

FORMULATION AND *IN VITRO* RELEASE TESTING OF RECTAL SUPPOSITORIES CONTAINING NIMESULIDE

ANCA CECILIA NICOARĂ¹, RADU GEORGE CAZACINCU^{2*}, DUMITRU LUPULEASA¹, DALIA SIMONA MIRON³, FLAVIAN ȘTEFAN RĂDULESCU⁴

¹University of Medicine and Pharmacy "Carol Davila" Bucharest, Faculty of Pharmacy, Department of Pharmaceutical Technology and Biopharmaceutics, 6 Traian Vuia street, 020956, Bucharest, Romania

²Ovidius University, Faculty of Pharmacy, 1 Aleea Universitatii, Constanta, Romania

³University of Medicine and Pharmacy "Carol Davila" Bucharest, Faculty of Pharmacy, Department of Pharmaceutical Physics and Informatics, 6 Traian Vuia street, 020956, Bucharest, Romania

⁴University of Medicine and Pharmacy Carol Davila Bucharest, Faculty of Pharmacy, Department of Drug Industry and Pharmaceutical Biotechnologies, 6 Traian Vuia street, 020956, Bucharest, Romania

*corresponding author: office@magistracc.com

Manuscript received: November 2014

Abstract

Rectal suppositories containing 100 mg nimesulide were prepared using lipophilic bases with different physico-chemical characteristics. The experimental protocol implemented for the evaluation of the pharmaceutical quality and *in vitro* performance included determinations of softening and disintegration times, as well as the release assessment using a dissolution equipment with compendial 40 mesh and Palmeri baskets. The mean profiles displayed a very low dissolution of the lipophilic drug, with release rates influenced on the melting and spreading pattern. The release kinetics was adequately described by the Higuchi model.

Rezumat

Supozitoare rectale conținând 100 mg de nimesulid au fost preparate utilizând baze lipofile cu diferite caracteristici fizico-chimice. Protocolul experimental implementat pentru evaluarea calității farmaceutice și a performanței *in vitro* a inclus atât determinări ale timpilor de înmuiere și de dezintegrare, cât și evaluări ale cedării, utilizând aparate de dizolvare cu două tipuri de coșulețe, compendiale de 40 mesh și Palmieri. Profilele medii au prezentat o dizolvare extrem de redusă a substanței medicamentoase lipofile, vitezele de cedare fiind influențate de caracteristicile de topire și etalare. Cinetica de cedare a fost descrisă adecvat de modelul Higuchi.

Keywords: nimesulide, suppositories, formulation, *in vitro* release

Introduction

The suppositories are considered by the International Pharmaceutical Federation as novel/special dosage forms [1], due to the particularities of their composition and manufacturing process, to the mechanism of release and the site of absorption, as well as to the lack of extensive experience in development and validation of adequate *in vitro* methodologies. The selection of testing equipment and conditions does not benefit from the existence of clear compendial recommendations. Therefore a case-by-case approach is usually implemented. The drug manufacturers have to develop adequate tests not only for routine quality control purposes, but also for selection of optimal candidates during research and development phases. One of the major issues is the variability of experimental data generated during the *in vitro* testing procedure. Specific phenomena, such as melting of the suppository

base, deformation, spring within the apparatus and dispersion in the release media, with or without inducing a biphasic system in the dissolution vessels, induce a high variability of the data.

The current compendial requirements for drug release methodologies do not refer to immediate release suppositories. A single specific monograph is included in the United States Pharmacopoeia, for indomethacin suppositories [2] and five more sets of conditions are provided in the FDA dissolution database [3]. Notably, testing temperature seems to vary largely, it is selected based on the physical properties of the suppository base and, in many instances, is outside the physiological range. For example, for mesalamine suppositories, the specification for the highest strength includes a temperature of 40°C. The European Pharmacopoeia describes a modified flow-through cell with three compartments inter-connected by capillary tubes [4]. Despite the advantage of increased biorelevance, controlled hydrodynamics and

theoretically unlimited *sink* conditions [5, 6], the previous experience in using this type of equipment suggested that the variability remains a major concern, due to the non-uniform behaviour of the dosage units during the procedure.

