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Preparation of quercetin delivery systems on the basis of amino-modified KIL-2 mesoporous silica

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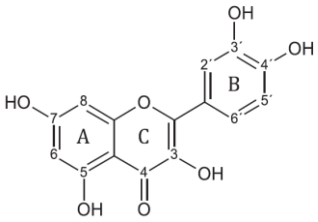
KIL-2 silica with textural mesoporosity was synthesized and modified with amino groups by post-synthesis method. Quercetin was successfully loaded on amino-modified KIL-2 by incipient wetness impregnation or solid-state method. Quercetin-loaded KIL-2NH_2 particles were coated by a polyelectrolyte polymer complex containing κ-carrageenan-chitosan-κ-carrageenan. The parent, amino-modified and quercetin loaded samples were characterized by XRD, N_2 physisorption, thermal gravimetric analysis and ATR-FT-IR spectroscopy. *In-vitro* release of quercetin from quercetin loaded formulations was studied in two acceptor media resembling physiological pH GIT (pH=1.2 and pH=6.8). The *in-vitro* release study showed slower quercetin release from polymer coated quercetin-loaded KIL-2NH_2 samples prepared by both methods compared to the uncoated ones.

Key words: Quercetin delivery, *In-vitro* release, Modified mesoporous silica KIL-2NH_2, Oral administration

INTRODUCTION

The interest in the recent years towards application of mesoporous silica materials as potential carriers for drugs is based on the advantages of these materials, such as biocompability, high specific surface and pore volume, tunable pore size, controlled particle size, morphology and possibility for surface functionalization [1-5]. Mesoporous silica carriers can overcome the problems associated with poor aqueous solubility, low bioavailability and chemical stability of the loaded bioactive substance. The appropriate surface modification of the silica matrix with organic functional groups can optimize the loading efficacy and the release kinetics of the drug [2, 4, 5].

In the recent years. natural flavonoids have attracted research interest due to their pleiotropic therapeutic potential [6-10]. Quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one) is one of the most common flavonols present in nature, a strong antioxidant and a major dietary flavonoid. It has been extensively studied because of its broad-spectrum pharmacological activities such as anticancer, antiviral, antimutagenic and lipid peroxidation inhibitory effects. Initially these effects have been attributed to the strong antioxidant properties of quercetin, because of its ability to scavenge free radicals and influence the intracellular redox status.



Scheme 1. Molecular structure of quercetin

It was found that quercetin can regulate cell cycle by modulating several molecular targets, including p21, cyclin B, p27, and topoisomerase [11]. In addition, quercetin displays specific inhibitory effects in various groups of kinases, including Janus kinases (JAK) and especially JAK-3 kinase, which is a non-receptor tyrosine kinase predominantly expressed in haematopoetic cells [12]. Quercetin is also known as an antiviral, anti-ageing, antimutagenic, anti-inflammatory, antiallergic and anti-psoriatic agent.

Unfortunately, the clinical realization of quercetin's therapeutic potential is greatly hampered due to unfavorable physicochemical and pharmacokinetic properties. Quercetin is characterized with very low aqueous solubility and with chemical instability, which respond in low bioavailability. To overcome these unfavorable characteristics of the drug a possible approach is

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the elaboration of carriers for optimized oral, systemic, site-specific or topical delivery of quercetin. Quercetin has been formulated in liposomes [13], nanoparticles [14], and metal ion complexes [15].

In the present study a novel approach to optimized oral delivery of quercetin is suggested. It is based on its encapsulation in amino-modified mesoporous silica materials. Two methods for deposition of quercetin have been applied, in particular loading from solution or by solid-state reaction into the pores of nanosized NH_2-modified KIL-2 mesoporous silica. Coating by a polymer complex of quercetin loaded mesoporous silica nanoparticles was used in order to modify the release properties. The adsorption capacity and the rate of release of the encapsulated quercetin in these delivery systems were assessed.

