Dissolution Profile of Nimesulide from Pharmaceutical Preparations for Oral Use

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Abstract

Nimesulide belongs to the group of semi-selective COX-2 inhibitors, widely used in solid oral formulations. In the present work, we studied the influence of surfactants among other drug excipients, as well as particle size of the active substance and the effects of medium pH on the dissolution profile of nimesulide from solid pharmaceutical forms. For that purpose, four different preparations containing 100 mg nimesulide *per* tablet and available on the market of Bosnia and Herzegovina (labeled here as A, B, C and D) were studied. The test for the assessment of dissolution profiles of the formulations was performed in surfactant-free dissolution medium pH 7.5. The dissolution profiles were compared by calculating difference (f1) and similarity (f2) factors. Increasing of the dissolution medium pH value from 7.5 to 7.75 resulted in a significant increase of nimesulide dissolution profile from the examined formulations. Also, the results showed that particle size affects to a great extent the dissolution profile and the best results were achieved with micronized nimesulide. The presence of the surfactants among the other excipients expressed a negligible effect on the dissolution profile.

Keywords: Nimesulide, dissolution profile, bioavailability

1. Introduction

Nimesulide belongs to the group of semiselective COX-2 inhibitors. It is applied after surgical and posttraumatic conditions, as a pain killer in cancer patients and in numerous pathological conditions (thrombophlebitis, dysmenorrhoea, fever and inflammations accompanied by respiratory and otolaryngological diseases).[1]

After oral application of a pharmaceutical in solid form, absorption rate of active substance through biomembranes is controlled by the slowest phase of the absorption process, most frequently just by the dissolution phase itself. Dissolution profile of an active substance contained in a drug and determining its bioavailability and thus its pharmacological action is influenced by numerous factors (*e.g.* physico-chemical properties of active substance itself, particle size and surface area, crystalline form, degree and disintergation rate of the pharmaceutical, additional ingredients and experimental approach employed for determination of dissolution profile).[2]

Solubility of an active substance represents a factor strongly influencing dissolution profile, *i.e.* increased solubility results in a higher dissolution profile. Frequently, absorption rate of a pharmacologically active substance is determined by its dissolution profile. On the other hand, dissolution profile can be directly correlated with efficiency of a pharmaceutical that may be the reason for the differences in bioavailability of different drug formulations containing the same active substance. Solubility of weak organic acids/bases depends on pH of the solvent medium and this fact explains the differences in the degree of dissolution profile in different parts of gastrointestinal tract (GIT).[3]

Since the amount of *in vivo* dissolved substance depends on both the dose and volume of the liquid taken with it, the dose number D_0 representing the basis for classification of pharmacologically active substances in Biopharmaceutical Classification System (BCS) as slightly and highly soluble ones can be calculated according to equation 1:

$$D_0 = M_0/V_0 * C_0 \quad ^{4,5} \qquad\qquad (1)$$

(M_0 – maximum therapeutic dose of an active substance; V_0 – volume of the liquid taken with the drug, usually a glass of water, *i.e.* 250 ml and C_0 – minimum solubility within physiological pH range). A medicinal substance is considered highly soluble when D_0 is less than unity and slightly soluble when D_0 is over unity.[4,5] Classification based on solubility means the highest dose effect of the drug applied.

In accordance with the Food and Drug Administration (FDA) directive, dissolution profiles are determined in water media within the pH range from 1.0 to 7.5, or from 1.0 to 6.8 as recommended by the European Medicines Agency (EMA).[6,7] Solubility of pharmacologically active substances is considered to be satisfactory if the highest therapeutic dose can be completely dissolved in 250 ml or less of water or liquid medium within these pH ranges.[6,7]

