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Original research paper

Influence of pH modifiers on the dissolution and stability of hydrochlorothiazide in the bi- and three-layer tablets

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During the past few years, the studies of bi- and multi-layered tablets increased due to the consumption of several different drugs per day by a patient and requests for appropriate patient compliance. The demographic shift toward older population increases the use of combination therapy as polypharmacy. Hydrochlorothiazide (HCTZ), as a model drug, is most commonly used in the treatment of hypertension, congestive heart failure and as a diuretic. The aim of the present study is to investigate the effect of the local environment on dissolution and stability behaviour of HCTZ in fixed multi-layered tablet combinations, which are commonly used in polypharmacy. For this purposes, three different systems were introduced: (*i*) two conventional tablets (HCTZ and pH modifying placebo), (*ii*) 2-layer tablets (HCTZ, pH modifying placebo) and (*iii*) 3-layer tablets (HCTZ, barrier and pH modifying placebo). Disintegration of tablets, dissolution of HCTZ from tablets and HCTZ related substances were monitored for all systems. Results showed that there was a significant difference between dissolution profiles of the conventional two-tablet system (HCTZ tablet and pH modifying tablet) and the 2-layer and 3-layer tablets, which include the pH modifying layer. In the case of the conventional two-tablets system, 85 % of HCTZ was dissolved in less than 15 minutes. The dissolution profiles of HCTZ from 2-layered and 3-layered tablets showed a decrease in the dissolution rate. In addition, during the stability studies, it has been confirmed that the typical degradation product of HCTZ is formed, impurity B (4-amino-6-chloro-1,3-benzenedisulfonamide), which implies formation of formaldehyde as hydrolytic impurity not reported in the *Ph. Eur.* (16). Both impurities are particularly raised in 2-layered tablets with alkaline and neutral placebo layers. Stability of HCTZ was improved in the case of the 3-layer tablet, where the intermediate separation layer of glycerol monostearate was present. It is presumed that the HCTZ dissolution rate was decreased due to formation of non-soluble substances as a result of HCTZ degradation in the presence of alkaline layer.

Keywords: hydrochlorothiazide, bi-, three-layer tablets, disintegration time, dissolution rate, stability

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HCTZ is known as a BCS class IV drug with low solubility and low permeability. Possible environments which could enhance HCTZ solubility can have a negative influence in its stability. The use of multi-layer tablets as drug delivery systems was increased during the last decade due to the therapeutic needs for combinations of drugs (1–6). Fixed combinations of oral drug delivery systems are substantiated by the increase of patient compliance and by the synergistic effect of such combinations (*i.e.*, diuretics and antihypertensives) (6). One of the demands of multi-layer tablets is to avoid chemical incompatibility of active substances, which is mainly achieved by physical separation of layers. The development of multi-layer formulations with combined biopharmaceutical and pharmacokinetic properties is substantiated from the therapeutical point of view (7). Multi-layer formulations are designed through physicochemical and geometric approaches in order to influence the performance of such drug delivery systems (8, 9). Different authors report mutual influences of layers resulting in quality attributes of the drug product (10, 13). The quality of multi-layer tablets is, however, strongly influenced by the excipients in the formulation, technology and manufacturing process. High tableting speeds, high compression forces, high elasticity of the components and non-optimal amount of binders result in increased tablet friability and lamination, caused by insufficient adhesion between the individual layers (11, 12). Particles cross-layer contamination is often present and may cause physicochemical incompatibilities manifested by changes in colour, separation of layers and formation of complexes between active substances and excipients (11, 12). The production of multi-layer tablets does not demand any special formulation approaches, since granules and tableting mixtures are prepared by well-known techniques. The key element is the preparation of tableting mixtures with sufficient flowability and mastering of the tableting process. Tableting equipment with two filling stations provides control of the individual layer mass during the tableting process.

Hydrochlorothiazide (HCTZ) is a diuretic drug frequently used for the treatment of hypertension in combination with some other active pharmaceutical ingredients (APIs) and is ideal for fixed combination formulations in hypertension treatment. HCTZ is slightly soluble in water and has therefore poor gastrointestinal absorption (14, 15). Its solubility is potentially improved by addition of weak organic and inorganic bases that increase pH of solution. On the other hand, HCTZ undergoes alkaline hydrolysis in the presence of heat and moisture (14, 15). Solubility of HCTZ in aqueous solutions is low, in the pH range from 1.0 to 7.4, ranging from 0.0608 to 0.103 g per 100 mL. Solubility in aqueous solutions within pH 10.2–11.6 changes to 1.79 and 2.2 g per 100 mL.