MATERIALS AND METHODS

Synthesis of KIl-2 silica material

Mesoporous disordered silicate KIL-2 was prepared by two-step synthesis in presence of the following compounds in a molar ratio of: 1 TEOS : 0.5 TEA : 0.1 TEAOH : 11 H_2O [16]. The template was removed by calcination at 773 K for 10 h in air flow.

Functionalization of KIL-2 with aminopropyl groups

Modification of the KIL-2 material with aminopropyl groups was accomplished by reaction with 3-aminopropyltriethoxysilane (APTES) in toluene (60^{o}C, 24 h) [17]. After completing the reaction, the samples were washed with several portions of toluene, methanol and finally water. 20 ml of APTES in 100 ml of toluene was applied to 1 g of silica. The APTES modified samples were designated as KIL-2NH_2.

Quercetin loading

The KIL-2NH_2 sample was loaded with quercetin by two different methods: deposition by incipient wetness impregnation and by solid-state reaction. KI-2NH_2 and quercetin in a weight ratio of 1.0:0.5 were stirred in 1 mL of ethanol till the total evaporation of the solvent. Then the powdered products were washed 3 times with 5 mL water, and dried at 40 °C overnight. For the solid-state method, the same loading ratio of quercetin to the KIL-2NH_2 material was used. Quercetin was mixed with the carrier at room temperature in a DDR-GM 9458 type vibrational ball mill mixer with a holder (ø = 24 mm) and one ball (ø = 9.5 mm), for 3 min without adding any additive for wetting. The obtained preparations by the two methods were designated as KIL-2NH_2/Qu(IW) and KIL-2NH_2/Qu(SS).

Coating by polyelectrolyte polymer complex

Solutions of chitosan (2 mg/ml, pH 5) and k-carrageenan (2 mg/ml, pH 5) were prepared. A "layer-by-layer" technique was applied to form a three-layer polymer coating around the particles. It involved alternating deposition of the oppositely charged polyelectrolytes starting with carrageenan. At each step the particles were dried at room temperature under reduced pressure. Coated samples were designated as KIL-2NH_2/Qu(IW)P and KIL-2NH_2/Qu(SS)P.

Characterization

X-ray powder diffraction patterns were recorded by a Philips PW 1810/3710 diffractometer with Bragg-Brentano parafocusing geometry applying monochromatized CuK_α (λ=0.15418 nm) radiation (40 kV, 35 mA) and proportional counter.

Nitrogen physisorption measurements were carried out at -196°C using Tristar 3000 Micromeritics volumetric adsorption analyzer. Before the adsorption analysis, the silica sample was outgassed under vacuum for 2 h at 200°C, while modified and drug-loaded samples were pretreated at 80°C for 5 h.

Thermogravimetric measurements were performed with a Setaram TG92 instrument with a heating rate of 5°C/min in air flow.

Attenuated total reflection infrared (ATR-FT-IR) spectra were recorded by means of a Varian Scimitar 2000 FT-IR spectrometer equipped with a MCT (mercury-cadmium-tellur) detector and a single reflection ATR unit (SPECAC “Golden Gate”) with diamond ATR element. In general, 128 scans and 4 cm^{-1} resolution were applied. For all spectra ATR-correction was performed (Varian ResPro 4.0 software).

In vitro release study

An *in vitro* quercetin release study was performed in buffers with pH = 1.2 and 6.8 at 37°C. The quercetin-loaded particles (2 mg) were incubated in 100 ml of 0.1N HCl (pH=1.2) and phosphate buffer (pH=6.8) at 37°C under stirring (100 rpm). At appropriate time intervals, 3-ml samples were withdrawn from the release medium and analyzed with UV-Vis spectroscopy at a wavelength of 367 nm. The concentration of the released quercetin was calculated according to the standard curves prepared in pH=1.2 and 6.8 solutions (r>0.9993).