Solubility of nimesulide was studied in media of different pH values ranging from 4.5 to 11.0. At pH < 4.0, the solubility curve demonstrated a slight solubility of nimesulide. This part of the curve corresponds to nimesulide natural molecular form log S_0, while the curve obtained at pH above 9.0 corresponds to anionic form of the molecule – log Si. Experimental curve obtained by regression analysis of nimesulide solubility showed linearity and a slope from pH values 6.4 to 7.4.[8] By its chemical structure nimesulide (4-nitro-2-phenoxymethanesulfonanilide), a derivative of *p*-nitrophenyl-methanesulfonamide represents a monosubstituted sulfonamide (Fig. 1).
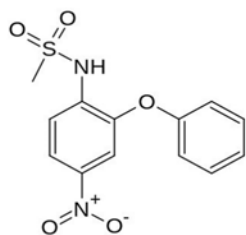

Figure 1. Structual formula of nimesulide

It is officinal according to Pharmacopoeia Europea 7, and appears as yellowish odorless powder soluble in acetone and almost completely insoluble in water.[9] The data from the available literature showed that its acidity constant (pK_a) ranges from 5.9 to 6.5. Nimesulide hydrosolubility of 0.01 mg/ml strongly depends on pH value of the medium.[10] Nimesulide molecule contains no assymetrical carbon atoms and lacks optical activity, as well as potential for chirality and isomery. It has been reported earlier that solubility of this drug under physiological pH range varies from 0.0078 mg/ml at pH 1.2 to 0.0829 mg/ml at pH 7.4.[1] Nimesulide has two polymorphic forms I and II, but only Form I is commercially available, due to

instability of Form II.[11] Both polymorphic forms of nimesulide were found to have very similar IR spectra and this was an unexpected result since the conformation of these two crystal structures differs.[8,12]

In the present study, dissolution profiles of nimesulide contained in the dose of 100 mg in four formulations of pharmaceutical preparations for oral use available on the market of Bosnia and Herzegovina and representing generic drugs were determined. The examinations described here were investigating the effects of different pH values of the dissolution medium (within the range from 7.5 to 7.75) and the influence of nimesulide particle size and the presence of surfactants among excipients on nimesulide dissolution profiles from above mentioned solid formulations of this pharmacologically active substance.

Content of nimesulide was measured by RP-HPLC method. The developed HPLC method is sensitive, simple, rapid and accurate. Early the method was successful used for monitoring the stability of nimesulide in different solid pharmaceutical dosage forms.[13] In this manuscript we showed successful use of this method for determination of dissolution profile of different solid pharmaceutical dosage forms of nimesulide.

2. Experimental

2. 1. Chemical and Reagents

Standards of nimesulide were obtained from EDQM, Council of Europe BP 907-F67029 Strassbourg CEDEX 1, Batch/Lot no. 1a.

TEA and formic acid (98–100%) of analytical purity were Merck (Darmstadt, Germany) products.

HPLC gradient-grade acetonitrile was supplied by Merck (Darmstadt, Germany).

Water for chromatography was obtained from Millipore Simplicity 185 purification system (Billerica, Massachusetts, USA).

All other reagents were of purity grade suitable for high-performance liquid chromatography.

Investigated nimesulid solid pharmaceutical dosage forms containing 100 mg nimesulide were formulated by the following pharmaceutical companies:
– By ReplekPharm (FYR Macedonia) (*VENTOR*®-labeled as formulation A, containing: dioctyl sodium sulfosuccinate as anionic surfactant (1.5 mg/tablet), hydroxypropylcellulose (0.80 mg/tablet), lactose monohydrate (153.70 mg/tablet), microcrystalline cellulose (100.00 mg/tablet), hydrogenated castor oil (8.00 mg/tablet), magnesium stearate (0.50 mg/tablet), and sodium starch glycolate (35.00 mg/tablet), as excipients; batch number 4847);
– By Actavis (Serbia) (*ACTASULID*®-labeled as formulation B, containing: lactose (80 mg/tablet), maize starch (46.32 mg/tablet), povidone K30 (9.40 mg/tablet), mag-

nesium stearate (3.60 mg/tablet) and colloidal anhydrous silica (0.68 mg/tablet) as excipients; batch number: 1206592)

