UNIVERSITY POLITEHNICA OF BUCHAREST

Faculty of Applied Chemistry and Materials Science

Doctoral School

Chemical Engineering

# Development of drug delivery systems based on natural compounds

## *PHD THESIS ABSTRACT*

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**Keywords:** polyphenols, mesoporous silica, ulvan, irinotecan, controlled release, targeted action, antioxidant activity, HPLC-PDA, chemical stability.

# Table of contents

# Introduction

The PhD thesis comprises two parts: a literature study divided into five chapters and original contributions with also five chapters, respectively. The first chapter of literature survey presents the application of mesoporous silica in drug delivery systems, being discussed the synthesis procedures, pore walls surface functionalization with either heteroatoms or through chemical modification, uses and biocompatibility. Further, the following two chapters present information about the raw materials used for polyphenolic extract preparation, grape pomace and *Aronia melanocarpa* fruits, being listed the main polyphenolic compounds found in phenolic extracts, the methods used for phytocomponents extraction, previous reports regarding encapsulation of extracts and their biological activity (antioxidant, chemo preventive, anti-inflammatory, antimicrobial and antidiabetic). Next chapter deals with the stability of individuals polyphenols or extracts. Finally, the last chapter presents the use of irinotecan, a semi-synthetic cytostatic agent, to target tumoral tissue *via* three strategies that were designed to valorize the benefits of natural compounds.

The original contribution part presents firstly the methods used for extracts, supports and materials with embedded extract characterization. Further, it is presented the obtaining of ethanolic extracts prepared from grape pomace and a commercial grape skin powder. The extracts with the best antioxidant properties were embedded in mesoporous silica modified with heteroatoms for which an enhanced stability was achieved through nanoconfinement in support mesopores. The next chapter emphasis the preparation of hydro-ethanolic extracts from a rose grape pomace from Mamaia cultivar for which the chemical profile and antioxidant activity were determined. The hydro-ethanolic extract with the best properties was embedded in MCM-41-type silica with organic groups linked on the inner pore walls. Antioxidant activity through ABTS and DPPH methods adapted for solid samples, as well as the reactive oxygen species on cellular level (NIH3T3 cell line) were determined. The fourth chapter presents the obtaining of an ethanolic extract from aronia fruits that have enhanced antioxidant and antimicrobial properties material and as well as antitumoral properties on human tumoral cell line (A375) through embedding in Zn-MCM-41, being less toxic on human keratinocytes cell line (HaCaT). The last chapter illustrates the development of irinotecan delivery systems. Innovative supports such as silica modified with folate moiety, mesoporous functionalized silica silica-ulvan (a natural sulphonated polysaccharide) composite or a MCM-41 mesoporous silica decorated with crystalline nanoparticles of ferric oxide were evaluated for irinotecan to modulate the cytostatic agent release kinetics.

# I. LITERATURE SURVEY

## 1. Mesoporous silica as carrier in drug delivery systems

Materials based on mesoporous silica were used as carriers in drug delivery applications due to its biocompatibility [1, 2], high adsorption capacity (high porosity that allows a large amount of therapeutic agent to be hosted), a possibility of silica nanoparticles surface modification through silanol groups on the surface that can react with organosilanes, and thus, being able to influence the adsorption of drug molecules and also their release [3-5].

## 2. Grape marc

Grape pomace, a mixture of skins, seed and stems resulted after pressing grapes in the winemaking process is an abundant by-product, representing about 20-30%wt of the processed grapes [6, 7].

Through the vinification process, sugars from grapes are converted in ethanol, but bioactive compounds such as polyphenols are not altered by this process, which leads to a preservation after processing of about 70%wt from the amount found in grapes [6, 8, 9]. Therefore, grape marc represents an important by-product with high amounts of various polyphenols unevenly distributed [10, 11].

## 3. *Aronia melanocarpa*

The main phenolic compounds found in *Aronia* extracts are phenolic acids from hydroxycinnamic groups: chlorogenic acid (3-caffeoylquinic acid, 23.12% from total polyphenols content) and neochlorogenic acid (5-caffeoylquinic acid, 11.23% from total polyphenols amount), flavonoids: anthocyanins, proanthocyanidins, flavonols and catechins. From the flavonol glycoside group quercetin-3-glucoside and quercetin-3-galactoside represent almost 10%wt from total polyphenols amount [12]. From the anthocyanins class in aronia extracts are found cyanidin glucoside (approximately 57%wt from total polyphenols amount), from which the main identified compounds are: cyanidin 3-*O*-galactoside and cyanidin 3-*O*-arabinoside [13].

## 4. Polyphenolic extracts stability

The polyphenolic extract efficiency is influenced by the preservation of chemical stability, bioactivity and bioavailability of their main components [14].

Usually, polyphenolic compounds are unstable during food processing, distribution, storage, or after ingestion, in gastro-intestinal tract, and their activity and health benefits are limited. Unfortunately, polyphenols are prone to oxidation processes that leads to browning or unpleasant odour instauration, with a significant antioxidant activity loss [15].

## 5. Irinotecan as cytostatic agent

Irinotecan, a semi-synthetic product, obtained from the natural alkaloid camptothecin, is a topoisomerase I inhibitor, which interferes with the replication stage of DNA, at duplication level. The cationic drug is an antineoplastic drug used since 1994 to treat different types of cancer and solid tumours (rectal, colon, ovarian cancer, small cells-cancers and glioblastoma) [16, 17].

The main issue of using irinotecan in the treatment of different types of cancers is represented by a high toxicity that leads to serious side effects such as neutropenia [18], severe diarrhea that determines a significant dehydration and potentially to death [17].

An ideal drug delivery system for an antitumoral agent needs to be designed to significantly improve the common treatments that use the drug by itself, avoiding drug degradation, which is related to an increased drug concentration that riches the tumoral sites. Moreover, the nanoparticles should be synthesized to ensure a selectivity accumulation in tumoral cells in order to reduce side effects on healthy cells [19].

# II. Original contributions

## Justification of topic choice

In the PhD thesis, "*Development of drug delivery systems based on natural compounds"* were used chemical engineering concepts, as well as knowledge of biomedicine (biocompatibility evaluation, antioxidant activity or cytotoxicity testing of proposed systems).

Natural resources contain numerous phytocompounds with important benefices such as antioxidant, anti-inflammatory, antidiabetic etc. Hence, in the last decade an important attention was focused on natural compounds due to their health benefits that are not associated with common side effects as in the case of synthetic drugs.

However, extracts are not used to their potential because of a complex and variable composition depending on vegetal material (type of cultivars, climatic conditions, and soil quality). Also, biological effects of extracts are not studied intensively and therefore, it is more difficult to be used because are more sensitive to environment and could easily degrade with a partial loss of their therapeutic properties.

Thus, it was proposed the embedding of polyphenolic extracts from grape marc or aronia fruits in mesoporous silica-type matrix to enhance their stability and so a preservation of antioxidant activity over time. Also, by obtaining polyphenolic compounds as a powder makes it easier to store and handle.

The last part of this PhD thesis presents the development of drug delivery systems for a controlled and targeted release of irinotecan using three different vehicles: functionalization with folate moieties of silica SBA-15-type carrier, the use of silica – ulvan composite-type carrier (ulvan is a natural sulphonated polysaccharide extract from green algae, *Ulva lactuca*) for tumoral cells internalisation and a support consisting in MCM-41 silica modified with ferric oxide nanoparticles for magnetic tumoral targeting. All these vehicles could be used to modulate the irinotecan release kinetics.

## PhD thesis objectives

- Synthesis and characterization of pristine and functionalized silica with heteroatoms or organic groups
- Studies on the extraction parameters (solvent, raw material, extraction method) to obtain polyphenolic extracts with high amount of valuable compounds
- Chemical profile determination of polyphenolic extracts from grape marc or *Aronia melanocarpa* fruits
- Development of two simple and reproducible procedures adapted for antioxidant activity for solid samples
- Evaluation of the polyphenolic extracts stability over time
- Development of nanoplatforms based on mesoporous silica for polyphenols-controlled release through the modulation of interactions between silica surface and extract phytocomponents
- Encapsulation of polyphenolic extracts to preserve or improve the extracts biological properties
- Valorization of natural compounds to target tumoral tissue.
- Modulation of cytostatic agent release kinetics from proposed mesoporous silica-type supports

## 1. Materials and Methods

Polyphenolic extracts were characterized using several spectrophotometric methods (Shimadzu UV-1800) to determine total amount of reducing substances, polyphenols, flavonoids and anthocyanins. The chemical profile of extracts was determined through high performance liquid chromatography (HPLC, Shimadzu Nexera 2).

The antioxidant activity of materials containing embedded extracts (DPPH assay) was evaluated though a method adapted for solid samples. Therefore, considering that mesoporous silica exhibits a high porosity and an ability to absorb organic molecules (DPPH free radicals) and the interaction between a material and free radical from solution is weak, the radical scavenger activity was determined after 24 h of samples incubation in DPPH ethanolic solution, in triplicate and it was compared with that of the free extract and corresponding support in the same amount as in the material containing extract using DPPH free radical solution degradation in time as control.

## 2. Polyphenolic ethanolic extracts from grape pomace characterization and embedding into mesoporous silica

Spectrophotometric methods used for the characterization of polyphenolic extracts, the determination of their composition by high performance liquid chromatography, but also the development of a procedure for testing the antioxidant activity of extracts incorporated in mesoporous matrices were reported in detail in Food Chem Toxicol. [20]. Then, the results of the analyses of the fractions obtained after each of the three extraction stages, but also of the global extract are presented either for conventional method or for microwave-assisted extraction (MW). Also, it was evaluated the stability of the extracts by DSC [21].

