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DISSOLUTION KINETICS STUDIES OF CLOPIDOGREL FROM SELECTED MULTISOURCE COATED TABLETS WITH APPLICATION OF CAPILLARY ZONE ELECTROPHORESIS METHOD

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Abstract: Resistance to an anti-platelet agent clopidogrel (CLP) and the growing number of products with the drug cause the need for comparison of their quality to assure patients safe and effective treatment. Therefore, the aim of the study was to compare *in vitro* dissolution kinetics of CLP immediate-release tablets, commonly used in anti-platelet therapy in Poland. For analysis of CLP in samples obtained from dissolution test a capillary zone electrophoresis (CZE) method was elaborated and validated. Separation of CLP and ticlopidine, used as an internal standard, was performed in silica capillary filled with phosphate buffer of pH 2.5, at the applied voltage of 20 kV. The CZE method fulfilled the validation requirements for determination of drugs in pharmaceutical matrices and was successfully applied for analysis of CLP dissolved from the tablets. Dissolution profiles were prepared for each product and mean dose fractions of CLP dissolved from tablets at 30 min were calculated. Kinetic parameters of the CLP dissolution from the studied products were compared. Analysis of variance (ANOVA) did not reveal differences between CLP fractions dissolved at 30 min time point from the tested drug products. However, ANOVA with Tukey multiple comparison test revealed significant differences in first-order dissolution rate constants and $t_{0.5}$ values (times at which 50% of drug is dissolved) of CLP among tested tablets. It was concluded that the studied CLP products met the acceptance criteria regarding dissolution test but differed with each other in dissolution kinetics.

Keywords: capillary electrophoresis, dissolution test, clopidogrel, pharmaceutical formulations, validation

Dissolution test is considered as one of the most important quality control procedures performed on pharmaceutical dosage forms to check batch-to-batch consistency and compliance with specifications, to determine the long-term stability and shelf life of a dosage form, and to assess the impact of post-approval changes in the formulation and manufacturing process. Besides, dissolution performance may provide valuable information when comparing different formulations and/or when identifying product and process quality critical attributes (1). In the case of immediate release oral solid dosage forms such as tablets, containing active pharmaceutical ingredients (APIs) poorly soluble but highly permeable across gastrointestinal membrane, dissolution tests can be prognostic of *in vivo* performance of such drug products (2, 3).

The analysis of dissolution test samples is usually carried out using UV spectrophotometry. However, simple UV measurements are often insufficient when analyzing dosage forms containing excipients that are strongly UV active or when products containing two or more active ingredients are being analyzed. Also separation and determination of enantiomers in formulations containing chiral drugs often demands more sophisticated analyzing tools. Therefore, separation techniques, such as HPLC or HPCE, offer an attractive alternative to the standard methods for determination of APIs in pharmaceutical dosage forms after their dissolution test. The main advantages of HPCE over HPLC are high efficiency and resolution, high speed of separation and the low solvent consumption. In recent years, the HPCE methods have been widely applied for analysis of drugs and their impurities in pharmaceutical formulations. El-Sabawi et al. elaborated a capillary electrophoresis (CE) method with electrokinetic injection for determination of amoxicillin

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released from capsules (4). There are also reports concerning an application of CE for determination of active ingredients in formulations with rimantadine hydrochloride (5), acetaminophen, phenylephrine and chlorpheniramine (6) and many others.

Clopidogrel (CLP), an anti-platelet and anti-thrombotic agent with an absolute S configuration at carbon 7 (7), is used worldwide for the long term prevention of atherothrombotic events. According to Biopharmaceutical Classification System (BCS), which is based on aqueous solubility and intestinal permeability, CLP is classified to the second class (II) of drugs – low soluble and highly permeable (8). Many clinical studies have shown that even 40% of patients treated with conventional doses of CLP do not display adequate antiplatelet response, which may lead to serious cardiovascular complications including stent thrombosis, myocardial infarction, stroke and death (9). Therefore, an introduction of the growing number of products with CLP into an anti-platelet therapy causes the need for comparison of their quality to assure patients safe and effective treatment. CLP is inactive pro-drug and hepatic activation by cytochrome P450 isoenzymes is necessary to induce expression of its anti-aggregating properties. In the biotransformation process, a small fraction of CLP is converted into the intermediate metabolite 2-oxo-clopidogrel, which is subsequently hydrolyzed to isomers of thiol metabolite (CTM). In patients' plasma, CTM is present as two isomers (H3 and H4) (10, 11) but only H4 is considered as pharmacologically active (11, 12). This compound reacts with the thiol of an amino acid of the platelet receptor and causes an irreversible blockade of ADP binding for the platelet's life span. The major metabolite of CLP is inactive carboxylic acid derivative of CLP, which represents 85% of circulating drug-related compounds in plasma (13) and is also regarded as one of the impurities present in pharmaceutical products of CLP (14).

