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Resveratrol loading on mesoporous silica and zeolite carriers by solid state method

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Dedicated to Acad. Dimiter Ivanov on the occasion of his 120th birth anniversary

The feasibility of mesoporous SBA-16 and MCM-41 materials to be used as carriers for the poorly water soluble compound Resveratrol (RES) was studied and compared with that of zeolite Y. A simple procedure of encapsulation in solid-state aiming amorphization of the drug which thus enhancing its solubility has been successfully applied. Investigations using powder XRD, N_2 physisorption, ATR FT-IR and TG analysis demonstrated the effective loading of RES on the surface of the carriers. RES release in buffers with pH=1.2 and pH=6.8 demonstrated faster dissolution of the loaded drug compared to non-loaded resveratrol.

Key words: resveratrol encapsulation, in vitro release, mesoporous silica, zeolite Y carrier

INTRODUCTION

The research and application of natural polyphenols have recently attracted great interest due to their potential in functional food and drug delivery [1-3]. Among these compounds, resveratrol (RES) is recognized mainly as an antioxidant, but also as antiviral, anticancer, an antiinflammatory, antihypersensitive, cardioprotective agent, etc. [4-6]. Despite the promising results in preclinical settings, the extensive use of RES has met only limited success, largely due to its insta-bility [7], poor solubility [8], inefficient systemic delivery and low bioavailability [9]. In this context, the encapsulation of RES into polymeric or lipid-based matrices is a major challenge, and nanotechnology represents a powerful strategy [10]. The development of innovative formulation strategies, capable of overcoming the physicochemical and pharmacokinetic limitations of this compound, is mainly based on discovering suitable carriers able to provide controlled release and protection for RES.

Mesoporous silica nanomaterials of the MCM-41 and SBA-series as well as the microporous zeolites, possess great surface area allowing an efficient drug loading and stabilization of labile drugs during storage or physiological administration. Further, these carriers could improve water solubility of poorly soluble drugs probably due to the increased surface and/or transformation of the crystal form into amorphous one. Ordered mesoporous silicate MCM-41 and SBA-15 have been widely evaluated as carriers for different poorly soluble compounds [11-15]. In contrast, there has been very limited work dealing with the employment of the cage-like SBA-16 materials as drug carriers [16,17].

The advantages of using zeolites for biomedical applications are their biocompatibility, low toxicity [18-20] and the ability to tune the zeolite properties by varying the SiO_2/Al_2O_3 ratio and the surface functional groups [21,22]. In addition, the smaller zeolite pore size (relative to mesoporous silica) more closely matches the drug molecule size and may better control the drug release kinetics.

The main method applied for loading of active molecules on these carriers is solvent impregnation. This method requires long incubation of the drug and the carrier, usually under heating in appropriate organic solvent. However, some drugs are thermosensitive or insoluble in solvents. Thus, because of this limitation, some new approaches should be developed for the achievement of efficient loading.

The aim of the present study was to evaluate the capacity of three carriers (MCM-41, SBA-16 and Y type zeolite) for RES loading by solid state method.

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The method was considered advantageous since no organic solvents or catalysts are required for the encapsulation process and the procedure could be easily performed. Furthermore, the solid state method could be applied as loading method especially for drugs with poor solubility and stability problems like resveratrol.

In our previous publication a thorough examination on the effectiveness of the newly introduced method for solid-state preparation of drug formulations composed of the purely soluble antioxidant resveratrol and nanosized silica materials has been made [23]. In the present contribution a comparison of the loading efficiency and the release kinetics of RES delivery systems based on mesoporous MCM-41 and SBA-16 silicas as well as on Y type zeolite support with different channel structures and morphology, formulated at dry mixing conditions has been made.