Another particularity is the limited information provided by additional physical tests, available in the general compendial monographs [7]. The evaluation of softening and disintegration times presumably provides valuable data for explaining the results of an *in vitro* release procedure or at least for adequate selection of operational parameters. In most of the cases, despite a claimed immediate release mechanism, the transfer of the active pharmaceutical ingredient is dependent only to a reduced extent on the melting or disintegration profile. Overall, with few exceptions, the release is slow; suggesting that after an initial time interval, the performance of the formulation is similar to the semisolid dosage forms.

The aim of the current study was to evaluate the role of lipophilic suppository bases from various sources and with distinct physico-chemical properties on the *in vitro* release of a low solubility, high permeability anti-inflammatory drug. The

compendial basket method and a specific adaptation (Palmieri baskets) were selected at the lowest stirring rate. In these conditions, it was assumed that the experimental protocol will provide insights on the relationship between the melting profiles of the pharmaceutical vehicles, the particularities of the compendial apparatus and the release kinetics.

Materials and Methods

In a preliminary study, the displacement value was determined for each suppository base. Briefly, a small amount of melted excipient was mixed with the active substance required for a suppository (100 mg nimesulide), the mixture was poured into one cavity of the mould, which was then filled with the molten excipient. After cooling, the suppository was weighed. The difference between the total mass of the suppository and the amount of nimesulide per suppository represented the excipient necessary for one suppository. The total amount of excipient was determined by multiplying the obtained value to the desired number of suppositories and by adding an excess amount.

Table I

The identification and main characteristics of the suppository bases (information collected from the technical specification of the manufacturer)

Excipient	Code	Batch no.	Aspect	Composition	Melting interval	I _{OH}	Observations
Estaram 299	E229	622954	Pellets	Semi-synthetic glycerides	33.5-35.5°C	2	I _{saponification} =240-255. Used in the delivery of drugs sensitive to hydrolysis.
Estaram H15	E H15	620434	Pellets	Semi-synthetic glycerides	33.5-35.5°C	5-15	Hard fat suppository base with low content of mono- and diglycerides.
Novata B PH	N B	8066318	Pellets	Semi-synthetic glycerides, coconut fatty acids triglycerides	33.5-35.5°C	20-30	-
Novata BC PH	N BC	7892170	Pellets	Semi-synthetic glycerides, coconut fatty acids triglycerides	33-34.5°C	30-40	-
Novata BCF PH	N BCF	7935538	Pellets	Semi-synthetic glycerides, coconut fatty acids triglycerides	35-37°C	20-30	-
Novata BD PH	N BD	8024357	Pellets	Semi-synthetic glycerides, coconut fatty acids triglycerides	33.5-35.5°C	5-15	-
Supoweiss S2	S S2	618203	Flakes	Semi-synthetic glycerides	34-36°C	15-25	I _{saponification} =235-245. High plasticity.
Suppocire AM	S AM	2E1205-2	Pellets	Semi-synthetic glyceride base comprising saturated C8-C18 triglyceride fatty acids.	35.0-36.5°C	5	Base with a low hydroxyl value to avoid interaction between free OH groups and acidic active ingredients. Suitable for high rate manufacturing but shock-cooling should be avoided.
Suppocire NAI 25A	S NAI	0E4204-2	Pellets	Semi-synthetic glyceride base comprising saturated C12-C18 triglyceride fatty acids.	33.2-35.2°C	20	The long chain triglyceride composition results in harder suppository units with rapid solidification properties and high mechanical resistance.
Suppocire BM	S BM	2E2104-2	Pellets	Semi-synthetic glyceride base comprising saturated C8-C18 triglyceride fatty acids.	36.0-37.5°C	5	Base with a low hydroxyl value to avoid interaction between free OH groups and acidic active ingredients. Suitable for high rate manufacturing, but shock-cooling should be avoided.
Wecobee FS	W FS	35437501	Block	Triglyceride derived from hydrogenated palm kernel oil vegetable oil.	37-43°C	5	-
Wecobee S	W S	S475245AB	Block	Triglyceride derived from fully hardened palm kernel and cottonseed oils.	43-47°C	-	-

I_{OH} - hydroxyl value; I_{saponification} - saponification value.

