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ORIGINAL ARTICLE

A new controlled release system of chlorhexidine and chlorhexidine:βcd inclusion compounds based on porous silica

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Abstract The purpose of this study was to prepare and characterize a controlled release system based on porous silica loaded with chlorhexidine (Cx) and its inclusion compounds in β-cyclodextrin (βcd), and to evaluate its antimicrobial activity. Acetate chlorhexidine (CxA), gluconate chlorhexidine (CxG), βcd:chlorhexidine acetate 2:1 (βcd:CxA) and βcd:chlorhexidine gluconate 2:1 (βcd:CxG) were incorporated into porous silica. Drug loading was characterized by FTIR, powder X-ray diffraction, thermal analysis and BET, and was shown to be in an amorphous state and porous matrix. The kinetics release parameter of the drug was established, which showed that the Cx systems release profile followed zero order release until 400 h and Higuchi model release until 750 h, after the burst effect at the first 8 h. Chlorhexidine therapeutic range was reached near first hour for all systems. The chlorhexidine porous silica system was biologically active against *Enterococcus faecalis* and *Candida albicans* in vitro. The systems showed an efficient Cx controlled release modulated by the presence of the β-cyclodextrin and by the porous silica matrices, providing effective antimicrobial activity.

Keywords β-Cyclodextrin · Inclusion compounds · Porous silica · Controlled release system · Chlorhexidine

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Introduction

Porous silica has been considered a very promising material for hosting as well as delivery of many molecules of pharmaceutical interest due to their ordered arrangement; a very narrow pore size distribution, large pores and surface areas, highly chemical and thermal stabilities, bioresorbability and biocompatibility [1–3]. Porous silica has shown unique physico-chemical properties, namely the well-defined surface properties, the high concentration of silanol groups on the surface and the specific surface area. These properties make this material suitable to be used not only for the drug, proteins and biogenic molecules, but also the drug controlled release matrix [4–7].

The drug controlled release systems present many advantages, such as the reduction of the total drug dosage introduced in the body, a precise drug setting in the target zone and the tuning of the drug release kinetics [2]. On the other hand, chlorhexidine (Cx) has been widely used in pharmaceutical formulations for its excellent broad-spectrum antimicrobial properties. It is an effective oral antimicrobial agent, and it is routinely used in caries prevention and periodontal therapy [8]. In addition, it has been described that Cx exhibits excellent antibacterial activity when used as medication during endodontic therapy—capable of preventing canal recontamination—due to its efficacy against *E. faecalis* and *C. albicans* [9–11].

In spite of the broad use of Cx in dentistry, drawbacks such as Cx low release from the matrix during use on composite restoration materials, methacrylate polymer systems, glass ionomers and resinous cements need to be resolved. These limitations could be due to the strong bonds formed between the drug and the polymeric matrix [12–14]. One strategy to circumvent these shortcomings is to use the host: guest strategy using cyclodextrin in order to module the drug release, as related in the literature [15–18], before its incorporation in the porous silica matrix. Based on mesoporous silica physico-chemical challenging drug carrier properties, the Cx broad spectrum antimicrobial activity and the host–guest strategy using cyclodextrin as a drug controlled release modulation, we decided to develop Cx and its β-cyclodextrin inclusion compound controlled release system from mesoporous silica and its physico-chemical characterization and antimicrobial evaluation.

Materials and methods

Materials

Porous silica particles (average diameter of $\sim$745 nm) were synthesized by sol–gel method using tetraethyl orthosilicate (TEOS), obtained from Merck Schuchardt, Germany, and used without further purification with ethanol and water in a 1:3:10 molar ratio with HCl and HF as catalysts. The samples were prepared in monolithic form and dried at 70 and 550 °C for 1 h each in accordance with the method described by Miranda et al. [19].

Gluconate of chlorhexidine 20% (CxG) was generously donated by Degussa, São Paulo, SP, Brazil. Acetate of chlorhexidine (CxA) was purchased from Stevia Commercial Exports, São Paulo, SP, Brazil and β-cyclodextrin from Xiamen Mchem, China, and used without further purification.

Inclusion compounds preparation

The inclusion compounds β-cyclodextrin:chlorhexidine acetate (βcdCxA) and β-cyclodextrin:chlorhexidine gluconate (βcdCxG) were prepared using the freeze-drying method. Briefly, βcdCxA and βcdCxG solution in a 2:1 (βcd:Cx compounds) molar ratio were mixed with a magnetic stirrer for 48 h. After, these solutions were frozen in liquid nitrogen and submitted to the lyophilization process as described elsewhere [20]. The formation of inclusion complexes were assessed by FTIR spectroscopy analysis using a Perkin Elmer 283B spectrometer. The spectra were measured over the range 4000–400 cm^{-1} at room temperature in KBr pellets. The differential scanning calorimetry (DSC) was carried out on a Shimadzu 50 termobalance using a platinum crucible. The heating rate was 10 °C min^{-1}, between 25 and 480 °C, in an N_2 atmosphere. NMR ^1H and NOESY (Bruker DRX Avance 400 MHz) experiments were developed as related previously by Denadai et al. [17].