### 2.1. Polyphenols extract from grape pomace. Valorisation through encapsulation into mesoporous silica-type matrices

Thirteen substances of the available standards were identified in the prepared extracts or in the extracts analysed after 4 months. The chromatograms of prepared extracts are presented in Figure 1.

High amounts (0.462 - 1.171 mg/g extract) of gallic acid, vanillic acid (0.368-1.088 mg/g extract), syringic acid (0.339-2.031 mg/g extract) and protocatechuic acid (0.104-0.489 mg/g extract), as well as low concentrations (0.019-0.083 mg/g extract) in *trans*-resveratrol were found in all samples. In the case of MW-assisted extraction a lower content of gallic acid, protocatechuic acid, vanilic acid, syringic acid and *p*-coumaric acid (not detected), and an enhanced concentration of rutin hydrate, (-) epicatechin, and *trans*-resveratrol were observed.

The *in vitro* cytocompatibility of CS and GS extracts free and encapsulated in mesoporous silica matrix modified with zinc oxide, Zn-MCM-41, and magnesium oxide, Mg-MCM-41 was tested on human normal keratynocytes, HaCaT cells, using MTT assay to assess the metabolic activity of cells after 24 h. MTT results showed high cytocompatibility, similar (GS) or higher (CS) when compared to control, in correlation with the antioxidant capacity. The cell viability was dependent on type of functionalization of the MCM-41 carrier. While ZnO modification of MCM-41 support slightly reduced the cells viability (94 ± 3%) when compared to control, MgO functionalization of silica significantly lowered the percentage of viable cells (69 ± 6%).

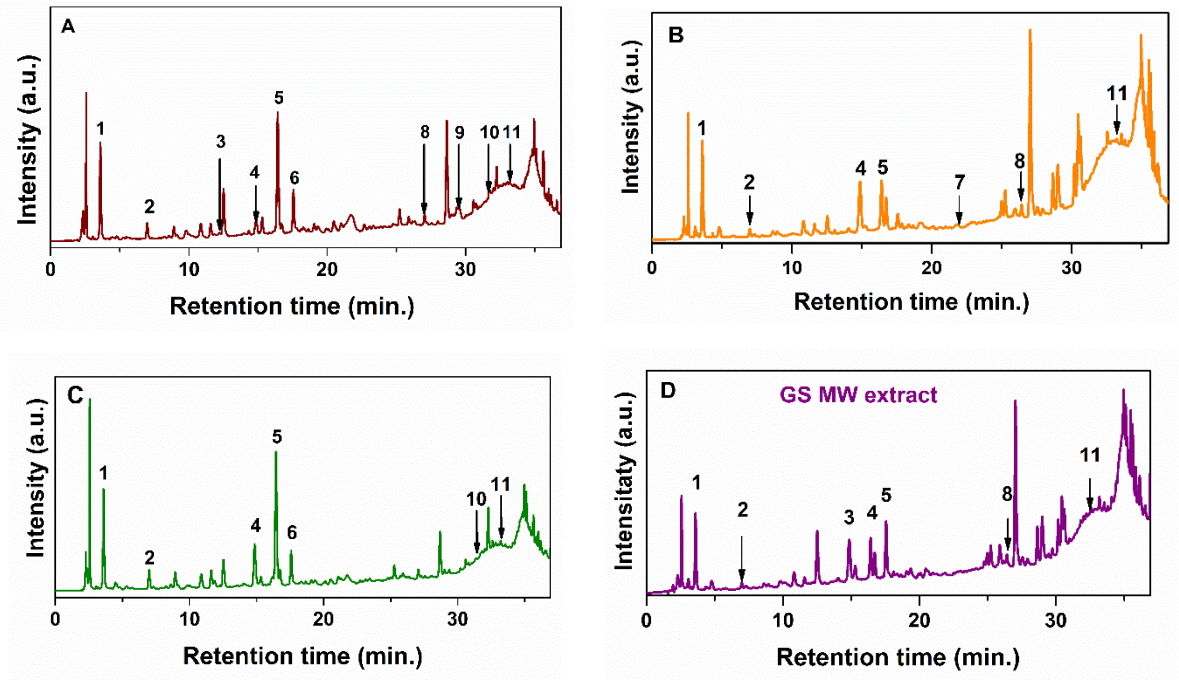



**Figure 1.** HPLC chromatogram at 279 nm for extracts from **A**- Cabernet Sauvignon (CS), **B**-grape skins (GS, Conv), **C**- Feteasca Neagra (FN) and **D**- grape skins (GS, MW) (1- gallic acid; 2-protocatechuic acid; 3-catechin hydrate; 4-vanillic acid; 5- syringic acid; 6- (-) epicatechin; 7-*p*-coumaric acid; 8-rutin hydrate; 9-pelargonidin chloride; 10-myricetin; 11- *trans*-resveratrol).

The most promising samples, CS free and encapsulated into Zn-MCM-41 matrix were also evaluated with regards to the Giemsa-stained cell morphology. Normal cuboidal morphological phenotype was maintained for the cell monolayer treated with free or encapsulated extract, similar to the control, untreated keratinocytes (Fig. 2).

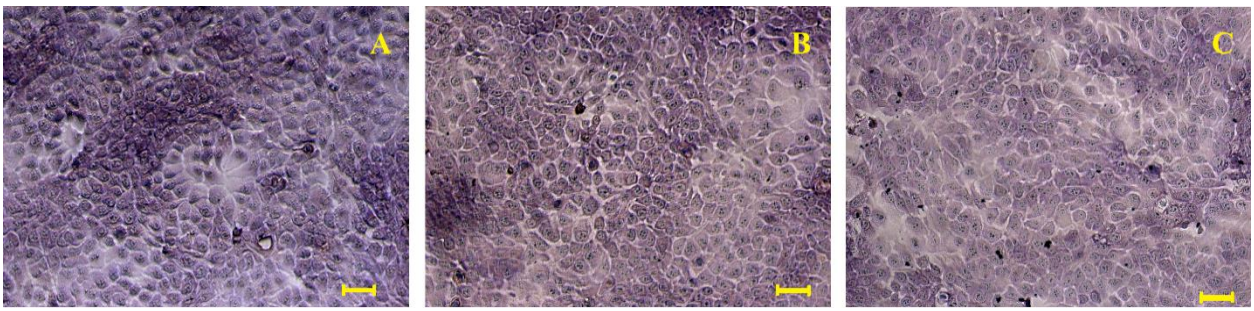



**Figure 2.** Giemsa staining of the human keratinocytes: **A** – Control, **B** – Cabernet Sauvignon extract, **C** – encapsulated extract (CS@Zn-MCM-41 sample). Scale bar: 50 µm.

## 2.2. Enhanced stability of polyphenolic extracts from grape pomace achieved by embedding into mesoporous silica-type matrices

The differential scanning calorimetry (DSC, Mettler Toledo DSC 3+) (Fig. 3) recorded on vacuum dried extracts showed stability up to 150°C. The residual solvent evaporation can be noticed during the first heating cycle for both extracts between 25 – 100 ºC. This endothermic

effect is greatly reduced during the 2nd heating cycle, and it is absent during the 3rd cycle, indicating a complete solvent removal. Two types of reversible transformations can also be noticed at -7 ºC and in the temperature range of 15 – 50 ºC, which did not affect the extracts stability.

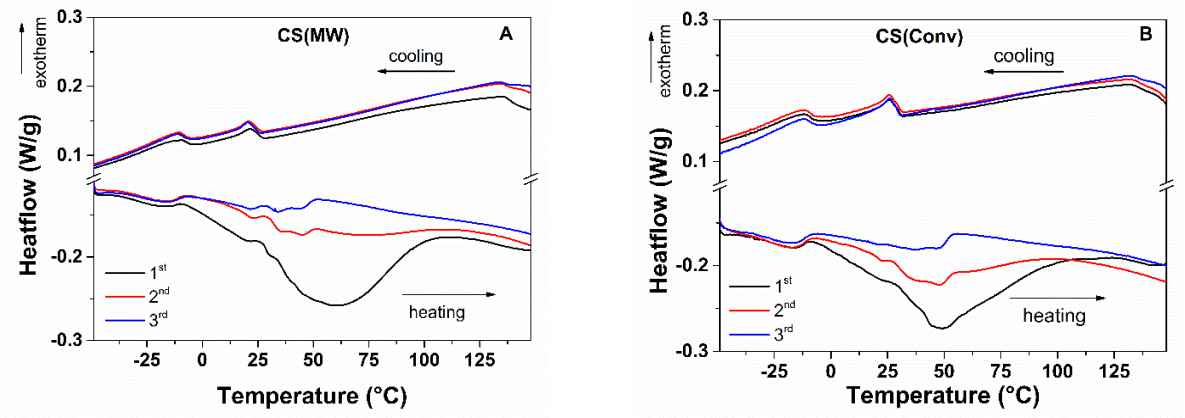



**Figure 3.** DSC analysis for CS(MW) (**A**) and CS(Conv) (**B**) free extracts dried in vacuum, showing the 1st, 2nd and 3rd heating – cooling cycle

## 3. Effect of nanoconfinement of polyphenolic extract from grape pomace into functionalized mesoporous silica on its biocompatibility and radical scavenging activity

In this chapter are reported the results related to the loading of a hydroalcoholic polyphenolic extract from grape pomace into mesopores of MCM-41-type silica functionalized with organic groups and how the acidic silica surface nature influences the release of polyphenolic compounds from carrier in phosphate buffer solution pH 5.7. It was observed a good cytocompatibility (MTS assay, where MTS is an abbreviation for 3-(4,5–dimethylthiazol–2-yl)–5-(3-carboxymethoxyphenyl)–2-(4-sulfophenyl)-2H-tetrazolium) and antioxidant activity on NIH3T3 cells line of materials containing polyphenolic extract that could be further used for the development of nutraceutical and cosmetic formulations [22].