According to USP 32, spectrophotometric method is encouraged for determination of CLP in dissolution test and HPLC should be used for analysis of drug impurities (14). Until now, only few methods for the determination of CLP in pharmaceutical formulations have been presented in the literature. Mitakos and Panderi reported a non-stereospecific HPLC method with UV detection for the determination of CLP in oral dosage forms. The method was applied to degradation studies under stress conditions (15). Moreover, a validated HPLC-UV method was reported for determination of CLP and its impurities in 19 drug products present on Asian and South American market (16). Recently, capillary electrophoresis method was presented for separation and determination of CLP and its impurities in commercial bulk samples (17) as well as for separation and determination of CLP and its metabolite in serum samples (18). However, up to now, capillary electrophoresis assay for the determination of CLP in samples obtained from dissolution test has not been published. Moreover, there is no data regarding kinetic parameters of dissolution process of CLP from the different drug products.

Therefore, the aim of this study was to compare the *in vitro* dissolution kinetics of CLP from the selected drug products commonly used in antiplatelet therapy in Poland. The usefulness of the val-

Table 1. Examined CLP coated tablets.

	Product[a]	Lot No.	Pharmaceutical company
1	Areplex®	DM045108	ADAMED Sp. z o.o. (Poland)
2	Clopidix®	081008	LEK-AM Sp. z o.o. (Poland)
3	Clopidogrel GSK®	F21162	GlaxoSmithKline Export Ltd. (UK)
4	Clopidogrel Teva®	C97036	Teva Pharma B.V. (Netherlands)
5	Clopinovo®	1101395	ARTI FARMA Sp. z o.o. (Poland)
6	Egitromb®	5041A0111	EGIS PHARMACEUTICALS PLC (Hungary)
7	**Plavix®**	3Y050	Sanofi (France)
8	Plavocorin®	CJ0903	Sandoz (Austria)
9	Trombex®	3570810	Zentiva (Czech Republic)
10	Zyllt®	P12484	KRKA Polska Sp. z o.o. (Poland)

[a] – originator product is marked in bold.

idated CZE method for determination of CLP in samples obtained from tablets dissolution tests was also examined.

MATERIALS AND METHODS

Materials

(+)-S-clopidogrel bisulfate (optical purity, o.p. 99.0%) was a generous gift of Pharmaceutical Research Institute (Warszawa, Poland). Ticlopidine hydrochloride (TCLP, IS) were purchased from Sigma-Aldrich (Steinheim, Germany). Hydrochloric acid 37–38% and potassium chloride were from POCH S.A. (Gliwice, Poland). NaOH (1.0 M and 0.1 M), water for HPCE and 50 mM phosphate buffer of pH 2.5 for HPCE (Agilent Technologies, Waldbroon, Germany) were used. Methanol (J.T. Baker, Deventer, The Netherlands) was of HPLC grade. Demineralized water (Simplicity UV, Millipore, USA) was always used to prepare buffer applied in dissolution test. The original product (Plavix®) and 9 generic products of CLP in the form of coated tablets containing 75 mg of the drug were included in the study. The commercial names and manufacturers are listed in Table 1.

Dissolution test

The *in vitro* dissolution studies were performed based on USP 32 monograph "Clopidogrel Tablets" method (14) on ten formulations of CLP tablets using USP apparatus 2 (DT60 dissolution tester, Erweka, Heusenstamm, Germany). The labeled amount of drug substance for each brand was the same (75 mg). The dissolution medium consisted of 1000 mL of a pH 2.0 hydrochloric acid buffer maintained at 37 ± 0.5°C. The buffer was prepared according to USP monograph "Buffer solutions" (14) by mixing 250 mL of 0.2 M potassium chloride solution with 65 mL of 0.2 M hydrochloric acid solution and further dilution with deionized water to 1000 mL. The buffer was filtered (0.45 µm regenerated cellulose membrane filter) by drawing a vacuum and deaerated by ultrasonic sound waves. The rotation speed of the paddles was set at 50 rpm. Samples (5 mL) were withdrawn at 5, 10, 15, 20 and 30 min by a syringe using an Erweka manual sampling manifold equipped with a tip filter with a porosity of 1 µm. After withdrawal of the sample, fresh dissolution medium was simultaneously replaced in the vessel to maintain a constant dissolution volume. The samples were then analyzed by the reference spectrophotometric method and HPCE method. Based on the acceptance criteria of the USP for CLP tablets (14), not less than 80% of the labeled amount of CLP should dissolve in 30 min. Subsequently, at the first stage of the test (S_1) the percentage of CLP dissolved for each of the six tablets examined might not be less than 85% of the theoretical drug content. If the tablets did not pass the test, another 6 units were examined. At the second stage (S_2) the acceptance criteria for 12 units implied the average content ≥ 85% and each unit content ≥ 65%. The third stage (S_3) with testing of another 12 tablets did not have to be performed.

