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Prolonged release of metformin by SiO_2 nanoparticles pellets for type II diabetes control

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ABSTRACT

Mesoporous silica nanoparticles (MSNPs) were synthesized and loaded with metformin hydrochloride (Metf), its adsorption has studied at different concentrations and pHs, optimal adsorption conditions were determined. Hybrid MSNPs-Metf were mixed with chitosan to compress them and form quasi-spherical pellets, were coated with five chitosan layers as a barrier to prolong metformin release. It showed that this pellet is useful for metformin controlled release since drug over time was significantly delayed by the chitosan coating and then, as metformin is electrostatically linked to MSNPs, it also controls the release of drug, releasing 170 mg after 17 h of exposure at pH 1.2. When pH is > 1.2, metformin release was significantly prolonged. Since 170 mg is 21% of a 850-mg metformin dose and previous studies report that 90% of metformin is recovered as unchanged drug in urine after 12 h of metformin intakes. These results suggest that MSNPs-Metf pellets, coated with chitosan, are an option to avoid excessive metformin ingest.

1. Introduction

Diabetes mellitus is a global health problem due to its elevated prevalence and disability produced. It is one of the main morbidity and mortality cause; nearly 8.3% of the world's population suffers from this disease, its origin lies in some factors such as obesity and sedentary lifestyle (Wild et al., 2004). *Diabetes mellitus* developing serious diseases affecting heart, blood vessels, teeth, kidneys, eyes, nerves and developing infections, which shortens life expectancy (Chacko, 2016), it is caused by a multifarious metabolic disorder characterized by defects in insulin secretion and/or the insulin action, causing hyperglycemia, where fasting plasma glucose concentration is above 1.26 g/L or above 2.00 g/L in blood at any time (Viswanatha et al., 2017).

Metformin hydrochloride (Metf) is one of the most widely used agents for *Diabetes mellitus* control, it is an oral antihyperglycemic that improves glucose tolerance in patients by decreasing basal and postprandial plasma glucose. Metformin acts through three mechanisms: first, reduces hepatic glucose production by inhibiting hypoglycemia and avoids hyperinsulinemia; second, it increases insulin sensitivity in muscle and improves peripheral glucose uptake and utilization; third, it delays the intestinal absorption of glucose. During controlled clinical trials of metformin (850 mg) three times daily, peak plasma levels are reached between 2.5 and 3.0 h after ingestion and do not exceed 590–1300 ng/mL with half-life between 1.5 and 4.5 h, reaching a lower concentration in blood than in plasma (Moffat et al., 2006). Metformin kinetics are characterized by slow and incomplete absorption (bioavailability 50–60%), from an oral dose of about 30 to 50% is excreted unchanged in urine within 24 h and about 30% in feces (Moffat et al., 2006).

Traditional doses to eliminate pain or infections require high medication levels, generating toxicity, to keep low drug level in serum lower levels than the required dose are provided, causing drug resistance. A local system that delivers medicament could correct these inconvenient of traditional dosing (Warren et al., 2008). A problem is presented when drugs are ingested orally, they could dissolve rapidly in the stomach producing blood level peaks and may not produce adequate levels. In addition, when drug blood level is adequate, it begin to decrease and becomes necessary to ingest another dose in a few hours. It is a problem since some drugs are irritating to the stomach or may produce undesirable side effects. The need to release drugs in a gradual or sustained way has given rise to exhaustive research that has generated different solutions, among them arise the possibility of coating the

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particles or tablets with molecules or polymers that dissolve slowly or do not dissolve in the gastric juice, so then, the drug is delivered in the intestines. An alternative is to mix the drug in a molten medium of grease, plastic polymer or wax, and then the composite is cooled to be granulated, encapsulated or compressed (Wilson et al., 2008). Another alternative is to adsorb the drug in ion exchange resins; or lately, in mesoporous silica nanoparticles. MSNPs can be used for drug storage due to its large surface area and pore volume, appropriate pore size, low density, thermal insulation, permeability, large porosity and does not swell in solvents (Vafayi and Gharibe, 2013). One of the most interesting MSNPs properties is the biocompatibility, this protein has been research subject as protein adsorption (Karlsson et al., 2003), cell adhesion (Sapelkin et al., 2006), biodegradability (Anderson et al., 2003), and tissue compatibility assessment (Rosengren et al., 2000), among others. The possibility of modifying the size of MSNPs gives advantages over other materials, also the pore walls surface chemistry may be changed and controlled, besides the possibility of functionalizing its surface to adapt it to certain medications (Schwartz et al., 2005). Many drug molecules delivered orally suffer for poor bioavailability (Brayden, 2003) due to small solubility and/or drug dissolution rate in the intestinal lumen, slow or inadequate drug permeability across the gastrointestinal wall and the high intestinal metabolism (Panchagnula and Thomas, 2001). In this sense, MSNPs are stable under the stomach and gastrointestinal tract pH without being eroded, their physico-chemical properties remain intact (Salonen et al., 2001), which allows them to transport and deliver the drug adequately.

Polymers are used in drug oral dosage forms to provide controlled and sustained release to enhance therapeutic prosperity and lower adverse side effects (Khandare and Haag, 2010). Among the approved polymers to control the drug release by U.S. FDA are polymethacrylate, polyvinyl acetate and ethyl cellulose, which are hydrophobic in nature and polyvinyl alcohol, polyethylene oxide, hydroxyl cellulose and chitosan, which are hydrophilic (Mansour et al., 2010). Chitosan, due to its nontoxicity, biocompatibility and biodegradability, has been widely used in medicine, agriculture, cosmetics, tissue engineering, food, pharmaceutics fields and so on (Qin et al., 2002; Kweon et al., 2003). Controlled release of medicament from an inert matrix presents some advantages such as efficiency and safety (Negoda et al., 2013).