The supports for loading of polyphenolic extracts were obtained though post-synthesis functionalization using cyanoethyl triethoxisilane and 3-mercaptopropyl triethoxysilane organosilanes (MCM-CN and MCM-SH), while MCM-$SO_3H$ and MCM-COOH were obtained through an oxidation or hydrolysis reaction accordingly to the scheme from figure 4.

The morphology of MCM-41-type silica supports was evaluated by scanning electron microscopy (SEM, Tescan Vega 3 LMH). The synthesized MCM-41E support consists of ellipsoid-shaped particles with a diameter in the range of 170–310 nm and dimensions ratio of about 2 (Fig. 5 A). All functionalized MCM-41 materials were obtained from commercial MCM-41; thus, they have similar morphology presenting particles that form agglomerates with irregular shape and dimensions between 0.5–1.5 µm (Fig. 5B–E). Because no significant differences in the FTIR spectra (Bruker Tensor 27) of MCM-SH and MCM-$SO_3H$ were observed, both functionalized supports were investigated by EDX analysis, which evidenced a slight difference of the sulphur content (Si/S = 30 for MCM-SH material and Si/S = 37 for MCM-SO3H sample) indicating that during the oxidation reaction some of mercaptopropyl moieties broke down.

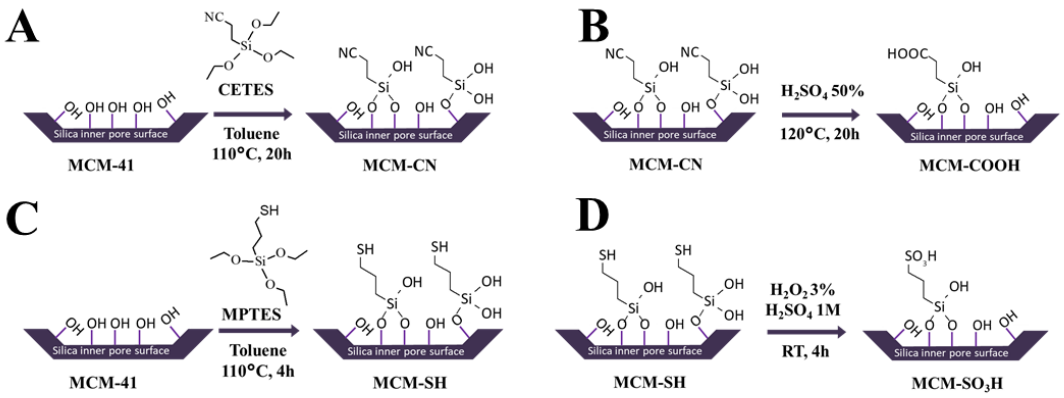


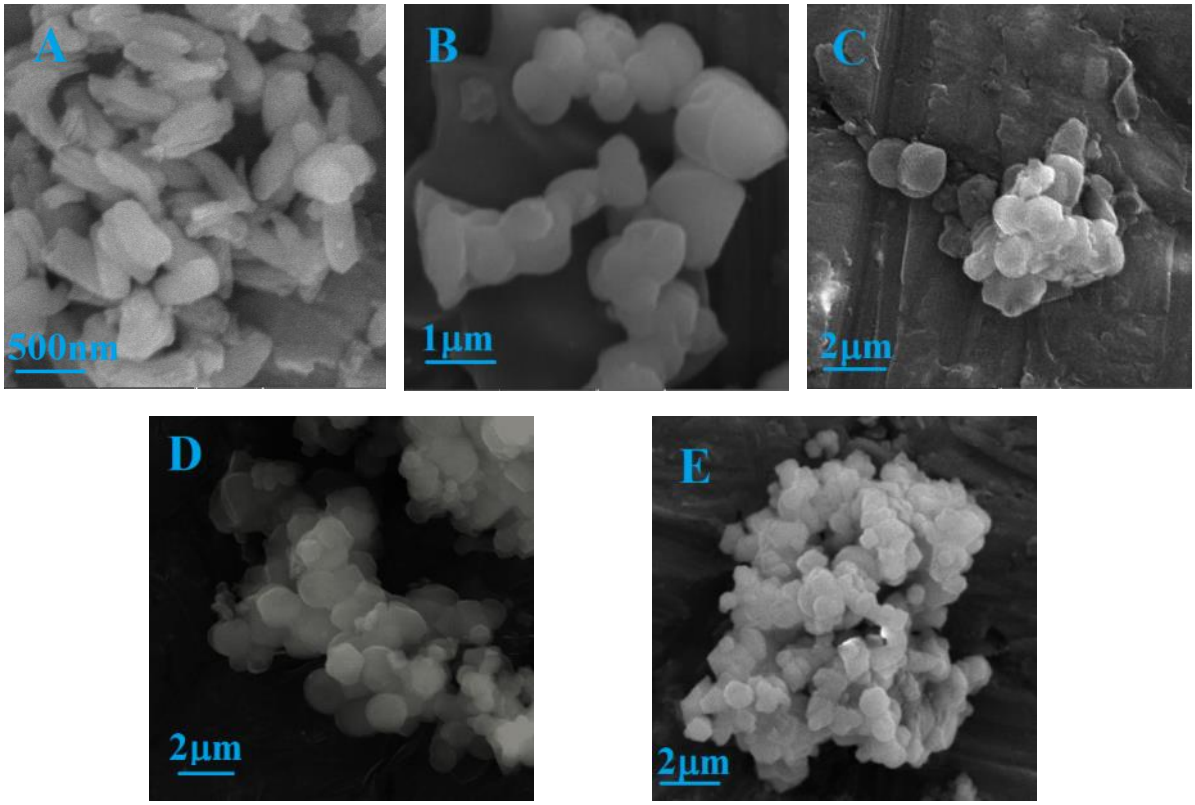

**Figure 4.** Synthesis of mesoporous silica type supports modified with organic groups for **A**-MCM-CN, **B**-MCM-COOH, **C**-MCM-SH and **D**-MCM-$SO_3H$, respectively.

The textural parameters of supports determined from $N_2$ adsorption-desorption isotherms are listed in table 1. After functionalization, the average pore size decreased compared to that of the commercial MCM-41 used for obtaining of functionalized mesoporous silica materials, more visible in the case of MCM-CN and MCM-COOH samples that have a higher amount of functional groups linked to the silica surface (between 7.5–8%wt) than MCM-SH or MCM-$SO_3H$. The functionalized materials have lower specific surface area (585–845 $m^2/g$) and total pore volume (0.74–0.43 $cm^3/g$) than pristine silica (976 $m^2/g$ and 0.85 $cm^3/g$, respectively).

The RSA values determined by ABTS assay are consistent with that determined through DPPH method, higher values were observed for MM(conv) extract embedded in all mesoporous supports after up to 9 months of storage in dark conditions, at 4 °C (Fig. 6 C,D).

The RSA values of embedded extracts obtained by using ABTS assay were higher when compared with that of the DPPH method because of different solvent, *p*H and nature of free radicals, which influence the scavenging capacity of polyphenolic compounds. However, the RSA values higher for ABTS method than for DPPH assay obtained for extract-loaded materials are consistent with the behaviour of the free MM(Conv) extract. The extract loaded in all MCM-41-type supports preserved significant better the radical scavenger capacity, being almost constant after several months of storage in dark conditions, at 4 °C than the free extract, whose antioxidant activity decreased over time (Fig. 6). These results demonstrate an improved stability of extract when confined in silica mesopores, considering that the supports did not exhibit an important effect on both DPPH• and ABTS•+ scavenging capacity. The mesoporous supports exhibited very low RSA values (higher up to 2.5% and 0.63% for DPPH• and ABTS•+ controls, respectively, except MCM 41E). The effect of MCM-41E support on radicals scavenging capacity (7.6% higher than DPPH control) can be explained by its higher adsorption capacity of organic species due to larger pore volume than that of functionalized silica carriers. Unlike the other materials containing embedded extract, a lower RSA was observed for the MM@MCM-CN sample than that for the free extract after two months of storage (Fig. 6A), probably because of the strong base nature of CN groups associated with most likely strong acid–base interactions between phenolic acids and cyanoethyl groups grafted on MCM-CN support. However, even though this support was less efficient in terms of interactions with phytocompounds, after six months of storage, the DPPH• scavenging capacity of MM@MCM-CN sample remained almost constant in comparison with that of the free extract, whose antioxidant activity decreased significantly. Also,

the ABTS•+ scavenging capacity of MM@MCM-CN sample after nine months of storage was higher that of the free extract.




**Figură 5.** Scanning electron micrographs (SEM) for MCM-41E (**A**), MCM-CN (**B**), MCM-COOH (**C**), MCM-SH (**D**) and MCM-$SO_3H$ (**E**) , respectively.

In order to evaluate the correlation between polyphenolic compounds delivery process and the nature of functional organic groups linked on silica-type support, the experimental data were fitted using a three-parameter model (Fig. 7) [64], which considers an equilibrium between adsorbed and desorbed organic molecules and the diffusion process, all having a first order kinetics that facilitate the transport of phytochemicals inside the support meso-channels and then into the release medium (eq.1). The free molar enthalpy, $\Delta G$, (eq.2) is a parameter that characterized the adsorption ($k_{on}$)/desorption ($k_{off}$) equilibrium, being proportional to the amount of phytochemicals released in the first stage of the release process (burst effect), when the molecules that interact weakly with the silica surface are able to diffuse rapidly into the release medium, and the rate constant for diffusion, $k_d$, is proportional to the polyphenols delivery rate [24].