Equipment and conditions for CE and spectrophotometric analysis

Analytes were determined on an Agilent model ^{3D}CE apparatus (Agilent Technologies, Waldbronn, Germany) with UV detector set at λ = 200 nm. The samples were automatically injected using hydrodynamic injection at the anode. The temperature of the capillary was maintained by a thermostatic system at 25°C. The separation was performed in a fused silica capillary, 35 cm × 50 mm i.d., 26.5 cm to the detector. The system was controlled by ChemStation software. All experiments were carried at the 20 kV and 50 × 5 mbar × s injection (12 nL injected volume). The volume of a sample loaded to capillary was calculated using Hagen-Poiseuille equation (19). BGE was composed of commercially available 50 mM phosphate buffer of pH 2.5 and water for HPCE (1 : 1, v/v). A new capillary was conditioned with 1.0 M sodium hydroxide, subsequently 0.1 M sodium hydroxide, water for HPCE and finally with BGE for 10, 10, 5 and 8 min, respectively. Before each run the capillary was rinsed out with 0.1 M sodium hydroxide, water for HPCE and BGE for 2, 5 and 5 min, respectively.

In spectrophotometric analysis the amount of CLP in filtered medium samples obtained from dissolution test was determined by employing spectrophotometer Spekol UV-Vis (Analytic Jena AG, Jena, Germany) at a wavelength of 200 nm.

Sample preparation for calibration curves

CLP and IS stock solutions of 1.0 g/L were prepared in methanol. Then, standard solutions of 5.0, 10.0, 20.0, 50.0, 75.0, 100.0, 120.0 and 150.0 mg/L CLP were prepared in a buffer of pH 2.0, used as dissolution medium in the dissolution test. Moreover, a solution of 50 mg/L IS in the above buffer was also prepared. For CE determination, the volume of 100 µL of the standard solution was transferred to a vial containing 100 µL of IS solution and injected into the capillary. For spectrophotometric method, samples with CLP were diluted with dissolution buffer (1 : 4, v/v) prior to analysis.

Preparation of the samples obtained from dissolution test

The sample of medium obtained from the dissolution test was passed through 0.45 μm filters to remove the particles present after dissolution. For CE determination, an aliquot of 100 μL of the sample was mixed with 100 μL of 50 mg/L IS solution. For spectrophotometric method, samples with CLP were diluted with dissolution buffer.

Validation parameters

Selectivity

Selectivity describes an ability of a method to discriminate the analyte among all potential interfering substances. To confirm selectivity of the elaborated CZE method, resolution of CLP and IS was performed in a buffer used as dissolution medium.

Linearity

Linearity of the calibration curve was estimated for the ratio of the peak area of CLP to IS as a function of the analyte concentration covering the range of 10–150 mg/L. The correlation coefficient r was calculated. Mandel's fitting test has been applied for the evaluation of the linearity of a straight line regression model, test value (TV) < F_{crit} means statistically non-significant differences (20).

LOD, LOQ, precision and accuracy

LOD of the analyte was determined as an S/N baseline ratio of 4 : 1. LOQ was defined as the lowest concentration of CLP determined by the method with the relative standard deviation (RSD) ≤ 2% of its nominal value.

Intra-day and inter-day precision of the method, expressed as %RSD, has been estimated for QC samples at concentrations of 20, 75 and 120 mg/L of CLP, prepared in six replicates analyzed over six different days. Accuracy was estimated for the same analyte concentrations as for evaluation of precision of the method and was expressed as relative error (%RE) equal to the percent relative difference between the mean determined concentration and the nominal concentration.

Stability

Stability of CLP in solutions, prepared in buffer used for dissolution test, was evaluated at concentrations of 20, 75 and 120 mg/L (in three replicates for each analyte concentration) after 24 h standing in autosampler and long-term storage for 3 months at –25 ± 3°C. The concentration of the analyte after each storage period was calculated using a calibration curve, obtained from freshly prepared samples in the same analytical run. The analyte was considered stable if the deviation from the nominal concentration was within ± 2%.