The HCTZ ionization constant (pK_a) ranges from 8.60 (potentiometric titration) to 8.81 (photometric titration) (14, 15). Solid HCTZ stored at room temperature for five years shows no degradation (14). The *Ph. Eur.* monograph (16) specifies impurities A, B and C. In aqueous solutions HCTZ undergoes hydrolysis, giving formaldehyde and 4-amino-6-chloro-1,3-benzenedisulfonamide (impurity B according to *Ph. Eur.*) (14–16). Hydrolysis of HCTZ is complete at pH higher than 12 (14, 15). At pHs below 2.5 and above pH 12 degradation is linear and shows first-order dependence of H^+ and OH^- concentration (14, 15). The degradation profile between pH 7 and 11.5 is probably the result of dissociation equilibrium. The decreasing slope in the degradation profile from pH 7 to 3 is explained by the absence of intermediates, due to one- or two-step reaction (14, 15). The pH in the micro-environmental scale (pH_M) is strongly influenced by the included excipients (17) and therefore the approaches to modify pH_M are useful for optimization of HCTZ stability and dissolution profile. However, they have limitations associated with the heterogeneity of the formulations (17).

According to the well-known Noyes-Whitney equation the dissolution rate is a function of the solubility of the drug and the surface area available for dissolution. Consequently, the performance of the slowly disintegrating formulation is affected by geometric configuration (17) and drug dissolution rate:

$$\frac{\mathrm{d}m}{\mathrm{d}t} = \frac{DA(c_s - c)}{L}$$

where $\frac{\mathrm{d}m}{\mathrm{d}t}$ represents the dissolution rate, A is the surface area of the solid compact, c_s is the saturated concentration of the drug in the diffusion layer surrounding the compact, c is the concentration of the solid in the bulk dissolution medium, D is the diffusion coefficient of the substance and L is diffusion layer thickness.

The purpose of the present study was to investigate the impact of different placebo compositions with included pH modifiers on the HCTZ dissolution and stability in 2- and 3-layer tablets. Systems of two conventional tablets, *i.e.*, HCTZ and pH modifying placebo tablet were compared to 2-layer and 3-layer tablet regarding the dissolution characteristics and stability. Such 2-layer and 3-layer tablets prototypes can be used as model formulations with HCTZ in combination with other APIs for different fixed combination formulations. It is expected that the micro-environmental pH could modify the solubility and dissolution rate of HCTZ and have an impact on the HCTZ stability in solid state. In order to assure erosion as the only mechanism of disintegration of 2-layer and 3-layer tablets, no disintegration excipients were used in any tablet formulation.

EXPERIMENTAL

Materials

The following API and excipients were used: HCTZ with the assay of 99.98 % (Krka, d.d., Slovenia), manitol Partec M 200 (Merck Millipore, Germany), lactose monohydrate 200 mesh (Meggle, Germany), polyvinylpyrrolidone (Povidone K30) (BASF, Germany), colloidal anhydrous silica Aerosil 200 (Evonik, Germany), hydroxypropylcellulose Klucel EF (Ashland, UK), meglumin (Merck), citric acid (Jungbunzlauer, Austria), tri-sodium citrate dihydrate (Merck), sodium hydroxide (Merck), ferric oxide (Rockwood Italia, Italy), glycerol monostearate (GMS) (BASF), crospovidone (Kollidon CL) (BASF), Mg stearate (Faci Spa, Italy), Na-stearyl fumarate (JRS Pharma GmbH&Co.KG, Germany).

Preparation of granules

The first step in the preparation of 2- and 3-layer tablets was the granulation of fillers with different pH modifiers (in placebo layer) and separate preparation of HCTZ granules. Polyvinylpyrrolidone solution (17 %) with addition of different pH modifiers was used as granulating solution in placebo layers and hydroxypropyl cellulose (10 %) served as granulating solution in case of HCTZ. Manitol and lactose monohydrate were used as fillers and different pH modifiers (citric acid/tri-sodium citrate dihydrate, sodium hydroxide/meglumine) for the purpose of achieving specific pH values (Table I). Granulating solution was sprayed over powder mixtures in a top-spray fluid-bed granulation procedure (Glatt, GPCG WSG-3, Germany). Granules were dried up to the required loss on drying (LOD)

value, and a combination of hydrophobic (Mg-stearate) and hydrophilic (Na-stearyl fumarate) lubricants was added to dried placebo granules. HCTZ granules were prepared with manitol as filler, hydroxypropylcellulose (HPC) as binder in the granulating solution, ferric oxide as colour pigment, colloidal anhydrous silica as glidant and Na-stearyl fumarate as lubricant. Several granule formulations were prepared and are shown in Table I.

