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Research paper

Effect of gliclazide immobilization into layered double hydroxide on drug release

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ABSTRACT

This paper deals with a new hydrotalcite-like compound used as a matrix to improve dissolution rate of the poorly water-soluble drug gliclazide and to administer at the same time micro- and oligo-elements useful to improve insulin performance. Gliclazide is a second-generation sulfonylurea compound used in the treatment of type II diabetes mellitus. As a consequence of the poor water solubility, its absorption is limited. Thus, a new hydrotalcite-like compound containing Zinc and Chromium, micronutrients directly involved in the physiology of insulin and in the carbohydrate, lipid and protein metabolism, was synthesized. The gliclazide-hydrotalcite-like clay nanohybrid was prepared via ion-exchange in its nitrate form and was characterized by inductively coupled plasma-optical emission spectrometry and thermogravimetric analysis. The drug loading resulted in 42.9% (w/w). As a consequence of the intercalation, the interlayer distance of the host increased from 0.89 nm (interlayer distance of nitrate form) to 1.5 nm. The intercalation product was submitted to solubility measurements and in vitro dissolution test. A remarkable improvement of the apparent solubility and dissolution rate in comparison to the crystalline drug was observed in acid fluids (pH 1.2 and 3). The presence of chromium and zinc cations was also found in the medium. These results showed that the hybrid nanostructure could represent a promising system to improve drug dissolution rate and to release cations involved in the performance of insulin.

1. Introduction

Gliclazide (GLI) (Fig. 1) is a second-generation sulfonylurea derivative, widely used for the treatment of type II diabetes mellitus. Prior reports state that the drug shows good general tolerability, low incidence of hypoglycaemia, low rate of secondary failure and may represent a better choice in long-term sulfonylurea therapy [1,2]. Rapid gastrointestinal absorption is required for oral hypoglycaemic drugs to prevent a sudden increase in blood glucose level after food intake in diabetic patients. The absorption rate of GLI appears to be slow and variable among subjects. Previous studies show that the time to reach peak plasma GLI concentration (t_{max}) is very variable and ranges from 2 to 8 h following tablet oral administration [3]. The slow rate of drug absorption can be correlated with either the poor drug dissolution from the formulation or its poor permeability across the gastrointestinal membrane. The solubility of GLI in distilled water at room temperature is very low (1.5 μg/ml) and is attributed to its hydrophobic structural nature [3,4]. Furthermore, it is a weak acid (pKa = 5.8) [5] whose solubility profile varies according to the pH of the dissolution medium [2]. As a consequence, the poor and slow dissolution rate represents the absorption rate-limiting step. GLI solubility is influenced by the pH values of gastric fluid and is conditioned by intersubject and intrasubject variability too. Therefore, many strategies have been proposed with the aim of improving GLI dissolution rate [6–9].

The aim of the present work was to use the nanohybrid material, formed by the inorganic host hydrotalcite-like anionic clay (HTlc), to improve dissolution rate of the organic guest GLI. Hydrotalcite-like compounds are anionic, inorganic-layered solids that can be easily synthesized in laboratory and have the general formula $[M(II)_{1-x}M(III)_x(OH)_2]^{x+}[A^{n-}_{x/n}]mS$, where M(II) is a divalent cation, such as Mg, Zn or Cu, M(III) is a trivalent cation, such as Al, Cr or Fe, A^{n-} is an anion of charge n such as NO_3^-, Cl^- or organic anion, and m is the mole of solvent co-intercalated per mol of compound [10–12]. Hydrotalcite layers have positive fixed charges that are balanced by counterions, e.g. NO_3^-. The counterions are positioned in the interlayer region and are exchangeable by other inorganic, organic and metallo-organic anions and/or whichever biologically active molecules containing ionisable acid group, such as GLI. At acid pH values (pH < 4), the HTlc dissolves and releases the drug in molecular form promptly suitable for absorption [13,14].

In this work, instead of the already known Zn–Al–HTlc [14], the ternary system Zn–Al–Cr–HTlc was used because Cr^{3+} and Zn^{2+} cations are directly involved in the performance of insulin and, thus, influence carbohydrate, lipid and protein metabolism.

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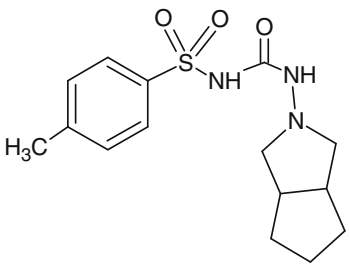

Fig. 1. Chemical formula of gliclazide.

Trivalent chromium, present in many foods and nutrient supplements, is an essential nutrient with very low toxicity. A recent study demonstrated that chromium may function as part of oligopeptide low-molecular weight chromium (LMWCr)-binding substance [15]. This oligopeptide is found on insulin-sensitive cells. LMWCr has been proposed to participate in the insulin signal amplification system; the result of this process is the activation of the insulin receptor. Moreover, chromium inhibits phosphotyrosine phosphatase, the enzyme involved in the extinction of the insulin signal. On the basis of these action mechanisms, it has been suggested that chromium enhances insulin binding, its internalization and β-cell sensitivity [15].

Several clinical trials suggest that the reduced Zn status is associated with diabetes and that Zn is involved in insulin synthesis, storage, secretion and signalling, and thus, in the consequent action of insulin on the metabolism. Zn ions bind insulin with a hexameric crystalline structure, which is stored in secretory granules [16]. Moreover, zinc ions have a physiological role in insulin signal transduction as a particularly sensitive target of zinc ions is the protein tyrosine phosphatase 1B, a key regulator of the phosphorylation state of the insulin receptor [17].

Furthermore, Zn and Cr are hypothesised to function as antioxidants [18]. A restored Zn and Cr status in type 2 diabetes mellitus patients may oppose the negative effects of oxidative stress and help to prevent complications associated with diabetes [19–22].