**Table 1.** Textural parameters of supports and their corresponding extract-loaded materials.

| Support type | Support | | | | MM@support | | | |
|---|---|---|---|---|---|---|---|---|
| | $n_{SiO2}$/ $n_{GF}$ | $d_{DFT}$ (nm) | $S_{BET}$ ($m^2$/g) | $V_p$ ($cm^3$/g) | Extract (%wt) | $d_{DFT}$ (nm) | $S_{BET}$ ($m^2$/g) | $V_p$ ($cm^3$/g) |
| MCM-41E | - | 3.93 | 781 | 0.69 | 42.0 | 3.66 | 97 | 0.11 |
| MCM-SH | 25 | 3.54 | 843 | 0.74 | 36.0 | 3.42 | 231 | 0.16 |
| MCM-$SO_3H$ | 50 | 3.66 | 798 | 0.59 | 38.5 | 3.54 | 141 | 0.10 |
| MCM-CN | 11 | 3.18 | 845 | 0.55 | 39.0 | - | 75 | 0.06 |
| MCM-COOH | 14 | 3.18 | 585 | 0.43 | 39.0 | - | 10 | 0.04 |

FG-functional groups; $d_{DFT}$-average pore size values computed from non-local density functional theory (NLDFT) method, $S_{BET}$- specific surface area determined by the Brunauer Emmett Teller (BET) method, $V_p$-total pore volume, measured at $p/p_0 = 0.99$. Commercial MCM-41 ($d_{DFT}$ = 3.66 nm; $S_{BET}$ = 976 $m^2$/g; $V_p$ = 0.85 $cm^3$/g).

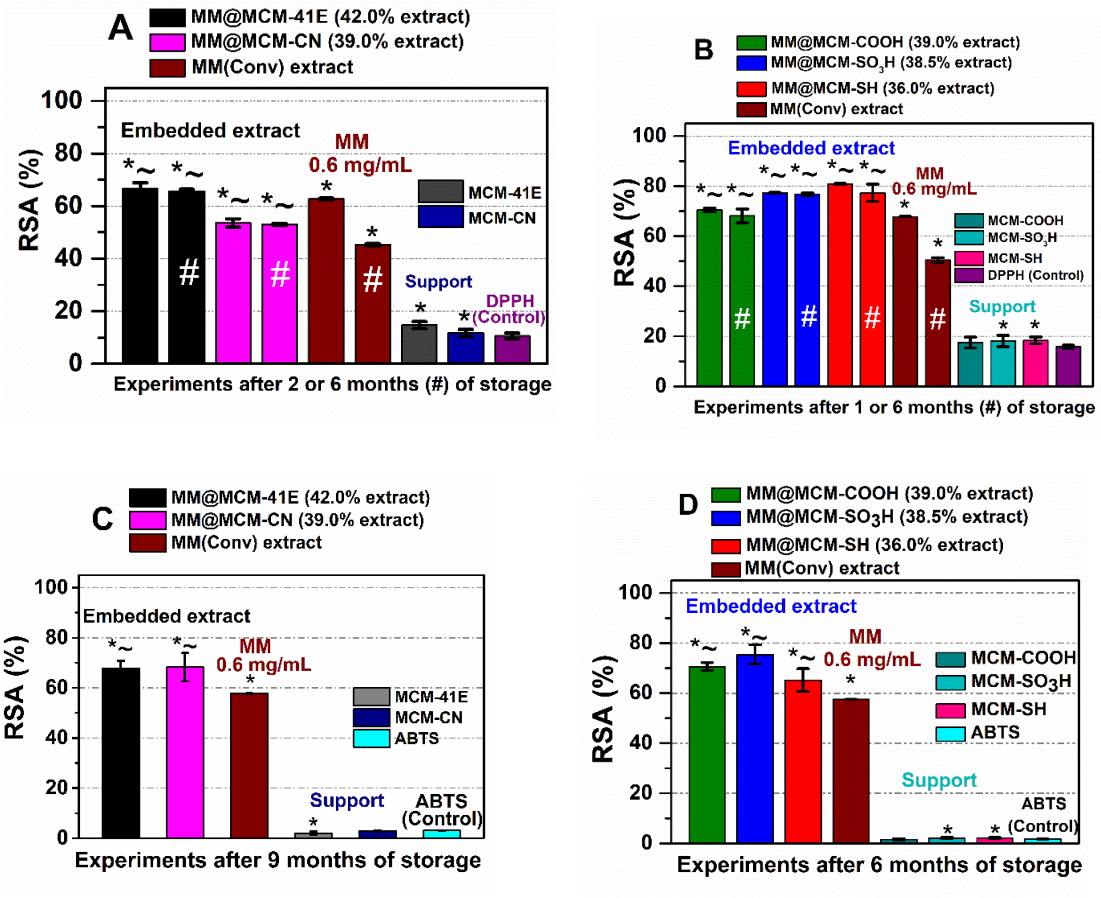



**Figure 7.** Radical scavenger activity on solid samples for embedded extracts in comparison with the corresponding support and free extract after 1–2 months or 6 months of storage (#), determined by DPPH assay (**A** and **B**) and after 6–9 months of storage using ABTS procedure (**C** and **D**). *$p$ < 0.05 compared to control; ~$p$ < 0.05 compared to extract (Student $t$-test).

$$\frac{m(t)}{m(0)} = \frac{\lambda_2 \cdot (k_d - \lambda_2)}{(k_{on} + k_{off}) \cdot (\lambda_1 - \lambda_2)} \cdot \left(1 - e^{-\lambda_1 \cdot t}\right) + \frac{\lambda_1 \cdot (\lambda_1 - k_d)}{(k_{on} + k_{off}) \cdot (\lambda_1 - \lambda_2)} \cdot \left(1 - e^{-\lambda_2 \cdot t}\right) \quad (1)$$

$$\Delta G = -k_B \cdot T \cdot \ln\left(\frac{k_{on}}{k_{off}}\right) \quad (2)$$

where: $m(t)$ and $m(0)$- amount of phytocompounds released at time t and initially, respectively. $\lambda_{1,2} = \frac{(k_d + k_{on} + k_{off}) \pm \sqrt{(k_d + k_{on} + k_{off})^2 - 4 \cdot k_d \cdot k_{off}}}{2}$ are negative values of the linear systems used to obtain eq. 1, $k_B$ - Boltzman constant and $T$ – absolute temperature.

Regarding ΔG values (Table 2), the lowest polyphenols amount delivered in the first stage of the release process is observed from pristine silica, followed by supports functionalized with organic groups with base nature (MM@MCM-CN and MM@MCM-SH). The highest burst effect was noticed for carriers with acidic groups, especially for the MM@MCM COOH sample for which the highest phenolic compounds recovery yield was reached (Fig. 7A).

The increase of acidity of organic groups linked on silica pore walls surface favoured the polyphenols delivery due to weaker acid–base interactions between support and phenolic acids that are in a high amount in MM(Conv) extract, the *p*Ka value of organic groups being inversely proportional with the phytochemicals amount delivered in PBS pH 5.7 (Fig. 7B).

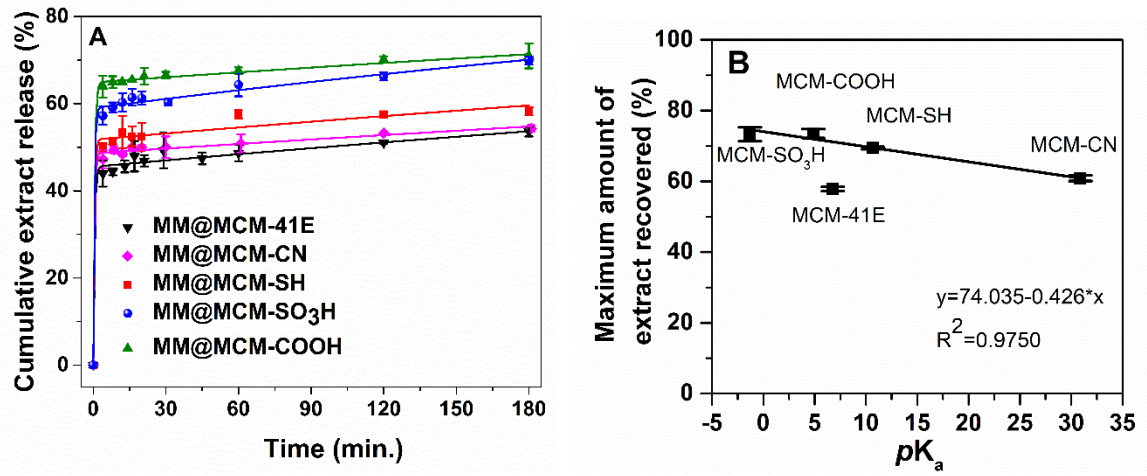



**Figure 6. A** - Polyphenols release profiles from MCM-41 silica-type supports fitted with three-parameters model. **B** - Dependence of maximum amount of phenolic compounds recovered on *p*Ka values of organic groups grafted on silica support.

The cellular viability experiments on NIH3T3 fibroblast cells proved no significant toxicity for polyphenols-loaded materials. The intracellular reactive oxygen species assay correlated well with the release in PBS of the polyphenolic compounds: the lowest cytosolic ROS production was observed in the case of MM@MCM-COOH material, which showed the highest amount of phytochemicals released in PBS pH 5.7. Moreover, the polyphenols-loaded MCM-41-type silica, especially MM@MCM-COOH sample, showed an improved stability over the time and a better in vitro antioxidant effect than the polyphenolic extract alone.