The active substance was added under continuous stirring, using a magnetic stirrer, into the melted excipient at a temperature corresponding to the melting point declared by the technical specification of the manufacturer. The identification and the main characteristics of the suppository bases are presented in Table I. The

homogeneous mixture was poured from a syringe into metal moulds, previously sprayed with ethanol. Constant temperature and stirring were maintained throughout the process to avoid sedimentation and uneven distribution of nimesulide in the dosage units. After cooling at room temperature, suppositories were stored in a refrigerator for 30

minutes to completely solidify. The suppositories were easily removed from the moulds, as all the excipients presented a good volume contraction. The uniformity of mass was evaluated on ten dosage units, individually weighed. The average weight and percentage deviation were calculated.

The softening time determinations and the disintegration tests were performed using a Copley suppository Disintegration Tester SDT 1000 (Copley Scientific, UK). The equipment consisted of an acrylic water bath, external thermo-stirrer heater, temperature measurement by a digital thermometer, 4 L glass beaker, suppository disintegration rig (containing a stainless steel basket attached to a transparent plastic sleeve). The adaptation for the assessment of the softening time consisted of three glass tubes placed on a rack, each closed with a rubber stopper at the bottom and three glass rods containing lead shot at a weight of 30 ± 0.4 g. The procedures were performed according to the recommendations of the European Pharmacopoeia (Chapter 2.9.2. Disintegration of suppositories and pessaries, respectively chapter 2.9.22. Softening Time Determination of Lipophilic Suppositories - apparatus B). During the disintegration test, the suppository was placed between the two perforated plates of the basket, which was inserted into the acrylic sleeve. The suppository disintegration rig was then placed in the glass beaker containing 4 L of purified water, thermostated at $36-37^\circ\text{C}$. Every 10 minutes, the basket was rotated manually through 180 degrees. To pass the test, all samples must have disintegrated within 30 minutes. For the determinations of softening times, the suppository was introduced with the tip downwards into the inner tube filled with 5 mL of water at $36.5 \pm 0.5^\circ\text{C}$ and onto that, the glass rod was placed. The time elapsed between this moment and the moment when the lower, rimmed end of the glass rod reached the narrowed part of the inner glass tube was recorded, as melting is considered to be complete.

In vitro drug release studies were performed on a Hanson SR8 Plus dissolution tester (Hanson Research Inc., USA) according to two experimental protocols, USP Apparatus 1 (compendial, 40 mesh baskets) and a non-compendial adaptation (Palmieri baskets). Volumes of 900 mL of phosphate buffer pH = 7.4, prepared according to recommendations of the United States Pharmacopoeia (USP), were used as dissolution medium, after degassing by filtration under vacuum (900 mbar; cellulose acetate membrane with a mean pore diameter of $0.45\mu\text{m}$, diameter 47 mm).

The tests were performed in triplicate, at $37 \pm 0.5^\circ\text{C}$ and using a stirring rate of 50 rpm. Based on preliminary evaluations on the reference product

(Aulin[®] 200 mg, Helsinn Birex Pharmaceuticals Limited, Ireland), two additional runs were performed using USP apparatus 2, at 50 and 75 rpm. Studies were carried out on three individual dosing units (three suppositories).

Samples of 5 mL were collected at 10, 20, 30, 45, 60, 90, and 120 minutes and replaced by equal volumes of blank media. Filtration was performed through polyethylene cannula filters, attached on stainless steel rods, immersed at halfway between the liquid surface and the upper part of the basket. The quantitative determination of nimesulide was performed by a spectrophotometric method ($\lambda_{\text{max}} = 392 \text{ nm}$), using a UV visible spectrophotometer (Agilent 8453 UV-Vis Spectrophotometer, Agilent Instruments, Germany). Undiluted probes were automatically analysed at ambient temperature by interpolation on the calibration curve ($R^2 = 0.999$).