Recently, studies have been carried out with the aim of obtaining GLI-controlled release formulations [23,24]. Incorporation of GLI in this kind of formulations may control its absorption from the gastrointestinal tract and overcome the variability problems, which are associated with GLI administration. Following these observations, hydrotalcite has been proposed as a matrix to produce GLI-controlled release enteric formulations after proper coating. In fact, drugs can be released after intercalation in a modified fashion by exchanging them with the ions present in the dissolution medium [14,25,26].

In light of these results, the present work was aimed to (i) evaluate the effects of GLI–HTlc intercalation on the drug dissolution rate to provide additional evidences of the utility to employ lamellar solid as carriers; (ii) obtain useful co-administration of oligo- and micro-elements (Zn and Cr); (iii) evaluate the possibility to obtain a drug-controlled release in the intestinal environment.

For these purposes, GLI was intercalated into HTlc and, after characterization, drug apparent solubility and its dissolution rate at different pH values were compared to that of the crystalline drug. The in vitro chromium and zinc release was evaluated as well.

2. Materials and methods

2.1. Materials

Chromium nitrate, zinc nitrate and aluminum nitrate were obtained from Riedel-de-Haën (Seelze, Germany). GLI was purchased from Sigma–Aldrich Chemical (Milan, Italy). Deionized water was obtained by reverse osmosis process with a MilliQ system (Millipore, Rome, Italy). Other reagents and solvents were of reagent grade and were used without further purification.

2.2. Methods

2.2.1. Synthesis of Zn–Al–Cr–HTlc

Zn–Al–Cr–HTlc was synthesized according to the procedure based on the precipitation of Zn(II), Al(III) and Cr(III) as hydroxycarbonates, accomplished by the hydrolysis of urea [27]. Solid urea (58.4 g) was poured into an aqueous solution obtained by mixing 500 ml of zinc nitrate 0.5 M with 189 ml of aluminum nitrate 0.5 M. The mixture was refluxed and when the solution became cloudy, 5.56 ml of an aqueous solution of chromium nitrate 0.5 M was dripped. The suspension was allowed under reflux for 24 h. The resulting solid was filtered, washed twice with degassed water and then dried at room temperature over P_4O_{10}. The resulting hydrotalcite was titrated with HNO_3 water solution (1 M) at pH = 5 to convert the carbonate form into the nitrate form. The titration of original HTlc–CO_3 with concentrated HNO_3 was performed to exchange carbonate anions with NO_3^- anions that are more easily replaceable than CO_3^{2-} [28,29]. Indeed, it is well known that the CO_3^{2-} anions are strongly held in the interlayer region because form double electrostatic bounds and it is very difficult to replace them with other anions using simple ion-exchange procedures [25].

The resulting solid product (HTlc–NO_3) was filtered, washed three times with degassed water under N_2 atmosphere, and finally dried at room temperature over P_4O_{10}. The resulting HTlc–NO_3 was characterized by XRPD, ICP-OES analysis, TGA and FT-IR.

2.2.2. Intercalation of GLI in HTlc–NO_3

GLI (3.56 g) was dissolved in a degassed water/acetone (1/3 v/v) mixture (100 ml) and then was converted into the sodium salt form by adding a carbon dioxide free NaOH 0.1 N solution (11.01 ml). Then HTlc–NO_3 (4 g) was added. This mixture was kept under magnetic stirring at room temperature for 24 h. The resulting solid (afterwards HTlc–GLI) was filtered in vacuo, washed twice with degassed water and with acetone under N_2 atmosphere, and finally equilibrated on a saturated NaCl solution (75% of relative humidity) at room temperature.

HTlc–GLI was characterized by XRDP, ICP-OES analysis and FT-IR. The drug content was determined by TGA and UV absorption spectrophotometer Agilent 8453 at λ_{max} = 227.0 nm.

2.2.3. Characterization

The X-ray powder diffraction (XRPD) patterns were taken by a computer-controlled PW 1710 Philips diffractometer (Philips, Almelo, The Netherlands), using Ni-filtered Cu Kα radiation.

FT-IR spectra in the range 4000–400 cm^{-1} were recorded in air, at room temperature on a Jasco FT/IR 410, 420 Herschel series (Jasco Corporation Tokyo, Japan) in KBr dispersion using the EasiDiff Diffuse Reflectance Accessory. Samples were prepared by gently grounding the powders with KBr.

Differential scanning calorimetry (DSC) analyses were performed using an automatic thermal analyser (Mettler Toledo DSC821^e Novate Milanese, Italy). Temperature calibrations were performed using an indium standard. Holed aluminum pans were employed in the experiments for all samples and an empty pan, prepared in the same way, was used as reference. Samples of 3–6 mg were weighted directly into aluminum pans and thermal analyses were conducted at a heating rate of 5 °C/min from 25 to 200 °C.

Thermogravimetric analyses (TGA) were carried out by a thermoanalyzer (TG-DTA Netzsch STA 490, Selb, Germany) at a heating rate of 10 °C/min with 30 ml/min air flow.

2.2.4. Preparation of GLI/HTlc–NO_3 and GLI/HTlc–Cl physical mixtures (PHYS-MIX)

GLI/HTlc–NO_3 physical mixture was prepared by mixing crystalline GLI and HTlc–NO_3 in the proper weight ratio with a spatula and was used, as a comparison, in XRPD, FT-IR and DSC analyses. GLI/HTlc–Cl physical mixture was used in vitro release tests and solubility assays, as HTlc–NO_3/GLI physical mixture shows an UV adsorption band due to released nitrates that interferes with GLI at λ_{max} 227.0 nm. For these purposes, in turn GLI/HTlc–Cl physical mixture was used. HTlc in chloride form was prepared according as follows: HTlc–NO_3 (3 g) was suspended in a degassed aqueous solution of NaCl (0.5 M) and the suspension was kept at room temperature under magnetic stirring for 24 h. The solid (HTlc–Cl) was recovered by filtration and was characterized by XRDP. The GLI/HTlc–Cl physical mixture was prepared as described above.