EXPERIMENTAL

Synthesis of mesoporous MCM-41 and SBA-16 silica materials. Zeolite Y support

MCM-41 with 100 nm particle size was prepared according to the procedure of Huh *et al.* [24]. This sol-gel procedure is carried out at 80°C without co-solvent, in water solution and applying NaOH as a catalyst. The relative molar composition of the reaction mixture was: 1 TEOS: 0.12 C_{16}TMABr: 0.31 NaOH: 1190 H_2O. The formed gel was aged at 80°C for 2 h, then washed with distilled water until neutral pH and dried at ambient temperature. Structure directing agent removal of MCM-41 materials was carried out in air at 550°C with 1 K/min rate and held at this temperature for 5 h.

SBA-16 has been synthesized according to Zhao *et al.* [25]. F127 triblock copolymer (BASF) is used as template and tetraethylortosilicate (TEOS) as silica source. The relative molar composition of the synthesis mixture is the following: 4 g $PEO_{106}PPO_{70}PEO_{106}$: 0.24 HCl: 0.04 TEOS: 7.86 H_2O. The synthesis mixture was stirred at room temperature for 20 h, than hydrothermally treated at 80°C for 48 h. Structure directing agent was removed by calcination in air at 500°C with a heating rate of 1 K/min.

Zeolite Y in its ammonium form was supplied by Zeolyst International (UWE Ohlrogge (VF), CBV 500) as a powder with the following characteristics: Si/Al molar ratio of 2.6, Na_2O = 0.2 wt.%, unit cell size = 24.53. The decomposition of the NH_4^+-form was performed by stepwise heating in dry N_2 as follows: heating up to 300°C, hold for 30min and then heating from 300°C to 550°C, hold 90 min. Heating rate was 8 K/min. The as-obtained initial material is designated as HY(2.6).

Characterization of the samples

X-ray patterns were recorded by a Philips PW 1810/3710 diffractometer with Bragg-Brentano para focusing geometry applying monochromatized CuKα (λ = 0.15418 nm) radiation (40 kV, 35 mA) and a proportional counter. Philips XPert-MPD apparatus was used for structure characterization of SBA-16.

Nitrogen physisorption measurements were carried out at 77 K using Quantachrome Autosorb 1C apparatus. The specific surface area was calculated by the BET method in the range of relative pressures from 0.01 to 0.1. The pore-size distribution was calculated from desorption branch of the isotherms with the BJH method. The silica supports were pre-treated at 350°C, whereas the drug loaded formulations were heated at 80°C for 5 h before measurements.

Thermogravimetric measurements were performed with a Setaram TG92 instrument. The samples (about 20 mg) were treated in situ in air flow up to 600°C (5°C/min) followed by a hold-up of 1 h. The detected weight loss corresponds to the amount of the deposited resveratrol.

Attenuated Total Reflection Infrared (ATR-FT-IR) spectra were recorded by means of a Varian Scimitar 2000 FT-IR spectrometer equipped with a MCT (mercurycadmium-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).

Resveratrol loading and in vitro release studies

For the solid state method of RES loading 1:1 weight ratio of RES to the parent mesoporous MCM-41 and SBA-16 materials was used. In case of the zeolite sample 0.8:1.0 ratio was applied. RES was mixed with the carriers at room temperature in a DDR-GM 9458 type vibrational ball mill mixer with stainless holder (ø = 24 mm) and one ball (ø = 9.5 mm), for 3 minutes without adding any additive for wetting. The as-prepared samples are designated as R/MCM-41(SS), R/SBA-16(SS) and R/Y(2.6)SS indicating their solid-state procedure of preparation. Resveratrol (>99.5%) was provided by Sigma-Aldrich.

For *in vitro* release studies, 5 mg of the drug loaded particles were incubated in 200 ml acid or phosphate buffers (pH=1.2 or pH=6.8, respectively) at 37°C under stirring (100 rpm). At appropriate time intervals, 3 ml samples were withdrawn and replaced by fresh buffer. The withdrawn samples were centrifuged at 15 000 rpm for 15 min and the concentration of the released RES into the supernatant was determined by UV-Vis spectrophotometry (Hewlett Packard 8452A) at a wavelength of 308 nm.