Table I. Composition of granules (%, m/m*) and pH values for 25 % granule water suspension*

Layer	A1g	A2g	A3g	NLg	NMg	BLg	BMg	Ug
	Acidic granules			Neutral granules		Alkaline granules		HCTZ
HCTZ	–	–	–	–	–	–	–	8.3
Povidone K30	5.0	5.0	5.0	5.0	5.0	5.0	5.0	–
Hydroxypropyl cellulose	–	–	–	–	–	–	–	0.5
Lactose monohydrate	–	–	–	93.0	–	86.6	–	–
Citric acid	1.4	1.7	0.3	–	–	–	–	–
Tri-sodium citrate dihydrate	–	1.8	3.8	–	–	–	–	–
Manitol	91.6	89.6	88.8	–	93.0	–	86.6	88.6
NaOH	–	–	–	–	–	1.4	1.4	–
Meglumine	–	–	–	–	–	5.0	5.0	–
Ferric oxide yellow	–	–	–	–	–	–	–	0.5
Silica colloidal, anhydrous	–	–	–	–	–	–	–	0.1
Mg-stearate	1.0	1.0	1.0	1.0	1.0	1.0	1.0	–
Na-stearyl fumarate	1.0	1.0	1.0	1.0	1.0	1.0	1.0	2.0
pH	2.4	4.2	6.4	7.0	8.2	11.3	12.1	7.2
LOD (%)	0.2	0.4	0.8	0.5	0.3	3.5	1.1	0.3

Characterization of granules

Loss on drying. – LOD evaluation was performed with HR73 (Mettler Toledo, Switzerland) apparatus. Granules (5–10 g) were dried for 5 min at 105 °C. Results are presented as percentage of sample mass loss.

pH of granulates. – Suspension (25 %) was prepared using different granulates and the pH value was measured with a pH meter (Mettler Toledo) (Table I).

Flow properties

All granules showed acceptable flow properties, confirmed by Hausner index calculations, based on the measurement of bulk and tapped density using a Jet Stampfvolumeter (STAV 2003, Gemini BV Laboratory, Germany). All granules showed comparable particle size distribution with the d50 values between 125–250 μm.

Compression of tablets

Tableting was performed using a column press SP 300 (Killian IMA, Germany). Round punches with 12 mm radius and bevelled edges were used. 2- and 3-layer tablets with 620 mg and 720 mg mass were pressed, respectively. In the case of 3-layer tablets an intermediate layer of glycerol monostearate in the amount of 100 mg was pressed between placebo and the HCTZ layer. It was added to separate the HCTZ layer and placebo layer in order to improve the solid state stability of HCTZ. Additionally, conventional HCTZ and placebo tablets with 310 mg mass were prepared, using round punches with 12 mm radius and bevelled edges. In order to assure comparability of the produced 2-, 3-layered tablets and conventional tablets, the compression force was adjusted to achieve a definite value of tablet porosity (*i.e.*, 22 ± 2 %). Tablet batches and their compositions are shown in Table II.

Table II. Designation of tablet batches and their composition

Code/name	Composition of tablets and layers
U	Conventional tablet with HCTZ granules Ug
A1, A2, A3	Conventional placebo tablet with granules A1g, A2g, A3g
NL, NM	Conventional placebo tablet with granules NLg, NMg (where NLg is designated for neutral placebo granules where lactose monohydrate is used as a filler while NMg is designated for neutral placebo granules where manitol is used as a filler)
BL, BM	Conventional placebo tablet with granules BLg, BMg (where BLg is designated for alkaline placebo granules where lactose monohydrate is used as a filler while BMg is designated for alkaline placebo granules where manitol is used as a filler)
2layer-A1, 2layer-A2, 2layer-A3	2-layer tablet with HCTZ and the A1g, A2g, A3g granule layer
2layer-NL, 2layer-NM	2-layer tablet with HCTZ and NLg, NMg granule layer
2layer-BL, 2layer-BM	2-layer tablet with HCTZ and BLg, BMg granule layer
3layer-A1, 3layer-A2, 3layer-A3	3-layer tablet with HCTZ, intermediate GMS layer and A1g, A2g, A3g granule layer
3layer-NM	3-layer tablet with HCTZ, intermediate GMS layer and NMg granule layer
3layer-BM	3-layer tablet with HCTZ, intermediate GMS layer and BMg granule layer

Characterization of tablets

For uniformity of tablet mass, the average value and standard deviation were determined for 20 tablets in the case of 2-layer tablets and 10 tablets in the case of 3-layer tablets.

Disintegration of tablets was tested according to *Ph. Eur.* at 25 ± 0.5 °C (16). This was done on three tablets purified water at two different temperatures, *i.e.*, at 25 and 37 °C.

Temperature of 25 °C was added in order to stipulate the influence of temperature on HCTZ solubility.

Uniformity of content and percentage of released HCTZ were assessed using HPLC (UV detector at 225 nm, Krka´s internal method fully validated in Krka, Novo Mesto, Slovenia). Uniformity of content was estimated on a set of three tablets for each batch.

Dissolution tests for HCTZ were performed using Apparatus 2, USP monograph for HCTZ tablets (23). The test was performed on three tablets from each batch in purified water at 22 ± 1 °C. The impact of the local pH microenvironment on the dissolution profile of HCTZ was tested. Two tablets were put into media and the release profiles of two conventional tablets (HCTZ + placebo) are presented in Fig. 1.