**Table 2.** Kinetic parameters for polyphenols release from MCM-41-type supports.

| Materials containing embedded extract | Three-parameter fitting equation | | | | | Maximum amount of extract recovered (%) |
|---|---|---|---|---|---|---|
| | $\Delta G$ ($10^{21}$ J) | $k_d$ ($min^{-1}$) | $\Delta G$ ($10^{21}$ J) | $k_d$ ($min^{-1}$) | $\Delta G$ ($10^{21}$ J) | |
| MM@MCM-41E | -0.76 | 1.940 | 0.899 | 1.073 | 0.9936 | 57.8 ± 0.6 |
| MM@MCM-SH | 0.31 | 2.256 | 0.985 | 0.917 | 0.9934 | 69.4 ± 0.4 |
| MM@MCM-$SO_3H$ | 1.57 | 2.256 | 1.756 | 1.218 | 0.9975 | 73.3 ± 1.9 |
| MM@MCM-CN | -0.07 | 2.256 | 0.721 | 0.732 | 0.9981 | 70.0 ± 1.4 |
| MM@MCM-COOH | 2.63 | 1.938 | 1.129 | 0.610 | 0.9993 | 73.5 ± 1.3 |

## 4. Biological evaluation of aronia extract free and embedded in mesoporous silica-type matrices

This part of PhD thesis presents the encapsulation of black chokeberry extract into MCM-41- type mesoporous silica (pristine and modified with zinc oxide nanoparticles). The aim of this study was to evaluate the activity of two different formulations containing encapsulated *A. melanocarpa* polyphenolic extract in comparison to that of the free extract based on their preliminary biological tests, such as their antiradical and antioxidant capacity, antimicrobial potential against selected strains, and in vitro effects on cancer cell viability (using A375 human melanoma cells) and normal skin cells (using HaCaT human keratinocytes).

This study [25] was performed in collaboration with a research group from University of Medicine and Pharmacy "Victor Babes", which evaluates the biological activity of ethanolic extract free and incorporated in mesoporous silica matrices.

The effects of *A. melanocarpa (Michx.) Elliott* extract, as well as the extract incorporated into the mesoporous silica-type matrices (i.e., Ar@MCM-41E and Ar@ZnMCM-41) and the MCM-41E and ZnMCM-41 matrices, were assessed on a human melanoma cell line (A375) and on a non-tumour cell line (HaCaT human keratinocytes). Concentrations in the range of 10-250 µg/mL were tested and in figure 8 are presented comparatively the cytotoxic effects for the highest dose tested on both cellular lines. After the 24 h treatment (Fig. 8A) one can observed that on healthy keratinocytes cell line both free and encapsulated extract or corresponding supports did not exhibit a significant cytotoxicity having cell viabilities above 80%, except MCMC-41E. The effect of the free or embedded extract on tumoral cell line (A375) is quite similar, for which an important cytotoxicity was noticed with values of cellular viability in the range of 39.5 – 43.8% compared to Control, a slightly higher being obtained for Ar@Zn-MCM-41 (39.5 ± 5.5% compared to Control); the carrier was responsible for only a slight reduction of cell viability (87.8 – 88.0% compared to Control). At a higher incubation time (48 h) one can observe a slightly enhanced selectivity of embedded extracts that are more cytotoxic on tumoral cells and significant less toxic on HaCaT cell line. This effect could be corelated with a better antioxidant activity in time for the encapsulated extracts compared to the free one whose radical scavenging activity diminished easily in time.

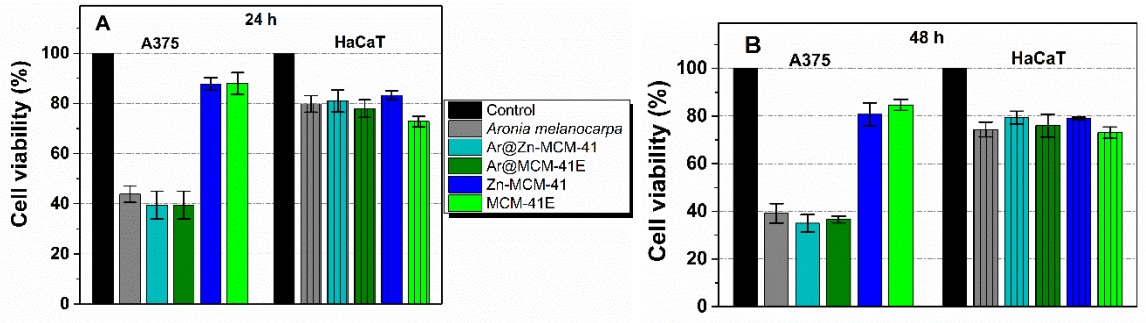



**Figure 7.** Cell viabilities for human melanoma cell line (A375) and on healthy keratinocytes (HaCaT) for a concentration of 250 μg/mL for different incubation time: 24 h (**A**), 48 h (**B**) for *Aronia* extract free and embedded in mesoporous matrices and corresponding supports.

The loading of the black chokeberry extract into mesoporous silica-type supports determined the preservation of its antioxidant capacity and antimicrobial potential on the tested Gram-positive bacteria. The encapsulation of the *A. melanocarpa* extract into the ZnMCM-41 support led to a slightly better antimicrobial capacity than the free extract due to ZnO synergistic effect.

## 5. Mesoporous silica carriers for irinotecan targeting tumoral tissue

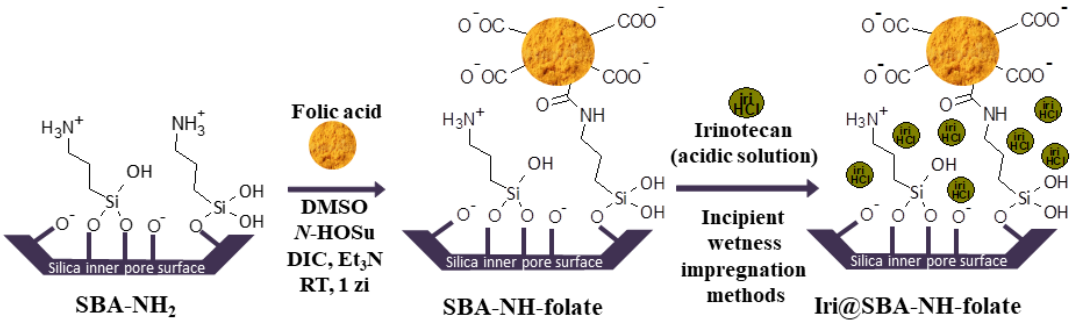
This chapter of the PhD thesis deals with the influence of vehicle used for irinotecan on its release kinetics, the study on how carriers' modification can target tumoral tissue, but also a modulation of irinotecan release kinetics from proposed supports in correlation with their biological activity.

### 5.1. Targeted action through folate moieties linked on silica surface

This study was performed in collaboration with a research group from University of Medicine and Pharmacy "Carol Davila" who studied the cytotoxic effect of irinotecan incorporated in pristine SBA-15 type mesoporous silica or modified with folate moieties (SBA-NH-folate), but also an investigation of drug delivery systems distribution at cellular level using Cytoviva hyperspectral microscope and cell image reconstruction using Matlab suite.

SBA-NH-folate support was synthesized through a two-stage process (Fig. 9). Firstly, the synthesis of SBA-$NH_2$ carrier using calcined SBA-15 silica and 3-aminopropyltriethoxysilane (APTES), in toluene (molar ratio of APTES/SBA-15/toluene=1/5/235), in inert and dry conditions for 15 h at 110°C was performed. The resulted solid was isolated through centrifugation and further washed with toluene to remove the unreacted organosilane, acetone, ethanol and then with HCl 1M aqueous solution to break hydrogen bonds between amine and silanol groups and then the sample was dried in air, at room temperature. The resulted material was used for obtaining of SBA-NH-folate. Firstly, folic acid was dissolved in anhydrous dimethylsulphoxide at approximatively 60 °C. Subsequently, *N*-hydroxysuccinimide, *N*,*N*-diisopropylcarbodiimide and triethylamine were added to the folic acid solution at room temperature, under inert atmosphere and the resulted mixture was homogenized for 3 minutes. Afterwards the corresponding amount of SBA-15-$NH_2$, previously outgassed at 60 °C (1°C/min, 12h) was added (molar ratio of folic acid / HOSu / DIC / $(C_2H_5)_3N$ / DMSO /SBA-15-$NH_2$ of 1/1.1/1.1/1.4/1400/1.66). The reaction mixture was kept at room temperature, under constant magnetic stirring for 24 h and then the solid was

separated by centrifugation and washed with warm DMSO to ensure that unlinked folic acid was removed and ethanol for several times. The resulted yellow powder (denoted SBA-NH-folate) was dried at room temperature. The obtaining of SBA-NH-folate is depicted in figure 9.




*DMSO – dimethylsulfoxide; N-HOSu – N-hydroxysuccinimide; DIC –carbodiimide; $Et_3N$ – triethylamine;*

**Figure 8**. Synthesis route for SBA-NH-folate and the loading of irinotecan onto SBA-NH-folate carrier.

The organic groups content, as well as the drug amount of mesoporous SBA-15-type carriers were determined using thermogravimetric analysis (Fig. 10) by considering the weight loss up to 800 °C after deducting the influence of physically adsorbed water, noticeable as the first endothermic event. Based on thermogravimetric analysis of drug-loaded samples, the irinotecan amount was determined after deducting the adsorbed water and the weight loss due to functional groups attached on silica surface. The content of irinotecan was in the range of 11.2-11.6%wt. One can observe that irinotecan was not completely released from supports mesopores based on the TG analysis performed on the isolated solid recovered after in vitro drug release experiments (Fig. 11 A-c and Fig. 11 B-b).