Determination of agreement between methods using Bland-Altman analysis

The spectrophotometric method was assumed as the reference method for determination of CLP in dissolution test. The degree of agreement between CE and spectrophotometric measurements was assessed by Bland-Altman analysis, which includes the use of a graphical method to plot the difference between two measurements against the mean for each sample. In the method, the 95% limits of agreement as the mean difference (± 2×SD) were evaluated for visual judgment of how well two methods of measurement agree. The smaller the range between these two limits, the better the agreement is (21).

Kinetic studies

To study the *in vitro* dissolution kinetics of CLP from the examined IR tablets, the obtained dissolution data were fitted to first-order model:

$$\%\text{diss} = 100[1 - e^{-kt}] \quad (1)$$

where %diss is the CLP percent dissolved at time t and k is dissolution rate constant.

The first-order model was fitted to six individual dissolution data (first stage of the dissolution test (S_1), CLP determined using CE assay) of each brand of CLP tablets with linear least-squares curve-fitting technique using DDSolver software (22). The first-order dissolution rate constants (k) and times at which 50% of drug is dissolved ($t_{0.5}$), were calculated (23, 24), and normal distribution of data was verified using the Shapiro-Wilk test. Next, the differences in kinetic parameters (k, $t_{0.5}$) were compared using a one-way analysis of variance (ANOVA) with Tukey *post hoc* multiple comparison test, at $\alpha = 0.05$. For all comparisons, $p \leq 0.05$ was considered to be statistically significant. Univariate statistical analyses were performed using the STATISTICA data analysis software system, version 10.0 (StatSoft, Inc., Tulsa, OK, USA). Unless stated otherwise, data are presented as the mean ± SD.

RESULTS

Validation of the CE method

The CE method was highly selective with no interferences from components of the buffer used in dissolution test or excipients from tablets (Fig. 1).

Standard curve estimated for CLP was linear in the range of concentrations 10–150 mg/L. Its equation and correlation coefficient are presented in

Table 2. The curve was determined as the mean of six calibration curves prepared for CLP. Statistical analysis using Mandel's test with results TV < F_{crit} confirmed linearity of the calibration curve. The curve was applied for the quantification of the analyte in samples obtained from dissolution test. In the worked out conditions, LOD at an S/N baseline ratio = 4 : 1 for CLP was found to be 5 mg/L and LOQ was 10 mg/L. Intra- and inter-day precision of the CE method, expressed as %RSD, was in the range of 0.8–1.9%, while intra- and inter-day accuracy of the method, expressed as %RE, was ≤ 2.0% (Table 2).

CLP proved to be stable in buffer solutions for 24 h standing in autosampler and for at least three months stored at -25°C, as demonstrated by %RE < 2%.

The validation parameters of the reference spectrophotometric method was also calculated and they comprise linearity in the range of CLP concentration 20–150 mg/L and %RSD and %RE ≤ 2.0%.

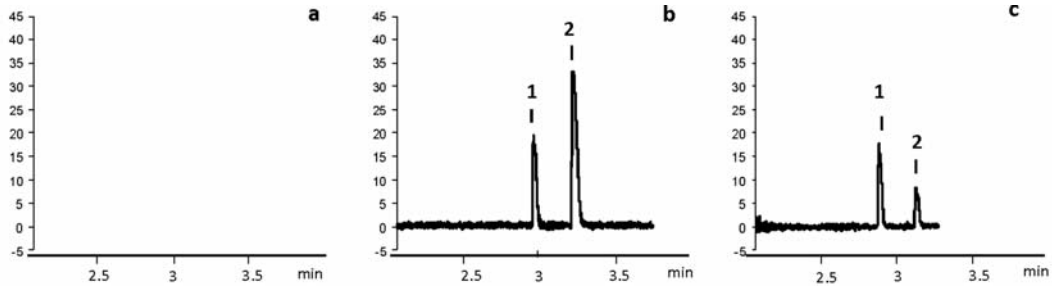

Figure 1. Electropherograms of: a – blank sample (dissolution buffer); b – sample of dissolution buffer spiked with 120 mg/L of CLP and 50 mg/L of IS; c – sample of dissolution medium after dissolution test spiked with 50 mg/L of IS (determined CLP concentration amounted to 27.3 mg/L). CE conditions: 0.025 M phosphate buffer of pH 2.5, 35 cm total length of fused silica capillary, 50 µm i.d., temperature 25°C, voltage 20 kV, current 30–35 µA. Peaks denoted: 1 – IS, 2 – CLP

Table 2. Validation parameters of the CE method for analysis of CLP in samples obtained from dissolution test.