– By Panacea Biotec (India) (*NIMULID*®-labeled as formulation C, containing: lactose monohydrate (151.5 mg/tablet), crosscarmelose sodium (7.50 mg/tablet), colloidal anhydrous silica (6.00 mg/tablet), maize starch (37.60 mg/tablet), povidone K30 (8.50 mg/tablet), sodium-docusate as anionic surfactant (6.80 mg/tablet), and polysorbate 80 (1.00 mg/tablet) as excipients; batch number 1932501);

– By Berlin-Chemie AG (Germany) (*NIMESIL*®-labeled as formulation D, containing: macrogol cetostearyl ether as non-ionic surfactant (8.00 mg/tablet), saccharose (1805.00 mg/tablet), maltodextrin (15.00 mg/tablet), citric acid anhydrous (30.00 mg/tablet), orange flavor (42.00 mg/tablet) as excipients; batch number 21193).

Particle size of nimesulide in the examined formulations varied from 10 μm to 15 μm (formulation C and D) and from 4 μm to 7 μm of micronized nimesulide (formulation A and B).

2. 2. Instrumentation and Chromatographic Conditions

Nimesulide content was determined by a HPLC/UFLC Shimadzu chromatograph, SPD-M20A prominence, CTO-20AC prominence oven, serial No. Shimadzu USA MPG INC. L202348 74875, with a diode array detector and 5 μL loop injector. Compounds were separeted using an Agilent Zorbax Extend C18 column (150 × 4.6 mm), particle size 5 μm (a part number 773450-902, packing lot B11159). Mobile phase and the solutions were degassed and vacuum filtered through 0,45 μm nylon membranes (Alltech Associates, Loceren, Belgium) before the use.

The mobile phase consisted of acetonitrile – TEA – water (45 : 0.5 : 54.5 *v/v/v*), and pH adjusted to 5.2 with formic acid. Isocratic elution was performed at a flow rate of 1.0 mL/min and UV detection at 302 nm. Before each injection, the column was equilibrated to stable baseline at a flow rate of 1.0 mL/min and temperature 40 °C.

Dissolution profile of nimesulide was determined by an Erweka DT 800 apparatus with an automatic sampling device 114650.1BBC. The other equipment included a Metrohm 827 pH LAB pH-meter, a model USK-RO-VEP ultrasound bath (EI Niš, Serbia), a Sartorius analytical balance (Sartorius AG Göttingen, Germany) and a Whatman 47 mm system for water filtration (a glass-mesh membrane carrier and 0.45 μm membrane filters).

Nimesulide dissolution profiles were examined in water media (6.8 g Na-monophosphate were dissolved in 900 ml purified water and 1.84 g NaOH were added). The pH values of the resulting solutions were adjusted to pH 7.5, 7.75, or 8.0 with 2 M NaOH or 2 M HCl solution and the volume adjusted to 1000 ml. The examinations were performed at 37 ° ± 0.5 °C with constant stirring (100 r.p.m.), for 60 min at time points of 10, 15, 25, 45 and 60 min.

The results of nimesulide dissolution profiles in the four formulations were compared using difference (f1) and similarity (f2) factors. The former factor is proportional to the mean difference of two dissolution profiles as shown by the equation 2:

$$f_1 = \{[\textstyle\sum_{t=1}^{n} |R_t - T_t|] / [\textstyle\sum_{t=1}^{n} R_t]\} * 100 \qquad (2)$$

(n – number of time points; R_t – cumulative value of dissolution profile for drug R in t time and T_t – cumulative value of dissolution profile for drug T in t time). Differences between the resulting curves that would indicate similarity in dissolution of an active substance in two different formulations should have f1 close to zero (0–15).

Similarity factor (f2) that is inversely proportional to square difference of two profiles with great differences for each time point and representing the measure of similarity of the two profiles is given by the equation 3:

$$f_2 = 50 * \log \{[1+(1/n) \textstyle\sum_{t=1}^{n} (R_t - T_t)^2]^{-0.5} * 100\} \quad (3)$$

The values of f2 should exceed 50 (50–100) to consider dissolution profiles of an active substance in two different formulations similar. In the case of two identical profiles f2 makes 100 and when average difference between the profiles is 10%, f2 is 50.[14,15]

The samples used to determining dissolution profiles were filtered (Whatman filter paper No 1) rejecting first 5.0 ml of the filtrate. Calibration curve was constructed for five experimental points using diluted stock nimesulide solution. Time intervals for linearity examinations were 12–120% of declared nimesulide concentration. Nimesulide stock solution contained 1.2 mg/mL. Concentration of nimesulide standard in working solution was 0.12 mg/mL.