RESULTS AND DISCUSSION

Material characterization

The low-angle powder XRD patterns of KIL-2 indicate that a mesoporous structure with textural mesoporosity was synthesized (not shown). For the amino-modified and quercetin-loaded silica carriers decreased intensity and some broadened reflections were observed which were indication of partial pore filling. XRD patterns at higher angles of quercetin-loaded amino-modified samples (Fig. 1) show the presence of crystalline quercetin which is more pronounced for the sample prepared by solid-state reaction.

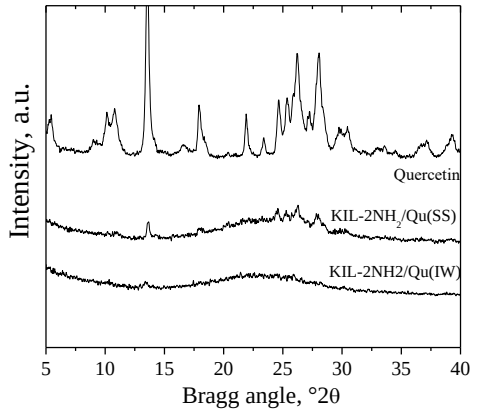

Figure 1. XRD patterns of quercetin-loaded parent and amino modified KIL-2 formulations compared to pure quercetin

This is un evidence that a part of quercetin can be found on the outer surface of the silica nanoparticles or in the voids among the particles.

The nitrogen physisorption isotherms of parent, and quercetin loaded amino-modified KIL-2 samples are presented in Fig. 2. Textural parameters are summarized in Table 1.

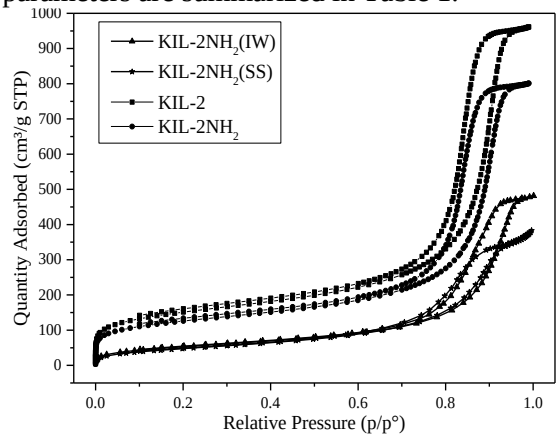

Figure 2. Nitrogen adsorption and desorption isotherms of the parent, amino-functionalized and quercetin-loaded samples.

Significant decrease of the textural parameters, such as surface area and total pore volume of the quercetin-loaded samples indicate pore filling by quercetin. This effect is similar for both quercetin-loaded formulations. Despite the smaller amount of loaded quercetin on the KIL-2NH_2/Qu(SS) sample, its partial deposition on the external surface leads to similar decrease in surface area and pore volume as that obtained for the KIL-2NH_2/Qu(IW) sample.

Table 1. Textural properties of the parent, amino-functionalized and quercetin-loaded mesoporous silicas

Samples	BET (m^2/g)	Pore volume (cm^3/g)	PD[a] (nm)	Quercetin amount (wt.%)
KIL-2	660	1.20	15.3	-
KIL-2NH_2	530	1.00	13.2	-
KIL-2NH_2/Qu(SS)	202	0.41	8.2	19.8
KIL-2NH_2/Qu(IW)	195	0.40	7.9	30.9