Nominal concentration (mg/L)	Mean assayed value (mg/L)	Accuracy (%RE)	Precision (%RSD)
Quality Control Samples (QCS)			
	Intra-day repeatability (n = 6)		
20	19.8	1.0	1.9
75	73.9	1.5	1.3
120	119.0	0.8	1.4
Inter-day reproducibility (n = 6)			
20	19.9	0.5	1.2
75	75.1	0.1	1.8
120	120.0	0.0	0.9
Calibration curve			
Inter-day reproducibility (n = 6)			
10	10.2	2.0	0.8
20	20.3	1.5	1.2
50	49.2	1.6	1.3
75	74.1	1.2	1.8
100	98.6	1.4	1.1
120	119.0	0.8	1.5
150	151.0	0.7	1.0

Equation of calibration curve: $y = 0.019 \cdot x$, $r = 0.999$

Bland-Altman analysis

To assess the agreement of CE with the reference spectrophotometric method, CLP concentrations were determined using both assays in all samples obtained from dissolution test. Figure 2a presents a simple plot of the values of CLP concentration in samples determined by spectrophotometric method against those determined by CE. The data points fall near the line of equality, suggesting there is agreement between methods. Moreover, the high value of determination coefficient confirmed that the results of both methods are highly related. To assess the degree of agreement between spectrophotometric and CE measurement, the Bland-Altman plot was constructed (Fig. 2b). The x-axis represents the average concentrations of CLP determined with both methods while the y-axis expresses the difference between the values. The SD of the difference scores was used to calculate the 95% limits of agreement presented on the plot as the dotted horizontal lines. The methods were considered equivalent because 95.4% of the differences lie between the limits of agreement.

Dissolution test

According to the acceptance criteria from USP 32, all the studied products passed the dissolution test at the first (S_1) or at the second (S_2) stage of the study, releasing at least 80% of the declared drug content in 30 min. Dissolution profiles obtained for the studied products at the S_1 stage with CLP determined using CE assay are presented in Figure 3. The highest mean amount of CLP was dissolved from Plavocorin® tablets (92.0%) while the lowest from Clopidix® ones (83.5%). For the reference product (Plavix®), a total of 86.1% of the labeled CLP con-

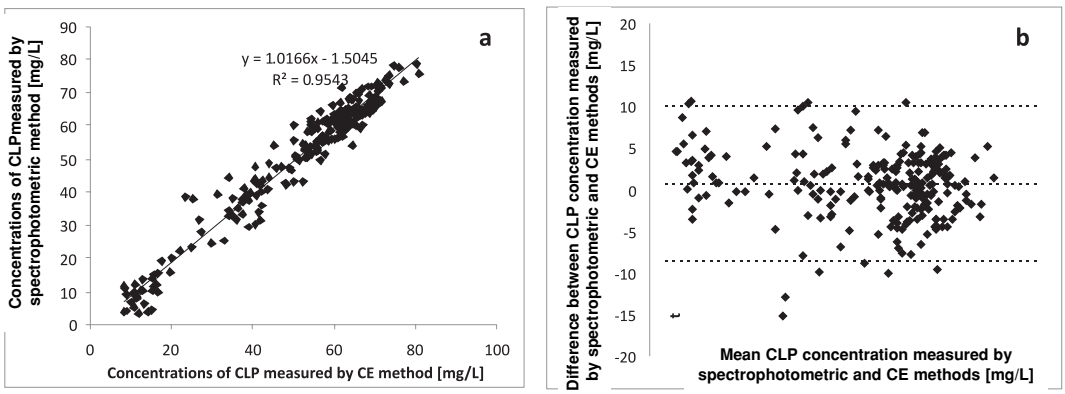

Figure 2. Plots for the Bland-Altman analysis. a – scatter plot of the CLP concentrations determined by UV spectrophotometric method versus those obtained by CE method; b – Bland-Altman plot of the difference between the CLP concentrations determined by UV spectrophotometric and CE methods against the mean of the concentrations

Table 3. Cumulative percentage of CLP dissolved from the tested tablets (mean ± SD, n = 6) at each time point.