2.2.5. Solubility studies

Drug solubility from the intercalated compound, from the physical mixture and from the crystalline drug powder, was determined in sovrasaturation condition and under stirring (140 rpm) using an orbital incubator (Gallencamp Incubator Type INR 2000, Leicestershire UK). The solubilization media were: (i) pH 1.2 ± 0.1 gastric juice (USP XX) without pepsin and NaCl at 37 ± 0.5 °C, (ii) pH 3 ± 0.1 fluid prepared by adding 100 μl of HCl (37%) to 400 ml of deionized water and adjusting the resulting volume to 1000 ml with deionized water. Excess amounts of each sample, in a manner that each preparation corresponded to 100 mg of GLI, were placed in series of closed flat-bottomed glass vessels containing 50 ml of the solubilization fluid pre-equilibrated at 37 °C ± 0.5 °C. At appropriate time intervals, aliquots of 2 ml were removed and filtered (25 mm nylon membrane, unit 0.45 μm Membrane Disc Filter Type HNN, mdi) with the Swinnex system. The removed aliquots were immediately replenished with the same volume of media equilibrated at 37 ± 0.5 °C. After filtration the drug concentration was determined by UV spectrophotometry (λ_{max} = 227.0 nm). Tests were performed in triplicate, the results were registered as an average and the error was expressed as standard deviation.

2.2.6. Dissolution tests

In vitro drug releases from HTlc–GLI, PHYS-MIX-1 and crystalline drug were performed by using the rotation paddle method, according to Farmacopea Ufficiale Italiana (FU XI ed.) at 70 rpm at 37 ± 0.5 °C. Tests were performed in 1000 ml of the following fluids: (i) pH 1.2 ± 0.1 gastric juice (USP XX) without pepsin, (ii) pH 3 ± 0.1, prepared as described above, (iii) pH 3 ± 0.1 saline fluid, prepared by adding NaCl (2 g) to pH 3 fluid, (iv) phosphate buffer pH 6.8 ± 0.1 (USP XX) and (v) simulated pH 7.5 ± 0.1 intestinal fluid (USP XX). Drug release profiles were monitored for 3 h at pH 1.2 and 3 and for 24 h at other pH values. Dissolution tests were performed in samples containing 80 mg of GLI. Four millilitres of dissolution fluid were removed from the vessel at predetermined intervals and replaced by the same volume of fresh dissolution medium. The samples were filtered through Membrane Disc Filter Type HNN, mdi 0.45 μm with the Swinnex system, and the GLI content was determined by UV spectrophotometry (λ_{max} = 227.0 nm). All experiments were performed in triplicate, the results were registered as an average and the error was expressed as standard deviation.

The release of chromium and zinc from HTlc was determined in acid fluids (pH 1.2 gastric juice and pH 3 with/without NaCl) by Inductively Coupled Plasma-Optical Emission Spectrometers (ICP-OES), Varian, Inc 710-ES, which permits the multi-element analysis of major, minor and trace elements in a variety of sample matrices. Samples were introduced as solutions into the instrument and their quantification was performed through external calibration.

3. Results and discussion

3.1. Preparation and characterization of the HTlc–NO_3 and HTlc–GLI

The synthesis of hydrotalcite in its nitrate form and its conversion into the GLI form have been performed as described elsewhere in this article. The desired hydrotalcite contained Zn, Al and Cr as elements.

The proper chromium molar fraction was determined on the basis of the following considerations: (i) in spite of the quantity admitted by the Recommended Daily Allowances for chromium, that is 20–200 μg/day [15], in the literature it is reported that in many clinical trials chromium supplementation may reach 1000 μg/day and its safety is supported by numerous papers [21], (ii) the clinically effective GLI dosage is 80–320 mg/day. Thus, supposing an administration of the maximum dosage of GLI (320 mg/day), the following chromium supplementation should not exceed the dose of 1000 μg per day.

After many attempts, the desired HTlc, whose chromium index was proper to permit a right cation maximum administration of ⩽1000 μg/die, was obtained. From ICP-OES analysis and TGA, its formula resulted $[Zn_{0.762}Al_{0.231}Cr_{0.007}(OH)_2](CO_3)_{0.119}{\cdot}0.4H_2O$.

Drug intercalation was performed by anionic exchange between HTlc–NO_3 $[Zn_{0.762}Al_{0.231}Cr_{0.007}(OH)_2](NO_3)_{0.238}{\cdot}0.4H_2O]$ and GLI sodium salt generated in situ. The chemical composition of the intercalated system by ICP-OES analysis and TGA resulted to be $[Zn_{0.762}Al_{0.231}Cr_{0.007}(OH)_2](GLI)_{0.238}{\cdot}0.66H_2O$. This proves that GLI intercalation has occurred due to the increase in the interlayer distance observed in the XRPD diffractogram (Fig. 2). It can be seen that, as a consequence of the intercalation, the interlayer distance increased from 0.89 nm (for HTlc–NO_3) to 1.5 nm (for HTlc–GLI). Since the thickness layer is 0.48 nm [30] the gallery height, i.e. the interlayer distance, increased to 1.06 nm (i.e. 1.5–0.48). The intercalation compound structural model (Fig. 3), obtained by optimizing the anion geometry by Hyperchem MM+ force field, suggests that the GLI ions are positioned in the interlayer region as a monolayer of species partially superimposed. This arrangement is due to π−π interactions between two benzene rings of GLI and ionic interactions between the C=O of the ureide group, where the negative charge is localized, and the HTlc positive charges.

The amount of GLI content was determined by UV and TGA. By spectrophotometric determination, the drug loading resulted to be 42.9 ± 0.9% (w/w).