Cx controlled release system preparation

Four solutions containing CxA, CxG, βcdCxA and βcdCxG with the powdered porous silica were prepared at weight ratio of 2:1 (Cx:silica) and were stirred vigorously for over 60 h. Thereafter, the samples were centrifuged for 10 min at 15.000 rpm, and rinsed with distilled water to remove unentrapped chlorhexidine. The obtained samples SCxA, SβcdCxA, SCxG and SβcdCxG were dried in vacuum condition at 46 °C for 3 h [21]. The Cx loading in the matrix was assessed by subtracting the amount of the drug remaining in the lyophilized supernatant obtained after the washing step, from the total amount added to prepare the initial samples.

Physico-chemical characterization of Cx controlled release systems

The characterization techniques were FTIR analysis, powder X-ray diffraction and Brunauer Emmet Teller technique (BET). FTIR analysis was performed using a Perkin Elmer 283B spectrophotometer. The spectra were measured over the range 4000–400 cm^{-1} at room temperature in KBr pellets. Powder X-ray diffraction patterns were carried out on crushed samples using a Rigaku Geigerflex diffractometer system using a CuKα radiation at 40 kV and 30 mA. Specific surface area and porosity parameters were determined using BET technique based on nitrogen gas adsorption in an Autosorb-Quantachrome Nova 1200. Prior to analysis, the samples were crushed and outgassed.

Cx and Cx:βcd inclusion compound release studies

10.0 $\pm$ 0.2 mg of porous silica loaded with Cx and Cx inclusion compounds with βcyclodextrin were placed into an eppendorf tube containing 1.5 mL of distilled water (pH = 6.0–6.5) at 37 °C and shaked on a Cientec orbital shaker (1.000 rpm) to ensure thorough mixing. After centrifugation at 10.000 rpm, to ensure sink conditions, the release medium was completely removed and replaced with 1.5 mL of fresh medium at pre-determined time intervals. Cx release was quantified by UV spectrometry (UV–vis HP8453) at λ = 230 nm for 2 weeks for SCxG, Sβcd-CxG and for 4 weeks for SCxA, Sβcd-CxA. The experiments were obtained in triplicate measurements and the data points are plotted as mean values with standard deviation, S.D.

Antimicrobial studies

The antimicrobial tests were performed using Pour Plate method. The microorganisms tested were two common oral microorganisms pathogens: a *Enterococcus faecalis* (ATCC 14508), and a fungus *Candida albicans* (ATCC 18803). The inhibitory activity of the prepared systems SCxA, Sβcd-CxA, SCxG or Sβcd-CxG were evaluated at three concentrations 25; 50 and 100 μg/mL and the materials CxA, βcd-CxA, CxG and βcd-CxG were tested for comparison simultaneously. The *Enterococcus faecalis* (*E.f.*) strain was resuspended in brain–heart infusion (BHI) and *Candida albicans* (*C.a.*) in Sabouraud Dextrose. The microorganisms were grown at optimal growth conditions in aerobic atmosphere for 24 h, having a final inoculum concentration 1.5×10^8 CFU/mL. The pour plate/traditional streak agar diffusion method was performed as described by DiFiore [22]. The microorganism strains were then seeded on Müeller Hinton agar culture plates prepared with the different antimicrobials. Agar plates were inoculated with *E.f.* and *C.a.*, and incubated aerobically at 37 and 34 °C respectively, for 24 h. A positive control was performed for each pathogen on a separate plate and mixed when the agar was warm and at the same concentration of tested drugs. The nonparametric Kruskal–Wallis test was used to determine the differences in efficacy of the systems.

Results and discussion

Characterization of βcd:Cx inclusion compounds

The formation of inclusion compounds between CxA and CxG with βcd was assessed by FTIR, Differential Scanning Calorimetry (DSC) and NMR ^{1}H and NOESY. The chemical structures of Cx, CxA, and CxG are presented in the Fig. 1. The IR spectra of the inclusion compounds