The suppository bases were kindly donated by the manufacturers (Estaram and Supoweiss, Croda; Novata, BASF The Chemical Company; Suppocire, Gattefossé; Wecobee, Stepan Company). All reagents used were of analytical purity (potassium dihydrogen phosphate anhydrous, Sigma Aldrich; sodium hydroxide, Riedel-de Haen; nimesulide, batch 126036-6, Cayman Chemical Company). The purified water was generated using an Ultra Clear UV plus purification system (SG Wasseraufbereitung und Regenerierstation GmbH, Germany).

Results and Discussion

The prepared suppositories (length, 3.5 cm; base diameter, 8 mm; weight, 2.77-2.81 g) had a glossy, smooth surface, without showing cracks, white or coloured spots, air bubbles, fat blooming or exudation. The colour went from off-white to light yellow, depending on the base used and the degree of dissolution of nimesulide in the base with regard to the lipophilic nature of the active substance. No individual mass exceed the range of $\pm 5\%$ of the average value (-1.06% and $+1.39\%$).

The softening and disintegration times varied largely, depending on the properties of the lipophilic base and on its interaction with the active pharmaceutical ingredient, having weak or non-acidic properties [8]. Within each group of excipients, the Estaram type displayed the lowest variability, the melting occurring within 12 minutes after the debut of the tests, whereas the disintegration was noted after 13 to 14 minutes. For the formulations based on triglyceride derived from fully hardened palm kernel and cottonseed oils (W S), disintegration didn't occur within 30 minutes and the hard consistency was maintained for more than 45 minutes (table I). It is to be noted that this type of compendial evaluations are not adequate for the assessment of product performance [7],

similarly to the disintegration tests performed on the oral solid dosage forms.

The analysis of *in vitro* release profiles showed an extremely low nimesulide release. The paddle method applied in case of the reference listed drug generated dissolved fractions between 0.19 and 2.57%, with an apparent dependence on the stirring rate. No disintegration or deformation phenomenon was noted throughout the evaluation. At the end of the tests, no major change in the consistency of the dosage units was concluded. The diffusional resistance opposed by the vehicle was considerable. Preliminary solubility evaluations were conducted at both 25 and 37°C, in the phosphate buffer system with a pH between 1.2 and 7.4. The results suggested that the maximum solubility is reached in the conditions simulating the distal part of the intestine (55.55 mg/L solubility at pH 7.4, 37°C). Therefore, it is reasonable to assume that the low values of the fraction released were related to the superficial interaction between the suspended particles of the drug and the release media. The suppository surface area (cm²) was calculated based on the formula proposed by Puffer HW et al [9], based on the diameter (d; cm) and length (l; cm):

$$S = \left(\frac{\pi d^2}{2} \right) + \pi dl$$

No significant changes in the contact area were assumed. The quantity released per surface unit was

plotted against the square root of time [10, 11]. Linear regression was performed on the resulting points, the slopes corresponding to the release rate. The values of the correlation coefficient were higher than 0.99, suggesting that the Higuchi model adequately described the process (Figure 1). Alternative release models (e.g. zero or first order kinetics, Krosmeier Peppas etc.) were applied, but didn't lead to an acceptable fitting of the experimental data.

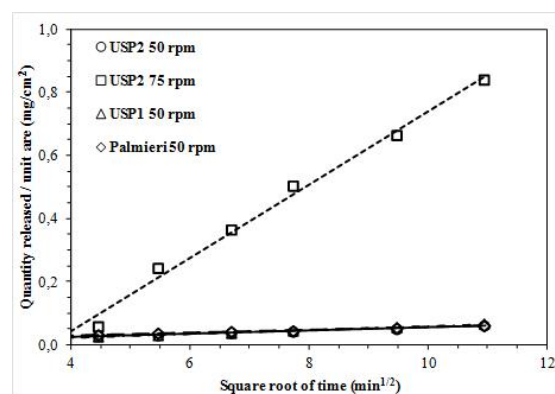
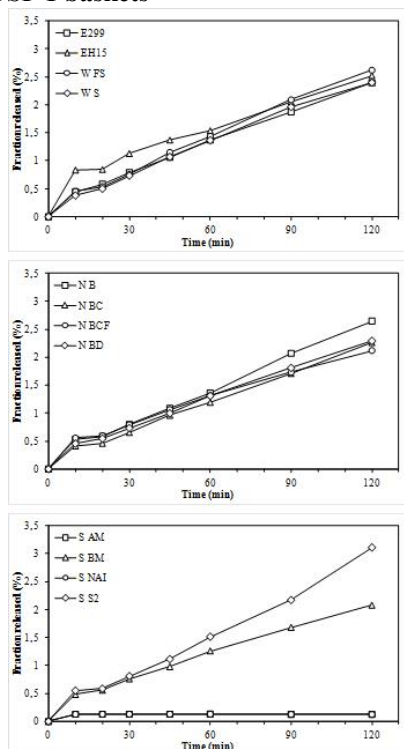