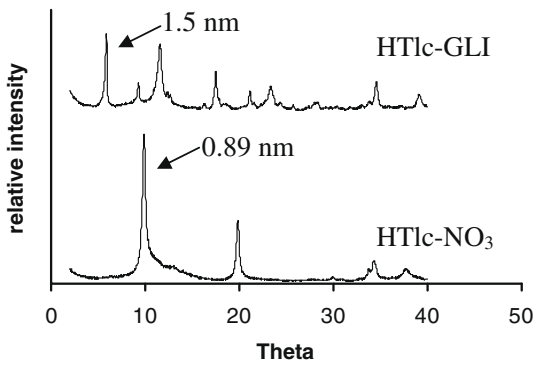

Fig. 2. XRPD patterns of HTlc–GLI and HTlc–NO_3.

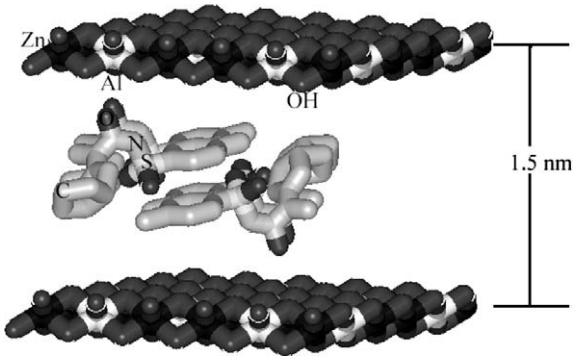

Fig. 3. Computer Generate Model of HTlc–GLI (some Al ions are substituted by Cr ions in the ratio of HTlc–GLI formula).

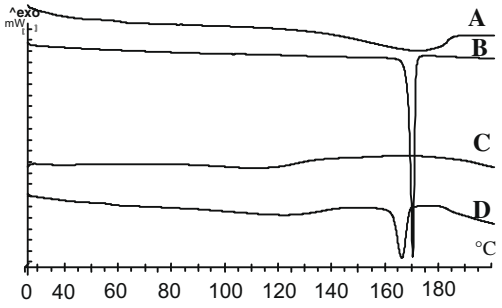

Fig. 5. Thermal profile of HTlc–GLI (A), GLI (B), HTlc–NO_3 (C) and PHYS-MIX (D).

TGA of HTlc–GLI (Fig. 4) shows the first endothermic mass reduction at ca. 180 °C due to the loss of intercalated water and two more weight losses (the first endothermic and the second exothermic) attributed to thermal degradation of intercalated GLI molecules and the inorganic sheets dehydroxylation confirming the calculated drug loading.

DSC thermal behaviours of HTlc–GLI, crystalline GLI, HTlc–NO_3 and PHYS-MIX are reported (Fig. 5). GLI thermogram shows an endothermic peak around 170 °C due to the fusion of the compound; in the thermogram of HTlc–GLI, the lack of the same endothermic peak is a proof of the formation of a new compound consequent to drug intercalation into the HTlc. In the physical mixture thermogram, the presence of GLI and HTlc–NO_3 peaks is diagnostic of the intactness of these two compounds. The drug melting peak was slightly shifted to lower temperature and becomes wider. This may be explained by weak interactions between the drug and HTlc in the physical mixture at high temperature.

FT-IR spectra of the samples crystalline GLI, HTlc–NO_3, HTlc–GLI and PHYS-MIX (Fig. 6) are reported as well. The spectrum of hydrotalcite in nitrate form is characterized by the absorption of the nitrate ions at 1400 cm^{-1}. In the intercalation product, this band disappears as a consequence of the complete anionic exchange between NO_3^- and GLI, while the GLI spectrum shows a peak at 3265 cm^{-1} related to NH ureidic group. The lack of this peak in HTlc–GLI is attributed to the presence of GLI in an anionic form. Moreover the carbonyl group, which is located at 1710 cm^{-1} in the GLI FT-IR spectrum, shifted in the HTlc–GLI spectrum to 1665 cm^{-1}. The sulphonyl group bands located at 1353 cm^{-1} (asymmetric vibration) and 1164 cm^{-1} (symmetric vibration) in crystalline GLI shifted to 1371 and 1146 cm^{-1}, respectively, in HTlc–GLI spectrum. The absorption variations of the functional groups of GLI in the intercalated compound are again attributed to the presence of GLI in its anionic form. The carbonyl shift is higher than the sulphonyl shift since the carbonyl group has a minor double-bond character [31]. For this reason, it was supposed that the negative charge is prevalently localized on the carbonyl oxygen rather than on the ureide nitrogen due to the mesomeric effect of the vicinal group. Similar changes were not present in the PHYS-MIX. Its IR spectrum, in fact, displays no interactions between drug and inorganic matrix.

3.2. Solubility measurement

Solubility of GLI from HTlc–GLI, PHYS-MIX and the crystalline drug was determined at pH 1.2 ± 0.1 gastric juice (USP XX) without pepsin and NaCl at 37 ± 0.5 °C (Fig. 7I) and at pH 3 ± 0.1 fluid at 37 ± 0.5 °C (Fig. 7II).

3.2.1. Solubility measurement at pH 1.2

At pH 1.2, in the case of the HTlc–GLI, after 5 min, the GLI concentration was ca. five times higher than that of the crystalline powder and higher values were maintained for 1 h. This higher concentration during the first hour is attributed to the lack of crystallinity of the intercalated drug, which is directly released in the molecular form by dissolution of HTlc in acidic medium. This was confirmed by the fact that PHYS-MIX drug concentration was always lower than that from HTlc–GLI.