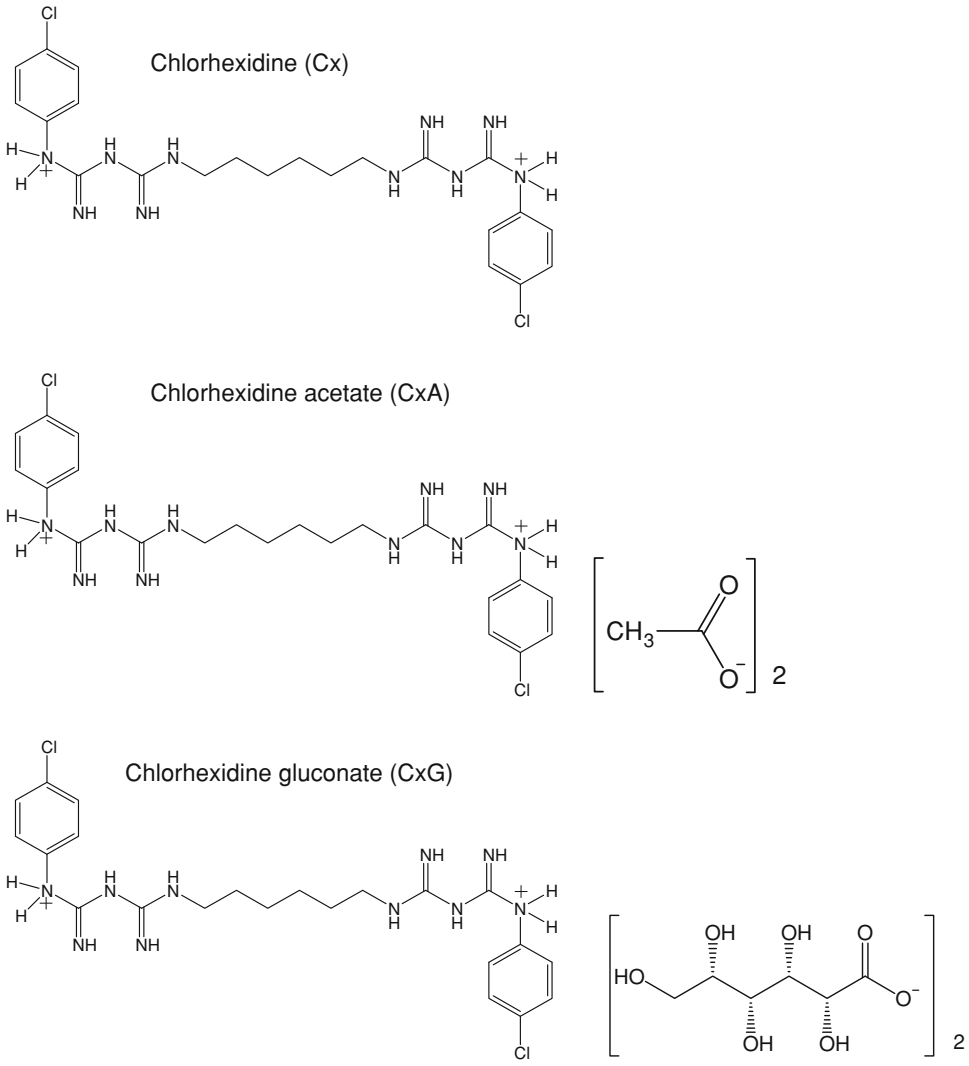

Fig. 1 The chemical structures of chlorhexidine (Cx), chlorhexidine acetate (CxA), and chlorhexidine gluconate (CxG)

revealed a strong band around 3400 and 1100 cm^{-1} associated with ν_{-OH} and ν_{-C-O-C}, respectively. These bands were significantly narrow in comparison to free β-cd, which suggests disruption of hydrogen bonds within the β-cd cavity upon inclusion. The formation of the inclusion compounds were further confirmed in DSC curves by absence of the CxA and CxG melting points around 153 °C, associated with pure Cx [16].

When analyzing the ^1H NMR spectra of the inclusion compound, one can observe a sharp coalescence of the aromatic ^1H NMR signals of CxA and CxG in the presence of βcd. These results could be ascribed to the disturbances in the electronic density of the Cx aromatic ring caused by unbounded electrons of the oxygen atoms from the cyclodextrin molecule, due to the formation of dipolar interactions. Correlations between aliphatic and aromatic hydrogens of Cx and hydrogens inside the βcd cavity can be observed. The results from the formation of inclusion compounds between CxA and CxG with βcd assessed by FTIR, DSC and NMR ^1H and NOESY confirm those previously related by [16, 23].

Physico-chemical characterization of Cx dried controlled release systems

Drug loading determination in the controlled release systems

Experimental percentage of CxA, CxG and their respective inclusion compounds in dried porous silica are depicted in Table 1.

One can observe higher loading percentages for the pure CxA (22.9%) and CxG (18.9%), when compared to the respective βcdCxA (21.0%) and βcdCxG (15.4%) inclusion compounds. In all systems, the content of Cx after drying was similar to literature's data regarding porous silica systems [3, 6]. As expected, the observed drug loading percentage decreased as the molecule or inclusion compound size increased, confirming that the drug loading in matrix depends on the molecular size, as described by [3, 24, 25].

Table 1 Experimental loading on the dried porous silica percentage of CxA, CxG and their respective inclusion compounds SβcdCxA and SβcdCxG

Systems	Experimental Cx loading (wt%)
SCxA	22.9
SβcdCxA	21.0
SCxG	18.9
SβcdCxG	15.4

FTIR

The FTIR spectra of pure silica and the silica Cx loaded systems (SCxA, Sβcd-CxA, SCxG or Sβcd-CxG) are depicted in Fig. 2a, b. The drug loading in the porous silica matrix has been qualitatively confirmed by FTIR spectra, and no significant new peaks were observed upon Cx loading, indicating a mainly Cx physical adsorption, not revealing any detectable changes in the porous network arising from the presence of chlorhexidine or its inclusion compound [26, 27].

