AAPS PharmSciTech, Vol. 10, No. 3, September 2009 (© 2009)
DOI: 10.1208/s12249-009-9298-y

Research Article

The Application of Modified Flow-Through Cell Apparatus for the Assessment of Chlorhexidine Dihydrochloride Release from Lozenges Containing Sorbitol

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Received 7 February 2009; accepted 27 July 2009; published online 11 August 2009

Abstract. The objective of this work was to apply a new apparatus for the assay of the drug release from lozenge tablet with a potential use in the treatment of oral candidosis and another conditions connected to microbial etiopathology in the oral cavity or as an antiplaque factor. Also, an approach to comparison of the applied method with the classical paddle apparatus method was performed. Tablets containing chlorhexidine dihydrochloride were formulated with granulated sorbitol of different grades (diameter of 110, 180, 480, and 650 μm, respectively), lactose, and magnesium stearate as excipients. Tablets were obtained through direct compression, and uniformity of weight, friability, breaking strength, disintegration, and release rate were evaluated. The disintegration times ranged between 10 and 21 min. In the next stage of the study, the release of chlorhexidine from lozenges prepared with granulated sorbitol grade 110 μm and different amounts of lactose and magnesium stearate was assessed. Two stages were observed during the release of chlorhexidine dihydrochloride from the lozenges, assayed by the classical paddle apparatus method II USP. In the first stage, release rates were between 2.6×10^{-2} and 4.7×10^{-2} min^{-1}, in the second stage between 1.7×10^{-3} and 7.7×10^{-3} min^{-1}. In the case of the in-house method, the release was near to first-order kinetics through the entire release experiment, with rate constants between 3.6×10^{-2} and 6.6×10^{-2} min^{-1}. The sorbitol granulate of granules with diameter 110 μm was found to be most suitable for the lozenges with chlorhexidine dihydrochloride and lactose. The in-house release method, proposed in this work, seems to be more realistic for the preliminary assessment of predicted drug concentrations in the oral cavity after the intake of a lozenge.

KEY WORDS: chlorhexidine; lactose; lozenges; magnesium stearate; release rate; sorbitol.

INTRODUCTION

Chlorhexidine is a potent oral antimicrobial agent that can suppress Mutans Streptococci levels and potentially reduce the caries increment in humans (1). It has a range of dental and dermatological application, according to its bactericidal activity against both Gram-positive and Gram-negative microorganisms, *Candida albicans* and *Mycobacterium tuberculosis* (2,3). It has been incorporated into mouth rinses and shown to be effective in inhibiting dental plaque and gingivitis in human subjects (4,5). According to the research of Koenig *et al*., the increased temperature has a beneficial effect on the activity of chlorhexidine applied as the mouth rinse. The temperature in mouth is in the range of 36.8°C, whereas during the infection increases up to 38.5°C. This is a factor which would increase the temperature of chlorhexidine applied in the form of lozenges (6). The crucial parameter in the formulation of a tablet with chlorhexidine would be the concentration of the drug in the oral cavity during the application of the lozenge. In the case of antimicrobial substances, like chlorhexidine salts, the prolonged presence of the active substance in the minimal inhibition concentration (MIC) or the minimal bactericidal concentration (MBC) is of great importance (7). During the dissolution of a solid drug form in the saliva, a solution of active substance in the mouth is developing. The concentration of the drug in the fluids of oral cavity depends of multiple parameters. The physical and chemical characteristics of the drug form and the physiology of the oral cavity both influence the amount of drug in the mouth. *In vitro* assays for the prediction of drug levels in the oral cavity are studied extensively, but as to date, these efforts are not much reflected by the pharmacopoeial practice. The number of parameters, like saliva flow in the range of 0.33–5.0 $ml\cdot min^{-1}$, and the periodic contact of the drug form with the air present in the drug cavity, disable the convenient mimicking of the environment of oral cavity (8).

A variety of designs of apparatus for dissolution testing has been proposed and tested, varying from simple beaker with stirrer to complex systems with lipid phases and lipid barrier, where an attempt is made to mimic the biological milieu (9). Over the last few years, there has been a great increase in interest for dissolve-in-the-mouth dosage forms. The characterization of dissolution in the

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mouth is not easily done by current methodologies, such as variants of the European Pharmacopoeia (Ph. Eur.) or the United States Pharmacopoeia (USP) dissolution tests. The reason for this is the rapidity of disintegration compared to typical dosage forms designed to be swallowed as a whole. It appears that there is a deficiency of *in vitro* systems to evaluate the buccal dissolution of such dosage forms. Hughes and Gehris described a dissolution testing system that is capable of characterizing buccal conditions (10). There is a number of factors that are unique to characterizing buccal dissolution that do not apply to gastrointestinal dissolution. They include small volume, short residence time, solids transfer, composition, and incomplete dissolution. Most of the Ph. Eur. or USP dissolution tests use large volumes of solution defined as sink conditions (11,12). For buccal dissolution, the volume of saliva is very small compared to that of the gastrointestinal tract.

The objective of this work was to apply a new apparatus for the assay of the drug release from lozenge tablet with a potential use in the treatment of oral candidosis and another conditions connected to microbial etiopathology in the oral cavity or as an antiplaque factor.