3.2.2. Solubility measurement at pH 3

At pH 3, the drug concentration from HTlc–GLI was higher than that of the crystalline powder during the first hour. The highest dif-

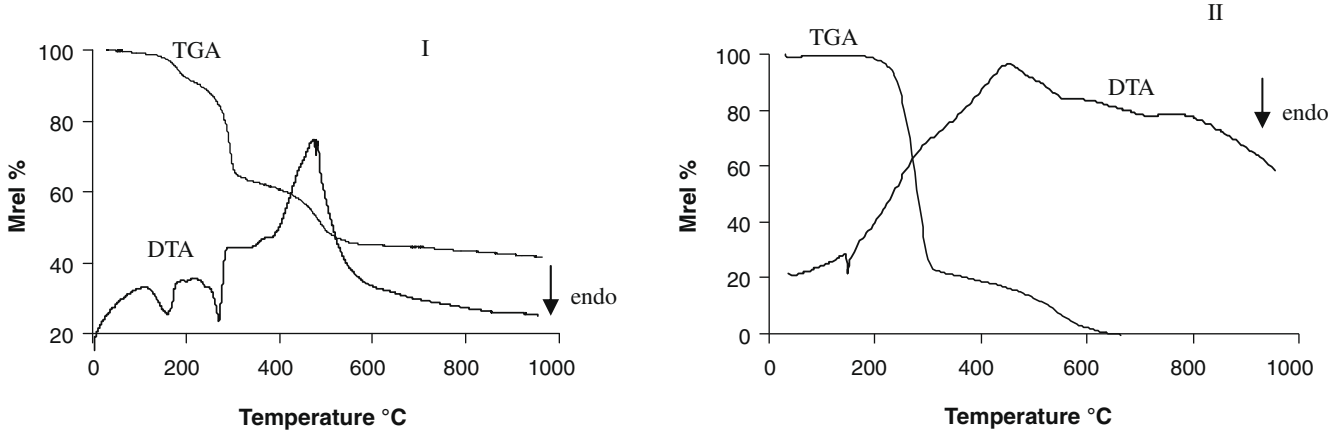

Fig. 4. TGA and DTA of HTlc–GLI (I) and GLI (II).

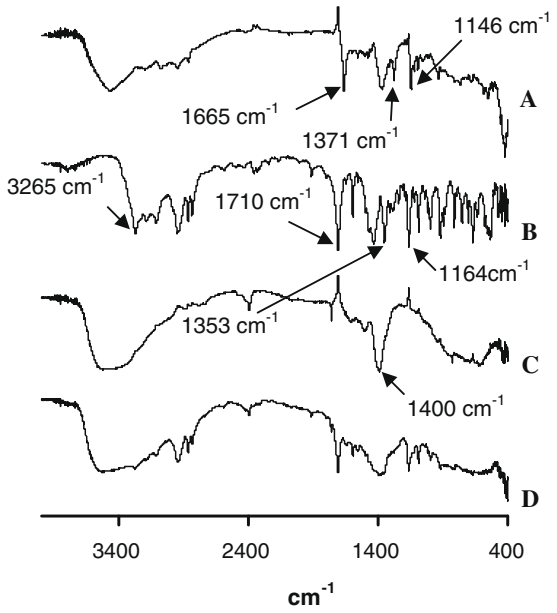

Fig. 6. FT-IR spectra of HTlc–GLI (A), GLI (B), HTlc–NO_3 (C) and PHYS-MIX (D).

ference in concentration was reached after 5 min (80 mg/l for HTlc–GLI and 22 mg/l for crystalline GLI, ca. 3.6 times higher) and was maintained for 20 min. After this time, the drug concentration gradually decreased and, after ca. 90 min, it was comparable to the value of the crystalline GLI. The higher apparent solubility during the first 20 min is due, as it was observed at pH 1.2, to the liberation of drug in a molecular form, whereas the following decrease in the apparent solubility from HTlc–GLI is due to HTlc destruction, which increased the pH from 3 to 5.2 (measured values). The pH variation of dissolution medium is responsible for the reduction in drug solubility. In fact, the same values of drug concentration were observed from HTlc–GLI and crystalline GLI at the end of the experiment because the intrinsic solubility of GLI is the same at pH 3.0 and 5.2 [2].

3.3. In vitro drug release

The GLI dissolution behaviour from its crystalline form, from HTlc–GLI complex and from PHYS-MIX, was observed at different pH values, to evaluate the influence of the inorganic matrix. Indeed, this matrix acts differently on drug release depending on the pH of the medium (above or below 4). In fact, at pH < 4, HTlc dissolves and thus GLI is released, whereas at pH > 4, GLI undergoes an ion-exchange procedure. Drug release studies were carried out in sink conditions in all fluids with the exception of that at pH 3 (with/without NaCl) because of the low GLI solubility. In this case, conditions reported in the literature [2] have been used.

3.3.1. In vitro drug release at pH 1.2 and 3

At pH 1.2 (Fig. 8I), as expected, a faster drug dissolution rate was obtained from HTlc–GLI than from crystalline GLI and PHYS-MIX, whereas comparable drug release profiles were obtained from GLI and PHYS-MIX-2.

At pH 3 (with NaCl), drug release from HTlc–GLI was higher than from GLI and physical mixture (Fig. 8II). It is interesting to note that GLI release from HTlc–GLI never reached 100% and that after an initial burst effect within 45 min it became gradual and slower. This behaviour is due to the consequence of both the hydrotalcite dissolution and the increase in pH from 3.0 to 5.0. At this pH , the hydrotalcite layers do not dissolve and the drug anions, not yet released, remain entrapped into the lamellar spaces. Therefore, it can be released only by anionic exchange with Cl^- present in the fluid. In order to confirm this hypothesis, the same test was performed in a NaCl-free dissolution medium. As reported in Fig. 8III, the drug release from HTlc–GLI at the end of the test reached 58.7% vs. the 71.7% obtained in the presence of the saline fluid. This is a proof that Cl^- anions contribute to drug release. It must be highlighted that as it was impossible to perform the release tests in sink condition at pH 3, this fact could affect the drug release profile. In the case of the PHYS-MIX, the release percentages were considerably lower than those from the intercalated product, but were higher than those of crystalline GLI. This could be explained by the formation of higher pH microenvironments, due to HTlc disruption, in which the GLI solubility is higher.

3.3.2. In vitro drug release at pH 6.8 and 7.5

The drug was released only during the ionic-exchange process of mono and dibasic phosphates present in the dissolution medium at pH 6.8 and 7.5. The drug release was monitored for 24 h although in Fig. 8IV–V data are reported for the first 8 h. At pH 6.8, the drug release from HTlc–GLI was always higher than that from crystalline GLI, probably because of the high ionic force of the fluid, but never reached a complete drug release. Since mono and dibasic phosphate ions have a higher affinity to hydrotalcite than the drug, they replace GLI with the lamellar structure by consequently increasing its release.