For clarification of the interaction between the quercetin molecule and the amino groups of the mesoporous silica carrier the quercetin-loaded samples were characterized by the ATR FT-IR method (Fig. 3). Pure quercetin shows characteristic IR bands of stretching vibrations of aryl ketonic carbonyl ($n_{C=O}$ at 1660 cm^{-1}) and of aromatic ring C=C (at 1605, 1555, 1511 and 1457 cm^{-1}). Band at 1350 cm^{-1} belongs to OH bending vibration of the phenols and the band around 1309 cm^{-1} can be assigned as in-plane bending vibration of aromatic C-H [18]. The modification of KIL-2 sample by APTES results in the appearance of the bands at 2929 cm^{-1} and at 1540 cm^{-1} (not shown) which are attributed to C-H stretching and N-H scissoring vibrations of aminopropyl residues anchored on the surface of the mesoporous support [19, 20]. In the ATR-FTIR spectra for quercetin-loaded KIL-2NH_2 a shift of $\nu_{C=O}$ at 1660 to 1668 cm^{-1} is registered, while the bands of aromatic ring C=C vibrations show up-shift. The band at 1350 cm^{-1} (belonging to OH bending of phenols) is shifted to higher wavenumbers.

The registered changes in the ATR-FTIR spectra are evidence for interaction between the NH_2 groups of the KIL-2 matrix and the phenolic OH groups of quercetin, which leads to a conjunction

loss among the aromatic and the pyrone ring in the quercetin molecule.

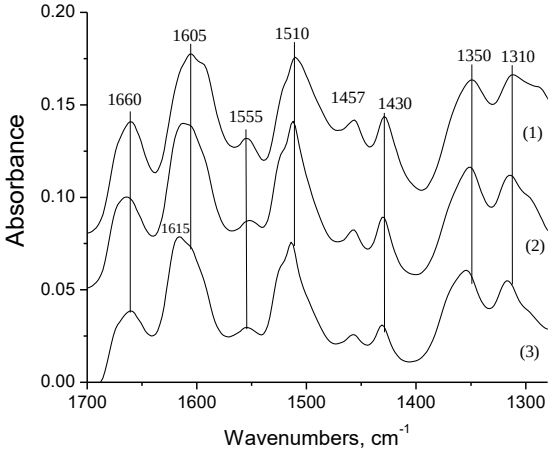

Figure 3. ATR FT-IR spectra of quercetin (1), KIL-$2NH_2$/Qu(SS) (2) and KIL-$2NH_2$/Qu(IW) (3) samples.

Quercetin loading and in vitro release

The amount of the functional aminopropyl groups, loaded quercetin and polymer coatings in the prepared samples were investigated by thermogravimetric method. The calculated amount of aminopropyl groups connected on the surface of KIL-$2NH_2$ is 6.5 wt.%. The TG analysis determined the actual amount of quercetin in the carriers after correcting the curves by water and aminopropyl content for KIL-$2NH_2$. TG data show that the loading of quercetin on KIL-$2NH_2$ samples by solid state and incipient wetness impregnation is around 19.8 and 30.2 wt.%, respectively (Table 1). The higher amount of loaded quercetin by the impregnation method can be explained by easier penetration of dissolved quercetin molecules in the pores of the support. Moreover, the quercetin loaded by solid state reaction is partially deposited on the external surface of KIL-$2NH_2$ as can be seen from XRD data. The calculated weight losses from TG analysis due to the decomposition of polyelectrolyte complex containing k-carrageenan – chitosan-k-carrageenan were 9.5 wt% for KIL-$2NH_2$/Qu(SS) and 8.8 wt% for KIL-$2NH_2$/Qu(IW).

Faster release of quercetin was registered for the KIL-$2NH_2$/Qu(IW) sample in both buffers compared to its analogue prepared by solid state reaction. The quercetin-loaded KIL-$2NH_2$ formulations show slower release in buffer with pH=6.8 than that in buffer with pH=1.2. Total quercetin release for KIL-$2NH_2$/Qu(IW) at pH=1.2 was achieved in 3 h whereas in buffer with pH=6.8 the maximum release of quercetin for the same sample reached 92 % in 4 h. This result can be explained by protonation of aminopropyl groups in acidic buffer facilitating the release of quercetin molecule as a result of the competitive adsorption between quercetin and water molecules. Faster release of quercetin from the KIL-$2NH_2$/Qu(IW) sample can be explained with the amorphization of quercetin during its penetration into the pore system of the carrier in comparison to the KIL-$2NH_2$/Qu(SS) material, in which the presence of crystalline quercetin was seen by XRD. The coating with a k-carrageenan –chitosan-k-carrageenan complex leads to decrease of the release rate of quercetin in both buffers for the KIL-$2NH_2$/Qu(IW)P and KIL-$2NH_2$/Qu(SS)P materials as seen in Fig.4.