A typical vibrational band can be observed in the pure silica FTIR spectra at 3400 cm^{-1}, a broad peak associated υ_{sSiOH} groups with hydrogen bond interaction with adsorbed H_2O molecules; a band at 1620 cm^{-1} assigned to water deformation mode δ_{H_2O}; a medium-intensity band at 1240 cm^{-1} and a sharp and intense band at 1100 cm^{-1} associated to $\upsilon_{asSiOSi}$ groups, in addition to a weaker band at 800 cm^{-1}. A band at 940 cm^{-1} could be attributed to υ_s Si–OH. The low energy bands at 570 and 490 cm^{-1} are also typical for mesoporous silica associated with network defects such as tetra- and trisiloxane rings. The Cx silica loaded FTIR spectrum has shown not only the characteristic peaks observed in the pure silica one, but also an additional new band at 1492 cm^{-1} characteristic of υ_{C-Cl} of the aromatic chlorhexidine moiety. The Cx typical bands at 3100–3300 cm^{-1} and at 1650 cm^{-1} associated respectively to υ_s and υ_{as} and δ_s NH group were not observed in porous silica Cx loaded spectra, probably due to overlapping with the broad υ_{sOH} band of cyclodextrin or through interactions between Si–OH and NH groups. It is noteworthy that the incorporation of Cx in the porous silica did not cause any noticeable shift in the silica bands. Conversely, when βcd-CxA and βcd-CxG inclusions were loaded, broadening of the $\upsilon_{asSiOSi}$ occurred at 1100 cm^{-1} as consequence of new hydrogen bonding interaction between the Si–OH of silica matrix and –OH of βcyclodextrin. In addition, a shift of approximately 100 cm^{-1} occurred in the FTIR spectra of Sβcd-CxG with the $\upsilon_{asSiOSi}$, in this case suggesting strong hydrogen bonding when compared to the Sβcd-CxA compound. After drug loading, the broadening of the band centered at approximately 3400 cm^{-1}, and the near disappearance of υ_s Si–OH band at 940 cm^{-1} suggests hydrogen interactions between silanol groups on the pore surface, Cx and their inclusion compounds, as suggested by [28, 29].

Powder X-ray diffraction

Powder XRD patterns diffraction of pure silica, CxA, CxG, βcdCxA and βcdCxG inclusion compounds and the porous silica Cx loaded systems, namely SCxA; SβcdCxA; SCxG; SβcdCxG compounds are shown in Fig. 3a, b. It can be

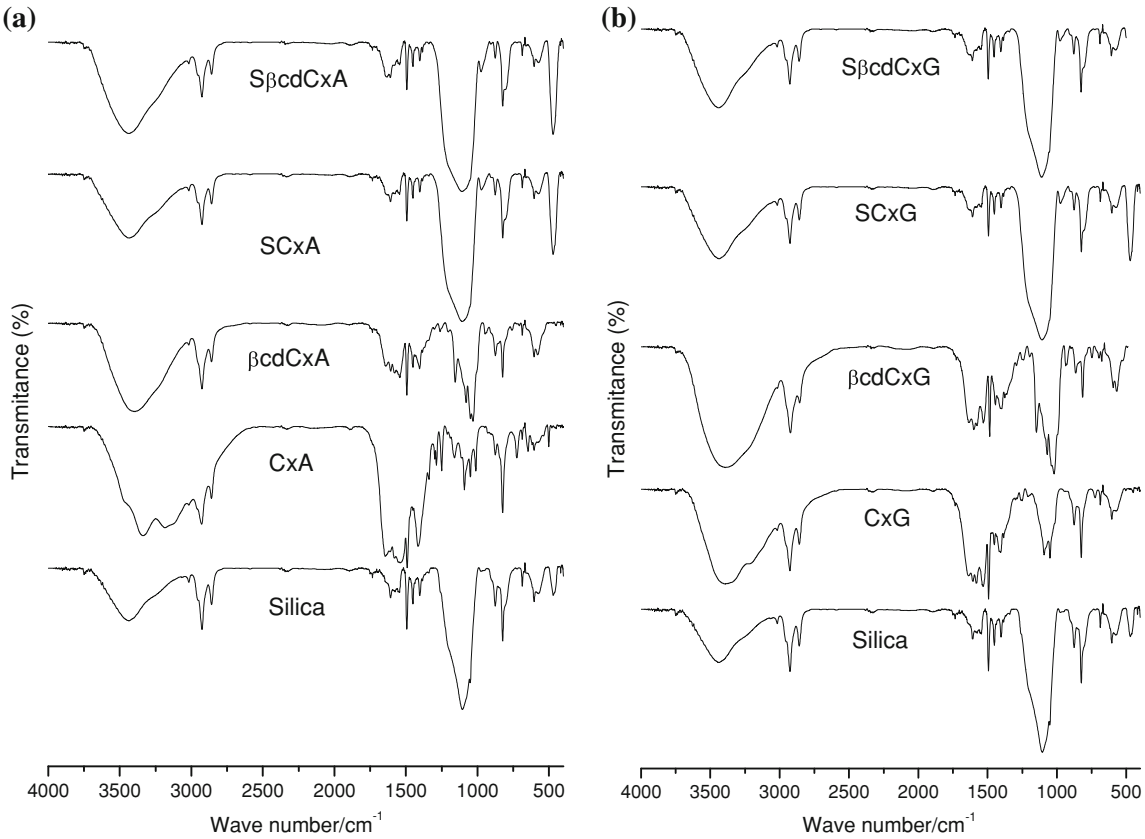

Fig. 2 FTIR spectra of **a** pure porous silica (S), chlorhexidine acetate (CxA), CxA:*β*cd inclusion compound (*β*cdCxA) and systems SCxA and S*β*cdCxA after loading and **b** pure porous silica (S), chlorhexidine gluconate (CxG), CxG:*β*cd inclusion compound (*β*cdCxG) and systems SCxG and S*β*cdCxG after loading

observed that the XRD pattern of the pure silica, CxG, *β*cd:CxA, *β*cd:CxG, S*β*cd:CxA and S*β*cd:CxG diffractograms have a profile predominantly amorphous, and the CxA and *β*cd show diffraction peaks characteristic of a poly crystalline compound. Although the *β*-cyclodextrin inclusion compounds show generally crystalline XRD patterns, others studies found that the chlorhexidine:*β*-cyclodextrin inclusion compounds show a more amorphous pattern when compared with the XRD of the free components, suggesting a disorder phenomenon upon inclusion [20].