The drug release profile was different at pH 7.5 than at pH 6.8. In this case, it was slower from HTlc–GLI than from crystalline GLI and PHYS-MIX and reached 90%. This different behaviour could be ascribed to (i) the higher ionic force of the phosphate buffer at pH

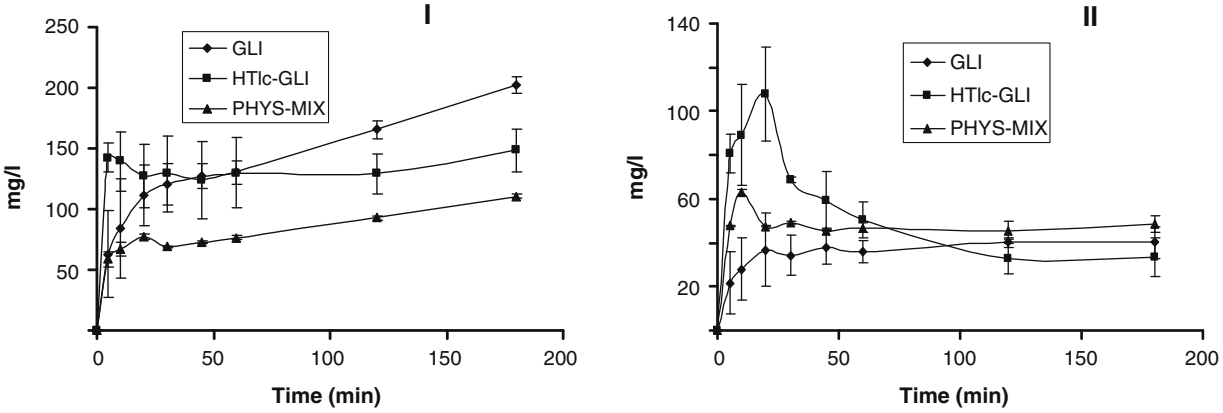

Fig. 7. (I–II)-Apparent Solubility of GLI (♦), HTlc–GLI (■) and PHYS-MIX (▲) at pH 1.2 (I) and at pH 3 (II).

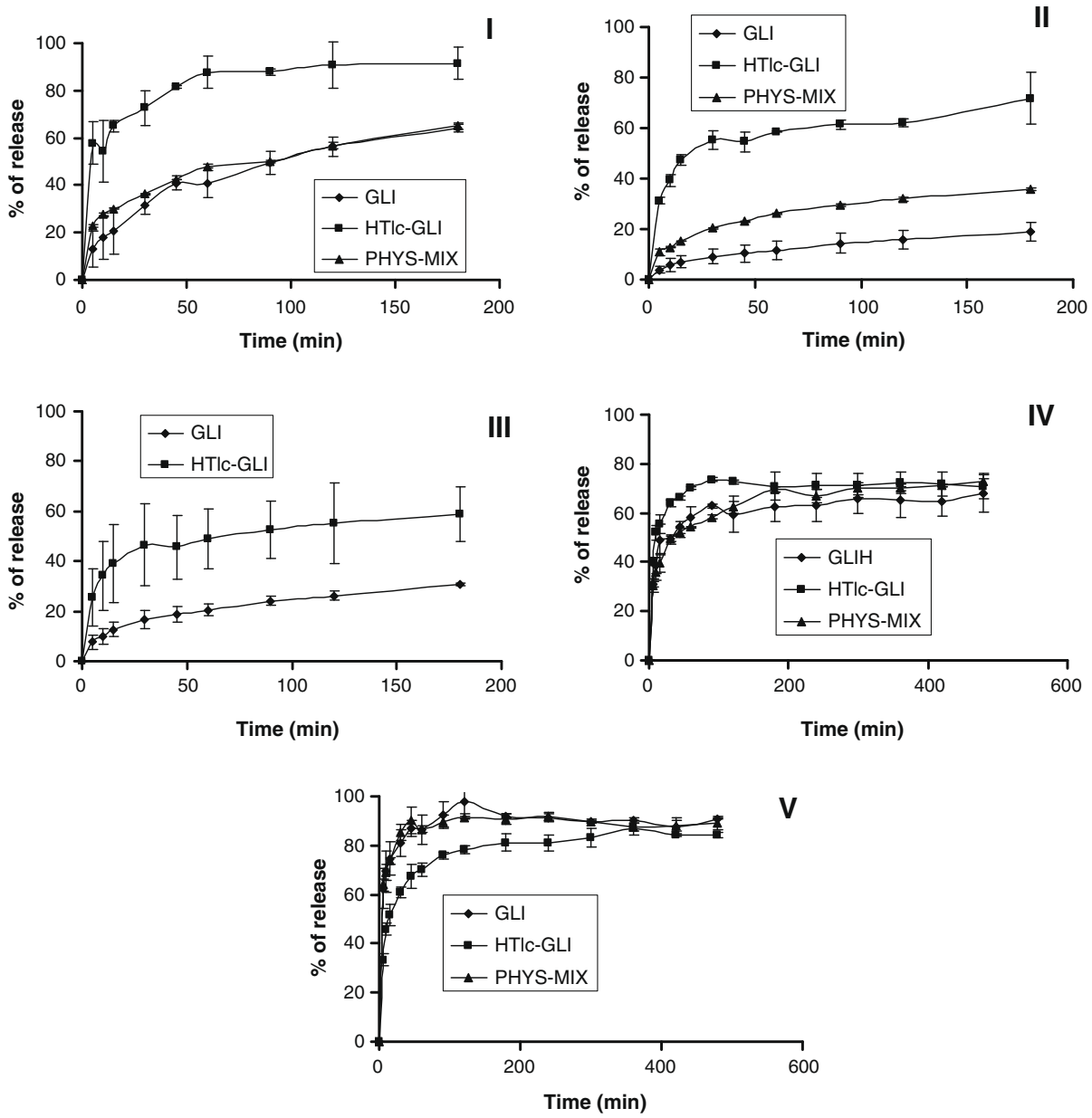

Fig. 8. (I–II)-Release Profile of GLI from crystalline GLI (♦), HTlc–GLI (■) and PHYS-MIX (▲) at pH 1.2 (I), at pH 3 with NaCl (II), pH 3 without NaCl (III), pH 6.8 (IV) and pH 7.5 (V).