RESULTS AND DISCUSSION

Materials characterization

The XRD reflections of the commercial RES compound and the grinded substance are presented in Fig. 1a. Decrease in the intensity of the diffraction peaks at $2\theta = 17^o$, 19^o, 22^o, 23^o, and 28.3^o compared to that of the commercial form of the drug is observed (Fig. 1a). This effect proves that treatment in the vortex mixer leads to partial amorphization of the antioxidant, which was the aim of applying this solid-state preparation procedure. Presence of crystalline RES phase is clearly visible, however, on all loaded samples (Fig. 1a-c). This is evidence that RES is not only introduced in the meso/micropores, but can be found on the outer surface of the nanoparticles or in the voids among them.

The low-angle powder XRD patterns of MCM-41 and SBA-16 show an ordered pore arrangement with the typical reflections at the low angles at $2\theta = 2.4^o$ (Fig. 1a) and $2\theta = 1^o$ (Fig. 1b) for MCM-41 and SBA-16 respectively which are not destroyed upon RES encapsulation. The X-ray reflections of RES loaded mesoporous carriers reveal clear indication for presence of small RES crystallites on the surface of both MCM-41 and SBA-16 materials.

The X-ray powder diffraction patterns of the calcined zeolite reveal the characteristic of the faujasite topology main diffraction peaks assigned to (1 1 1) and (5 3 3) faces at $2\theta = 6.2^o$ and 15.3^o respectively (Fig. 1c) the intensity of which decreeses to some extent compared to the parent HY zeolite. This effect indicates that the microporous structure was retained, although, the crystallinity has decreased, together with that of RES during the solid-state preparation of the RES containing zeolite formulation (Fig.1c).

The parent and resveratrol loaded MCM-41, SBA-16 and Y samples were characterized by the method of low temperature nitrogen adsorption and the data are presented in Table 1 and in Fig. 2.