3. Results and Discussion

Nimesulide has two absorption spectra maxima at 240 nm and 301 nm (Figure 2 and 3). Due to the interference of triethanolamine (TEA) present in mobile phase (cut off for TEA occurs at 235 nm) with nimesulide, the absorption peak at 240 nm was unclear and the detection was performed at 301 nm (peak index 0.99, limit value of each point 0.99, index of minimum peak purity 1241) (Figure 2 and Figure 3).

The curve was constructed using the statistics of the least squares line fit to the data. Statistical data confirming a satisfactory linearity are presented in Table 1:

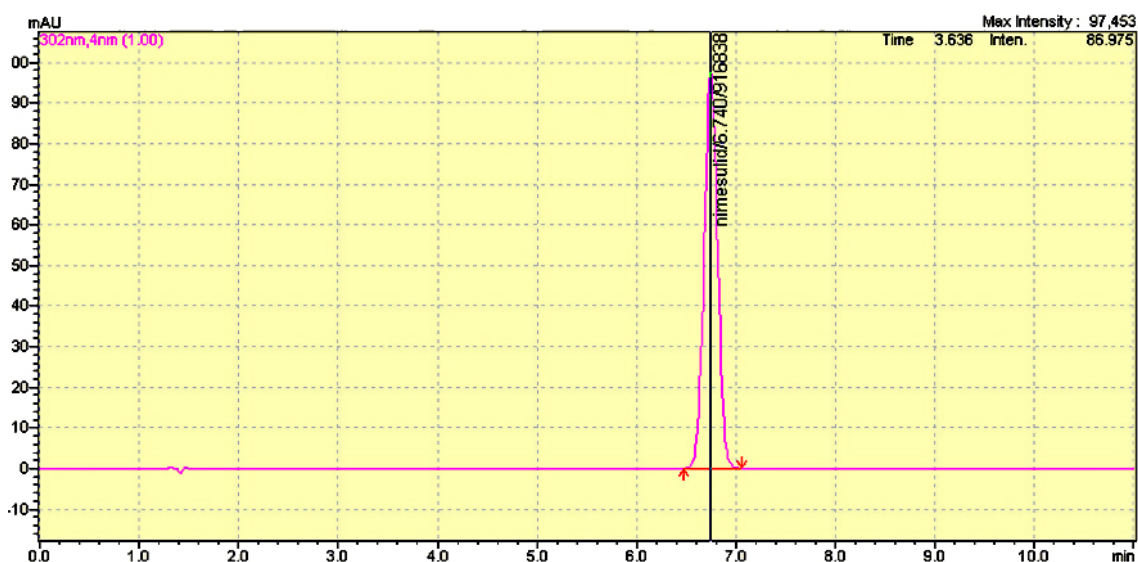**Figure 2.** Absorption spectra of nimesulide (maxima at 240 nm and 301 nm)

Figure 3. Chromatogram of nimesulide

Table 1. Parameters of the regression analysis.

Concentration range	0.012 – 0.120 mg/ml	
Regression equation	y =((0.000000141*x) – 0.000693406)	
Statistical parameters	r = 0.9999	r² = 99.98%
	t = 2.35	t$_{tab}$ < 2.44
	Sa = 23.39	Sb = 225.05

Sa – standard deviation intercept; Sb – standard deviation of the slope; *r* – correlation coefficient

Calculated dose nimesulide number of 51.3 demonstrated a slight solubility of this compound. Nimesulide belongs to II BCS class, corresponding to good permeability and low solubility.