MATERIALS AND METHODS

Materials

The bulk powders of different granulated sorbitol grades of diameters of: 110, 180, 480, and 650 μm, respectively, were obtained from Roquette (Lestrem, France, batch no. 640343, 028117, 632894, and 638297, respectively) and used as delivered. Chlorhexidine dihydrochloride (CHX) was received from Sigma–Aldrich (Steinheim, Germany, batch no. 00802H). Pharmaceutical grade lactose (Pharma Cosmetic, Krakow, Poland, batch no. 612845/7) was used as diluent. Magnesium stearate of analytical grade (MgSt, POCH, Poland, batch no. 4826/05) was used as lubricant. All other chemicals were of analytical grade.

Composition of Tablets and Compression Process

Sixteen tablet formulations, A1–D4, containing different amounts of granulated sorbitol, lactose, and MgSt, and 5 mg of CHX were prepared by direct compression (Table I). The total weight of the compressed tablets was maintained at 811±2 mg, according to the added amounts of MgSt—4 or 8 mg.

All ingredients were weighed accurately according to the experimental design (batch size 200 g) and mixed well in a twin-shell mixer Turbula mixer (Turbula WAB, Systems Schatz, Switzerland) at 28 rpm for 10 min. All of the MgSt was added 1 min before the end of mixing. The powder mixtures were compressed to tablets using an eccentric compressing machine (Hanseaten Exacta E 1, W. Fette, Schwarzenbek, Germany). The machine, its measurement devices for punch forces and their calibration, was described earlier in detail (13,14). Flat-faced punches of 10 mm diameter were used. The appropriate amount of material, weighed on an analytical balance (Analytical Balance AE 166/9, Mettler, Giessen, Germany), was filled manually into the die and was compressed at a machine speed of 30 strokes per minute to a maximum upper punch pressure of 73.5 Mpa.

Tablets Properties

The uniformity of mass, friability, breaking strength, disintegration time, and release of CHX were determined according to the Ph. Eur. (10)—the appropriate numbers of methods are stated in the text.

Breaking Strength

The radial breaking strength was measured according to the Ph. Eur. 2.9.8 method 24 h after preparation of the tablets. A calibrated (14) crushing strength tester (TBH 28, Erweka Apparatenbau, Heusenstamm, Germany) was used at 25±1°C. Ten tablets of each composition were tested.

Uniformity of Mass

Twenty units were taken randomly and individually weighed (Analytical Balance AE 166/9, Mettler, Giessen, Germany). The average mass was determined, compared to the limits, according to the Ph. Eur. method 2.9.5, and SD values were calculated.

Friability

The friability test was performed according to the Ph. Eur. method 2.9.7. A drum with an internal diameter of ca. 286 and 39 mm in depth, made of transparent polyacrylate polymer, rotating at 25 rpm, was applied. Ten tablets were used for the assay; before weighing of the residual tablet mass, dust was removed on a sieve with the aid of pressurized air.

Disintegration Test

Disintegration tests were performed according to the Ph. Eur. method 2.9.1. The method selected is critical, and the method stated in the Ph. Eur. for conventional tablets may not be appropriate. The analogous method in USP 28 states that method 701 is provided to determine compliance with the limits on disintegration stated in the individual monographs except where the label states that the tablets or capsules are intended for use as troches or are to be chewed. According to the USP, Ph.Eur. and International Pharmacopoeia, there is no specified disintegration time limit for tablets for use in the mouth, i.e., lozenges and chewable tablets, as they are formulated to effect a slow release and local action of the active ingredient (10,11,15). The apparatus for the Disintegration of Tablets and Capsules was applied to elucidate the disintegration times of prepared lozenges.

Dissolution

To determine the release rate for CHX from prepared formulations containing different amounts of sorbitol, lactose, and MgSt, the dissolution tests were applied. To develop a more proper method for the *in vitro* assay of CHX release

Table I. The Composition of Studied Lozenge Formulations (Absolute Mass [g] Per Batch)

Components	S110	S180	S480	S650	Lactose	MgSt	CHX
Formulation	[mg]						
A1	600.0	–	–	–	200.0	4.0	5.0
B1	600.0	–	–	–	200.0	8.0	5.0
C1	400.0	–	–	–	400.0	4.0	5.0
D1	400.0	–	–	–	400.0	8.0	5.0
A2	–	600.0	–	–	200.0	4.0	5.0
B2	–	600.0	–	–	200.0	8.0	5.0
C2	–	400.0	–	–	400.0	4.0	5.0
D2	–	400.0	–	–	400.0	8.0	5.0
A3	–	–	600.0	–	200.0	4.0	5.0
B3	–	–	600.0	–	200.0	8.0	5.0
C3	–	–	400.0	–	400.0	4.0	5.0
D3	–	–	400.0	–	400.0	8.0	5.0
A4	–	–	–	600.0	200.0	4.0	5.0
B4	–	–	–	600.0	200.0	8.0	5.0
C4	–	–	–	400.0	400.0	4.0	5.0
D4	–	–	–	400.0	400.0	8.0	5.0