FT-IR spectra were recorded using a Spectrum 100 (Perkin Elmer, USA) with DTGS – deuterated triglycine sulphate detector. Spectra were recorded between 4000 and 400 cm^{-1}. Five-mg samples were mixed with 200 mg KBr and pressed into a pellet.

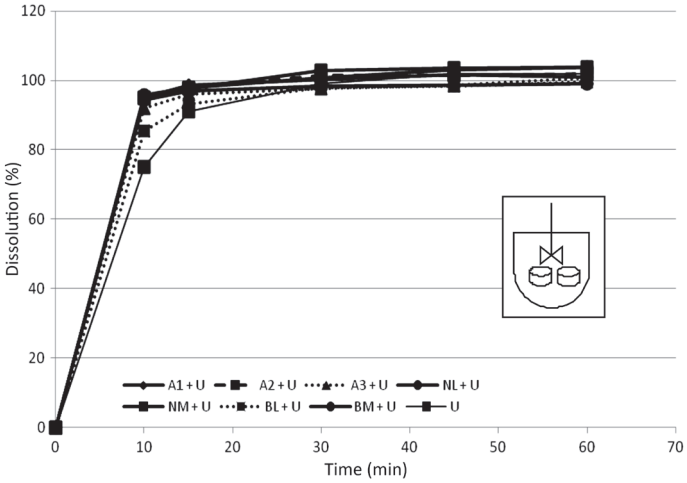

Fig. 1. HCTZ dissolution profiles for systems of two conventional tablets (HCTZ tablet designated as U, and placebo tablet with one of different pH modifiers: A1, A2, A3, NL, NM, BL, BM). Scheme in the figure represents the performance of the test with two conventional tablets. The test was performed on three tablets from each batch (RSD_{max} 5.0 %).

Stability testing

Conventional HCTZ, 2-layer tablets and 3-layer tablets (n = 6) were exposed to room (25 °C, 60 % RH) and stress conditions (50 °C, 75 % RH). Tablets were loaded to the control chambers (Kambič, Slovenia) to achieve and maintain their exposure to set conditions.

Quantification of related substances was performed by HPLC (UV detector at 225 nm, Krka´s internal method). Results are presented as % of individual impurity B and % of total impurities. HCTZ known impurities according to the *Ph. Eur.* (16) are impurity A: 6-chloro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide (chlorothiazide), impurity B: 4-amino-

-6-chlorobenzene-1,3-disulfonamide (salamide) and 6-chloro-*N*-[(6-chloro-7-sulfamoyl-2, 3-dihydro-4*H*-1,2,4-benzothiadiazine-4-yl 1,1-dioxide)methyl]-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide.

Maillard reaction occurs between the reducing sugar (lactose) added into the formulation as filler, and a primary or secondary aliphatic or aromatic amino group (in HCTZ). It is known, that Maillard reaction does not take place with non-reducing sugars (*e.g.*, mannitol). During the Maillard reaction, formation of melanoidins as brown-colored products occurs. Two approaches were tested, one using lactose monohydrate and the other mannitol, both in placebo layers. Evaluation of Maillard reaction was performed qualitatively by visual inspection of tablet samples only. Colour change was evaluated after first exposing tablets to 14- and 60-days periods under room conditions and after keeping tablets 14 days at 50 °C, 75 % RH and comparing to fresh tablets.

Maillard reaction was presumed when initial tablet colour appearance changed to brownish tones. While taking colour photographs, object exposure was defined by manually defining the amount of flash intensity; photographs were further standardized by the usage of black, white and grey standard cards.

RESULTS AND DISCUSSION

Characterization of prepared granules

The measurements of pH in 25 % granule suspension showed slightly alkaline pH of the HCTZ granules. Placebo granules (acid and alkaline types) achieved the desired pH values in all ranges. Moisture can affect particle flowability in two ways: by changing the surface properties of the particles and by linking of particles into larger clumps through liquid bridges (22). The high moisture content as in the case of BL is not desired due to the reduction of flow properties. Increased moisture content is probably due to the basic pH modifier content.

Characterization of tablets

Tablets were compressed using the compression force, which assured the average porosity about 21 % and comparable tensile strengths (ranging from 1.09 to 1.94 MPa).

Tablets of all batches met the requirements for uniformity of mass of single dose pharmaceutical dosage forms from *Ph. Eur.* (16).

The average content uniformity of active substances was determined for all batches of tablets and was found to be between 96.7 and 98.6 % of the nominal HCTZ value.

