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Formulation of β-Cyclodextrin Nanosponges by Polycondensation Method: Application for Natural Drugs Delivery and Preservation.

Y.Nait Bachir[1], M.Medjkane[2], F.Benaoudj[3], N.Sahraoui[4], A.Hadj-ziane[1]

1. Chemical Engineering Laboratory, Process Engineering Department, Faculty of Technology, University of Saad Dahlab-Blida 1, Blida, Algeria.
2. Laboratory of Natural Bio-Resources, Department of Biology, Faculty of Science, Hassiba Benbouali University Chlef , BP. 151, Chlef 02000, Algeria.
3. Laboratory of Vegetal Production, Department of Vegetal Biotechnology, Faculty of Agronomy, University of Saad Dahlab-Blida 1, Blida, Algeria.
4. Transfer Phenomena Laboratory, Mechanical and Process Engineering Faculty, University of Science and Technology Houari Boumediene, BP32, El-Alia,16111, Bab Ezzouar, Algiers, Algeria.

ABSTRACT — Nanosponges are a novel class of encapsulating nanosystems based cross linked polymeric materials with a porous surface, used as a promising delivery system of synthetic and natural bioactive molecules. The aim of the present work was to prepare a stable nanoemulsion based on β-cyclodextrin nanosponges for enhance physicochemical stability of *salvia officinalis* essential oil. In first, new β-cyclodextrin nanosponges were synthesized by polycondensation using naphthalene dicarboxylic acid as cross-linking agent, the latter system was characterized by FTIR spectroscopy, BET and powder XRD. afterwards, nanoemulsion stabilized by nanosponges was prepared, its physicochemical properties were determined (particles size, zeta potential, viscosity, turbidity and essential oil content) and its stability was studied at different storage temperatures (4°C, 20°C and 40°C) during 30 days. Finally, pharmaceutical application of prepared nanoemulsion was investigated by in-vitro antimicrobial activity. Reaction yield of nanosponges based β-cyclodextrin and 2,6-naphtalene dicarboxylic acid was 42.51%. Characterization of nanosponges by FTIR, BET and XRD showed the enhancement of their porosity and solubility compared to natural β-cyclodextrin's ones, these results clearly demonstrated their high capacity to encapsulate hydrophobic substances. Oil-in-Water nanoemulsion of *salvia officinalis* essential oil stabilized by these nanosponges presents a very high stability; this nanoemulsion presents very high stability at all storage temperatures (4°C, 20°C and 40°C) during storage period (30 days), these results permit to broaden the industrial applications of sage essential oil. Investigation of pharmaceutical application of this engineered nanoemulsion by in-vitro antimicrobial activity study proved the increase of essential oil bioactivity.

***Keywords**: Nanosponge, β-cyclodextrin, Polycondensation, Nanoemulsion, Sage essential oil, Physicochemical stability, in-vitro antimicrobial activity.*

I.Introduction

Cyclodextrin-based nanosponges can be obtained by cross-linking different types of cyclodextrins with a carbonyl or a dicarboxylate compound as cross-linker agents [1].

***Corresponding author**: Yacine NAIT BACHIR, Research field: Nanotechnology, Pharmaceutical Engineering, Drug Delivery Systems, Natural Drugs. Adress. Chemical Engineering Laboratory, Process Engineering Department, Faculty of Technology, University of Saad Dahlab-Blida 1, Blida, Algeria.*
E-mail: phd.nait.bachir.yacine@gmail.com

They are biocompatible nanoporous nanoparticles with spherical morphology and the capacity of encapsulating active molecules due to the cooperation of cyclodextrin cavities and cross-linker network [2-5].

Natural β-cyclodextrins have been the most widely used of all the cyclodextrins [6]. β-cyclodextrins and nanosponges based on β-cyclodextrins are encapsulating type of nanoparticles which encapsulates the natural and synthetic drug molecules within its core for

enhancement of their solubility [7-12], bioavailability [11,13] and therapeutic efficacy [14-16].

The objective of the work was preparation of stable nanoemulsion stabilized by Cyclodextrin-based nanosponges content *Salvia officinalis* essential oil as natural bioactive substance for enhancement of its therapeutic activities and its stability during storage.

II. Experimental details

II.1. Materials

β-cyclodextrin (BCD) and 2,6-Naphthalene dicarboxylic acid (NDCA) were procured from sigma-Aldrich. *Salvia officinalis* essential oil was extracted in our laboratory by hydrodistillation using Clevenger apparatus. All other materials used were of pharmacopeial grade.