6.8; (ii) the grafting reaction that occurs at pH 6.8 between the ZnAl–HTlc layer hydroxyls and the acidic $H_2PO_4^-$ anions, giving rise to the formation of a layered hydroxyphosphate [27]. In this strongly bounded form, phosphates are no longer exchangeable and can impede further diffusion of the intercalated GLI that remains entrapped between the HTlc layers.

3.4. In vitro chromium and zinc release

The choice of Zn–Al–Cr–HTlc was most wanted because of the utility of the chromium and zinc supplementation in people with diabetes [20–22]. As the destruction of the matrix lamellar structure occurs [10] at pH < 4 and thus the relative constituent metals are released, the chromium release was monitored in dissolution tests at acid pH values (pH 1.2 and 3).

At pH 1.2, the hydrotalcite quickly dissolves; therefore, in this medium, the complete chromium release (0.488 ± 0.029 ppm) was obtained after 5 min. This value was maintained during the experiment (3 h). Similar behaviour was seen in zinc, whose release was almost complete after 10 min (44.4 ± 1.1 ppm).

At pH 3 (with/without NaCl), the profile of chromium and zinc releases had different behaviours than at pH 1.2 fluid. The chromium and zinc dissolution profiles in saline and NaCl-free pH 3 media are reported (Fig. 9). In both cases, a rapid chromium release was observed in the first 30 min, which reached a plateau of ca. 33–35% without achieving complete release. Possible explanations are that chromium and zinc ions are released from the lamellae as long as the HTlc layers are dissolved. The registered increase in pH was responsible for the stopping of the matrix dissolution and the lack of the metal release. As chromium release is not influenced by the presence of Cl^-, since it does not undergo anionic exchange process, the release curves in both dissolution fluids at pH 3 (with/without NaCl) were superimposable.

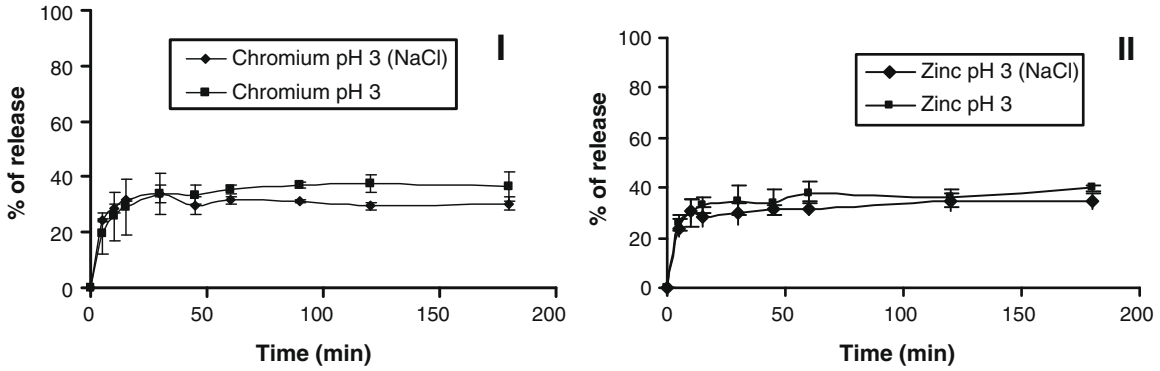

Fig. 9. Release profile at pH 3 of chromium (I) and Zinc (II) (with/without NaCl).

4. Conclusions

In this work, a new approach for improving GLI apparent solubility and dissolution rate is described. This method is based on the hydrotalcite feasibility of intercalating drugs, not arranged in crystals between its layers, and on its characteristic of dissolving it at acid pH (< 4) in molecular form.

This approach has different advantages:

1. The GLI anions through an exchange process gave rise to a Zn–Al–Cr–HTlc intercalation compound with a drug loading of 42.9% w/w.
2. Dissolution test at both pH 1.2 and 3 showed a rapid and improved drug release as a consequence of the intercalation process in the matrix.
3. Concurrent Cr^{3+} and Zn^{2+} releases were detected during all dissolution tests.

On the basis of these results, Zn–Al–Cr–HTlc can be considered a good matrix to improve GLI solubility and dissolution rate in the stomach, to overcome its variability in bioavailability (C_{max} and T_{max}), which is related to its poor water solubility, and to permit a co-administration of Cr^{+3} and Zn^{+2} cations as well.