Disintegration

The tests were performed by comparing two conventional tablets (one placebo and one HCTZ) *vs*. 2-layer tablets. On average, conventional tablets disintegrated 25–30 % faster than 2-layer tablets. The simple explanation is the fact that in the case of conventional tablets (system of two tablets) the surface area is about 60 % higher than in the case of 2-layer tablets. All batches except the 2-layer NL tablet, met the disintegration requirement

according to *Ph. Eur.* (16), *i.e.*, less than 15 minutes. It was established that HCTZ layer disintegrated faster than any of the placebo layers, which was most probably due to the different binders used.

Dissolution tests

Two tablets together give a mass of 620 mg, equivalent to the mass of a 2-layer tablet. The approach simulates the intake of 2 drugs simultaneously like in the case of polypharmacy. But, in order to study only the effect of pH environment on HCTZ behaviour, the study was limited to the case where placebo would prevail over the influence of the active substance in the second layer.

All batches of two conventional tablets completely disintegrate and dissolve within 15 minutes. HCTZ tablet always disintegrates before the placebo tablet. For all combinations, the release satisfies the criteria of the guideline from the European Medicines Agency requirement for immediate release tablets, *i.e.*, more than 85 % of API is dissolved in 15 minutes (19). Results of the dissolution profiles imply that the influence of the placebo pH modifying tablet of 900 mL has no significant impact on the dissolution rate of HCTZ.

The dissolution profiles of 2-layer tablets are shown in Fig. 2.

All batches of 2-layer tablets disintegrated slower than the combination of two conventional tablets. As already discussed, this is due to the higher total surface area of conventional two-tablet systems in comparison with the multi-layered tablets. However, dissolution was completed in the time frame of approx. 30 minutes. The differences in the dissolution rates for each batch of 2-layer tablets are larger than in the group of the conventional two-tablet systems. This impact on the dissolution rate can be attributed to the fact that the 2-layer tablets of HCTZ have a physical barrier on one side of the HCTZ layer,

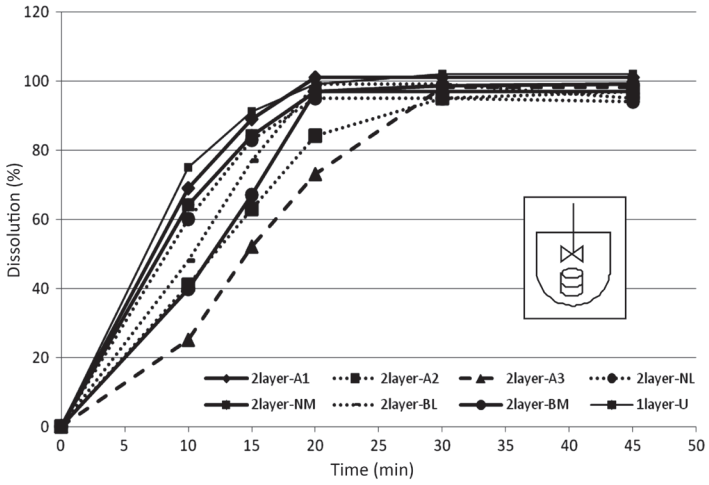

Fig. 2. HCTZ dissolution profiles for 2-layer tablets consisting of a HCTZ layer and different pH modifying layers (mass 620 mg). Comparison is made with a pure HCTZ tablet. The test was performed on three tablets from each batch (RSD_{max} 9.3 %).

which retards HCTZ dissolution. It can be also assumed that the HCTZ layer, which is in direct contact with different placebo layers and different micro pH values in the tablet, undergoes different degradation reactions (15, 20). During the study, it became evident that the degradation reactions and degradation products could influence the dissolution rate of HCTZ.

HCTZ release profile rates for all three systems with acidic placebo layers of 2-layer tablets showed a decrease with the increase of pH values of the corresponding placebo layer suspensions (Table I). Further, unexpectedly, a slow rate of HCTZ release was observed also for 2-layer tablets with an alkaline layer (BM, BL).

In order to exclude possible direct interaction between placebo (acid or alkaline) and the HCTZ layer, an additional layer was introduced into the 2-layer system.

Three-layer tablets consisted of a HCTZ layer, a layer of glycerol monostearate as the separating middle layer, and different placebo layers: A1, A2, A3, NM, NL, BM, BL. Tablet mass was 720 mg and the HCTZ dose was the same as in previous systems. Dissolution results are presented in Fig. 3.

It can be observed that some batches of 3-layer tablets did not dissolve completely in 45 min. The differences in the release profiles from 3-layer tablets are larger than in the cases of 2-layer and conventional two-tablet systems (HCTZ and placebo). All three systems with acidic placebo layers, *i.e.*, A1, A2 and A3 are consistent with the results of 2-layer tablets. In spite of inclusion of the separation layer, the alkaline formulation (3-layer BM) showed again, unexpectedly, slow and incomplete release of HCTZ. This result might correlate with the prolonged disintegration of these tablets during the dissolution test.