The results on dissolution profile of nimesulide from pharmaceutical preparation A under experimental conditions applied and in three media of different pH are summarized in Figure 4, and clearly illustrate a strong influence of medium acidity on this parameter.

Each data point represents the mean ± RSD of 3 measurements.

The results of nimesulide dissolution profile in all four analyzed pharmaceuticals at pH 7.5 are listed in Figure 5.

Each data point represents the mean±RSD of 3 measurements.

The highest values were obtained with the formulation B, and factors of difference and similarity (f1 and f2, respectively) of the remaining formulations were calculated in relation to formulation B.
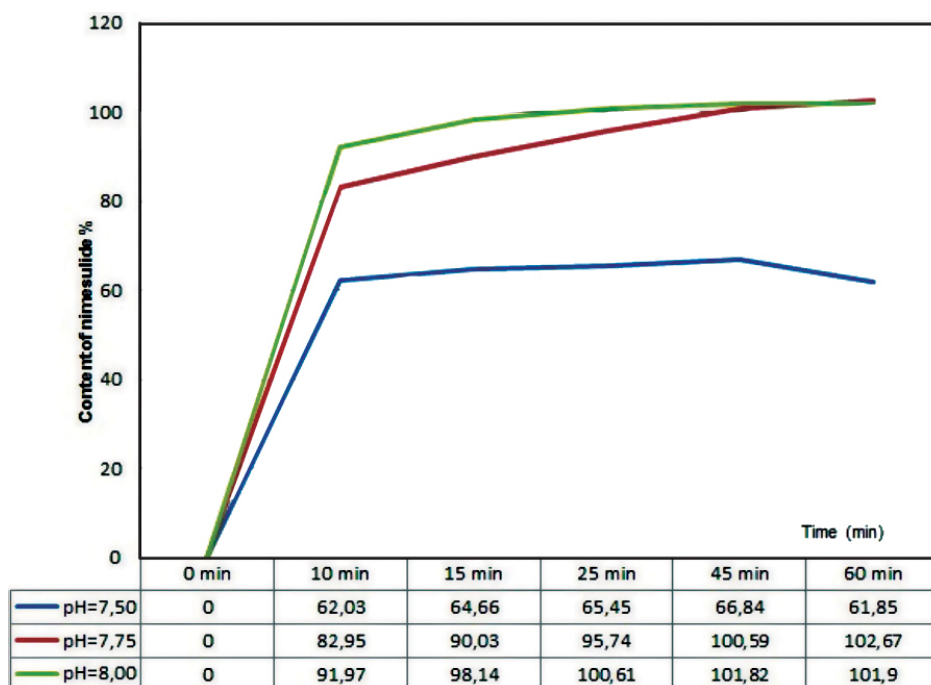
Figure 4. Dependence of nimesulide dissolution profiles from pharmaceutical preparation A on the pH 7.5 of the dissolution

	0 min	10 min	15 min	25 min	45 min	60 min
pH=7,50	0	62,03	64,66	65,45	66,84	61,85
pH=7,75	0	82,95	90,03	95,74	100,59	102,67
pH=8,00	0	91,97	98,14	100,61	101,82	101,9