Figure 1.

The *in vitro* release profiles of nimesulide from the reference product (mean values, n=3; standard deviation not displayed for clarity)

USP 1 baskets



Palmieri baskets

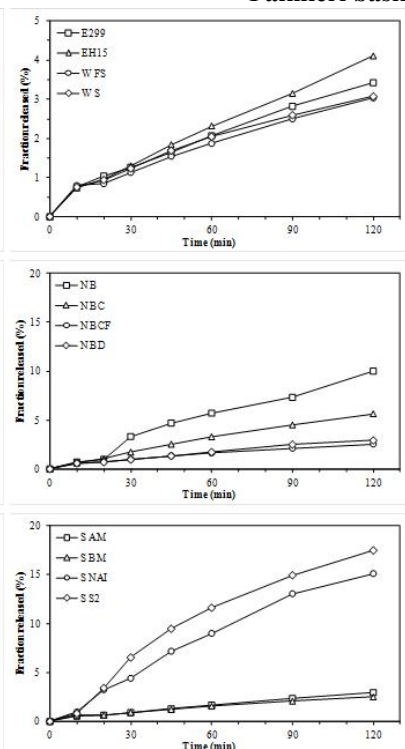


Figure 2.

The *in vitro* mean release profiles of nimesulide from experimental formulations prepared with different suppository bases (n=3; standard deviation not displayed for clarity)

Setting the paddle apparatus to 75 rpm led to a 23 fold increase in the release rate, but the cumulative quantities of nimesulide were still below 1%. Obviously, none of these experimental conditions are to be applied for quality control purposes. Noteworthy, the pattern of the variability was atypical. The value of the coefficient of variation was low for the early experimental time points, whereas a continuous increase was noted by the end of the study. The explanation relies on the distinct behaviour within each group of three dosage units that were tested, despite the same initial positioning inside the baskets.

In case of the experimental formulations, the single difference was the nature of the suppository base. The same quantity of particles of the same size was used, the formulations had the same age at the time of testing and the manufacturing parameters were standardized to a reasonable extent. The maximum value of the released fraction was obtained for S S2 base (17.47% of the content, using Palmieri baskets), corresponding to the rapid changes in consistency. For S AM and S NAI bases, the amount of nimesulide recovered in the media was very low and relatively constant throughout the test conducted on compendial apparatus 2 (0.14 µg/mL), suggesting an initial burst in release, followed by no apparent consecutive dissolution (Figure 2).

Melting and advanced deformation of the bases induced a time dependent accumulation in the upper section of the basket. Due to the relative low stirring rate, the vehicle was not expelled to a significant extent through the meshes and formed a cylinder-shape layer, of approximately constant

area. For the Palmieri basket, a partial accumulation of the melted vehicle at the surface of the media was observed at the end of the tests. A fraction of the melted base was slowly expelled from the immersing element through the longitudinal slots. The increase in the contact surface with the release media can explain the higher cumulative amounts of nimesulide obtained in this setup (Figure 3). Data were consistent with previous reports [12], but also suggested some limitations in terms of the stirring rate. The use of non-physiological temperatures, up to 40°C [13], as the single parameter which will likely have an impact on the consistency of lipophilic bases with higher melting points, would have dramatically reduced the relevance of the results.