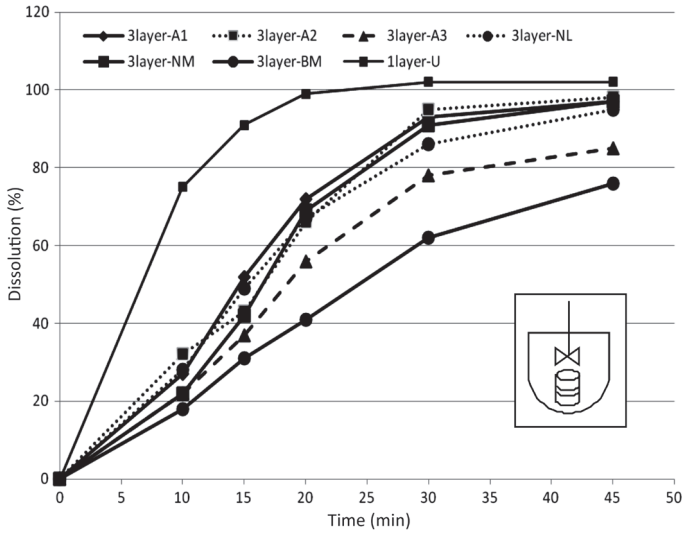

Fig. 3. HCTZ dissolution profiles of 3-layer tablets in distilled water where one layer consists of HCTZ granules, the intermediate layer with GMS and the third placebo layer with different pH modifiers A1, A2, A3, NL, NM, BM, compared to the HCTZ tablet as reference. The test was performed on three tablets from each batch (RSD_{max} 10.8 %).

Physical separation of the HCTZ layer and placebo layer with GMS decreases the dissolution rate of HCTZ. This can be also caused by GMS not being soluble in water and constituting a physical barrier between dissolution medium and HCTZ layer. The dissolution rate in the combination of two conventional tablets (HCTZ + pH modifying placebo tablet) is much higher than in the case of 2- or 3-layer tablets. These differences are in accord with the Noyes-Whitney equation (1), at least as the systems differ in the specific surface area.

Stability tests

The stability behaviour of HCTZ in 2-layer and 3-layer tablets was checked in comparison with HCTZ conventional tablets using HPLC. Impurity B, as a typical degradation impurity, and total impurities were measured. Quantity of impurity B is in the same molar ratio as formaldehyde according to the reversible chemical reaction of HCTZ degradation (15). Two-layer and 3-layer tablets were analyzed immediately after manufacturing (t = 0 days) and after 14 days under stress conditions of 50 °C and 75 % RH in closed vials. Results for impurity B and total impurities (Fig. 4) are presented as an increase of impurity values after storing formulations under stress conditions for 14 days.

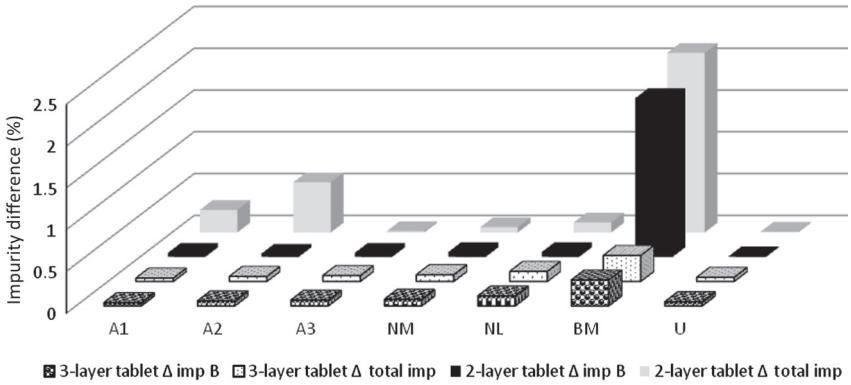

Fig. 4. Difference in impurity B and total impurities level in 2- and 3-layer tablets in comparison with the tablet U with HCTZ after 14 days, 50 °C, 75 % RH, in closed vials.

The contact between HCTZ and placebo layer in 2-layer tablets was only through the interface. From the results obtained after 14 days at 50 °C, 75 % RH, it is evident that HCTZ, in the presence of an alkaline placebo layer BM, with manitol as filler in the placebo layer, increased the content of impurity B, and consequently, formaldehyde and total impurities. In the case of sample BL, where lactose monohydrate is used as filler, complete degradation of the sample occured during stability testing and the results are not given in Fig. 4. The residue of the tablet can be seen in Fig. 5.

The results of the peak area of impurity B after 14 days under stress conditions show that the minimum peak area of impurity B was observed in contact with an acidic placebo layer. However, formulations A1, A2, A3, NL and NM exhibit comparable stability (Fig. 4).