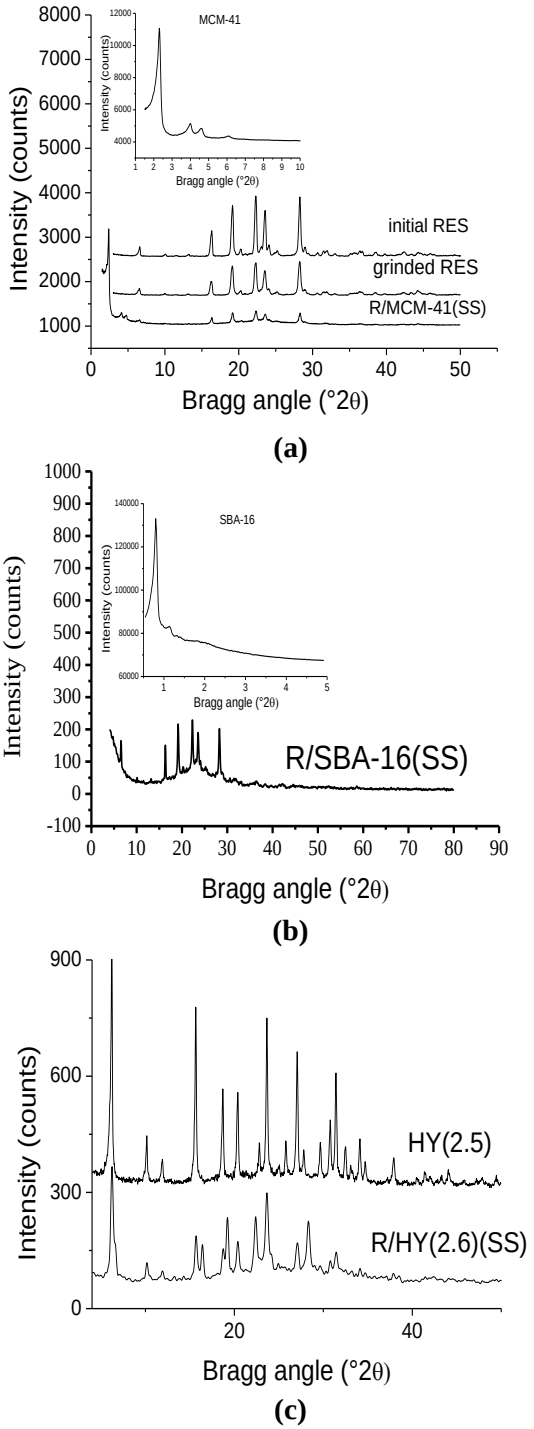

Fig. 1. Powder X-ray diffraction patterns of: (a) initial and grinded RES and RES/MCM-41(SS); (b) RES/SBA-16(SS); (c) parent and RES loaded, RES/HY(2.6)(SS).

As the results reveal, compared to the parent carriers, all loaded with resveratrol compositions exhibit substantial decrease in the surface area and pore volume, as well as some pore diameter reduction in case of the mesoporous carriers (Table 1, Fig. 2). It can be suggested that the pores of the latter supports are not filled entirely with drug, however.

Table 1. Physicochemical properties of the supports and their formulations with RES and concentration of the released RES in pH=1.2 and pH=6.8.

Samples	BET (m^2/g)	Pore diameter (nm)	Pore volume (cm^3/g)	TG data for loaded RES (%)	Released RES* (%)	
					in pH=1.2	in pH=6.8
MCM-41	1175	2.7	0.97	-	-	-
R/MCM-41(SS)	306	2.5	0.27	50.6	36.7	31.8
SBA-16	1128	2.5-7.9	0.64	-	-	-
R/SBA-16(SS)	105	2.5-4.4	0.10	45.5	36.8	33.5
HY(2.6)	758	0.11	0.34	-	-	-
R/Y(2.6)(SS)	119	n.d. **	0.04	37.1	27.0	31.7

*Determined by TGA by heating from 180°C to 600°C; **no presence of pores detected.

As the results reveal, compared to the parent carriers, all loaded with resveratrol compositions exhibit substantial decrease in the surface area and pore volume, as well as some pore diameter reduction in case of the mesoporous carriers (Table 1, Fig. 2). It can be suggested that the pores of the latter supports are not filled entirely with drug, however.

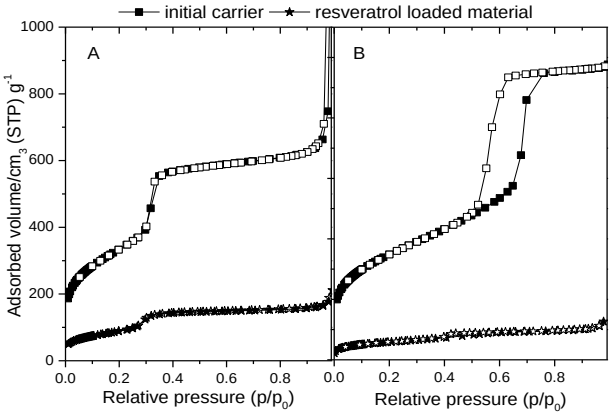

Fig. 2. Nitrogen adsorption/desorption isotherms of the parent materials and resveratrol loaded MCM-41 (a) and SBA-16 (b) composites, respectively.

The change in the isotherms for the loaded Y zeolite, compared to the parent carrier is an indication for total micropore filling or rather plugging. The microporosity of this material with much narrower channel system was actually disappeared and the total pore volume was strongly reduced.