The silica powder XRD diffraction remained in the amorphous profile after CxA and *β*cd:CxA inclusion compound loading process. This may suggests that the incorporation of the CxA and *β*cd:CxA compound in the silica did not change the crystallite size, or the drug loading was a disordered process in the porous silica matrix [30, 31]. The appearance of amorphicity could be explained by assuming that the pores present in the silica carrier are too small to allow formation of critical nuclei, which are necessary for the crystallization process to begin, either from saturated solution or from the amorphous state-once formed, as shown elsewhere by [6, 25, 32]. While all systems prepared in this study contain amorphous Cx or Cx:*β*cd inclusion compounds, there are differences in the composition and in the structure of individual systems that may have an impact on their mechanical properties and behavior during dissolution.

Specific surface area and pore size distribution

The Barret, Joyner and Halenda (BJH) pore size distribution of pure porous silica and porous silica Cx loaded systems SCxA; S*β*cdCxA; SCxG and S*β*cdCxG obtained by nitrogen desorption method are shown in Fig. 4. The results depicted narrow pore size distribution with an average pore diameter of 150 Å for pure silica. One can observe that the Cx loading process in the porous silica matrix caused a lower pore distribution when compared to the pore silica.

The BJH pore distribution was around 90 Å for S*β*cd-CxA and 120 Å for SCxA, SCxG and S*β*cd-CxG. As expected, the BJH analysis has shown that the presence of Cx molecules in the silica pores leads to corresponding decreases in pore diameter, according to literature [24, 29, 33]. Nitrogen isotherms obtained for pure silica and the Cx

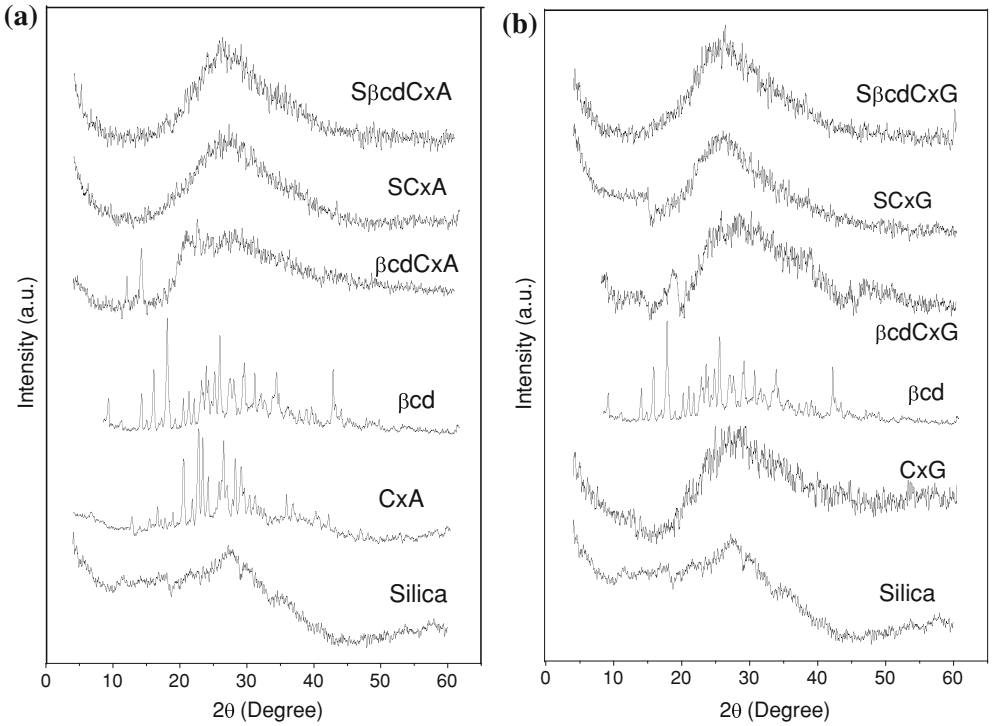

Fig. 3 XRD diffractograms of **a** pure porous silica (S), chlorhexidine acetate (CxA), *β*cyclodextrin (*β*cd), CxA:*β*cyclodextrin inclusion compound (*β*cdCxA) and SCxA and S*β*cdCxA systems; and **b** pure porous silica (S), chlorhexidine gluconate (CxG), *β*-cyclodextrin (*β*cd), CxG: *β*cyclodextrin inclusion compound (*β*cdCxG), and SCxG and S*β*cdCxG systems

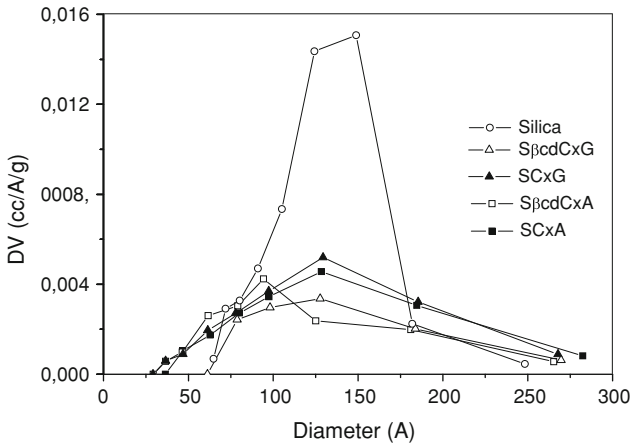

Fig. 4 The Barret, Joyner and Halenda (BJH) analysis of the pore size distribution in the pure porous silica (*open circle*) and porous silica Cx loaded systems SCxA (*filled square*); S*β*cdCxA (*open square*); SCxG (*filled triangle*) and S*β*cdCxG (*open triangle*) obtained by nitrogen desorption method

and Cx inclusion compounds loaded silica matrices are presented in Fig. 5a, b. All systems exhibited similar isotherms profiles, such as the pure silica, and could be classified as type IV profile, according to the IUPAC classification, as mesopores materials.