Tablets (n = 6)[a]	CLP dissolved (%)[b]				
	5 min	10 min	15 min	20 min	30 min
Areplex®	21.19 ± 8.33	51.44 ± 8.09	68.07 ± 5.84	74.48 ± 5.80	83.80 ± 3.46
Clopidix®	17.23 ± 6.41	32.65 ± 6.89	51.61 ± 3.84	70.49 ± 10.15	83.49 ± 2.83
Clopidogrel GSK®	15.28 ± 3.81	70.76 ± 10.38	80.20 ± 4.05	84.10 ± 1.97	87.32 ± 2.16
Clopidogrel Teva®	42.10 ± 8.76	70.46 ± 3.98	73.75 ± 4.20	78.65 ± 4.55	83.27 ± 3.98
Clopinovo®	52.02 ± 5.12	64.98 ± 5.39	72.24 ± 5.46	79.83 ± 4.09	87.07 ± 3.93
Egitromb®	15.16 ± 3.06	58.45 ± 12.23	81.32 ± 8.91	84.95 ± 8.91	86.98 ± 8.93
Plavix®	36.64 ± 9.74	55.48 ± 2.75	72.49 ± 8.80	80.59 ± 10.73	86.12 ± 10.56
Plavocorin®	22.73 ± 3.74	55.53 ± 8.10	79.44 ± 4.85	87.02 ± 3.39	91.96 ± 1.83
Trombex®	19.20 ± 2.23	48.07 ± 7.09	68.35 ± 6.99	81.83 ± 4.61	89.01 ± 3.34
Zyllt®	20.48 ± 10.49	39.89 ± 9.40	61.37 ± 14.54	74.37 ± 9.93	87.47 ± 5.50

[a] - originator product is marked in bold, [b] - data taken from the first stage (S_1) of the dissolution test, CLP determined using CE assay.

Table 4. Kinetic study results of CLP dissolution from the examined tablets (k is the first-order dissolution rate constant; $t_{0.5}$ is time at which 50% of drug is dissolved: $R^2_{adjusted}$ is the adjusted coefficient of determination).

Tablets (n = 6)[a]	First-order dissolution rate parameters[b]		
	k [min^{-1}]	$t_{0.5}$ [min]	$R^2_{adjusted}$
Areplex®	0.068 ± 0.007	10.31 ± 1.06	0.9613 ± 0.0225
Clopidix®	0.051 ± 0.006	13.63 ± 1.58	0.9492 ± 0.0221
Clopidogrel GSK®	0.090 ± 0.009	7.82 ± 0.85	0.9039 ± 0.0153
Clopidogrel Teva®	0.096 ± 0.012	7.29 ± 0.99	0.9330 ± 0.0215
Clopinovo®	0.100 ± 0.016	7.04 ± 1.09	0.9322 ± 0.0390
Egitromb®	0.083 ± 0.019	8.65 ± 1.86	0.9177 ± 0.0197
Plavix®	0.082 ± 0.012	8.58 ± 1.27	0.9353 ± 0.0705
Plavocorin®	0.086 ± 0.010	8.13 ± 0.88	0.9625 ± 0.0124
Trombex®	0.071 ± 0.009	9.88 ± 1.35	0.9683 ± 0.0082
Zyllt®	0.063 ± 0.018	11.67 ± 2.86	0.9588 ± 0.0227

[a] - originator product is marked in bold, [b] - calculation based on data taken from the first stage (S_1) of the dissolution test, CLP determined using CE assay.

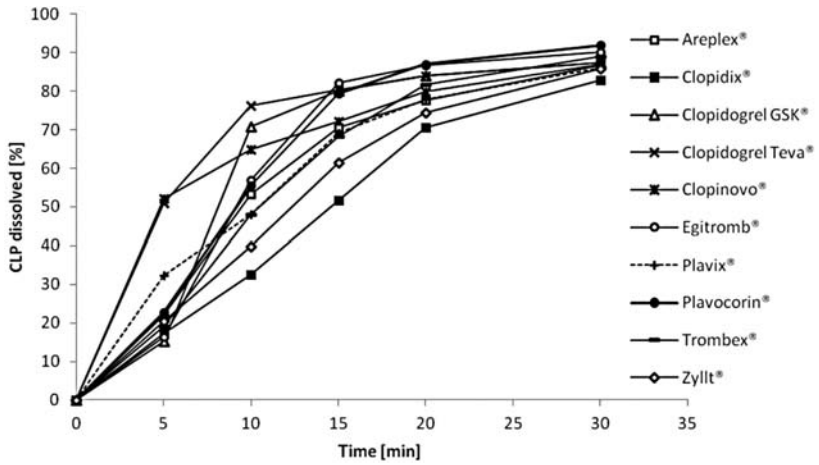

Figure 3. Mean dissolution profiles (n = 6) of ten CLP 75 mg products (apparatus 2, 50 rpm, 1000 mL of pH 2.0 hydrochloric acid buffer, first stage (S_1) of the dissolution test, CLP determined using CE assay)

tent was dissolved in 30 min (Table 3). The differences were not statistically significant (ANOVA at $\alpha = 0.05$, $p = 0.18$).