ATR FT-IR method was used to investigate whether some interaction between the adsorbed RES molecule and the nanosized porous carriers (Fig. 3) has occurred upon the drug encapsulation. As was already observed in our previous study [23] that the FT-IR spectrum of resveratrol shows three characteristic strong bands at 1606, 1585 and 1384 cm^{-1}, belonging to RES double bond C–C stretchings of olefinic and aromatic groups, and of ring C–C stretching, respectively. The bands at 1509, 1443 and 1324 cm^{-1} can be assigned to in plane and out of plane C–H bending, respectively [26]. After deposition into silica/zeolite framework, a slight blueshift of the resveratrol IR bands corresponding to vinylidene group and a decrease in intensity of the vinylidene C–H in plane band (1324 cm^{-1}) can be observed. These spectral features due to perturbation at vinylidene dipole moments suggest an interaction of resveratrol C=C with the MCM-41 silica and the zeolite framework. For all cases, the out of plane C–H bending of RCH=CHR (965 cm^{-1}, not shown), diagnostic for *trans*-configuration around the di-substituted double bond [27], remains unchanged, indicating that the *trans*-resveratrol configuration is preserved during encapsulation over all supports used.

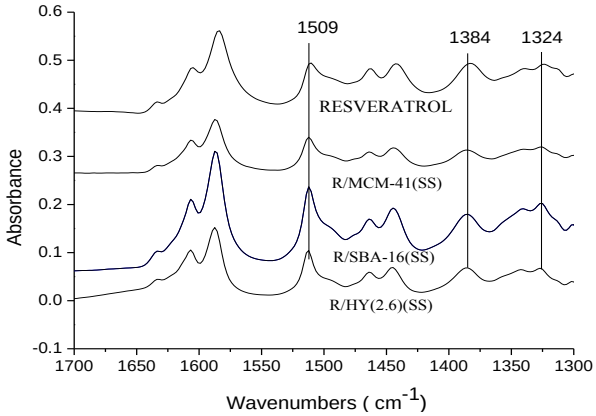

Fig. 3. FT-IR spectra of RES and RES loaded mesoporous and zeolite supports.

Resveratrol loading

The adsorbed amount of RES on the loaded preparations was evaluated by thermogravimetric analysis (TGA) considering the weight loss at heating the formulations in air up to 600°C (Fig. 4). The TGA profile of the pure grinded RES reveals two distinct weight changes starting at 250°C and 400°C which can be attributed to the onset of

melting followed by the decomposition of the RES molecule, respectively. At temperatures below 200°C the drug-host formulations lose the physic-sorbed water. Above 230°C the onset of a short temperature interval of melting of the supported substance follows, and then fast drug decomposition takes place.

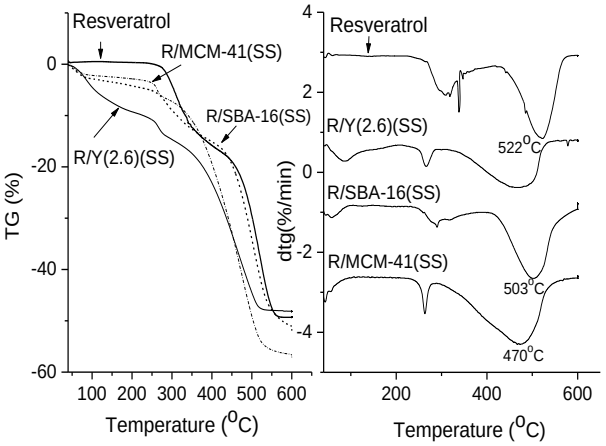

Fig. 4. TGA (a) and DTG (b) profiles of the RES loaded materials.

The calculated from the TGA data values for the amount of encapsulated RES are obtained by subtracting the weight loss due to water released from room temperature to 230°C from the total weight loss registered after heating for 1 h at 600°C.