In this work, mesoporous silica nanoparticles (MSNPs) were synthesized and used as matrix for metformin adsorption (MSNPs-Metf). The MSNPs surface charge, allowing the protonated metformin ($Metf^{1+}$) depending pH is objet of study, obtaining a stable system since it is adsorbed on surface particles by electrostatic forces and/or covalent bonds. To achieve successful metformin delivery, pH should be taken into account, the sudden release of metformin in the stomach should be avoided, since stomach is not considered the optimum site for metformin delivery. Prolong the metformin release, is one objective, even at pH 1.2 (stomach pH), which is the lowest pH that the MSNPs-Metf will be submitted. To prevent an early metformin release, MSNPs-Metf and chitosan were mixed and compressed to pellets, and then coated with five chitosan layers. These pellets are an attractive material as metformin carriers over a wide range of acid pH.

2. Materials and methods

2.1. Materials

Hydrochloric Acid (HCl, 37%), ethanol (C_2H_5OH, 99.5%), sodium Hydroxide (NaOH, 97%), powder quartz (SiO_2, 99.5%), sodium carbonate (Na_2CO_3, ≥99.0%), Pluronic P123 (Poly(ethylene glycol)-*block*-poly(propylene glycol)-*block*-poly(ethylene glycol)), citric acid (≥99.5%), and medium molecular weight chitosan (75–85% deacetylated) used were from Sigma Aldrich, metformin hydrochloride from Merck and Ultra-pure water with resistivity ≥18.3 MΩ cm was obtained after treatment with a RiOs5™-MilliQ® system (Millipore Ltd., UK). All chemical reagents were used as received.

2.2. Synthesis of SiO_2 particles

Mesoporous silica (MSNPs) synthesis was carried out according to the process of Dong et al. (2017). Briefly, powder quartz and sodium carbonate (molar ratio of 1:1) were mixed and molten (1460 °C) for 2 h to form carbon dioxide and sodium silicate (Na_2SiO_3), then this solid (4.7 g) was dissolved in water into a high-pressure reactor. A second solution was prepared, 2.4 g of poly(ethylene glycol)-*block*-poly(propylene glycol)-*block*-poly(ethylene glycol) was dissolved in 120 mL of HCl 3 M and stirred at 40 °C until get a transparent solution, then the sodium silicate solution was slowly incorporated, stirred for 1 min and kept unstirred for 20 h at 40 °C. The slurry was heated at 100 °C in a teflon-flask for 24 h. The solid was collected by filtration, washed with water, dried, calcined for 6 h at 550 °C and grinded in an agate mortar.

2.3. Characterization of SiO_2 particles

To evaluate the zeta potential and particle diameter distribution of MSNPs, Dynamic Light Scattering (DLS) method was carried out by a Laser Zeta meter (Malvern Instruments, Zetasizer MPT-Z) equipped with a 633 nm He−Ne laser and operating at a scattering angle of 173° at 25 °C. To determine the diameter distribution, the samples were prepared in plastic cuvettes by addition of 0.001 g of MSNPs particles and dispersed in 4 mL of ethanol; components were gentle blended after each addition. To evaluate the zeta potential, the MSNPs were dispersed in deionized water, pH was controlled with an auto-titrator (Malvern Instruments, model MPT-Z) with feeding solutions of sodium hydroxide or hydrochloric acid at 0.25 M. To obtain an infrared spectrum of MSNPs and thus determine the qualitative composition of the synthesized sample, an spectrophotometry analysis was carried out using a Fourier Transform Infrared Spectrophotometer (FTIR) trademark Agilent Technologies, model Cary 600, spectra were performed in a range of wavelengths from 4000 to 400 cm^{-1}. To determine the porosity of MSNPs and also to determine the presence of metformin in the sample once they have been exposed 24 h to metformin solution, Nitrogen adsorption-desorption isotherms were measured at −196 °C by a Micrometrics porosimeter, model ASAP 2020. Before each nitrogen adsorption-desorption measurement, the samples were subjected to degassing process at less 10 h at 200 °C under vacuum, the adsorption and desorption of nitrogen in solid particles allows determination of total pore volume and pore size using Brunauer, Emmett and Teller model (BET). To determine the MSNPs morphology, micrographs and elemental analysis (EDX) were performed using a Transmission Electron Microscopy (TEM, JOEL, JEM 1230) equipped with energy dispersive X-ray spectroscope.

2.4. Drug loading

Metformin adsorption studies were performed by mixing 0.1 g of MSNPs with 10 mL of metformin solution at different concentrations (10, 50, 100, 200 and 400 ppm) and at different pH values (1, 7 and 13). Lower pH values were reached by adding drops of HCl (0.1 M) and higher pH values were reached by adding NaOH (0.1 M). The metformin mixture and MSNPs was left in agitation during 0.5, 1, 2, 4, 8 and 24 h. To separate the non-adsorbed metformin from the MSNPs, vacuum filtration was performed, and both the metformin solution and the silicon oxide particles were recovered. In each experiment the absorbance of the recovered metformin solution was measured to determine, the concentration of metformin not adsorbed by the MSNPs using calibration curves, previously constructed at different pH and in the range from 0 to 400 ppm. By difference of concentrations, the quantity of loaded metformin (Q) into MSNPs was calculated using from the following equation:

$$Q = \frac{(C_0 - C)V}{m}, \quad (1)$$

where Q is in mg/g, C_0 is the initial metformin concentration (mg/L), C is metformin concentration after adsorption (mg/L), V is the volume of the solution (L) and m is MSNPs mass (g). The absorbance analysis was carried out with an UV–Vis Spectrophotometer (Agilent Technologies, Cary 60) at 232 nm.