S110, 180, 480, 650: Sorbitol granules of the respective diameter [μm]
MgSt magnesium stearate, *CHX* chlorhexidine dihydrochloride

from prepared lozenges, the classical paddle apparatus according to the Ph. Eur. method 2.9.3 (Apparatus II USP) and the in-house made apparatus were used and the acquired data were compared. The latter apparatus consisted of a thermostatic glass cell, with glass balls of diameter in the range of 5 mm on the bottom, as depicted in a scheme in Fig. 1. The water-bath coat maintained the required temperature of the release process. The concept of this in-house release device is that the small amount of acceptor medium, in droplets, just covers the surface of the tablet, and the release process may proceed. The highest diameter of the biconical device is ca. 50 mm, and the smallest one is ca. 8 mm, with a mesh over the outlet to fix the glass balls. The inlet orifice, from which the droplets of acceptor fluid are delivered, is ca. 1 mm and is regulated by a valve. The water pressure was maintained, applying the water column in the vessel connected to the device. The acceptor medium flow of temperature 37°C was 1.9 ml min^{-1}. The critical parameters were assessed in our lab and validated in consequently repeated measurements, including: the fixed acceptor medium flow, the fixed temperature, and the inlet and outlet water stream velocity. Water was applied as the acceptor medium, and the supernatant from collected samples was assayed by spectrophotometry.

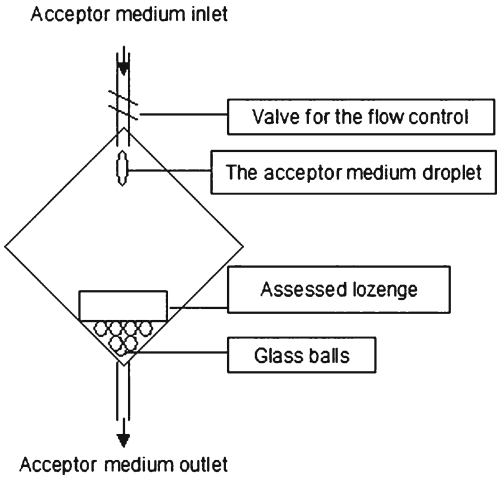

Fig. 1. The scheme of the in-house made flow-through cell apparatus

An UV-Vis spectrophotometer (CECIL CE 5501, Cecil Instruments, Great Britain) confirmed the absorption peak of CHX at 254.5 nm. A linear relationship between absorbance peak height obtained from UV-Vis spectrophotometry and the CHX concentration in reference solutions, containing ten graded concentrations of CHX in water 5 to 50 mg·L^{-1}, with five samples prepared for each concentration grade, was established. With the aid of a statistical program (16), both linearity and significance of the difference between the extrapolated intersection with the abscissa of the linear regression line and "zero" were tested. Homogeneous regression (n=50, p=0.05) resulted in the characteristic value of slope of the regression line—0.0495 mg L^{-1}; the limit of determination was calculated to be 1.74 mg L^{-1}.

RESULTS

Tablets Properties

The mean breaking strengths were in the range 35.2–124.1 N. The respective values with SD for ten tablets are presented in Table II.

In Table II, average weights of prepared tablets and their standard deviation are presented. All of the tablets fulfilled the pharmacopoeial standards of the uniformity of mass. The average tablet weight ranged between 0.803±0.021 g (mean and SD) for formulation A1 and 0.826±0.015 g for formulation C1. The results of the friability assay are included in Table II. The values of the weight loss ranged from 0.9% for the formulation C4 to 1.3% A4.

Disintegration times are presented in Table III. Tablets containing sorbitol of 110 μm grade and 50% of lactose with 1% of MgSt—formulation D1—were characterized by the highest disintegration time with an average of 21 min and

Table II. The Average Mass, Friability, and Breaking Strength of Studied Lozenges

Formulation	A1	B1	C1	D1	A2	B2	C2	D2	A3	B3	C3	D3	A4	B4	C4	D4
Average mass [g]	0.80	0.81	0.82	0.81	0.82	0.81	0.82	0.82	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.80
SD [g]	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Mass loss[a] [g]	0.08	0.09	0.10	0.09	0.08	0.10	0.08	0.10	0.08	0.09	0.08	0.08	0.11	0.10	0.07	0.09
% of mass loss	1.00	1.10	1.20	1.10	1.00	1.20	1.00	1.20	1.00	1.10	1.00	1.00	1.30	1.20	0.90	1.10
Breaking strength [N]	35.2	40.3	45.6	51.4	57.9	66.5	72.4	79.6	85.3	93.0	96.5	99.5	103.7	113.3	117.1	124.1
SD [N]	6.2	8.5	9.6	10.4	12.10	10.3	8.9	9.6	10.2	8.9	10.0	11.5	11.2	10.1	9.3	5.4

SD standard deviation
[a] For ten tablets

33 s. The fastest disintegration—average 9 min and 54 s—was observed in the case of formulation A4 with sorbitol grade 650 μm, 25% of lactose, and 0.5% MgSt. The disintegration time increased with the decreasing diameter of sorbitol granules, with the increase of lactose percentage in the formulation, as is depicted in Fig. 2, and with the increase of MgSt content.