II.2. Preparation of nanosponges

Nanosponges were prepared by reaction of BCD with NDCA in the presence of catalytic sulfuric acid, NDCA and sulfuric acid 98 % (2 drops) were added to 25 ml of BCD (200 mmol/l) aqueous solution under stirring and temperature was increased to 100 °C during 48h reaction time [17]. The molar ratio of BCD: NDCA was fixed at 1:10. Chemical structure of 2,6-naphthalenedicarboxylic acid is shown in figure 1.

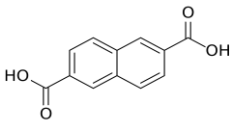

Fig. 1 Chemical structure of 2,6-naphthalenedicarboxylic acid.

II.3. Characterization of nanosponges

Prepared nanosponges are characterized using FTIR spectroscopy, powder XRD and BET.

FTIR spectra of BCD, NDCA and nanosponges were recorded at room temperature on a Tensor 27 FT-IR spectrophotometer.

ASAP 202 MICROMIRITICS surface analyser instrument was used for determination of the surface area measurements of nanosponges compared with natural BCD by measurements of Brunnauer, Emmet and Teller (BET) Nitrogen adsorption isotherm.

X-ray diffraction patterns of natural BCD and nanosponges were obtained using a Philips PW 3710 X-ray Diffractometer equipped with X powder software.

II.4. Preparation of nanoemulsion

Nanoemulsion was prepared by high energy method using Ultra Turrax high shear mixer. 2.5 ml of *Salvia officinalis* essential oil is dispersed in bidistilled water (at 5% w/w) containing nanosponges, using an Ultra Turrax (T25 IKA Labortechnik, Germany) high shear mixer at 24,000 rpm for 5 min. Composition of prepared nanoemulsion is given in table 1.

Table 1. Composition of nanoemulsion.

Compounds	***Content on nanoemulsion***
Salvia officinalis essential oil	5%
Nanosponges	5%
Water	90 %

II.5. Characterization of nanoemulsion

Prepared nanoemulsion is characterized by laser particles size analysis, zeta potential, viscosity, turbidity and essential oil content estimation.

Laser particle size analysis of nanoemulsion was determined using Nanotrac TM250 (Microtrac Inc, PA, USA) Instruments after 1/100 dilution with bidistilled water, The mean diameter of the volume distribution (MV) was calculated by the following equation [18]:

$$MV = \frac{\sum V_i d_i}{\sum V_i}$$

Where 'Vi' is the volume percentage between droplet sizes and 'di' is diameter of droplets.

Zeta potential of nanoemulsion was determined using Nanotrac wave (Microtrac Inc, PA, USA) Instruments and calculated using Smoluchowski's equation [19]:

$$\zeta = \frac{4\pi\eta}{\varepsilon}\frac{v}{\frac{U}{L}}$$

Where 'U' is the voltage and 'L' is the distance between two electrodes, 'ε' and 'η' are the dielectric constant and the viscosity of pure water, respectively, and 'ν' is the mobile velocity of SEO droplets in the electric field.

Viscosity of nanoemulsion was measured using a Brook Field Viscometer (For LV-II viscosity range), the applied shear rate was fixed at 170 s-1.

Turbidity of nanoemulsion was determined by measuring the absorbance of diluted sample 1:100 with bidistilled water at 600 nm using Agilent 8453 UV-Vis spectrophotometer.

Extraction of total essential oil from nanoemulsion was realized by hexane [20], 1ml

on nanoemulsion, 20 ml of distilled water and 10 ml of hexane was added in glass tubes. The prepared systems were sonicated during 20 min at 60 °C using X-TRA 30H ULTRASONIC BATH and the organic phase (hexane + Salvia officinalis essential oil) was separated from the aqueous phase by centrifugation at 4500 rpm for 10 min. SEO content in NEs was determined by spectroscopic quantification of SEO in the organic phase extracted previously using Agilent 8453 UV-Vis spectrophotometer at 256 nm. SEO content in NEs was calculated as follows:

$$\text{SEO Content (\%)} = \frac{\text{SOE content in 1ml of NEs}}{0.05} \times 100$$

The measurements are triplicated and all experiments were carried out at 25 °C.

II.6. Stability study of nanoemulsion

After preparation, nanoemulsion was stored at 4oC, 25oC and 50oC. nanoemulsion samples were observed every 5 days during 30 days for determination of creaming and/or sedimentation for each nanoemulsion.