2.5. Preparation of pellets

For the pelleting process, MSNPs particles previously subjected to metformin adsorption were selected, with an initial metformin concentration of 400 ppm and pH 7. Pellet preparation was carry out by mixing with spatula of 1.17 g of hybrid MSNPs-Metf and 0.3 g of chitosan solution (1 g of chitosan and 20 mL of 1% w/w acetic acid solution, agitated for 12 h), the paste was poured into cavities of polytetrafluoroethylene molds, 6 pellets were generated with these portions. The molds were stored 1 h at 37 °C, the pellets were extracted from the molds and dried on polytetrafluoroethylene mesh at 37 °C for 12 h. To coat the pellet with a chitosan layer, according to the method of Warren et al. (Warren et al., 2008), the pellet was immersed into chitosan solution (1 g of chitosan and 50 mL of 1% w/w acetic acid solution, stirred for 12 h) under very slow stirring, then placed and drained on a polytetrafluoroethylene mesh, washed with deionized water and placed on the mesh for drying during 2 h at 25 °C and 1 h at 37 °C. The pellet immersion into the chitosan solution until drying the pellet was repeated five times to increase the pellet coating layers and thus delay metformin release. The pellets surface morphology was characterized using a scanning electron microscope (JEOL JSM-820).

2.6. Release studies

To determine the metformin release, for every three pellets hydrochloric acid solution pH (1.2, 3, 6 or 7) in ratio 0.1 g/5 mL were added. The release profiles were obtained *via* independent samples and reading were taken every 30 min, during 17 h, simulating the metformin process desorption in human organism; it remained under very slow stirring. After set time had elapsed, the acid solution with metformin was recovered by means of vacuum filtration. The filtered liquid absorbance was measured and the metformin concentration was determined by UV–Vis absorption using an external standard calibration curve and with the absolute value obtained from Eq. (1), where m is still MSNPs mass (g), three pellets contain 0.5 g of MSNPs.

3. Results and discussions

3.1. Characterization of SiO_2 particles

The MSNPs size was determined with the DLS technique. Different MSNPs particle size was obtained, but the diameter average was 220 ± 64 nm, the histogram is shown in Fig. 1a. Particle size is very important since small size implies a greater surface area of silicon oxide, allowing greater contact with metformin. In addition, to use MSNPs as a transport system for a drug within human body, particles sizes smaller than 400 nm are required to pass through human system conducts (Mishima et al., 2006). MSNPs particles meet the requirements for drug release in human body and can be considered as nanopharmaceutical agents that can evade the immune system and penetrate barriers, such as the wall of the gastrointestinal tract and hemotoencephalic, used by the body to prevent the penetration of unwanted or foreign substances (Bawa, 2009). Fig. 1b shows the N_2 adsorption isotherms of MSNPs, the shape is characteristic of isotherm of type IV, which occurs in porous adsorbent materials that contain a pore radius range of approximately 15–1000 Å. The adsorption slope increases at high relative pressures (P/P_0) due to an increase of N_2 when the pore is filled, the inflection point of the isotherm occurs near the first monolayer. It presents a hysteresis at a relative pressure of 0.57, the slope of the adsorption is high and the desorption slope is much

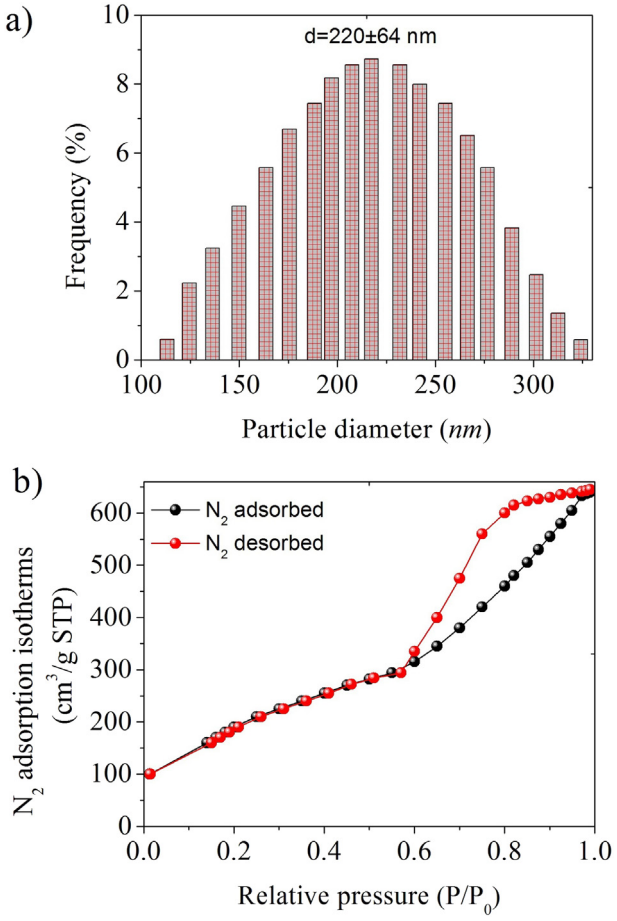

Fig. 1. MSNPs: a) Particle size distribution and b) nitrogen adsorption isotherms.

Table 1
Structure parameters of MSNPs and MSNPs-Metf.