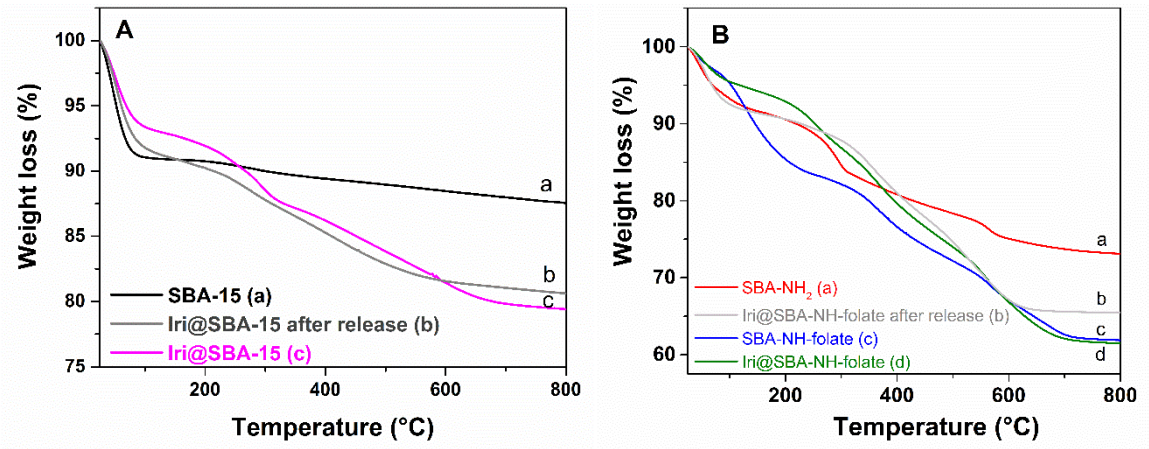



**Figure 9.** Thermogravimetric analysis of SBA-15 and Iri@SBA-15 samples (A) and for SBA-NH-folate and Iri@SBA-NH-folate (B).

In both cases, the amount of released irinotecan was low up to 40.5 ± 0.3%(wt) after 54 h, slightly higher in the case of SBA-NH-folate carrier (Fig. 11 A). However, for irinotecan release from SBA-15 material in PBS pH 7.6, one can observe that the same amount of drug was delivered in a shorter period of time, 32 h. The drug release kinetics from SBA-15-type carriers was slow, and the values of ΔG parameter emphasised a reduced burst effect, thus, a low amount of drug was released in the first stage of the release process. One can observe that the zero-order kinetics describes better the irinotecan release from SBA-15 experiment data ($R^2$=0.9994) and highlighted that a slow drug delivery was obtained considering that the $K_0$ value determined from the model is very low (Fig. 11B).

Poor internalization of SBA-15 particles and Iri@SBA-15 samples was observed in contrast to SBA-NH-folate support, the functional groups facilitating the penetration of particles into both cytoplasm and nucleus, which explained the low toxicity of the support on tumoral cells and high cytotoxicity in the case of irinotecan-loaded SBA-NH-folate.

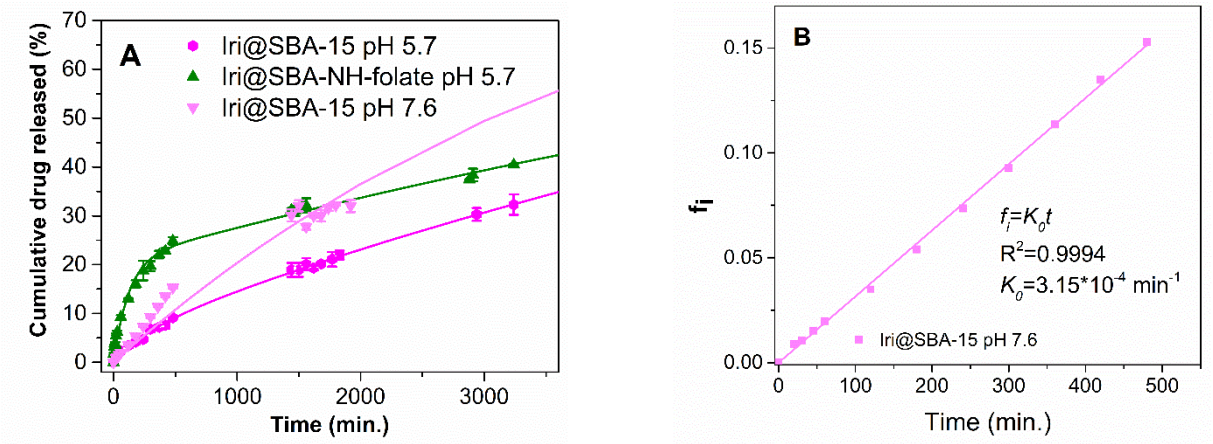



**Figure 11. A**-*In vitro* irinotecan release profile from drug-loaded materials in PBS (with straight line is represented the three-parameter fitting eq.), **B**-zero-order kinetics for irinotecan release from Iri@SBA-15 in phosphate buffer pH 7.6 (with straight line is depicted zero-order fitting eq.)

### 5.2. Targeted action through ulvan modification of functionalized silica carriers

To obtain an extract rich in polysaccharides, the crushed plant material of *Ulva lactuca* was pre-treated in acetone to remove chlorophyll and then in methanol to remove polyphenolic compounds and the rest of the chlorophyll. After each extraction stage (30 minutes), the algae were separated by filtration, followed by centrifugation.

The plant residue was treated with 50 mL of ultrapure water at reflux for 3h / 650 rpm for recovery of ulvan, and the scheme for isolating the ulvan from green algae is shown in figure 12.

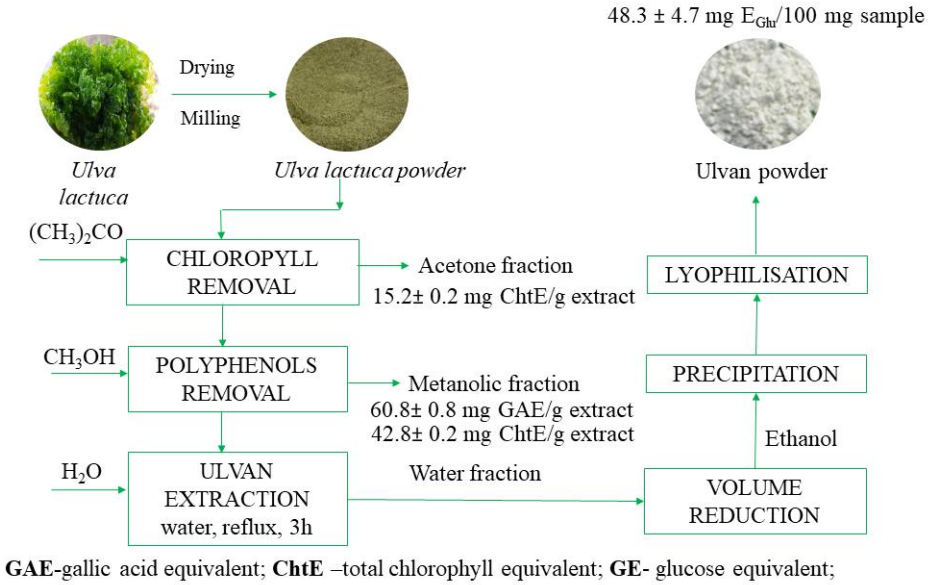



**Figure 10.** Ulvan extraction from green algae, *Ulva lactuca*.

One can observe that irinotecan from both silica-ulvan-type carriers is completely released in 8h (Fig. 13). However, a slower release kinetics ($\Delta G < 0$) is obtained for the ulvan@SBA-$NH_2$ support, most likely due to a higher polymer content on silica surface (6%). However, the diffusion rate value ($k_d$) is higher in the case of ulvan@SBA-$NH_2$ carrier due to larger pore size values than for ulvan@MCMB-$NH_2$ support with ball-like structure Also, in the case of Iri@Ulvan@SBA-$NH_2$ sample, a higher tendency of drug re-adsorption into the silica pores ($k_{on}$>$k_{off}$) was observed.

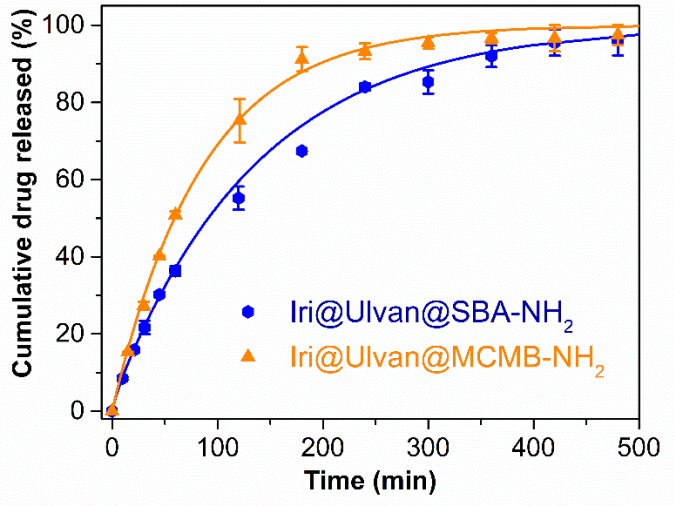



**Figure 11.** *In vitro* release profile for irinotecan from silica-ulvan matrices (with straight line is represented the three-parameter fitting eq.).