The overall N_2 adsorption amounts decrease according to the sequence pure silica > SCxA > SCxG > S*β*cdCxA > S*β*cdCxg, indicating the influence of compound size and its affinity with the silica matrix during the impregnation [1, 34]. In all Cx and chlorhexidine inclusion compound loaded silica matrices, hysteresis loops were observed at relative pressure (p/p_0) ranges of 0.6–0.8, reinforcing the mesoporous characteristic of the matrices after loading. Lower N_2 adsorption for the Cx and Cx:*β*cd loaded matrices (350–400 cc/g) was observed when compared to pure silica (750 cc/g). Table 2 summarizes the textural parameters specific surface areas (S_{BET}), pore diameter (d_P) and pore volume (V_p) of porous silica before and after Cx and Cx:*β*cd loading process.

It was observed that highest specific surface areas, lower pore volume and pore diameter of Cx and Cx:*β*cd loaded matrices, as compared to the pure silica. The adsorption of Cx and its inclusion compounds leads to lower pore diameter, pore volume [24, 29, 33], reinforcing the presence of the drug in pores after the loading process. The unexpected increase of specific surface area after drug loading may be largely attributed to the partial filling of macropores of the silica, changing itselves into mesopores, and these mesopores were then identified and measured by BET technique. On the other hand, one could observe some quantitative differences among the substrates in the effectiveness of Cx compound pore filling. These differences may be being reflected as changes in the physico-chemical

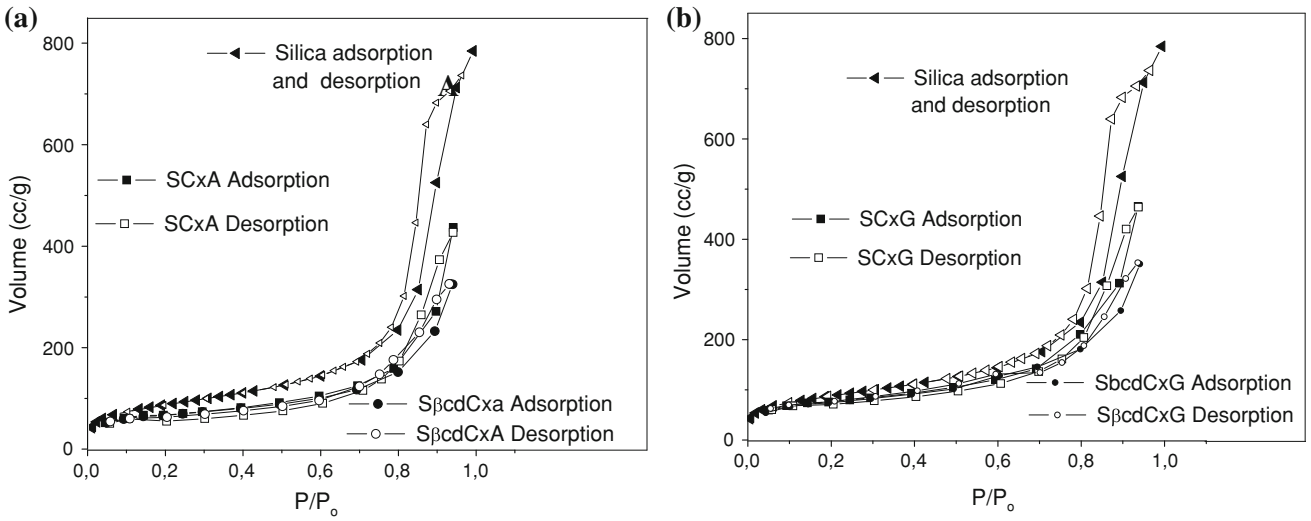

Fig. 5 N_2 sorption isotherms **a** for the pure porous silica (*filled left-pointing triangle*), silica:chlorhexidine acetate(SCxA *filled square*) and silica:chlorhexidine acetate inclusion compound (S*β*cdCxA *filled circle*) systems after loading and **b** for the pure porous silica (*filled left-pointing triangle*), silica:chlorhexidine gluconate (SCxG *filled square*) and silica:chlorhexidine gluconate inclusion compound (S*β*cdCxG *filled circle*) systems after loading

Table 2 Textural parameters

Sample	S_{BET} (m^2/g)	V_p (cc/g)	d_P (Å)
Silica	92.04	1.213	527.4
SCxA	227	0.674	118
S*β*cdCxA	212	0.492	93
SCxG	260	0.719	112
S*β*cdCxG	261	0.544	84

Specific surface area obtained by BET plot (S_{BET}); Total pore volume (V_p) and Pore diameter (d_P) obtained by the BJH method (Barret Joyner Hallenda) for the pure silica matrix and SCxA, S*β*cdCxA, CxG, S*β*cdCxG loaded silica matrices

affinity of the drug or inclusion compounds by the silica matrix. The adsorption process is not only a surface phenomenon as a function of the surface area of the matrix, but also to the chemical affinity between the silica matrix and the Cx or inclusion compounds. Adsorption could result either as the universal van der Waals interactions (physical adsorption), or it can have the characteristics of chemical hydrogen bonding interaction [3, 35, 36].