Sample	BET surface area (m^2/g)	Pore volume (cm^3/g)	Pore size (nm)	Porosity (%)
MSNPs	540	0.99	6.1	39.87
MSNPs-Metf	281.4	0.67	3.6	36.01

higher at high relative pressures. BET surface area, pore volume and pore diameter of samples MSNPs and its hybrids with metformin (MSNPs-Metf) are summarized in Table 1. The hybrid samples used for the porosity test are those that were immersed 24 h in metformin at initial concentration of 400 ppm. Comparing the pore diameter values found for MSNPs with those of MSNPs-Metf, a big decrease in average pore diameter and total pore volume is observed. The decrease of average pore diameter and total pore volume is due to the loading of the drug in the inner pores. This confirms that metformin was loaded on MSNPs external surfaces, but mainly inside the pores. To determine the porosity of the material the following equation was used:

$$Porosity\ (\%) = \frac{V_{pore} \times 100}{V_{pore} + \left(\frac{1}{\rho}\right)}, \quad (2)$$

where V_{pore} is the total pore volume and ρ is the particles density ($\rho_{MpSiO_2} = 0.67$ g/cm^3 and $\rho_{MpSiO_2-Metf} = 0.84$ g/cm^3). Sample MSNPs has a porosity of 39.87% and sample MSNPs-Metf 36.01%. Porosity data are summarized in Table 1. Due to the metformin adsorption, it covers the pores of the MSNPs causing the porosity to decrease. The large specific surface area and large pore volume allows a high

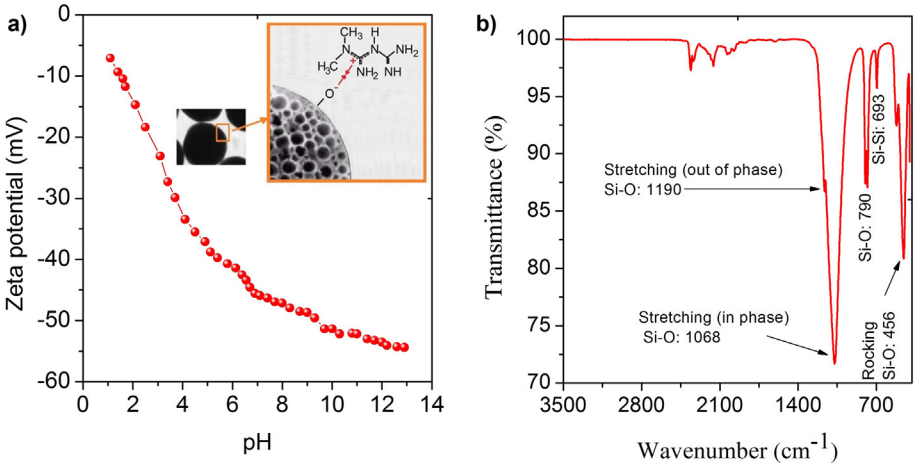

Fig. 2. MSNPs a) Zeta potential depending on pH, the inset is a representative metformin adsorption on MSNPs by electrostatic charges and b) FTIR spectra for structure confirmation of MSNPs.

concentration of metformin on the MSNPs surface. As the MSNPs do not show swelling and due to their structural properties such as particle size, high surface area and its porosity are not affected by pH changes, these nanoparticles help to protect the active ingredients of drugs, preventing denaturation caused by changes in pH and high temperature.

An important aspect of solid-liquid interfaces is the appearance of a double electric layer; this is a result of the interaction between the solid surface (MSNPs) and the aqueous phase. This aspect plays an important role in the drug adsorption process since the nano-pharmaceutical transporting agents are delivery vehicles that are directed to the action sites through physicochemical mechanisms such as adsorption (Goldberg et al., 2007). Fig. 2a shows the zeta potential evolution depending on pH, in the pH ranging from 1 to 13, the MSNPs surface is negatively charged, while metformin is positively charged. Under these conditions, the interaction between MSNPs particles and metformin is electrostatic; so that the metformin release is verified through the ion formation pairs that cross the cell membrane (Wang et al., 2013). The MSNPs zeta potential sample is promising for the loading efficiencies and drugs stability such as protonated metformin, since it can be adsorbed on the particles surface by electrostatic forces; see the inset of Fig. 2a. The fact that the MSNPs nanoparticles can be used as a vehicle for the metformin release lies in the adsorption phenomenon that takes place on the surface and inside the particles pores, which allows the metformin lodging until its release under appropriate conditions through interactions and/or covalent bonds. The chemical and physical properties of these MSNPs particles allow new applications in pharmacology as a matrix for the active ingredients release of drugs that allow transporting them to the interest place controlling the dose without causing collateral damage. The synthesized MSNPs particles spectrum is shown in Fig. 2b. In the spectrum there is a shoulder around 1190 cm^{-1}, it is characteristic of Si—O vibration. The vibration bands in the range of 1060–1098 cm^{-1} and 780–806 cm^{-1} correspond to the asymmetric and symmetric stretching of the Si—O bonds, respectively (Patiño-Herrera et al., 2016; Amlouk et al., 2006). The bands at 456 and 442 cm^{-1} are attributed to symmetrical elongation of Si—O (Dong et al., 2017; Shim et al., 2015), which confirms the success of MSNPs synthesis.

Fig. 3a shows a Transmission electron microscopy (TEM) image, allows visualizing the morphology and size of MSNPs particles. The micrograph shows semispherical particle with average diameter around 220 nm, some agglomeration is observed. The particle size allows easy endocytosis in plant and animal cells. The elemental analysis (EDX) is shown in Fig. 3b, which allowed corroborating the adequate incorporation of reagents to form MSNPs particles with composition of 41.53% w/w silicon and 58.47% w/w oxygen. This type of nanostructured materials represents an alternative to the organic type matrices that are often used; however, for consider these nanostructured materials as medicines transporters must meet certain requirements such as low toxicity, optimum characteristics for transport and long to medium life times (Svenson and Tomalia, 2005; Eaton, 2011). In addition, SiO_2 shows low cell toxicity and does not cause cell death, generates low apoptosis and does not generate reactive oxygen species or membrane alterations (Bhattacharyya et al., 2011).