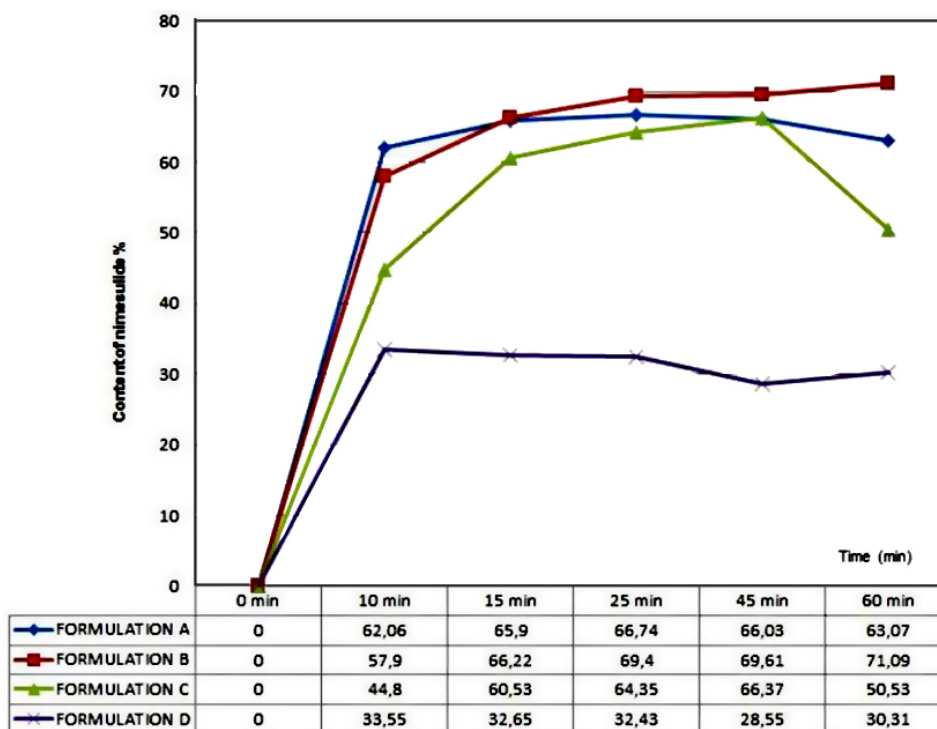|	0 min	10 min	15 min	25 min	45 min	60 min
FORMULATION A	0	62,06	65,9	66,74	66,03	63,07
FORMULATION B	0	57,9	66,22	69,4	69,61	71,09
FORMULATION C	0	44,8	60,53	64,35	66,37	50,53
FORMULATION D	0	33,55	32,65	32,43	28,55	30,31

Figure 5. Nimesulide dissolution profiles from the examined formulations at pH 7.5

The formulation A had satisfactory similarity factor/difference factor in relation to reference formulation B, the former being f2 = 66.8 and the latter f1 = 5.6. On the contrary, f1 of 14.3 in formulation C was satisfactory in relation to reference formulation B, while f2 of 46.9 was at the upper limit of acceptability. Similarly, formulation D with f2 of 22.3 and f1 of 52.9 was unsatisfactory.

So far, specifications for nimesulide dissolution profiles from solid pharmaceutical formulations have been unavailable in any of current Pharmacopoeas. Based on the dose number obtained throughout the present study (D_0 = 51.3), it can be concluded that nimesulide is slightly soluble and this is a limiting factor for its dissolution (both the extent and the rate) from the examined formulations. Dissolution profiles in media of different acidity (pH 4.5–11.0) showed that nimesulide solubility decreases parralel to pH decrease, *i.e.* solubility of this substance was increasing parralel to pH increase and the best results were obtained at pH 7.4. This is in agreement with the findings of the other authors.[8] Also, dissolution profile of nimesulide displayed the same trend and this parameter was increasing parralel to pH increase to provide the best results at pH 7.2.[16] Producers of the nimesulide-containing pharmaceuticals examined here declared that the assays for determining nimesulide dissolution profiles were performed at pH 8.0 and this is the highest permitted value in the regulatory sense.[8] It has been reported earlier that surfactants such as polysorbate and sodium lauryl sulphate, as well as co-solvents (polyethylene glycol – PEG and dimethylsulfoxide – DMS) present in dissolution medium acted by strongly increasing nimesulide solubility.[17] However, there is no data on the influence of surfactants on nimesulide dissolution profile when these substances were present together with other excipients in ready-to-use pharmaceuticals. So, the discrepancy of the results presented here and previous data of the others[17] could be ascribed to interference of additional ingredients with surfactants in pharmaceuticals examined throughout this work.

pH of body fluids in GIT sites of pharmacologically active substances absorption varies from pH 1.2 (gastric juice) to pH 7.8 (terminal GIT parts). After oral application, 40% of nimesulide are absorbed in stomach and proximal intestine part, 50% in distal intestine part and a low amount in colon.[11] Due to its high permeability, nimesulide absorption is quite saisfactory, but its slight solubility represents a limiting factor in its bioavailability. Since absorption of this drug mostly proceeds in distal GIT part at pH of about 7.5, comparative examinations of nimesulide dissolution profiles presented in this work were done at this pH value of the medium. The results demonstrated a strong influence of pH of the medium on dissolution profile and a conspicuous difference of this parameter was observed at pH 7.5 and that at pH 7.75. However, further increase of medium pH to 8.0 led to insignificant change in dissolution profile.