Release of chlorhexidine from porous silica matrix and kinetics studies

The in vitro release profiles of chlorhexidine and chlorhexidine:*β*cd inclusion compounds from the porous silica loaded systems have been performed in distilled water at 37 °C for 350 h for CxG and its respective inclusion compound, and 700 h for the CxA and its respective inclusion compound (Fig. 6).

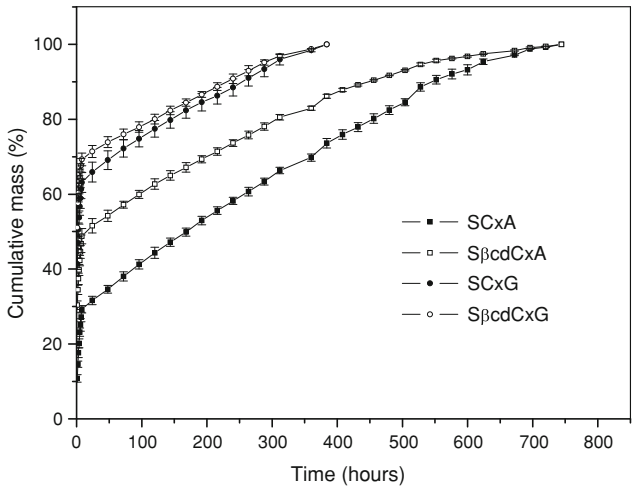

Fig. 6 Cumulative chlorhexidine release from the SCxA (*filled square*), S*β*cdCxA (*open square*), SCxG (*filled circle*) and S*β*cdCxG (*open circle*) silica porous systems from 0 to 700 h

Initially a burst effect of 30, 45, 60 and 75% for SCxA, S*β*cdCxA, SCxG and S*β*cdCxG, respectively during the first 8 h of the experiments was observed. Analyzing the Cx release profile from the porous silica systems, one can observe different profiles with typically controlled release pattern [3, 34]. These results confirm that porous silica can be applied as drug vehicle for controlled release systems. It is interesting to note that the amount of drugs loaded in systems SCxA and S*β*cd-CxA were similar, but higher than the amount found in SCxG and S*β*cd-CxG systems. The initial burst effect could be explained by the excessive amounts of the drug that were weakly adsorbed inside the pores or located at the outer surface of matrix [4].

Subsequently, the slow release of the Cx could be attributed to the strong interactions between entrapped drug molecules and SiOH groups on the pore surface (hydrophilic or electrostatic interactions). In addition, other interactions, such as SiOH and –OH of βcd or –NH of the Cx, could help to modulate the Cx as related by [1, 4, 6, 33]. The contents of Si–OH groups are very important in dissolution behavior, as they can increase the hydrophilic surface and wettability in aqueous solutions [6]. The initial higher jump observed for SCxG and SβcdCxG could be attributed mainly to the higher solubility of CxG in water when compared to SCxA and SβcdCxA. The Cx concentration in the release medium is zero, at early stage of the process, and a large concentration gradient between Cx and the water would prompt the fast release of the drug for these systems [1].

Drug release from soluble matrices such as porous silica sol gels can either be diffusion controlled, dissolution controlled or both. In previous studies, the drug release from these matrices was described as a diffusion controlled process [33, 34, 36]. Drug release from porous silica was modeled using Higuchi Eq. 1 which states that diffusion-mediated release should be proportional to the square root of time [37]. In addition to the Higuchi model, the data were analyzed using the zero order release Eq. 2, which states that the drug dissolution varies linearly with the time, and the k constant is the line inclination. The respective correlation coefficients (R_c) obtained by regression analyses are depicted in Table 3. The fitting procedure used a least-squares method that minimized the differences between experimental and theoretical values.

$$a = kt^{1/2} \quad (1)$$

$$a = kt \quad (2)$$

The modeling of the release from porous silica loaded with chlorhexidine and their inclusion compounds showed an excellent fit with zero order kinetics ($R_c > 0.995$) over a period of 24 h until 400 h for all systems. From 24 h until 750 h for SCxA and SβcdCxA, the Higuchi model fits better than the zero order. The present observation is in line with the fact that significant deviation is possible beyond 50% of release. The kinetics of the release of drugs from porous carrier materials is frequently described as the Higuchi model. According to this model, the release of a drug from an insoluble, porous carrier matrix can be described as the square root of a time-dependent process based on Fickian diffusion [2]. The amount of drug released (a) per unit of exposed area at time (t) can be then described by the simple relation, where k is the release rate constant for the Higuchi model. When the drug is dispersed in the matrix and diffusion occurs through solvent-filled pores, the formulation of the constant is:

$$k = f(D, \varepsilon, \tau, C, A) \quad (3)$$

where D is the diffusivity of the drug in the solvent, τ the tortuosity factor of the system, ε the porosity of the matrix and C is the solubility of the drug in the solvent used [3]. Thus, for a purely diffusion controlled process, the amount of drug released exhibits a linear relationship if plotted against the square root of time. When examining the Higuchi square root of time plots, shown in Fig. 7, a linear behavior after burst effect, characteristic of a zero order release system, is clearly observed.