3.2. Drug loading

Metformin adsorption is highly dependent on pH and is affected by the surface charge of MSNPs. Metformin has two acid dissociation constants $pK_{a1} = 2.8$ and $pK_{a2} = 11.6$ (Hernandez et al., 2015) and depending on pH three different species are produced. The concentration of each species was determined by the following equations,

$$[Metf^{2+}] = \frac{C_{Metf}}{1 + 10^{-pKa_1+pH} + 10^{-pKa_1-pKa_2+2pH}} \quad (3)$$

$$[Metf^{+}] = \frac{C_{Metf}}{10^{-pH+pKa_1} + 1 + 10^{-pKa_2+pH}} \quad (4)$$

$$[Metf^{0}] = \frac{C_{Metf}}{10^{-2pH+pKa_1+pKa_2} + 10^{-pH+pKa_1} + 1} \quad (5)$$

where $[Metf^0]$ is the neutral metformin concentration, $[Metf^{2+}]$ is the metformin concentration in its bi-protonated specie, $[Metf^{1+}]$ is the metformin concentration in its mono-protonated specie, there are two possible mono-protonated species (Hernandez et al., 2015) ($Metf_1^{1+}$ and $Metf_2^{1+}$), C_{Metf} is the total metformin concentration (100 ppm). Eqs. (3), (4) and (5) are derived from definition of the acid dissociation constants ($Ka_1 = \frac{[Metf(+)][H^+]}{[Metf(2+)]}$, $K_{a2} = \frac{[Metf^0][H^+]}{[Metf^+]}$) and the total metformin concentration is $C_{Metf} = [Metf^{2+}] + [Metf^{+}] + [Metf^{0}]$. Fig. 4 shows the pH effect on metformin concentration species, at pH 1 the bi-protonated species are found, at pH 3 coexist the mono-protonated and bi-protonated species 50% and 50%, respectively. At pH 11.6 co-exist the bi-protonated and neutral species 50% and 50%, respectively; while at pH 14 the neutral specie is found. Between pH 4.8–9.5 almost all metformin is found in mono-protonated form ($Metf^+$), since the surface of the MSNPs is negatively charged, it is expected that mono-protonated form of metformin will be the one that generates the highest adsorption.

Fig. 5a shows the adsorption metformin kinetics by MSNPs depending on pH metformin solution. To examine all metformin species, the pH values studied were 1, 7 and 13, all experiments show the bulk

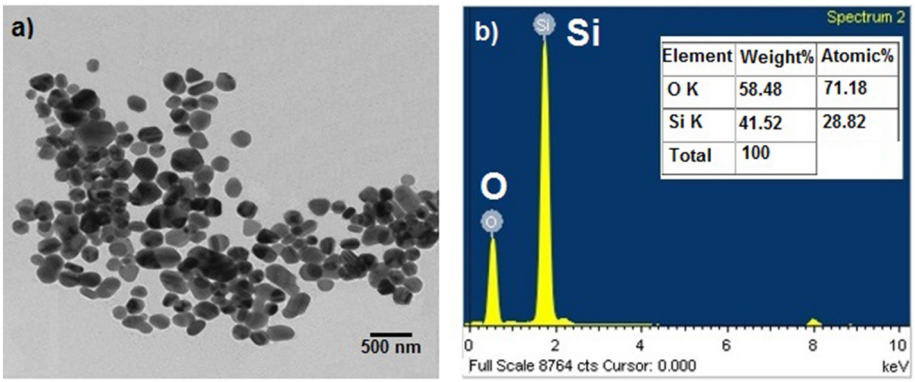

Fig. 3. a) TEM image of MSNPs and b) elemental analysis of MSNPs.

of the adsorption occurring at pH 7, indicating that the mono-protonated species generates greater adsorption efficiency affinity. This is because the silicon oxide particles have a negative surface charge and since at pH 7 the metformin is mono-protonated, it is sorbed on the MSNPs by electrostatic forces. At pH 1 the adsorption decreased by 67.26% with respect to pH 7, while at pH 13 the adsorption decreased 80.81%. At this pH (13) there is a little mono-protonated metformin, so it is possible that the adsorption is due to this specie and not to the neutral one and it can be concluded that the best affinity was reach at pH 7. It means that the maximum metformin loading by MSNPs is achieved in its mono-protonated form. Adsorption metformin capacity by MSNPs was conducted at five initial concentrations 10, 50, 100, 200 and 400 ppm, see Fig. 5b. During first two hours of metformin adsorption, all solutions have an ascending semi-linear behavior, the initial adsorption velocity is rapid since the process is controlled by metformin diffusion in the boundary layer surrounding the MSNPs particles and the adsorption is produced on the external MSNPs surface (Wu et al., 2009). Subsequently, the adsorption rates decrease markedly in the next two hours as occurs in an external diffusion mechanism; the curves slope decreases because the metformin diffusion slows over time. Then the adsorption kept a slow growth as happens in the intra-particle diffusion. The porosity of the MSNPs can generate that the external active sites are first saturated while the adsorption is remarkably rapid and then the intra-particular diffusion develops along 83% of the adsorption time with a slow decrease of the adsorption rates. Adsorption is greater at higher initial metformin concentration. Also it is evident that metformin adsorption capacity at 50 ppm of initial concentration reaches almost four times the adsorption capacity compared to the concentration of 10 ppm, being less significant between the range of 50 to 400 ppm. In brief, at any time, increasing the metformin concentration increases the metformin absorbed amount.