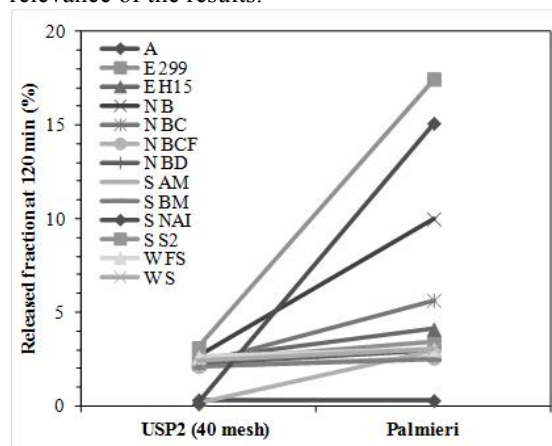


Figure 3.

Comparison of the released fractions at the end of the test between the two experimental setups

Table II

The mean values of the pharmacotechnical and *in vitro* release data (Higuchi model)

Suppository base (code)	Softening time (min)	Disintegration time (min)	USP Apparatus 1 (40 mesh)			Palmieri Basket		
			R ²	Release rate (µg/cm ² /min ^{1/2})	Lag time (min ^{1/2})	R ²	Release rate (µg/cm ² /min ^{1/2})	Lag time (min ^{1/2})
E 299	12.08	13.97	0.9957	87.17	2.70	0.9954	117.73	2.10
E H15	12.01	13.17	0.9945	78.69	1.28	0.9955	141.64	2.86
N B	11.05	14.75	0.9906	100.12	3.12	0.9924	360.78	2.97
N BC	12.80	12.45	0.9941	86.02	3.15	0.9997	221.40	3.06
N BCF	24.42	29.05	0.9991	24.05	2.10	0.9998	28.44	1.83
N BD	15.23	14.55	0.9958	84.93	2.80	0.9967	111.54	2.76
S AM	10.17	10.53	-	-	-	0.9977	37.76	3.00
S BM	10.08	8.17	0.9976	73.52	2.32	0.9990	89.33	2.31
S NAI	12.83	16.30	-	-	-	0.9985	183.15	3.02
S S2	8.88	8.63	0.9833	119.17	3.54	0.9949	665.03	2.56
W FS	19.25	18.52	0.9971	33.21	3.10	0.9989	32.69	1.70
W S	>45	>30	0.9984	30.35	2.99	0.9998	34.31	1.96

The application of compendial metrics was not suitable, the fraction released being in many instances lower than 10% of the theoretical amount

of nimesulide. In order to perform a meaningful comparison of the mean release profiles, the release rate was calculated for all the experimental

formulation, according to the procedure previously described for the reference listed drug.

For the bases that melted at the testing temperature, the surface was calculated by considering that the dosage forms adopt a cylinder shape with the diameter equal to the cross-section of the basket, i.e. 3.2 cm². The results for the two experimental setups are comparatively presented in Table II, together with the corresponding values of the softening and disintegration times. No direct relationship was concluded between the release or the lag time and the results of the two pharmacotechnical assessments, but a rank order was apparent in each group of bases. The consistency might be inversely related to the release rate.

The stirring rate cannot be increased, due to previously mentioned methodological limitations of the basket apparatus. The main concern was not the clogging of the meshes, but the expulsion and consecutive floating of the vehicle. Moreover, this approach leads to a decrease in the discriminatory character. The use of tensioactive agents would have increased the wetting and solubilisation of the drug [14], but this process was not the rate-limiting step. The low amounts of nimesulide released made very difficult the interpretation of possible *in vivo* consequences, especially if one considers that the same behaviour was noted in case of the reference listed drug. A major impact on the absorption pattern of suppositories is assumed for the rectal motility [15]. The mechanical stress could induce a particular spreading onto mucosa, the absorption depending on both contact surface and consistency [16]. Perhaps the suppository formulations have to be approached in a manner similar to the topical semisolids, i.e. they should be assessed as extended release products and the model-dependent approaches must be adopted for evaluation of *in vitro* similarity.