**Table 3.** Kinetic parameters for irinotecan release from silica-ulvan matrices.

| Encapsulated drug materials | Three-parameter fitting equation | | | | | Total cumulative drug released (%) |
|---|---|---|---|---|---|---|
| | $\Delta G$ ($10^{21}$J) | $k_d$ ($min^{-1}$) | $k_{off}$ ($min^{-1}$) | $k_{on}$ ($min^{-1}$) | $R^2$ | |
| Iri@Ulvan@SBA-$NH_2$ | -3.36 | 0.031 | 0.015 | 0.033 | 0.9978 | 96.1 ± 3.9 |
| Iri@Ulvan@MCMB-$NH_2$ | 1.70 | 0.020 | 1.483 | 0.996 | 0.9975 | 97.4 ± 2.6 |

### 5.3. Targeted action through magnetic guidance

The support (FeMCMB) exhibited an ordered hexagonal pore array proved by the small-angle XRD analysis (Fig. 14 - inset), being identified the three Bragg-reflections specific to the MCM-41 mesoporous silica-type material. The wide-angle XRD pattern evidenced the irinotecan encapsulation in support mesopores in amorphous state, not being identified diffraction peaks associated with cytostatic agent crystalline phase, the only crystalline phase being ferric oxide, the nanoparticles which decorate the silica surface (Fig. 14).

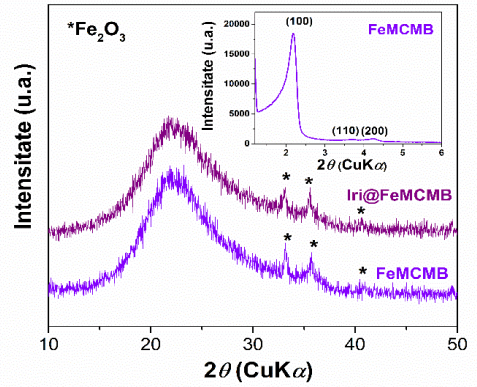



**Figure 12.** Wide-angle XRD patterns for support and irinotecan-loaded material and Inset- small-angle XRD for FeMCMB.

The kinetic parameters of irinotecan release from FeMCMB determined using the three-parameter model (Table 4) showed a slow process ($\Delta G < 0$), the slowest of all proposed systems. The diffusion rate constant is three orders of magnitude lower than the adsorption / desorption ones, which showed that the diffusion process is negligible compared to the adsorption / desorption ones. Also, the adsorption process is favoured, kon>koff, which can be correlated with an extremely small amount of drug released (9.5%).

To better describe the experimental data, zero-order kinetics was also used for fitting the experimental data, which showed an extremely slow release, with an apparent rate constant for the dissolution process $k_0$ of $5,047 \cdot 10^{-5}$ $min^{-1}$, lower than that for the release of irinotecan from the support SBA-15 at the same *p*H value of the release medium ($3,150 \cdot 10^{-4}$ $min^{-1}$).

The release of irinotecan from the FeMCMB matrix was the slowest of all the systems analysed, with a total cumulative drug delivery extremely small (9.5% in 32 h).

**Table 3.** Kinetic parameters of the irinotecan release from FeMCMB using the three-parameter model and the total cumulative drug release.

| Embedded drug materials | Three-parameter model | | | | | Total cumulative drug release (%) |
|---|---|---|---|---|---|---|
| | $\Delta G$ ($10^{21}$J) | $10^3 k_s$ ($min^{-1}$) | $k_{off}$ ($min^{-1}$) | $k_{on}$ ($min^{-1}$) | $R^2$ | |
| Iri@FeMCMB | -5.42 | 0.240 | 1.414 | 5.022 | 0.9906 | 9.5 ± 0.2 |

## Conclusions and perspectives

During doctoral studies were synthesized pristine silica and functionalized with heteroatoms or organic groups that were used either for the incorporation of polyphenolic extracts to achieve an increased stability thus, to maintain beneficial properties of phytocompounds or irinotecan delivery systems, in which case three strategies have been used to facilitate the targeting of tumoral tissue.

Polyphenolic extracts were prepared by conventional method or microwave-assisted technique. Through conventional method, ethanolic extracts were obtained from the Cabernet Sauvignon and Feteasca Neagra (red wine cultivars) and Mamaia (rosé wine cultivar) grape pomace, hydro-alcoholic ($EtOH/H_2O$ = 4/1, v/v) from the Mamaia grape marc and by microwave extraction from Cabernet Sauvignon and Mamaia grape marc from 2017 (Black Sea-Murfatlar area). To make a comparison, ethanolic grape powder extracts were also prepared by conventional and microwave-assisted extraction. The amount of extract depended on grape pomace cultivar being in the range of 9.2-19.3% for ethanolic extracts (the highest amount being obtained for the microwave extract from Cabernet Sauvignon rich in stems and seeds) and 14.4-15.9% for hydro-alcoholic extracts of the Mamaia variety (the higher value being obtained for the conventional extract).

For ethanolic polyphenolic black chokeberry extract prepared by the conventional method, the obtained amount of extract was much higher (53.8%) than in the case of extracts from grape marc, which can be explained through the use of dried aronia fruits and not a by-product.

The spectrophotometric methods allowed the determination of total content of polyphenols, flavonoids and anthocyanin pigments, as well as the antioxidant activity by ABTS and DPPH assays. The total polyphenol content of the extracts was high, the highest value being obtained for Feteasca Neagra ethanolic extract (279.64 ± 4.52 mg GAE / g extract), the highest flavonoid amount being observed in the case of ethanolic extract from grape skins (18.96 ± 0.02 mg QE / g extract), and the highest anthocyanin content was obtained in the case of ethanolic and hydro-ethanolic extract of Mamaia variety (13.27 ± 0.47 mg ECG/g extract). The antioxidant activity was influenced by vegetal material, pomace cultivar, solvent, as well as the extraction method. Therefore, it was noticed that the obtained values by both methods are consistent keeping the same order and highlighting the preparation of extracts with high antioxidant potential. Among the ethanolic extracts, the highest antioxidant effect was observed for CS (Conv) extract with an antioxidant activity of 344 ± 4 mg TE / g (DPPH assay) and 230 ± 7 mg TE/ g (ABTS method), respectively. Mamaia hydro-ethanolic extract also showed a high antioxidant activity (289.96-313.35 mg TE/ g), determined by both methods mentioned above, especially for an extract prepared from a rosé grape pomace.

The ethanolic extract of aronia showed a total content of polyphenols and flavonoids in accordance with the literature data and a lower anthocyanin content probably because it was prepared at high temperature (at reflux) that hindered the extraction of anthocyanins.

As respects the chemical profile of polyphenolic extracts, it largely depends on the pomace variety and its composition. However, a maximum of thirteen polyphenols (from the twenty-three available standards) were identified in all the extracts analysed, from which gallic, protocatechuic, vanillic and syringic acid were quantified in the ethanolic extracts, and myricetin was identified only in grape pomace extracts. For Cabernet Sauvignon grape pomace rich in stems and seeds, (-) epicatechin and pelargonidin chloride were additionally identified in all extracts. In the case of Mamaia hydro-ethanolic extracts, in addition to the compounds identified in the ethanolic extracts, several flavonoids were also identified, such as catechin hydrate, (-) epicatechin, rutin hydrate and quercetin, while myricetin was no longer identified.

For the incorporation of polyphenolic extracts, pure silica supports modified with heteroatoms or organic functional groups were chosen. These supports were chosen due to their high porosity and the possibility to interact with polyphenolic compounds. A higher stability of phytocompounds through embedding into mesoporous matrices was ensured and thus, a preservation of their beneficial properties over time.

The pristine and heteroatoms functionalized silica supports exhibited a large total pore volume (0.47-0.88 $cm^3$/ g), high specific surface area (510-796 $m^2$ / g), and a similar average pore diameter of about 2.66 nm (determined by the BJH method), which allowed the incorporation of high amounts of polyphenolic extract prepared from either grape pomace or black chokeberry fruits (30.5-49.7% extract in the resulting composites), depending on the total pore volume of the support.

For the hydro-alcoholic extracts prepared from Mamaia grape pomace, were employed supports functionalized with organic groups prepared by post-synthesis approach using commercial MCM-41 silica and organosilanes, resulting materials with a total pore volume of 0.43-0.74 $cm^3$/g and pore size ranging between 3.18 and 3.66 nm (determined by NLDFT), which allowed the incorporation of high amounts of phytocompounds (36-42% extract in the composite materials).

The antioxidant activity of embedded extracts was determined by two developed methods for solid samples, evaluated against the same concentration of free extract and support as in the material containing extract having as control the degradation of the DPPH free radical solution. DPPH assay is easier and more reproducible than ABTS method because the embedded extracts and supports are more stable in ethanolic solution but requires a longer analysis time (24 h). The ABTS method, which is preferable for antioxidants that are not soluble in ethanol, a shorter reaction time has been proposed to avoid hydrolysis of the silica matrix. Moreover, the better reproducibility of the DPPH method is also due to the nature of the reagent (DPPH •), which is commercially available as a free radical, unlike the radical carbocation ABTS•+ that must be generated using $K_2S_2O_7$.

In all the studies, the extracts with the highest antioxidant activity and the highest amount of phytocompounds were chosen to be incorporated into mesoporous silica-type supports to preserve the beneficial properties over time (up to 9 months) for potential use in nutraceuticals or cosmetics. Thus, the CS extract embedded in the Zn-MCM-41 or Mg-MCM-41 Zn supports and grape skins extract incorporated in the MCM-41 kept much better the radical scavenger capacity of extract for 1-5 months of storage (a RSA decrease of 0.82-3.2% for incorporated extracts compared to 17.2-32.0% for free extracts). CS extracts prepared by the conventional and microwave method from a grape marc rich in stems and seeds were incorporated into three types of heteroatoms modified silica and showed a high extract stability up to 150 °C, demonstrated using differential scanning calorimetry. In the case of these extracts, after 6 months of storage, the

incorporated extracts showed a higher free radical scavenging activity by 9.2-24.5% and 27.1-37.5% compared to the free conventional and microwave extract, which were more prone to degradation. The best long-term stability was observed when incorporating the extracts into the Zn-MCM-41 support, and the lowest, but still significantly higher when compared with the free extract was obtained for carrier decorated with crystalline phase $CeO_2$ (Ce-MCM-41).