Metformin adsorption sites by MSNPs were determined with an infrared spectroscopy analysis, see Fig. 6. The infrared metformin spectrum shows a strong intensity bands appearing at 3370, 3290 and 3147 cm^{-1} which are assigned to N—H stretching vibration, while the strong intensity bands of N—H deformation vibrations occur at 1625, 1571, 1476, 1455 and 1417 cm^{-1}. The medium to weak intensity bands appearing at frequencies 1169, 1043 and 1033 cm^{-1} are assigned to C—N stretching vibrations. A weak band at 806 cm^{-1} has been assigned to NH_2 rocking vibrations, also N—H wagging vibrations appear at 744 and 703 cm^{-1}. C-N-C deformation appears as medium to strong intensity bands at 580, 541 and 418 cm^{-1} (Sheela et al., 2010). After metformin adsorption (initial concentration of 400 ppm) by MSNPs for 24 h, the hybrid particles (MSNPs-Metf) were subjected to an FTIR analysis, it confirms metformin adsorption on MSNPs. The N—H stretching vibration appearance of the amino group at 3370 cm^{-1} and the N—H deformation vibrations at 1625 cm^{-1} indicates the amino groups were adsorbed by MSNPs. The increase in the hybrid intensity band (MSNPs-Metf) located at 1070 cm^{-1} is due to the vibrations superposition of Si-O-Si bonds and C—N bonds of metformin. Also, the increases in intensity of the band located around 776 cm^{-1} is due to the overlap of the Si—O and N—H bonds vibrations. The Si—Si bonds vibrations around 690 cm^{-1} match with the N—H bonds vibration at 703 cm^{-1}. Finally, the band around 454 cm^{-1} of the Si—O vibration is increased by the presence of metformin C-N-C vibrations located at 418 cm^{-1}. Thus, it can be concluded that a sequential modification on

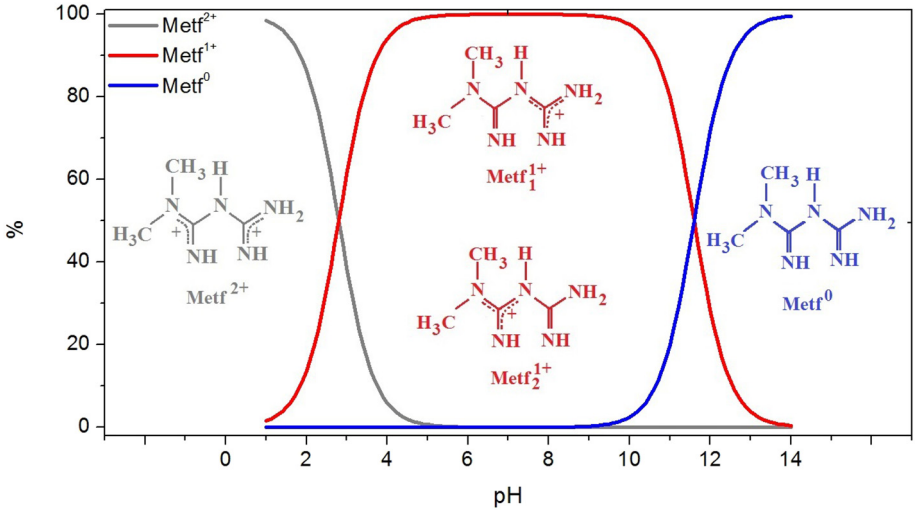

Fig. 4. Speciation diagrams of metformin depending on pH, neutral form ($Metf^0$), mono-protonated forms ($Metf_1^{1+}$ and $Metf_2^{1+}$) and bi-protonated form ($Metf^{2+}$).

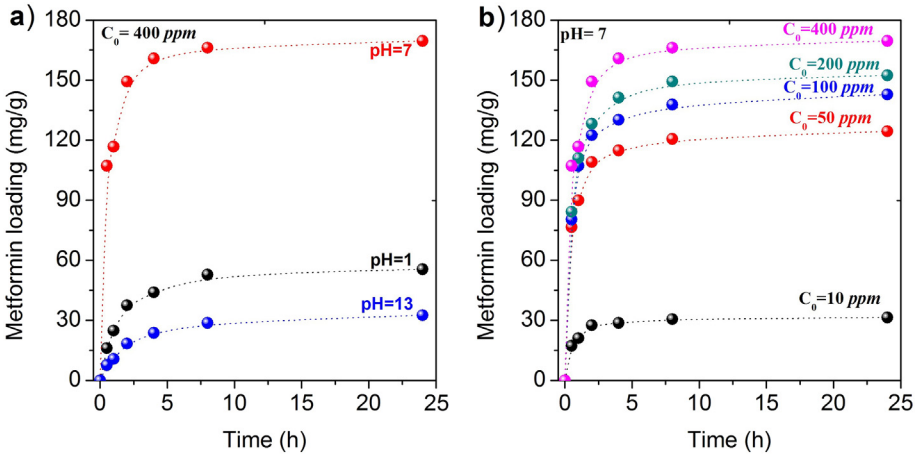

Fig. 5. Metformin adsorption capacity by MSNPs: a) depending on pH and b) depending on initial concentration.

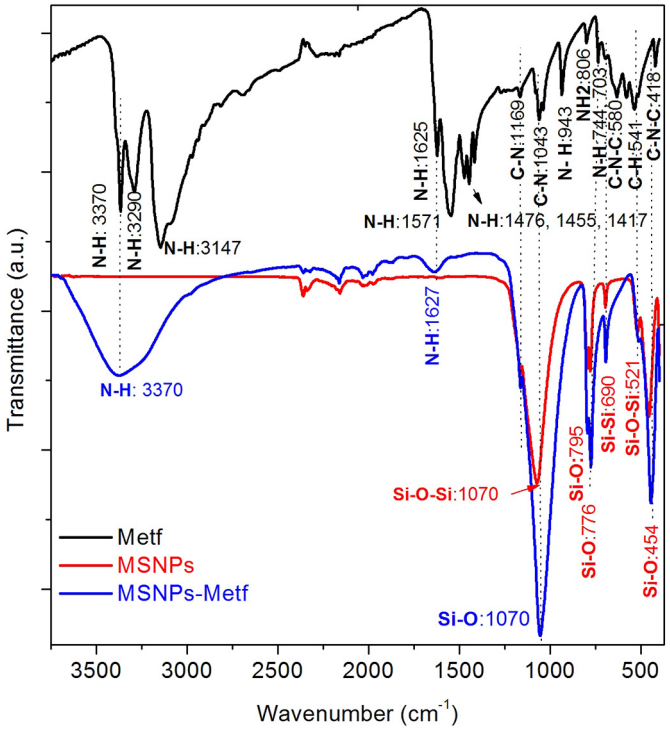

Fig. 6. FTIR metformin hydrochloride spectrum, MSNPs and hybrid MSNPs-Metf (after 24 h of adsorption).

the MSNPs sample takes place due to metformin adsorption.