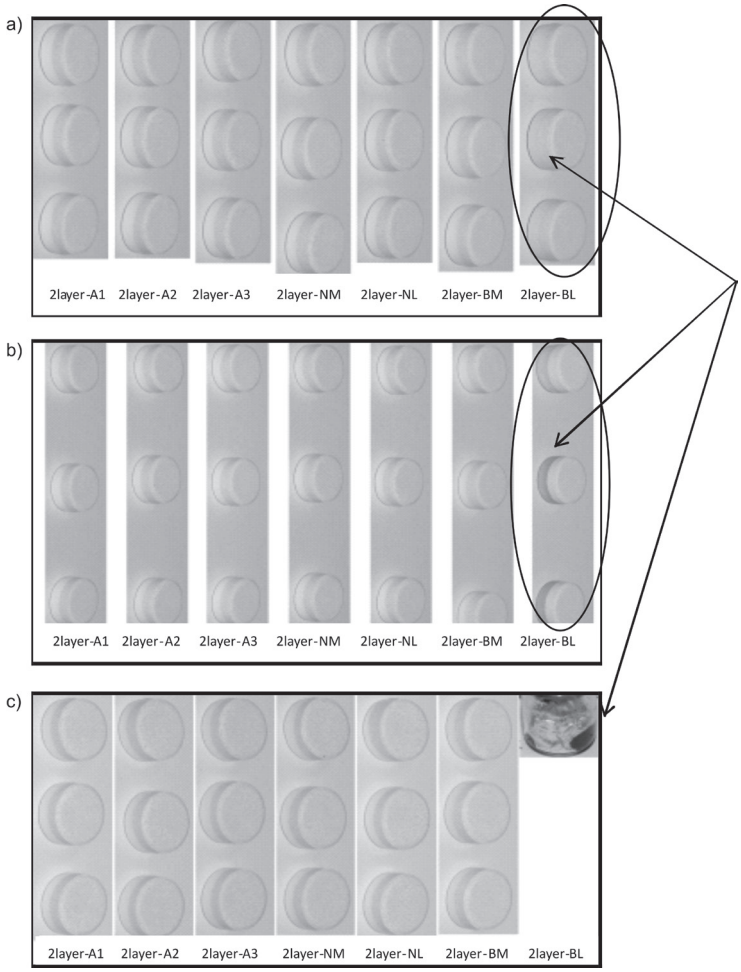

Fig. 5. Isometric perspective of 2-layer tablets (three samples of the same batch are presented) at: a) $t = 0$, b) 14 days under room conditions and c) 60 days under room conditions, 14 days at 50 °C, 75 % RH. Black arrows show the change in colour, which points to the Maillard reaction.

Complete chemical stability of HCTZ in terms of an increase of impurities cannot be achieved only by using physical separation of the HCTZ and placebo layers with a GMS barrier layer, as can be concluded from both sets of results (Fig. 4) denoted to 2-layers and 3-layers approach. The 2-layer BM tablet represents the most critical case for the stability of HCTZ and chemical stability is not ensured.

The levels of impurity B and total impurities rose above the *Ph. Eur.* (16) limits and could not provide the desired quality of the drug. The third layer was inserted in order to elucidate the efficacy of physical separation between layers and to enhance the stability of HCTZ formulation. From the results in Fig. 4 it is evident that the presence of the alkaline

placebo layer promoted the formation of impurity B. However, in the case of 3-layer tablets total impurities are present to a much lower extent than in 2-layer tablets, within acceptable limits. In case of 3-layer tablets, total impurities mainly consist of impurity B.

The stability results for 2-layer BM and 3-layer BM under accelerated conditions show that HCTZ degradation pathways are not routed only by the contact between layers but also by additional impact of temperature and local pH microenvironment enhancing HCTZ hydrolysis. Microenvironment is influenced by the chemical properties of the excipients and water in the formulation. Humid environment facilitates hydrolytic reactions and formaldehyde is formed besides impurity B. Formaldehyde is, however, not identified as an impurity in *Ph. Eur.* (16). We should consider formaldehyde as a highly reactive substance that could react with other tablet components, giving water insoluble products (15, 20, 21). Addition of acidic additives into the formulation reduces the hydrolysis of HCTZ and lowers the formation of formaldehyde (20). In this way, improvement in stability and dissolution may be achieved for all kinds of tablets. This is due to the fact that the hydrolysis process of HCTZ is pH dependent (20, 21).

It is evident that the stability of HCTZ with impurity B level below 0.5 % is achieved by physical separation of the drug layer by the intermediate 3rd layer between HCTZ and placebo layers and by the use of acidic or neutral placebo layers. The presence of formaldehyde in the tablet, especially in the case of alkaline formulation, might explain the impact on the slower dissolution rate and the increase of total impurities.

Slightly brown coloration of the 2-layer tablets could be seen already after 14 days of storage under room conditions. It was shown that with improper composition (alkaline millieu, presence of lactose) the Maillard reaction occurred even under mild conditions of storage and in a relatively short period of time. Maillard reaction can be observed in the sample of 2- layer BL, which underwent the chemical reaction of lactose in an alkaline environment and a secondary amine (pH modifier – meglumine).