Compared to the pure RES the loaded RES is released, with the exception of R/SBA-16(SS), at lower temperature, in both regions (Fig. 4). This is an indication for easier release corresponding to the enhanced solubility of the loaded compound compared to the non-supported one. Almost complete adsorption of the deposited RES, corresponding to the envisaged (1:1 for the mesoporous supports and 0.8:1 for the zeolite) RES: carrier weight ratio is registered for the three studied formulations. This result points to the high effectiveness of the simple solid-state procedure of RES deposition used for the preparation of such drug delivery systems.

In vitro drug release

The *in vitro* release process was studied in acid and phosphate buffer with pH values of 1.2 and 6.8, respectively. As shown in Fig. 5, the release rate of RES from the porous carriers was faster than its solubility in the same media. These results were probably due to the transformation of RES from crystal to amorphous state during the loading via grinding procedure. The slightly faster release in acid buffer was associated with the protonation of the carriers that was previously reported [28,29].

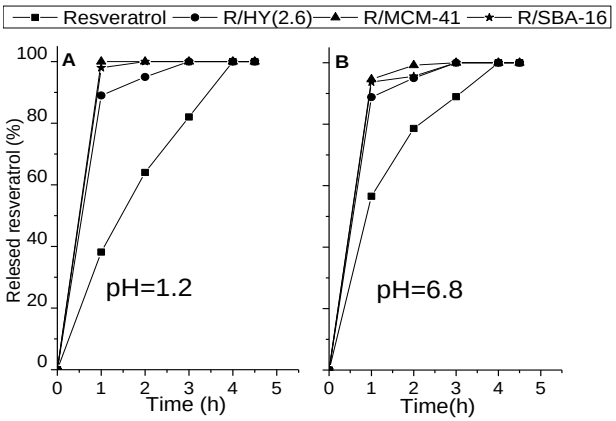

Fig. 5. In vitro release of resveratrol in pH=1.2 (a) and in pH=6.8 (b) from the supported samples.

Comparing the profiles of the three carriers, faster release was observed from both mesoporous carriers (MCM-41 and SBA-16) compared to the microporous Y type zeolite. This behavior could be perhaps explained with the slower diffusion of RES through the narrower zeolite pores. Thus, it appeared that the different pore size (mesoporous for the silica materials and microporous for the zeolite) influenced the release drug kinetics, in accordance to the previously reported findings of M. Vallet-Regi *et al.* [12].

CONCLUSION

Solid, silica-based spherical mesoporous MCM-41 and SBA-16 as well as microporous zeolite Y materials differing in morphology and pore size distribution were used as carriers for the preparation of resveratrol-loaded delivery systems by dry mixing of resveratrol and the respective carrier in solid state. This preparation procedure resulted in better resveratrol solubility compared to the free resveratrol, regardless of the carrier structure. The applied deposition method proved to be effective for encapsulation and stabilization of the drug as well as for its enhanced solubility.

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НАТОВАРВАНЕ НА РЕСВЕРАТРОЛ ВЪРХУ МЕЗОПОРЕСТИ СИЛИКАТНИ И ЗЕОЛИТНИ НОСИТЕЛИ ЧРЕЗ ТВЪРДОФАЗЕН МЕТОД

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(Резюме)

Възможността за използване на мезопорестите SBA-16 и MCM-41 силикатни материали като носители за слабо разтворимия във вода ресвератрол (RES) беше изследвана и съпоставена с тази на зеолит тип Y. Успешно беше приложена опростена процедура за натоварване в твърда фаза, съпроводена с аморфизиране на лекарственото вещество и повишаване на разтворимостта му. Изследванията с прахообразна рентгенова дифракция, азотна физисорбция, инфрачервена спектроскопия при пълно вътрешно отражение и термогравиметричен анализ показаха ефективно натоварване на ресвератрола върху повърхността на всички използвани носители. Резултатите от освобождаването в буфери с pH=1.2 и pH=6.8 показаха по-бързо разтваряне на ресвератрола, натоварен в подбраните носители, в сравнение с изходния ресвератрол.