According to Fig. 2, all formulations with 50% of lactose, with exception of C3, were characterized by disintegration times overlapping 15 min. Also in the case of formulation B1, a disintegration time higher than 15 min was observed.

Release Rates Assayed by the Paddle Apparatus According to the Ph. Eur. Method 2.9.3 (Apparatus II USP)

For the next stage of the work, three formulations with disintegration times exceeding 15 min—B1, C1, and D1—were chosen. Five assays were performed for every formulation. The absorbance of the samples were converted to the quantities of CHX released based on the linear relationship. The results are illustrated in Fig. 3. As in the case of tablets, the release rate is connected to the disintegration time; the release rate was parallel to the disintegration rate. The release rates were high in the case of B1 tablets with 25% of lactose and decreased in the case of formulations C1 and D1 with 50% content of lactose.

The average pharmaceutical availability of CHX from lozenges B1, with the weight relation sorbitol 110 μm to lactose of 2:1, was 60.7±0.20%, and the release was completed in ca. 37 min. In the case of lozenges C1 with sorbitol 110 μm and lactose weight relation 1:1, the pharmaceutical availability was higher, in the range of 66.8±0.22%, through the total effective release time of about 40 min. According to the depicted curve on Fig. 2, the percentage of the CHX released from formulation D1, with higher content of MgSt comparing to C1, acquired the value 46.9±0.15%. The dissolution was completed in 51 min, but the percentage of released CHX after ca. 30 min did not change significantly.

Release Rates Assayed by the In-House Made, Modified Flow-Through Cell Apparatus According to the Ph. Eur. (Apparatus IV in USP)

In the in-house method, based on the modified flow-through cell apparatus, the concentrations were measured in 9.5 ml samples taken in 5-min intervals. Data are gathered in Fig. 4.

The maximum observed concentration of CHX was 101.9 mg L^{-1}, and the pharmaceutical availability of CHX released from the lozenges B1 was assessed as 80.2±0.54%. According to the graph on Fig. 4, the maximum CHX concentration was observed ca. 15 min of release process. The pharmaceutical availability of CHX in the case of C1 tablets was 80.6±0.28% with the maximum reached concentration of 69.2 mg L^{-1}. The concentration of CHX in the first 20 min maintained the level 65 mg L^{-1} and then rapidly decreased. The maximum concentration was observed after 15 min, and the dissolution duration was ca. 37 min. Formulation D1 was characterized by decreased pharmaceutical availability of CHX −72.5%. The maximum concentration of

Table III. The Influence of the Lozenge Composition on the Disintegration Time (Mean Value ± SD)

	25% L+0.5% MgSt	25% L+1.0% MgSt	50% L+0.5% MgSt	50% L+1.0% MgSt
	(A)	(B)	(C)	(D)
SGD 110 μm (1)	713±16	908±36	1,182±11	1,293±13
SGD 180 μm (2)	726±36	821±41	1,092±41	1,218±34
SGD 480 μm (3)	674±86	818±40	869±37	1,020±62
SGD 650 μm (4)	594±24	695±11	930±91	1,027±68

(A), (B), (C), (D), and (1), (2), (3), (4), are respectively batch prefix codes, and batch suffix codes
SD standard deviation

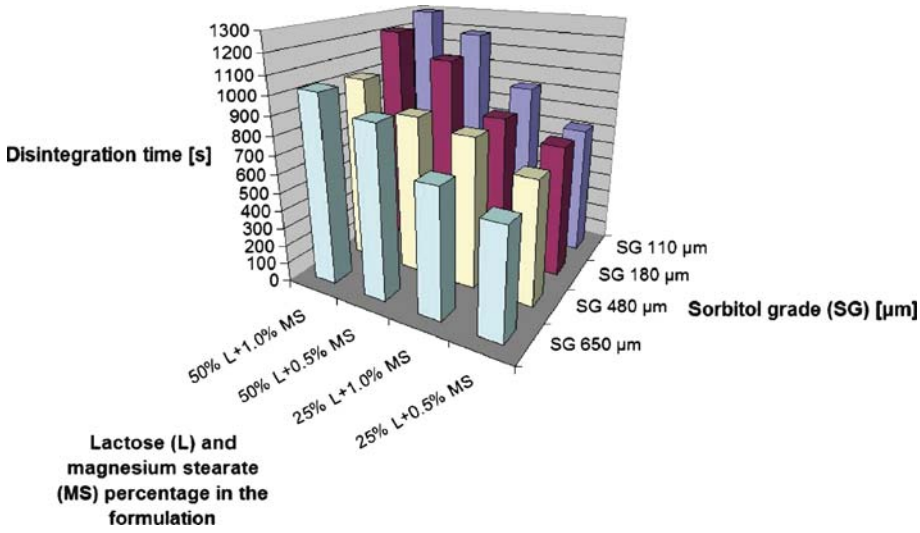

Fig. 2. The influence of sorbitol grade on the disintegration time of lozenges with chlorhexidine hydrochloride

62.7 mg L^{-1} was obtained in 10 min of the process. The average dissolution time was assessed as 40 min.