Formation of insoluble substances

In order to confirm the presence of related insoluble substances in 3-layer tablets, three different mixtures were prepared additionally. The three mixtures comprised GMS, HCTZ, Kollidon CL and addition of either NaOH or citric acid and sodium citrate dihydrate mixture in the ratio similar to the composition of 3-layer tablets. These mixtures were compressed into monolithic tablets named Tablets 1, Tablets 2 and Tablets 3 using comparable compression force as in the case of 3-layer tablets (Table III).

The disintegration test results for Tablets 1 show significantly longer disintegration time when compared to neutral (Tablets 3) and acidic formulations (Tablet 2). These results are in accord with the dissolution profiles of 3-layer tablets.

After disintegration of 6 tablets from samples of Tablets 1, Tablets 2 and Tablets 3, the undissolved flakes were filtered through filter paper and dried. The dried powder was analyzed using IR spectroscopy (Fig. 6). The presence of possible new substances was looked for in the IR spectra of Tablets 1, 2 and 3 disintegration residues. No typical peaks of HCTZ spectra were present in the disintegration residue of Tablets 1, which is not the case of Tablets 2 and 3 (wavenumbers around 1600 cm^{-1}). The peak at this wavenumber corresponds to N–H bond of primary amines and its absence predicts the formation of another substance. The result again confirms the alkaline system differs from the acidic

Table III. Composition of prepared tablets (%, m/m*) and the corresponding disintegration times*

Composition	Tablets 1	Tablets 2	Tablets 3
HCTZ	20.0	20.0	20.0
Glycerol monostearate (GMS)	74.5	72.0	76.0
Crospovidone (Kollidon CL)	4.0	4.0	4.0
NaOH	1.5	–	–
Citric acid	–	2.0	–
Tri-citrate dihydrate	–	2.0	–
Disintegration time (min)	18	2.5	2.5

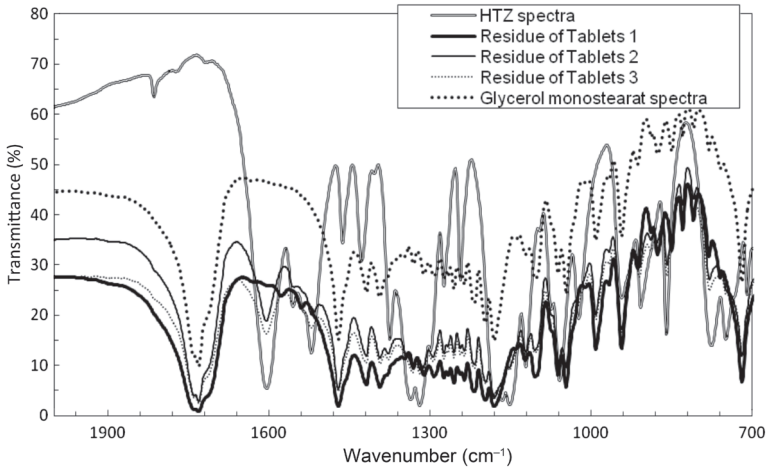

Fig. 6. IR spectra of residues of Tablets 1, Tablets 2 and Tablets 3 after dissolving in water.

and neutral ones and accelerates the degradation of HCTZ. Hydrolysis of hydrochlorothiazide can cause interruption of N-H, formation of formaldehyde and reaction with Kollidon CL. We can also only assume the formation of hydrochlorothiazide salt, which has to be proven by synthesis of the hydrochlorothiazide salt standard and evaluated with an additional technique such as FT-IR, LC-MS and mass spectroscopy.

CONCLUSIONS

The study confirmed the dependence of the dissolution profile and stability of HCTZ on placebo layer composition in 2- and 3-layer tablets.

The presumed HCTZ enhanced dissolution through the local alkaline pH environment was not confirmed as an optimal approach. It was shown that during storage the presence of alkaline pH and humidity caused the hydrolytic reaction of HCTZ. Impurity

B as a typical degradation impurity was increased in 2-layer tablets with alkaline placebo layers. It was proven that the content of impurity B can be reduced by using 3-layer tablets.

It is supposed that impurity B and formaldehyde released after decomposition of HCTZ most probably react with other ingredients in the tablet formulation and as a result the formation of non-soluble compounds might be responsible for the decrease of HCTZ release rate. It was confirmed that the intermediate 3rd layer decreased, HCTZ dissolution rate of 3-layer tablets with an alkaline placebo layer; however, at the same time the stability was improved. It is note worthy that in the case of 3-layer tablets, no Maillard products were observed.

Having in mind the differences in HCTZ behaviour in 2- and 3-layer tablets in comparison with the conventional tablet systems, which mimic the concomitant application of drugs, development of multi-layered formulations has to be carefully considered with regard to the drug dissolution and stability.

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