3.3. Pellets morphology

Pellet surfaces were studied using scanning electron microscope. As shown in Fig. 7a SEM micrographs of MSNPs-Metf pellets with quasi spherical shape, no cracks are observed in the surface, it is rough and full of pore structure, which probably facilitates the aqueous solution entrance into the pellet, allowing metformin diffusion out of the MSNPs-Metf, thus making metformin ready for absorption by the human organism. Based on SEM images, but mainly in the digital vernier readings when measuring 20 pellets, the pellet size was found to be 3000 ± 90 μm. The pellet surface, after it was coated with chitosan (Fig. 7b) was much smoother than the pre-coated pellet. The fact of having metformin adsorbed in the silicon oxide nanoparticles mixed with chitosan inside the pellet, which is also covered by chitosan layers, will allow the metformin release at slower and more controlled rate, maintaining a higher metformin concentration for a longer time. Thus, it is possible to administer smaller amounts of metformin, especially taking into account that most of this medicine is expelled from the human body 24 h after its intake without having been processed by the patient body.

3.4. In vitro drug release

To achieve successful metformin delivery, it shall be kept from abruptly released into the stomach, stomach is not considered the optimum site for metformin delivery. To address that problem, pH-sensitive carriers is an alternative for oral drug delivery. In this way, metformin delivery can last up to 17 h, since, at pH 1.2, this is the time required for the metformin contained inside the pellet to desorb. The pH of the stomach at rest is 3.5 to 4, while during digestion is 1.2 to 1.3; the intraluminal pH rapidly changes to pH ~6 in the duodenum, then gradually increases in the small intestine from 6 to 7.4 in the terminal ileum, and falls off to 5.7 in the caecum; then progressively increases to reaches 6.7 in the rectum (Fallingborg, 1999). To study potential application of MSNPs containing metformin as a medicament, the desorption pHs studied (1.2, 3, 6 and 7) were selected simulating the pH of the organism of healthy people and using citric acid solutions as acid medium. The drug released from MSNPs-Metf carrier (170 mg of metformin loaded in 1 g of MSNPs) as a function of time is shown in Fig. 8. Metformin concentration released was quantified every 30 min by UV–vis spectrophotometry. Fig. 8a show the *in vitro* release profiles, under different pH conditions, of metformin adsorbed in MSNPs. If pH was 1.2, there was an initial release of about 47%; while at pH 7 was approximately 11%, at this pH metformin release was sustained with only 41% of the drug released after 17 h; However, at pH 1.2 after 12 h, all metformin previously adsorbed had been released. Fig. 8b, due to the MSNPs-Metf pellets coating with chitosan there is a period of time in which there is no metformin release, its release begins at 4 h of exposure to the solution of pH 7; while at pH 1.2 its release begins at 1.5 h of exposure to the acid solution, this is due to the fact that metformin acquires the bi-protonated form at this pH, making the binding with the MSNPs particles unstable. After the chitosan disappearance coating, the MSNPs-Metf particles decompaction begins, (see inset Fig. 8b), and then the metformin release begins almost linearly. During six to eight hours food enters the stomach and passes through the small intestine where most of the digestion carries out, nutrients absorption, and the food conversion into energy take place. It can be concluded that the MSNPs carrier delayed the metformin release and can be employed in drug delivery application. After 17 h, at pH 1.2, 170 mg of metformin per gram of MSNPs was released, while at pH 7 only 44 mg of metformin was released, this is 26% of the total metformin loaded in the MSNPs. The digestion time of food varies from person to person and is