Calculation of the Release Rates

The absorbance peak heights of the replaced solutions at 254.5 nm were converted to the quantities of CHX released based on the linear relationship. Also, the logarithmic conversion was applied to check the order of observed release processes. The courses of the release processes—presented as the function of natural logarithm of CHX residual in drug form *versus* time—are depicted on Figs. 5 and 6.

The course of the drug release in the case of Paddle Apparatus, depicted on Fig. 5, could be interpreted as a two-stage process, where the first and the second stages may be labeled as a first-order process. The release rates are calculated by different authors considering various important parameters (17); however, in this case, comparative values were necessary to evaluate the difference between both applied methods. According to Wagner, the release process of active substance from a tablet may be considered as a pseudo-first-order path (18,19). The data obtained in the release experiments were compared assuming the first-order release process. The square of Pearson's coefficient (r^2) (20) on a semilogarithimic scale was used to establish adherence to a first order. As presented in Table IV, the r^2 values were higher applying the semilogarithmic scale to prove the idea of first-order process for the CHX release from the sorbitol–lactose compacts. The kinetic equation $K=(\ln C_1 - \ln C_2)/(t_2 - t_1)$ was applied, where K is the release rate, and C_1 and C_2 are the assessed concentrations of CHX in respective time intervals t_1 and t_2.

The calculated first-order release rate constants for the B1, C1, and D1 in the first stage were 4.69×10^{-2}, 4.41×10^{-2}, and 2.64×10^{-2} min^{-1} and in the second stage 7.7×10^{-3}, 7.6×10^{-3}, and

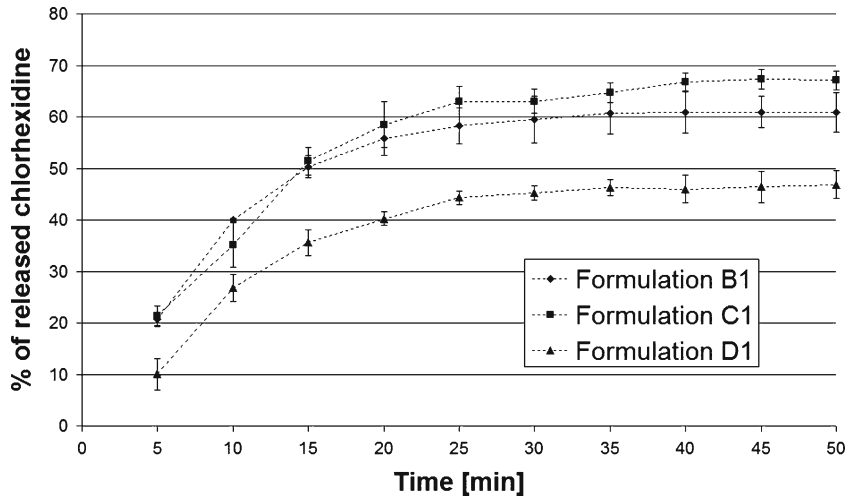

Fig. 3. The chlorhexidine release from sorbitol lozenges with different amounts of lactose and magnesium stearate assessed by paddle apparatus method

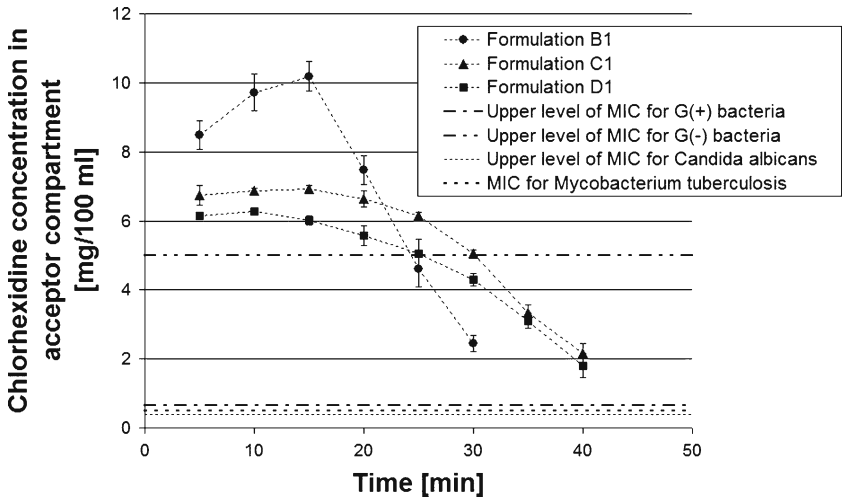

Fig. 4. The levels of chlorhexidine acquired in the assay of its release from sorbitol lozenges applying the in-house flow cell method

1.70×10^{-3} min^{-1}, respectively. The modified flow-through cell apparatus, however, results in a one-stage first-order process, which is clearly demonstrated by Fig. 6. Calculated release rate constants for formulations B1, C1, and D1 gained values between 3.6×10^{-2} and 6.6×10^{-2} min^{-1}.

DISCUSSION

In the USP XXVIII and Ph. Eur., there are three methods proposed for the assay of active substance release from tablets—basket apparatus, paddle apparatus, and flow-through apparatus. Besides, in USP, there is also an apparatus for the assay of the drug release from chewable gums.

