II)-RLMP complex dissolved could be predicted by the cubic model. The results have confirmed that the design technique can be successfully employed for designing a tablet with desirable physical properties. The obtained results showed that the most significant factor for dissolution profile of Cu(II)-RLMP from tablets was the resistance to crushing. The acceptance criterion for this factor was 50 N. In order to confirm the exact mechanism of drug release from these tablets, the data were fitted in accordance with the first order equation.

Acknowledgments

The authors are grateful to the colleagues of Pharmacy and Chemical Industry Zdravlje-Actavis, Serbia for the given practical help. This work was supported by the Ministry of Science and Technological Development of the Republic of Serbia under the project TR-34012. Ivan Savic is a recipient of a scholarship granted by the Ministry of Science and Technological Development of the Republic of Serbia.

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Received: 20. 05. 2011