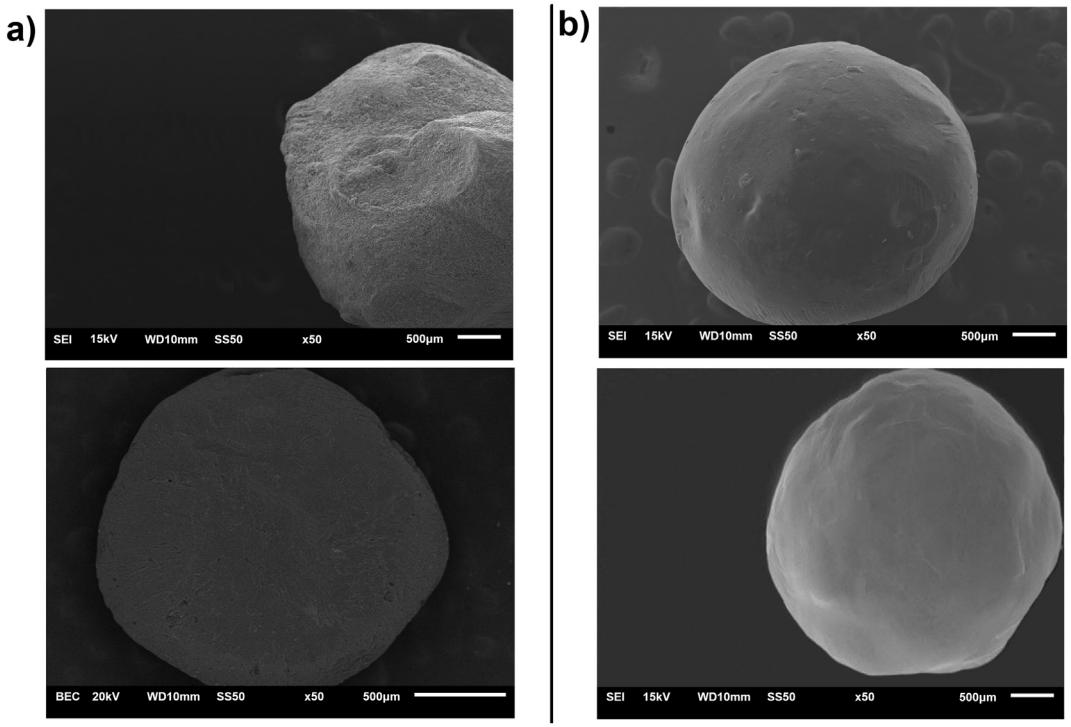

Fig. 7. a) SEM image of MSNPs-Metf pellet and b) SEM image of MSNPs-Metf pellet chitosan coated.

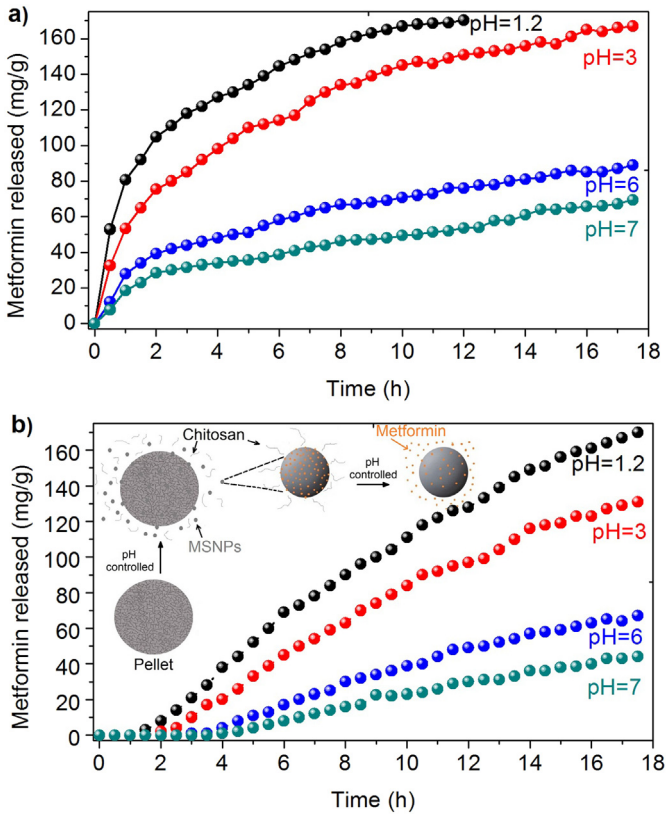

Fig. 8. *In vitro* metformin release profile from: a) MSNPs-Metf, b) MSNPs-Metf pellets. The inset is a representative metformin release from pellets.

affected by the amount of eaten food, food combination, physical activity and metabolism. Among the pills most used by type II diabetics are the 500, 850 and 1500 mg of metformin, with the possibility of taking 1 to 3 daily doses depending on the medical prescription; however, *in vivo* experiments showed that metformin is not metabolized, it was rapidly eliminated at a rate of approximately 90% at 12 h, it was recovered as unchanged drug in the urine (Robert et al., 2003). Taken orally, pellets have advantages over suspension, tablets and capsules; they can be divided into desired dosage without formulation or process changes, they disperse easily in the gastrointestinal tract, maximizing the metformin absorption and avoiding peak plasma fluctuation; the local irritation of the mucosa could be minimized due to the small metformin quantity available in a pellet (Jawahar and Anilbhai, 2012). To date the action mechanism against *Diabetes mellitus* of metformin has not been disbanded and then it is not convenient to supply large amounts of metformin. Instead, it is preferable to supply small amounts of drug and to delay its release as long as possible through the pellets formed with MSNPs-Metf and coated with chitosan, as an option to avoid unnecessary excessive intake of medication.

4. Conclusions

FTIR analysis corroborated the successful silicon oxide synthesis, its characterization showed particles of nearly spherical shape with an average diameter of 221 ± 64 nm, surface area of approximately 540 m^2/g, porosity of 38.87% and pore size of around 6.1 nm, which indicates that these are mesoporous particles. Porosity facilitates the metformin entry into the mesoporous silica nanoparticles (MSNPs) and the subsequent metformin adsorption onto its surface and inside the pores. It was found that the metformin adsorption into the mesoporous silicon particles increases proportionally to the concentration of the drug; 400 ppm was the highest concentration tested and it was possible to adsorb 170 mg/g at a pH of 7 since under this condition metformin is in its mono-protonated form ($Metf^+$), which generates a suitable environment to be attracted by the negative surface charge of the MSNPs. Addition of small chitosan quantity improves the pellets formation of MSNPs-Metf and the pellets coating with chitosan layers delays the metformin release depending on the pH (1.2 to 7) from 1.5 to 4 h. Maintaining pH at 1.2, 170 mg of metformin per gram of MSNPs are released in 17 h. If pH is > 1.2, the metformin release is significantly prolonged, due to the electrostatic equilibrium of the mono-protonated

form of metformin and the particles MSNPs; and because quitosan does not dissolve at pH above 5. The pellets formed with MSNPs-Metf and coated with chitosan are an option to avoid unnecessary excessive medication intake, compared to the 90% of metformin that is recovered as unchanged drug in urine after 12 h of metformin ingest with traditional excipients (Robert et al., 2003).

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