The dissolution in the mouth is not easily characterized by current methodology, such as variants of the Ph. Eur. dissolution tests. Various factors influence the buccal dissolution, which do not apply to the gastrointestinal conditions of dissolution, like small volume, short residence time, composition, and incomplete dissolution. Most of the Ph. Eur. and USP dissolution tests use large volumes of solvent media—500 or 900 ml, defined as sink conditions, to get complete dissolution of the biologically active substance. Machida used an apparatus to measure the dissolution rate by keeping the drug form in a rotating basket at 100 rpm in 900 ml of purified water (21). For the buccal conditions, the volume of the saliva is much smaller than the volume of fluids in the gastrointestinal tract.

The flow-through cell apparatus described as Apparatus IV in the USP has gained recent acceptance for dissolution tests for its versatility in the testing of novel dosage forms where traditional dissolution apparatus and methods have failed. Beside, during sucking of a lozenge, some air is present in the oral cavity, so the solid drug form is wetted by the saliva, although in the mean time, it is in contact with air. It must be underlined that even if saliva is excreted at about 2 ml min^{-1}, the tablet will be in contact probably only with part of this volume, in contrast to being immersed in gastric or intestinal fluids. According to the lack of a specific apparatus for release assays in the case of lozenges, the authors presented an in-house method, supported by the idea of the flow-through apparatus. As it was stated before, in

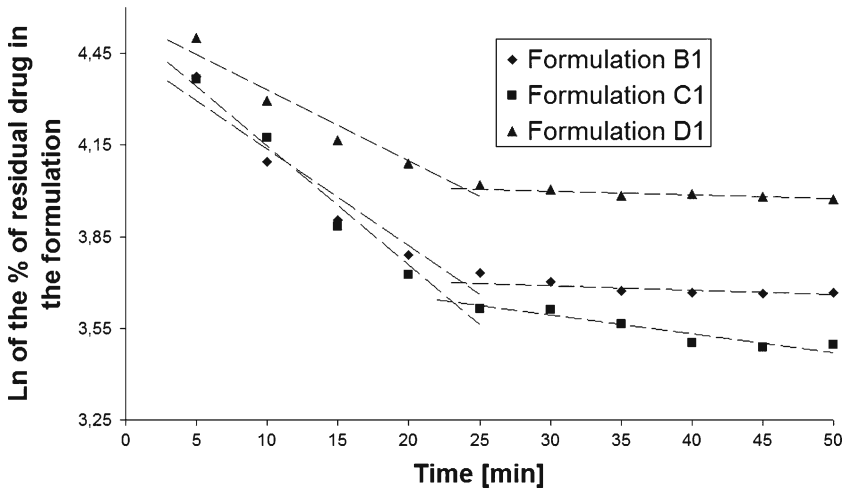

Fig. 5. Semilogarithmic graph of the chlorhexidine release from sorbitol lozenges with different amounts of lactose and magnesium stearate assessed by paddle apparatus method

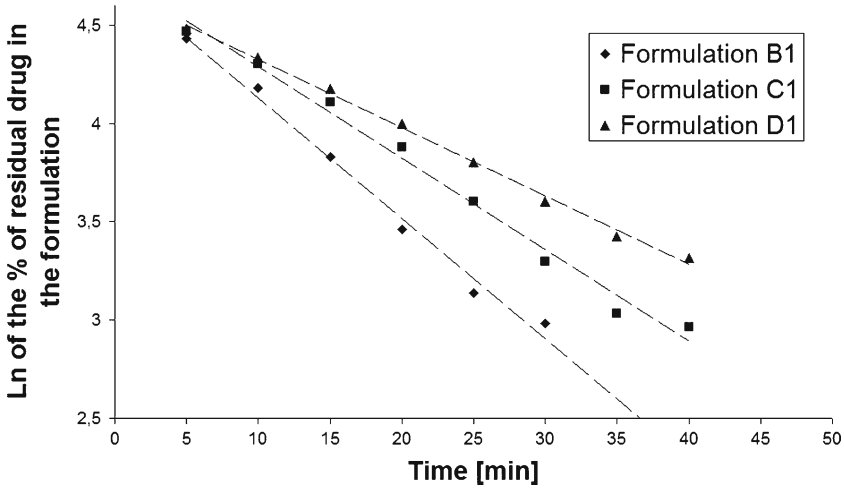

Fig. 6. Semilogarithmic graph of the chlorhexidine release from sorbitol lozenges with different amounts of lactose and magnesium stearate assessed by the in-house flow cell method

conditions mimicking the oral cavity area, the most important parameter for drug release is the small volume of acceptor fluid. Secondly, the stable, gradual, and slow flow is of high importance (22). The most proper method for the assay from the biopharmaceutical point of view would be the flow method.