Kinetic studies

The first-order model was fitted to six individual dissolution data of each brand of CLP tablets using the DDSolver software. This model provides a good fit for all of the brands ($R^2_{adjusted} > 0.9$), indicating similar drug release mechanisms for CLP drugs under different brands. Kinetic parameters (k, $t_{0.5}$) calculated for each drug product are presented in Table 4.

The highest value of k were noticed for Clopinovo® while the lowest one for Clopidix® tablets. The model parameters were compared using a one-way analysis of variance (ANOVA). The results of ANOVA ($p = 0.00000004$ and $p = 0.0000075$ for k and $t_{0.5}$, respectively) showed that the drug products were significantly different in terms of first-order kinetic parameters, implying that the dissolution profiles of these products were not similar. As for *post hoc* procedures, the results of pair-wise comparisons among tested products by Tukey multiple comparison test are given in Table 5. It was found that the first-order dissolution rate constant (k) of the CLP from Clopinovo® tablets was significantly higher than from Clopidix®, Zyllt®, Areplex® and Trombex® tablets. In opposite, the k of the CLP from Clopidix® tablets was significantly

Table 5. Significant differences in dissolution rate constants (k) and $t_{0.5}$ values of CLP among tested tablets.

A. k [min^{-1}]										
Tablets (n = 6)[a]	Areplex®	Clopidix®	Clopidogrel GSK®	Clopidogrel Teva®	Clopinovo®	Egitromb®	Plavix®	Plavocorin®	Trombex®	Zyllt®
Areplex®	-			**	**					
Clopidix®		-	***	***	***	**	**	***		
Clopidogrel GSK®		***	-							*
Clopidogrel Teva®	**	***		-					*	**
Clopinovo®	**	***			-				**	***
Egitromb®		**				-				
Plavix®		**					-			
Plavocorin®		***						-		
Trombex®				*	**				-	
Zyllt®			*	**	***					-
B. $t_{0.5}$ [min]										
Tablets (n = 6)[a]	Areplex®	Clopidix®	Clopidogrel GSK®	Clopidogrel Teva®	Clopinovo®	Egitromb®	Plavix®	Plavocorin®	Trombex®	Zyllt®
Areplex®	-	*		*	*					
Clopidix®	*	-	***	***	***	***	***	***	**	
Clopidogrel GSK®		***	-							**
Clopidogrel Teva®	*	***		-						***
Clopinovo®	*	***			-					***
Egitromb®		***				-				*
Plavix®		***					-			*
Plavocorin®		***						-		**
Trombex®		**							-	
Zyllt®			**	***	***	*	*	**		-

[a] – originator product is marked in bold. * Different from each other (ANOVA, Tukey multiple comparison test at $\alpha = 0.05$, $p < 0.05$), ** Different from each other (ANOVA, Tukey multiple comparison test at $\alpha = 0.05$, $p < 0.01$), *** Different from each other (ANOVA, Tukey multiple comparison test at $\alpha = 0.05$, $p < 0.001$).

slower compared to Plavix®, Egitromb® Plavocorin®, Clopidogrel GSK®, Clopidogrel Teva® and Clopinovo® tablets. Similar pattern of similarities/dissimilarities of pair-wise comparisons for $t_{0.5}$ parameter was observed (Table 5).

DISCUSSION

The high inter-subject variability of pharmacokinetic parameters and pharmacodynamic effect demonstrated in patients with cardiovascular diseases treated with CLP (25, 26) requires explanation with reference to the factors which contribute to the drug behavior in human body. In the case of CLP, which is the poorly soluble but highly permeable drug, dissolution tests can be prognostic of its absorption (2). As the CLP formulations contain different salts of the drug, their performance in dissolution process may provide valuable information on the product equivalency and further effectiveness.

For determination of CLP in samples obtained in dissolution test, CZE method was elaborated and validated. The method was adequately accurate and precise and fulfilled the validation requirements for pharmaceutical analysis (27). Moreover, the CZE method was equivalent with the spectrophotometric reference method, as has been confirmed by the Bland-Altman analysis (Fig. 2), and therefore it could be applied in the dissolution study of CLP from the commercial drug products.