From the comparison of the release profiles of the uncoated and coated particles it can be calculated that in the first 30 min the latter system releases twice less amount of drug. In conclusion, by the applied formulation method an efficient delivery system could be developed, providing controlled release of quercetin.

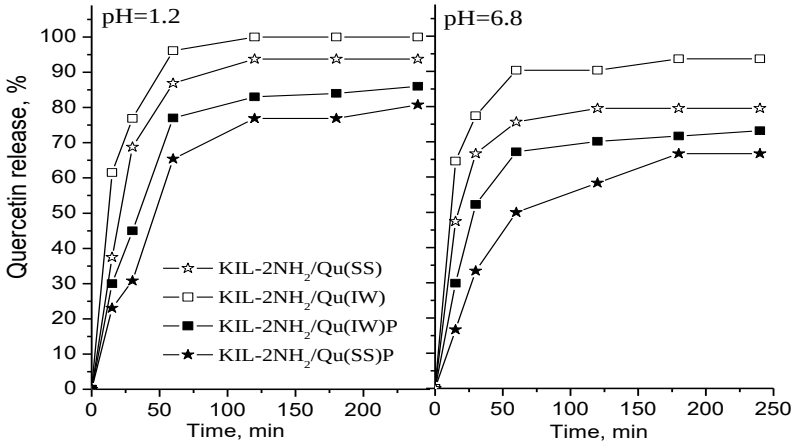

Figure 4. *In-vitro* release profiles of quercetin loaded amino modified KIL-2 samples at pH=1.2 and 6.8.

CONCLUSIONS

Mesoporous KIL-2 silica type was synthesized and modified with aminopropyl groups by a post-synthesis method. Incipient wetness impregnation and solid-state reaction methods were used for quercetin loading on the amino-modified KIL-2 samples. High loading capacity (20-30 wt. %) was registered on the KIL-2NH_2 by both methods. The *in-vitro* release process at pH=1.2 and 6.8 showed faster quercetin release from KIL-2NH_2 sample prepared by impregnation in comparison to that prepared by solid state reaction. It was shown that the release of quercetin loaded on the mesoporous nanocarrier can be additionally controlled by formation of a polyelectrolyte polymer complex.

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ПРИГОТВЯНЕ НА СИСТЕМИ ЗА ДОСТАВЯНЕ НА КВЕРЦЕТИН НА ОСНОВАТА НА АМИНО-МОДИФИЦИРАН KIL-2 МЕЗОПОРЕСТ СИЛИКАТ

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(Резюме)

KIL-2 силикат с текстурална мезопористост е синтезиран и модифициран с аминогрупи чрез следсинтезен метод. Кверцетин е нанесен върху амино-модифицирания KIL-2 чрез импрегниране с омокряне или чрез твърдо-фазна реакция. Частиците от KIL-2NH_2 с нанесен кверцетин са обвити с полиелектролитен полимерен комплекс, съдържащ к-карагенан-хитозан-к-карагенан. Изходните, амино-модифицираните и кверцетин-съдържащите образци са охарактеризирани чрез XRD, азотна физисорбция, термогравиметричен анализ и ATR-FT-IR спектроскопия. *In-vitro* освобождаването на кверцетин от образците е изследвано в две физиологични среди с pH GIT (pH=1.2 and pH=6.8). Резултатите от *in-vitro* освобождаването показа, че кверцетина се освобождава по-бавно от образци, получени по двата метода и обвити с полимер, в сравнение с необвитите.