Formulation A contains micronized nimesulide crystals and also a surfactant among additional ingredients. Our results obtained with this formulation pointed to a very fast achievement of a maximum dissolution profile, already within 15 min when saturation was reached and somewhat later a negligible decrease of nimesulide content was recorded. Formulation B also contains micronized nimesulide crystals, but contains no surfactant among excipients. As a result, dissolution of nimesulide proceeded gradually to reach maximum at 60 min time point. Formulation C has no micronized crystals but contains two surfactants. Nimesulide dissolution profile, *i.e.* content of dissolved nimesulide from this formulation during the time course of our experiments was lower comparing to those obtained with formulations A and B. These results led to the conclusion on stronger effect of drug particle size than the presence, *i.e.* the absence of surfactants. It is also worth mentioning that an abrupt decrease of nimesulide content at the end of the experimental time course was recorded. The reason for this phenomenon is unclear at the moment and calls for further examinations. When preparation D was examined, a very low dissolution profile of nimesulide was observed. This formulation contains both non-micronized nimesulide crystals and a non-ionic surfactant macrogol cetostearyl ether.

The results of examinations on nimesulide dissolution profiles from four different pharmaceutical formulations were comparatively studied by determining similarity and difference factors. The data obtained throughout the present study clearly demonstrated the effect of the pharmacologically active substance particle size on dissolution profile and thus its bioavailability. So, better results were achieved with the formulations containing micronized nimesulide crystals. In addition, particle size expressed much stronger effect on nimesulide dissolution profile than the presence/absence of surfactants in the formulations.

4. Conclusions

During production of pharmaceuticals a great attention has been always paid to active substance particle size and to attain their optimum solubility some additional ingredients are added for that purpose. Acidity changes of a medium for determination of dissolution profile are strictly defined by current regulations and in accordance with these regulations extreme pH values for examinations of drug solubility. *i.e.* dissolution profiles are not recommended.

Dissolution profile of nimesulide depends more on particle size than on the presence/absence of surfactants among excipients. Also, acidity of the medium used for measurements of dissolution profile of nimesulide expressed a strong effect. Our results clearly pointed to the need for further studies to justify employment of experimental media with pH values above 7.5 for examinations of dissolution profiles of pharmaceuticals.

5. References

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Povzetek

Nimesulid spada v skupino semi-selektivnih COX-2 inhibitorjev in se široko uporablja v trdnih oralnih farmacevtskih oblikah. V predstavljenem delu smo preučevali vpliv prisotnosti surfaktantov med pomožnimi substancami, pa tudi velikost delcev aktivne substance in učinek pH medija na profil raztapljanja nimesulida iz trdnih farmacevtskih oblik. V ta namen smo preučevali štiri različne pripravke, ki vsebujejo 100 mg nimesulida na tableto in so tržno dostopni v Bosni in Hercegovini (označeni kot A, B, C in D). Testiranje za oceno profilov raztapljanja formulacij smo izvajali v mediju za raztapljanje brez dodanih surfaktantov in s pH 7,5. Profile raztapljanja smo primerjali z izračunom faktorjev različnosti (f1) in podobnosti (f2). Povišanje pH medija za raztapljanje s 7,5 na 7,75 je povzročilo znatno povečanje profila raztapljanja nimesulida iz preiskovanih formulacij. Rezultati so tudi pokazali, da velikost delcev v veliki meri vpliva na profil raztapljanja. Najboljše rezultate smo dobili z mikroniziranim nimesulidom. Prisotnost surfaktantov med ostalimi pomožnimi substancami je imela zanemarljiv učinek na profil raztapljanja.