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References

[1] A.D. Harrower, Comparison of efficacy, secondary failure rate, and complications of sulfonylureas, J. Diabetes Complicat. 8 (1994) 201–203.
[2] S.S. Hong, S.H. Lee, Y.J. Lee, S.J. Chung, M.H. Lee, C.K. Shim, Accelerated oral absorption of gliclazide in human subjects from a soft gelatin capsule containing a PEG 400 suspension of gliclazide, J. Control. Rel. 51 (1998) 185–192.
[3] K.J. Palmer, R.N. Brogden, Gliclazide – an update of its pharmacological properties and therapeutic efficacy in non-insulin-dependent diabetes mellitus, Drugs 46 (1993) 92–125.
[4] Y. Zhao, W. Chen, Q. Cai, S. Wang, J. Bo, C. Wu, Erosion induced controllable release of gliclazide encapsulated inside degradable polymeric particles, Macromol. Biosci. 4 (2004) 308–313.
[5] C.S. Winters, L. Shields, P. Timmins, P. York, Solid-state properties and crystal structure of gliclazide, J. Pharm. Sci. 33 (1994) 300–304.
[6] M.J. Arias-Blanco, J.R. Moyano, J.I. Perez-Martinez, J.M. Gines, Study of inclusion of gliclazide in α-cyclodextrin, J. Pharm. Biomed. Anal. 18 (1998) 275–279.
[7] J.R. Moyano, M.J. Arias-Blanco, J.M. Ginés, F. Giordano, Solid-state characterization and dissolution characteristics of gliclazide-β-cyclodextrin inclusion complexes, Int. J. Pharm. 148 (1997) 211–217.
[8] Y. Özkan, T. Atay, N. Dikmen, A. Işimer, H.Y. Aboul-Enein, Improvement of water solubility and in vitro dissolution rate of gliclazide by complexation with β-cyclodextrin, Pharm. Acta Helv. 74 (2000) 365–370.
[9] K.A. Alkhamis, H. Allaboun, W.Y. Al-Momani, Study of the solubilization of gliclazide by aqueous micellar solutions, J. Pharm. Sci. 92 (2003) 839–846.
[10] V. Rives, Layered Double Hydroxides: Present and Future, Nova Science Publishers, New York, USA, 2000.
[11] F. Trifirò, A. Vaccari, Hydrotalcite-like anionic clays (layered double hydroxides), in: G. Alberti, T. Bein (Eds.), Solid-State Supramolecular Chemistry: Two and Three-Dimensional Inorganic Networks, of Comprehensive Supramolecular Chemistry, vol. 7, Pergamon Elsevier Science Ltd. Press, Oxford, 1996, pp. 1–17.
[12] F. Leroux, C. Taviot-Guého, Fine tuning between organic and inorganic host structure: new trends in layered double hydroxide hybrid assemblies, J. Mater. Chem. 15 (2005) 3628–3642.
[13] V. Ambrogi, G. Fardella, G. Grandolini, M. Nocchetti, L. Perioli, Effect of hydrotalcite-like compounds on the aqueous solubility of some poorly water-soluble drugs, J. Pham. Sci. 92 (2003) 1407–1418.
[14] U. Costantino, V. Ambrogi, M. Nocchetti, L. Perioli, Hydrotalcite-like compounds: versatile layered hosts of molecular anions with biological activity, Micropor. Mesopor. Mater. 107 (2008) 149–160.
[15] W.T. Cefalu, F.B. Hu, Role of chromium in human health and in diabetes, Diabetes Care 27 (2004) 2741–2751.
[16] C.G. Taylor, Zinc, the pancreas, and diabetes: insights from rodent studies and future directions, BioMetals 18 (2005) 305–312.
[17] H. Hajo, M. Wolfgang, Protein tyrosine phosphatases as targets of the combined insulinomimetic effects of zinc and oxidants, BioMetals 18 (2005) 333–338.
[18] E.F. Rostan, H.V. DeBuys, D.L. Madey, S.R. Pinnell, Evidence supporting zinc as an important antioxidant for skin, Int. J. Derm. 41 (2002) 606–611.
[19] R.A. Anderson, A. Roussel, N. Zouari, S. Mahjoub, J. Matheau, A. Kerkeni, Potential antioxidant effects of zinc and chromium supplementation in people with type 2 diabetes mellitus, J. Am. Coll. Nutr. 20 (2001) 212–218.
[20] J.R. Komorowski, D. Greenberg, V. Juturu, Chromium picolinate does not produce chromosome damage, Toxicol. In Vitro 22 (2008) 819–826.
[21] F.C. Lau, M. Bagchi, C.K. Sen, D. Bagchi, Nutrigenomic basis of beneficial effects of chromium(III) on obesity and diabetes, Mol. Cell. Biochem. 317 (2008) 1–10.
[22] H.E. Barlett, F. Eperjesi, Nutritional supplementation of type 2 diabetes: a systematic review, Ophthalmic Physiol. Opt. 28 (2008) 503–523.
[23] R.S. Al-Kassas, O.M.N. Al-Gohary, M.M. Al-Faadhel, Controlling of systemic absorption of gliclazide through incorporation into alginate beads, Int. J. Pharm. 341 (2007) 230–237.
[24] J.K. McGavin, C.M. Perry, K.L. Goa, Gliclazide modified release, Drugs 62 (2002) 1357–1364.
[25] V. Ambrogi, G. Fardella, G. Grandolini, L. Perioli, Intercalation compound of hydrotalcite-like anionic clays with antiinflammatory agents – I. Intercalation and in vitro release of ibuprofen, Int. J. Pharm. 220 (2001) 23–32.
[26] V. Ambrogi, G. Fardella, G. Grandolini, L. Perioli, M.C. Tiralti, Intercalation compounds of hydrotalcite-like anionic clays with anti-inflammatory agents, II: uptake of diclofenac for a controlled release formulation, AAPS PharmSciTech 3 (3) (2002) article 26.
[27] U. Costantino, F. Marmottini, M. Nocchetti, R. Vivani, New Synthetic Route to Hydrotalcite Like-Compounds. Characterization and Properties of the Obtained Materials, Eur. J. Inorg. Chem. (1998) 1439–1446.
[28] W.T. Reichle, Synthesis of anionic clay minerals (mixed metal hydroxides hydrotalcite), Solid State Ionics 22 (1986) 135–141.
[29] S. Miyata, Anion-exchange properties of hydrotalcite-like compounds, Clays Clay Miner. 31 (1983) 305–311.
[30] N.T. Whilton, P.J. Vickers, S. Mann, Bioinorganic clays: synthesis and characterization of amino- and polyamino acid intercalated layered double hydroxides, J. Mater. Chem. 7 (1997) 1623–1629.
[31] A.R. Vogel's, B.S. Furniss, A.J. Hannaford, P.W.G. Smith, A.R. Tatchell, Textbook of Pratical Organic Chemistry, fifth ed., Longman Scientific & Technical, Great Britain, 1989.