The pharmacopoeial assembly based on the device invented by Pernarowski (23) was developed in Deutsche Arzneimittel Codex (24) and accepted in modified form by Ph. Eur. (10) and USP (11). It consists of a reservoir containing the release medium, a pump that forces the release medium upwards through the vertically positioned flow-through cell, and a thermostatic water bath. The flow rate delivery capacity is between 4 and 16 ml min^{-1}. The pump usually has typical flow rates of 4, 8, and 16 ml min^{-1}. Thus, the flow is opposite to that in the natural in the oral cavity (25). The bottom cone of the cell is filled with small glass beads of about 1 mm diameter and with one bead of about 5 mm diameter positioned at the apex to protect the fluid entry tube, whereas a glass-fiber filter is positioned at the inner top. Collins applied an apparatus consisting of a water jacket and an internal compartment containing 50 ml of simulated saliva as a dissolution medium to evaluate the release of cetyl piridinium chloride tablet by placing it in a metal die sealed at the lower part by paraffin wax (26). In contrast, the in-house-made apparatus consisted of a thermostatic glass cell, with glass balls on the bottom, as depicted in Fig. 1. The acceptor medium flow was 1.9 ml min^{-1} at a stable temperature of 37°C. The acceptor medium moved in the direction similar to the flow of the saliva in the oral cavity. The droplets of the acceptor medium overflowed the tablet, so the release of the active substance was actually performed to a thin layer of water present on the surface of the tablet, which is the main difference between the USP and our in-house device. The performance studies were performed, and we conclude that the flow of the acceptor medium is stable and repeatable in the maintained conditions. Solid particles in the samples collected at the outflow were removed by centrifugation, and the supernatant was assayed by spectrophotometry. The presented procedure enabled the observation of CHX concentration changes, during the release from a lozenge, in a regimen mimicking the oral cavity conditions.

According to the disintegration test results, the composition of the excipients influenced the disintegration behavior of prepared lozenges. With the increase of the diameter of the sorbitol granules, the disintegration time decreased. This relationship was more evident with higher

Table IV. Squares of Pearson's Coefficient (r^2) for Regression of Values Obtained, Assuming Zero-Order and First-Order Kinetics for the Release Process of Chlorhexidine from Sorbitol–lactose Compacts

				III	
Formulation	Method	I	II	1st stage	2nd stage
B1	USP	0.6534	0.7191	0.9294	0.5970
C1		0.7378	0.8115	0.9756	0.8307
D1		0.6860	0.7346	0.9437	0.7605
B1	In-house	0.9459	0.9895	–	
C1		0.9661	0.9885	–	
D1		0.9752	0.9968	–	

I Square of Pearson's coefficient (r^2) for regression assuming zero-order process in one stage, *II* Square of Pearson's coefficient (r^2) for regression assuming first-order process in one stage, *III* Square of Pearson's coefficient (r^2) for regression assuming first-order process in two stages

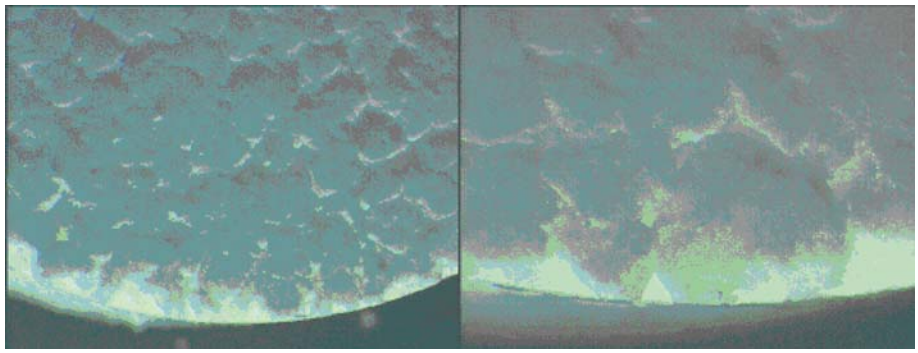

Fig. 7. The surface of a lozenge of formulation B4—16-fold magn. and 40-fold magn. in microscopic view

amount of lactose. The phenomenon could be attributed to the arrangement of the sorbitol granules in the tablet matrix; during compression, the granules of high diameter would leave more pore space in the tablet for the water access, so the processes of disintegration and dissolution would be more rapid. The surface of an example tablet is presented in Fig. 7. This item will be examined in detail in further works. In the case of 25% of lactose in the formulation, the difference in disintegration between lozenges with sorbitol 110 and 650 μm was in the range of 5 min and 46 s. The difference in the case of formulations with 50% of lactose was almost two-fold higher, i.e., 9 min and 40 s. The presence of lactose in the lozenges, similarly to MgSt influence, results in an increase of the disintegration time.

In the release assay, using the pharmacopoeial method with apparatus II USP, the pharmaceutical availability was different for the assessed formulations: 60.7%, 66.8%, and 46.9% for B1, C1, and D1 lozenges, respectively. In the dissolution test performed by the pharmacopoeial method, a release process with two stages of first-order kinetics was observed. More research in this field is intended to explain this behavior in detail. The low pharmaceutical availability was attributed to the adsorption of CHX on MgSt. To prove this, the influences of MgSt, lactose, and sorbitol on the concentration of CHX in the supernatant were assessed. According to the data in Table V, two mixtures of tablet components in 500 ml of water were prepared. In the same volume of water, two kinds of tablets were dispersed. The percentage of CHX in supernatant received from the dispersion of the components of the tablet without MgSt was 100±1.4%. In the presence of MgSt, the assessed amount of CHX in supernatant decreased to 79±5.5%. A similar effect was observed for the dispersed tablets of respective composition, with results of 91±3.1% and 68±5.3%, respectively.