Some deviation from overall linearity is probably related to the silica matrices degradation during in vitro measurements [2, 5, 36]. It is also known that silica degradates by hydrolysis of the Si–O–Si through the entire network,

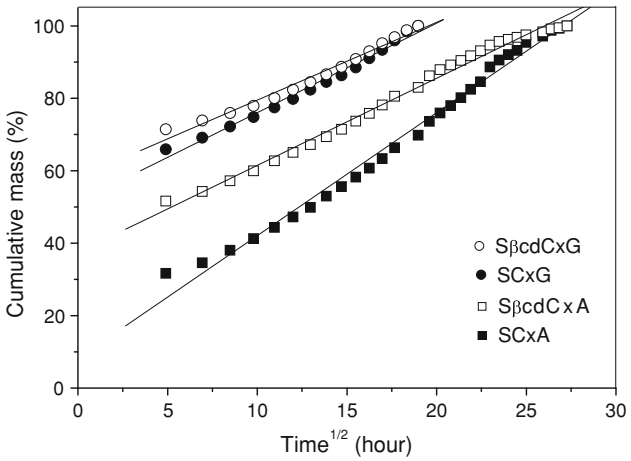

Fig. 7 Release of Cx from the SCxA (*filled square*), SβcdCxA (*open square*), SCxG (*filled circle*) and SβcdCxG (*open circle*) porous silica systems vs. the square root of time ($h^{1/2}$) from 24 h until 700 h

Table 3 Correlation coefficient (R_c) for the relationship between Cx and Cx:βcd inclusion compounds release from porous silica systems from 24 h until 400 h and until 750 h for SCxA and SβcdCxA systems

Model equation	R_c for the systems release					
	SCxA		SβcdCxA		SCxG	SβcdCxG
	Until 400 h	Until 750 h	Until 400 h	Until 750 h	Until 400 h	Until 400 h
Higuchi	0.99251	0.99471	0.99403	0.99516	0.99563	0.99213
Zero order	0.99842	0.99300	0.99746	0.97651	0.99580	0.99569

Table 4 Inhibitory concentration (μg/mL) of SCxA, S*β*cdCxA, SCxG, S*β*cdCxG systems and CxA, *β*cdCxA, CxG, *β*cdCxG against *Enterococcus faecalis* (ATCC 14508) and *Candida albicans* (ATCC 18803) determinated by "Pour Plate Method"

Microorganisms	Inhibitory concentration of antimicrobial agents (μg/mL)							
	SCxA	CxA	S*β*cdCxA	*β*cdCxA	SCxG	CxG	S*β*cdCxG	*β*cdCxG
E.f.	100	50	50	25	100	50	25	50
C.a.	50	50	50	25	50	50	50	25

and it is smallest in the early stages, increasing with the time releasing [19, 36]. It should be noted that the higher deviation was shown by SCxA and S*β*cd-CxA respectively, suggesting that the silica matrix degradation has some influence on the drug release rates. These results can be understood on the basis of the chemical structure of Cx acetate, gluconate and its interaction with the silanol groups present in the silica matrix, as suggested in previous studies for model drugs [1, 6, 33].

Antimicrobial studies

Antimicrobial evaluation in vitro, although difficult to directly correlate to clinical results, is justified in its use for its simple comparisons, screening of materials and application techniques. *E.f.* and *C.a.* are considered to be the most resistant microorganisms in endodontic infections, and are implicated as a possible cause of root canal treatment failure [38].

The inhibitory concentration (μg/mL) obtained for *E.f.*, and *C.a.* are presented in Table 4.

All porous silica systems containing the Cx or Cx:*β*cd inclusion compounds showed satisfactory inhibition against *E.f.* and *C.a.*, as well as the antimicrobial agents (without silica). Among the systems, the S*β*cd-CxG showed better inhibitory concentration (25 μg/mL) when compared to S*β*cd-CxA (50 μg/mL), SCxA (100 μg/mL) and SCxG (100 μg/mL) against *E.f.* The inhibitory concentration of 50 μg/mL was also observed against *C.a.* for all systems, contrary to the lower inhibitory concentration observed for *β*cd-CxA and *β*cd-CxG groups 25 μg/mL, as expected, having the highest inclusion compound solubility. Based on Kruskal–Wallis test, there were no statistical significant differences in efficacy ($p < 0.05$) between the systems for both microorganisms. Cx has been shown to be effective in eliminating *E.f.* and *C.a.* infection inside dentinal tubules, and has been suggested as an irrigant in endodontic therapy [39]. In this study, the silica Cx loaded systems exhibited excellent inhibitory concentration in microorganisms evaluated considering that the drug present in the medium was provided by controlled release, and not by direct contact with antimicrobial agent like the groups containing pure CxA, CxG and its inclusion compounds [8]. Based on data of controlled release of the systems after 2 days, it would probably have less than 10% of the total drug loaded in the culture medium concentrations, as compared to the disposable quantities for the others samples. It was also observed that the inclusion compounds provided a efficient sustained release and antimicrobial activity [16].

Conclusion

A new delivery system of chlorhexidine inclusion compounds based on porous silica shows improved controlled release profile, providing effective antimicrobial activity. Because the excellent properties of silica this system could have important applications in the disinfection of dentinal tubules of the root canal and other infections caused by this microorganisms.

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