For hydro-alcoholic extracts of MM adsorbed in pure or organic groups functionalized silica, it was shown that they present a higher stability for up to 9 months, tested comparatively by both DPPH and ABTS methods than the corresponding free extracts. Comparable results were obtained by both developed methods during the PhD studies, which proved reliability. Using the *t*-Student statistical test, it was demonstrated that embedded extracts retained its radical scavenger capacity over time as being significantly better than the free extract for all materials containing extract. The release profiles of MM extract from the functionalized silica supports were correlated using a semi-empirical model, the three-parameter model. A good correlation was obtained between the surface acidity (*p*Ka) and the maximum amount of extract released, the functionalization of the pore walls surface of the support with organic groups led to a modulation of phytocomponents release from the proposed supports, and the best results were obtained for MM@MCM-COOH, which showed a good preservation of the radical scavenging activity for up to 6 months.

In the case of ethanolic black chokeberry extract, it was observed that after 1 month of storage, the extract embedded in pristine silica, MCM-41E, did not show a significantly different RSA than that of the free extract, unlike in the case of extract-loaded Zn-MCM-41, but after six or nine months of storage, the extracts embedded in Zn-MCM-41 and MCM-41E preserved the beneficial properties of the phytocompounds, both having a significantly higher RSA value than the free extract (*t*-Student test), which highlights the beneficial role of the extract encapsulation.

Biological tests on free and embedded into silica-type mesoporous matrices extracts were performed to evaluate the effectiveness of the proposed formulations for their potential application as antioxidants in nutraceuticals or cosmetics.

In the case of ethanolic extracts of grape pomace, cytocompatibility tests performed on the keratinocyte line (HaCaT) demonstrated good cytocompatibility for CS and GS extracts, especially for CS extract, which stimulated cell proliferation. The best results were obtained for CS@Zn-MCM-41 that showed the best cytocompatibility and RSA over time.

The incorporation of the *Aronia melanocarpa* extract in mesoporous matrices led to the preservation of RSA and antimicrobial potential against on Gram-positive bacteria. From the proposed carriers, as in the case grape marc embedding, incorporation into Zn-MCM-41 was the most beneficial and moreover led to a slight improvement in antimicrobial activity due to the synergistic effect of polyphenolic extract and ZnO nanoparticles that decorate the silica pore walls surface. At the same time, a pronounced antiproliferative and antimigratory effect was obtained on human melanoma A375 cells, without having a higher toxicity on the human keratinocyte cell line (HaCaT).

The conventional hydro-alcoholic extract from MM was evaluated based on cell viability tests performed on NIH3T3 murine fibroblasts, highlighting the lack of toxicity for the embedding polyphenolic extracts. ROS values determined at the cellular level correlated well with *in vitro* release experiments of polyphenols in phosphate buffer. For MCM@MCM-COOH sample the lowest cytosolic production of ROS was obtained correlated with the highest amount of polyphenols released in PBS *p*H 5.7, showing a high stability over time and a higher *in vitro* antioxidant activity than that of free polyphenolic extract.

In the last part of the PhD thesis, strategies for irinotecan targeted action of tumoral tissue were approached, with obtaining various behaviours of the cytostatic release process from the proposed mesoporous matrices. Thus, *in vitro* release experiments in PBS with two *p*H values (5.7 and 7.6) showed a reduced desorption of irinotecan from SBA-15 carrier or modified with folate moiety, higher in the case of SBA-NH-folate support (about 40% of the amount of adsorbed drug) for which a better internalization of the cytostatic was achieved, evidenced by experiments performed by using Cytoviva hyperspectral microscope and images processing with the Matlab suite.

Moreover, functionalized silica decorated with ulvan supports were proposed as an alternative to the targeted release, platforms that ensured the complete release of the cytostatic agent in 8 h, the process being much faster than the delivery from the folate functionalized silica support (higher $\Delta G$ values). However, a higher ulvan content in the support resulted in a slower release kinetics and a higher value of the diffusion rate that could correlate with the textural parameters of the ulvan@SBA-$NH_2$ carrier. The release of irinotecan from the FeMCMB support was very slow, following zero order kinetics.

The obtained systems could be used in different contexts. Hence, Iri@SBA-NH-folate would be recommended for a targeted tumoral tissue with reduced side effects based on a very good internalization at the cellular level. Also, when a complete release of the cytostatic is desired in a shorter time, Iri@ulvan@SBA-$NH_2$ material could be recommended.

## *Main original contributions*

- Encapsulation for the first time of polyphenolic extracts in pristine, modified with heteroatoms or functionalized with organic groups MCM-41-type mesoporous silica and proving a preservation of radical scavenger activity for embedded. It was demonstrated that an increased bioavailability and a better biological activity through a modulation of silica surface and extract phytocomponents interaction were achieved.
- Development of two methods for radical scavenger activity determination (DPPH and ABTS) adapted for solid samples, which compares the activity of materials containing extract with the free extract and support. These procedures are reproducible and simple and could be used for any type of solid samples that contain a substance with radical scavenger activity.
- For the first time, carriers for irinotecan comprising of silica decorated with ulvan, a natural polysaccharide extracted from green algae (*Ulva lactuca*) or functionalized with folate residue were proposed to target tumoral tissue.

## *Perspectives*

I. The use of materials containing extract (from grape marc and aronia) with the best properties to develop cosmetics with topical applications;

II. Tailoring the extraction parameters to obtain extracts with a higher amount of polyphenols (acidic solvent, lower temperature, solvents mixture);

III. Biocompatibility evaluation of Iri@SBA-15 and Iri@SBA-NH-folate samples;

IV. Development of irinotecan delivery systems containing also polyphenols to achieve a synergism and cytotoxicity reduction.

## Publications list

### *Articles*

- V. Buda, **A. M. Brezoiu**, D. Berger, I. Pavel, D. Muntean, D. Minda, C. Dehelean, C. Soica, Z. Diaconeasa R. Folescu, C. Danciu, Biological evaluation of black chokeberry extract free and embedded in two mesoporous silica-type matrices, *Pharmaceutics* **2020**, 12(9), 838; IF=6,321;

- **A. M. Brezoiu**, L. Bajenaru, D. Berger, R.-A. Mitran, M. Deaconu, D. Lincu, A. Stoica Guzun, C. Matei, M. Moisescu, T. Negreanu-Pirjol, Effect of nanoconfinement of polyphenolic extract from grape pomace into functionalized mesoporous silica on its biocompatibility and radical scavenging activity, *Antioxidants* **2020**, 9(8), 696; IF=6,312;

- **A. M. Brezoiu**, D. Lincu, M. Deaconu, R.-A. Mitran, D. Berger, C. Matei, Enhanced stability of polyphenolic extracts from grape pomace achieved by embedding into mesoporous silica-type matrices, *U.P.B. Sci. Bull.*, Series B **2020**, 82(3), 2020;

- **A. M. Brezoiu,** C. Matei, M. Deaconu, A. M. Stanciuc, A. Trifan, A. Gaspar-Pintiliescu, D. Berger, Polyphenols extract from grape pomace. Characterization and valorisation through encapsulation into mesoporous silica-type matrices, *Food Chem. Toxicol.* **2019**, 133, 110787; IF=6,023.

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- **A. M. Brezoiu**, V. Buda, M. Deaconu, D. Lincu, A. Mocanu, C. Danciu, C. Matei, D. Berger, Chemical profile and biological activities of polyphenols extracts from black chokeberry free and embedded on silica-type supports, 1st Advances in Food Chemistry Conference, Online, România, 15-17 Aprilie, **2021**.

- **A. M. Brezoiu**, V. Buda, D. Lincu, C. Danciu, C. Matei, D. Berger, Investigation of Embedding Black Chokeberry Extract in Mesoporous Silica Supports on Its Beneficial Effects- Priorities of Chemistry for a Sustainable Development (PRIOCHEM), București, România, 28-30 Octombrie **2020**;

-**A. M. Brezoiu**, L. Bajenaru, M. Deaconu, D. Lincu, R.-A. Mitran, C. Matei, Daniela Berger, *Antioxidant activity of polyphenolic extract from rosé wine grape marc loaded on functionalized mesoporous silica*, International Chemical Engineering and Materials Symposium (SICHEM 2020), București, România, 17– 18 Septembrie **2020**;

- **A. M. Brezoiu**, M. Deaconu, D. Lincu, L. Bajenaru, C. Matei, D. Berger, *Embedding Polyphenols Extract from Grape Marc into Inorganic Supports with Enhanced Stability*, Priorities of Chemistry for a Sustainable Development (PRIOCHEM), București, România, 30 Octombrie-1 Noiembrie **2019**;

- **A. M. Brezoiu**, C. Matei, M. Deaconu, R.-A. Mitran, A. Trifan, A. M. Stanciuc, D. Berger, *Enhanced radical scavenger properties of polyphenolic extracts from grape marc through encapsulation into mesoporous silica-type matrices*, Romanian International Conference on Chemistry and Chemical Engineering (RICCCE), Constanța, România, 4-7 Septembrie **2019;**

- **A. M. Brezoiu**, C. Matei, M. Deaconu, A. Trifan, A. M. Stanciuc, D. Berger, *Valorization of grape pomace through encapsulation of polyphenols extract into mesoporous silica type matrices*, International Conference on Natural Products Utilization (ICNPU), Albena, Bulgaria, 29 Mai-01 Iunie **2019**.

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