In the case of our flow-through cell, the release of CHX from the lozenges prepared proceeded in one stage, interpreted as first-order kinetics. The pharmaceutical availability was higher, i.e., 80.2%, 80.6%, and 72.5%, respectively, for formulations B1, C1, and D1. It could be explained by the lower level of MgSt in the acceptor fluid during the assay, while throughout the release in the Apparatus II USP, all the MgSt is present in the vessel, through the entire assay time. Inversely, in the adapted flow-through cell method, during the assay, only part of MgSt was present in the consecutive samples of released CHX, taken every 5 min.

The method for the assessment of CHX release in a flow-through cell applied in this work enables the estimation of actual levels of CHX concentrations in the oral cavity. Comparing the estimated concentrations with known MIC and MBC, the effectiveness of the prepared lozenges against bacteria and molds could be evaluated. The MIC of CHX for *Streptococcus pyogenes*, one of the main pathogens in respiratory tract, is in the range 0.5–64 μg ml^{-1}, i.e., 0.5–64 mg L^{-1}. The effective concentration against this pathogen would be observed in the mouth for ca. 20 min in the case of formulations B1 and C1. The MIC for G(−) and G(+) bacteria and for *Candida albicans* is maintained in the acceptor fluid almost through the entire time of release (Figs. 2 and 3). Also, the tuberculostatic concentration is provided: in the case of B1 for 25 min, for D1 and C1 for 25 and 30 min, respectively. The inhibition concentration for *C. albicans* growth would be maintained for 25, 30, and 33 min, respectively. The G(−) bacteria are more resistant to

Table V. The Influence of Magnesium Stearate on the Assessed Chlorhexidine Content in the Prepared Lozenges and in the Respective Mixture of Components, Dispersed in Water

Assessed composition[a]		Assessed chlorhexidine content[b]	SD
Lozenges	In absence of MgSt	91.8	3.1
	In presence of MgSt	68.1	5.3
Powder mixture	In absence of MgSt	100.1	1.4
	In presence of MgSt	79.4	5.5

MgSt magnesium stearate
[a] The percentages were the same as formulation D1, the amounts of five tablets were applied in 500 ml of water for every assay
[b] Compared to 100% of initial content

CHX than G(+) microorganisms. The concentration of CHX is in the bottom level of MIC for these bacteria. Also the MBC values in the oral cavity could be estimated by the assessed levels of CHX in the acceptor fluid. In the case of formulation B1, the concentration is near to the upper level of MBC for G(+) bacteria.

Beyond direct release data, also the mechanical properties of lozenges play an important role in drug release from the solid drug form. In the mentioned conditions, the increase of breaking strength with the diameter of the applied particles could be the elucidated by the increased distance done by the punch in the matrix, in the presence of powders—compare Table II. With the increase of the diameter of powder particles, the matrix was filled with increased apparent volume of the powder. This could result in increased temperature for longer time, so the melting of the powders (melting point of sorbitol 95°C, of lactose 202°C) was performed in higher temperature, and the interpenetration of lactose and sorbitol was more consistent in the tablet matrix—the melted surface we can see on the macrophotographs on Fig. 7. The tablets were more resistant to the mechanical force, when the powders were melted to higher extent. The second reason could be the presence of some humidity inside of sorbitol granules—higher in larger granules, what enables better cohesion of the powders. However, disintegration time assessment is performed in the aqueous conditions, so we suggest that the water affinity was higher in the case of tablets made with particles of higher size, what is in agreement with general statements. The explanation of the parallel increased breaking strength is hard to evaluate, but there are available information about sorbitol, as an excipient for fast release solid drug forms, with sufficient, high mechanical resistance (27).

The proposed device for the assay of the release of active substance from lozenges enables the direct evaluation of the levels of the bactericidal and bacteriostatic substances which are present in the oral cavity after the application of tablet. The received data would avail the development of new drug forms for controlled delivery of active substances within the mouth. The tablets evaluated in this work could be applied as CHX carriers in the prophylaxis of superficial dental caries. The presented pharmaceutical model enables the *in vitro* preliminary assessment of this drug and selection of optimal candidates for the *in vivo* assays.

CONCLUSIONS

The presented pharmaceutical model enables the *in vitro* preliminary assessment of tablets with CHX presumed for the prophylaxis of superficial dental caries and selection of optimal candidates for the *in vivo* assays. In the case of traditional assessment with Apparatus II USP, the observed release process has two stages. During the assay in the in-house flow-through cell apparatus, the release consisted of one stage. The disintegration time increases with the diameter of applied sorbitol granules, whereas the increase in lactose content and in MgSt percentage in tablet results in the prolongation of disintegration. The MgSt present in the tablets adsorbs up to 20% of chlorhexidine released to the acceptor fluid. The levels of chlorhexidine agreed with MIC and MBC for the often recognized bacterial pathogens of the oral cavity and the upper part of the respiratory tract.

ACKNOWLEDGEMENTS

The work was partially supported by the British Stipendium Fund of Doctor Emil Niedzwirski. We thank Mr. Michal Zieba for the assistance in the measurements.

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