Conclusions

The influence of the composition on the results of the pharmacotechnical evaluations and on *in vitro* release parameters was assessed for rectal suppositories containing 100 mg nimesulide. The dependence between the softening or disintegration times and rate of the release process was not linear, suggesting the interplay of other factors such as the drug solubility in the base at physiological temperature, its relative affinity between the lipidic and aqueous media or the structure of the melted vehicle or the spreading surface. The kinetic particularities were adequately described by the Higuchi model, confirming the combined role of the contact surface and diffusional resistance.

Acknowledgements

This work was supported by UMF "Carol Davila" Bucharest projects number 28491/30.10.2012 (ACN) and by the programme *Partnerships in priority domains* - PNII, implemented with the help of MEN - UEFISCDI (PN-II-PT-PCCA-2013-4-1903). We acknowledge the support of the manufacturers of the excipients employed in the formulation of the suppository formulations (BASF The Chemical Company, Croda, Gattefossé and Stepan Company).

References

1. Brown C.K., Friedel H.D., Barker A.R., Buhse L.F., Keitel S., Cecil T.L., Kraemer J., Morris J.M., Reppas C., Stickelmeyer M.P., Yomota C., Shah V.P., FIP/AAPS joint workshop report: dissolution/*in vitro* release testing of novel/special dosage forms. *AAPS PharmSciTech.*, 2011; 12(2): 782-794.
2. United States Pharmacopoeia 36 / National Formulary 31. United States Pharmacopoeial Convention, Rockville, MD 20852.
3. Food and Drug Administration FDA-Recommended Dissolution Methods. Available at: <http://www.accessdata.fda.gov/scripts/cder/dissolution/>. Last accessed: July 07th, 2014.
4. Council of Europe. European Pharmacopoeia. 6th ed. Strasbourg: Council of Europe; 2007.
5. Woyczkowski B., Szulc J., Sznitowska M., Janicki S., Pilichowski J., Urbańska A., Feasibility of the Ph.Eur. flow-through cell for dissolution testing of the compounded rectal suppositories containing indomethacin or sodium diclofenac. *Acta Pol. Pharm.*, 2003; 60(3): 169-172.
6. Fotaki N., Flow-through cell apparatus (USP Apparatus 4): Operation and features. *Diss. Tech.*, 2011; 18(4): 46-49.
7. Janicki S., Sznitowska M., Zebrowska W., Gabiga H., Kupiec M., Evaluation of paracetamol suppositories by a pharmacopoeial dissolution test--comments on methodology. *Eur. J. Pharm. Biopharm.*, 2001; 52(2): 249-254.
8. Bernareggi R., Clinical pharmacokinetics and metabolism of nimesulide. *Inflammopharmacol.*, 2001; 9:(1-2): 81-89.
9. Puffer H.W., Crowell W.J., Salicylate release characteristics of selected polyethylene glycol suppositories. *J. Pharm. Sci.*, 1973; 62(2): 242-245.
10. Roseman T.J., Derr G.R., Nelson K.G., Lieberman B.L., Butler S.S., Continuous flow bead-bed dissolution apparatus for suppositories. *J. Pharm. Sci.*, 1981; 70(6): 646-651.
11. Akala E.O., Adedoyin A., Ogunbona F.A., Suppository formulations of amodiaquine - *in vitro* release characteristics. *Drug. Dev. Ind. Pharm.*, 1991; 17(2): 303-307.
12. Palmieri A., Suppository dissolution testing: apparatus design and release of aspirin. *Drug Dev. Ind. Pharm.*, 1981; 7(2): 247-259.

-
13. Baichwal M.R., Lohit T.V., Medicament release from fatty suppository bases. *J. Pharm. Pharmacol.*, 1970; 22(6): 427-32.
 14. Draganescu D., Popa D.E., Lupuleasa D., Burcea Dragomiroiu G.T.A., Miron D.S., Rosca A.C., Chivu R.D., The *in vitro* release mechanism of ibuprofen from conventional and special dosage forms. *Farmacia*, 2013; 61(4): 811-819.
 15. Tukker J.J., de Blaey C.J., Charbon G.A., Rectal motility and bioavailability. *Pharm. Res.*, 1984; 1(4): 173-178.
 16. de Blaey C.J., Fokkens J.G., Drug release from suppositories. *Pharm. Res.*, 1985; 2(2): 61-64.