One point sampling is very common for immediate release (IR) products in the USP monographs. The choice of one time point to collect samples represents a substantial data reduction of the kinetic process of dissolution (i.e., time *vs*. amount dissolved relationship). This reduction does not allow to fully characterize the biopharmaceutical properties of the formulation. For example, a film-coated tablet may required more precise observation at the early phase of the dissolution test to determine whether dissolution of the film is the rate-limiting step for subsequent release processes. To study the *in vitro* dissolution kinetics of CLP from the examined IR tablets, we generated dissolution profiles by taking samples at 5, 10, 15, 20 and 30 min during dissolution test. Dissolution profiles obtained for the studied formulations exhibit slight differences with the highest amount of CLP dissolved from Plavocorin® tablets and the lowest from Clopidix® ones (Fig. 3). However, both the original product and the generic formulations with CLP released at least 80% of the declared drug content and passed the pharmacopoeial dissolution test acceptance criteria (14). In contrast, Gomez et al. (16) observed an evident disparity between dissolution profiles of CLP from Plavix® and 18 generic products. They found that 95% of CLP was dissolved from the reference product, while two formulations failed the test with less than 61% of CLP. Moreover, many generics exhibited different, non-homogeneous dissolution profiles in comparison with Plavix®. The study did not include the dissolution kinetics of CLP (16).

Dissolution data can be represented by mathematical models which allow to describe the rate of the drug release from the formulation (23, 24). The approach requires a suitable mathematical function that can be linear or nonlinear to describe the dissolution data. Nonlinear models tend to be more reliable as they predict responses outside the observed range of data, whereas linear models are linear in their parameters (28). The dissolution profiles of CLP were compared and evaluated in terms of the first-order model parameters. We selected this model because it is based on the relationship of the ln function of the percent of undissolved drug *versus* time, and it is mainly related to immediate-release formulations, where the amount of drug released is proportional to the remaining amount in the dosage form, which decreases over time (1, 23). Based on calculated mean fitted kinetic parameters (k, $t_{0.5}$) it can be concluded that the fastest dissolution of CLP occurred from Clopinovo® tablets with $k = 0.100 \pm 0.016$ min^{-1} and $t_{0.5} = 7.04 \pm 1.09$ min, and was almost two times faster than CLP dissolution from Clopidix® tablets ($k = 0.051 \pm 0.006$ min^{-1}, $t_{0.5} = 13.63 \pm 1.58$ min) (Table 4). The faster dissolution of CLP from Clopinovo® tablets is probably due to different API salt used in this product. Clopinovo® tablets contain clopidogrel besylate compared to clopidogrel bisulfate in all the other tested tablets. Literature data regarding therapeutic equivalency of the CLP salts are inconsistent. Kim et al. (29) found that the pharmacokinetic/pharmacodynamic profiles of CLP besylate in healthy volunteers were not significantly different from those of CLP bisulfate. Similar results were obtained by Chamilos et al. (30), who did not find any differences for antiplatelet response between the patients with stable coronary artery disease treated with CLP bisulfate or CLP besylate. This suggests that both forms of the drug may be clinically equivalent. However, Meves et al. (31) observed the worse effect of the antiplatelet therapy in patients with ischemic stroke after switching them from the CLP bisulfate formulation to the product containing CLP besylate. Patel et al. (32) suggested that differences in the drug's absorption from formulations containing various

CLP salts may be related to their ionization status in the gastro-intestinal tract. CLP is a basic ionizable molecule and has a pKa value of 4.55, whereas the bisulfate and besylate counter-ions have pKa values of 1.92 and 2.54, respectively. At pH 1-5 both the drug and the counter-ion are ionized and absorption of an ion-pair between the drug and its salt in solution could be observed. Differences in dissociation constant of the two counter-ions may lead to a different amount of ion-pair at any given pH and result in significant differences in bioavailability (32).

CONCLUSIONS

According to our knowledge, this is the first study on dissolution kinetics of CLP from immediate-release commercial film-coated tablets, commonly used in anti-platelet therapy. Our results confirmed that all studied products with CLP met the acceptance criteria regarding dissolution test, releasing at least 80% of the declared drug content within 30 min. However, significant differences between the kinetic parameters of dissolution of the CLP salts from the studied formulations were noticed. Moreover, in the study the usefulness of the validated CZE method was confirmed for determination of CLP in samples obtained in dissolution test. Therefore, the CZE method can be applied alternatively to the spectrophotometric method in the study concerning pharmaceutical availability of formulations containing CLP.

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