II.7. In-vitro antimicrobial activity

Antimicrobial activity is determined measuring Minimum Inhibitory Concentrations (MIC) and Minimum Fungicidal Concentration (MFC) of *Salvia officinalis* essential oil and prepared nanoemulsion. The MICs/MFCs were examined as the inhibitory effects against the growth of tow fungal strains supplied by Algeria Pasteur Institute. Tested strains are *Microsporum canis* and *candida albicans*.

Minimum Inhibitory Concentrations were determined by the Agar dilution method that approved by the NCCLS and modified by [21,22]. Briefly, a series of twofold dilutions of each oil, ranging from 2% (v/v) to 0·03% (v/v), was prepared in sabouraud dextrose agar (SDA) with 0.5% (v/v) tween 20. Plates were dried at 35°C for 30min prior to inoculation with 1–2 ml spots containing approximately 104 cfu of each organism. SDA with 0.5% (v/v) Tween-20 but no oil, was used as a positive growth control.

Inoculated tubes were incubated at 25°C for 48h. Minimum inhibitory concentrations (MICs) were determined after 24h. The MICs were determined as the lowest concentration of oil inhibiting the visible growth of each organism on the agar tubes.

MFCs were determined by the inoculation of agar tubes that doesn't present visible growth of each organism on MIC determination. The MFCs were determined as the lowest concentration of oil inhibiting the visible growth of each organism on the agar tubes.

III. Results and Discussions

III.1. Preparation and characterization of nanosponges

The value of reaction yield is 42.51%; the obtained nanosponges are white amorphous powder present high solubility in water.

FTIR spectra of BCD, NDCA and nanosponges are shown in figure 2.

The peaks appearing at 3381, 1929 and 1638-1604 cm-1 approximately in FTIR spectrum of nanosponges (Fig. 2.C) are characteristics of (=C-H), (C=O) and (C=C) bonds respectively, which are specific for NDCA and that does not exist in BCD, the representative peaks of these bonds in pure NDCA are observed at 1811 and 1487-1619 cm-1, respectively (Fig. 2.B).

The carboxylic acid functions react totally (displacement of the bond from 3154 cm-1 in pure NDCA to 3422 cm-1 in nanosponges) according to the reaction of polycondensation of 2,6-naphthalenedicarboxylic acid (Fig. A) and BCD, while it remains free alcohol functions of the BCD [23].

The apparition of two characteristic bonds of ester function formation (C-O) at approximately 1067 cm-1 (Fig 2.C) resulting from reaction between aromatic carboxylic groups of NDCA (characteristic bond C-O at 919 cm-1, shown in Fig. 2.B) and primary alcohol groups of BCD (characteristic bond C-O at 1089 cm-1, shown in Fig. 2.A).

The BET results of BCD and nanosponges are <0.01 and 17.31 m2/g, respectively. This porosity enhancement is due to the formation of nanoporous material based on BCD content cyclodextrin cavities and cross-linker network.

X-ray powder diffractograms of nanosponges (Fig 3.A) and natural BCD (Fig 3.B) show clearly the reduction of crystallinity.

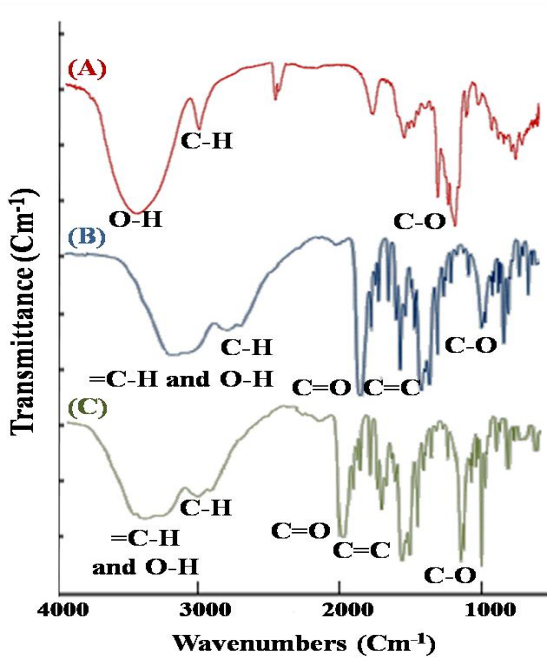

Fig. 2 FTIR spectra of BCD, NDCA and Nanosponges.

The crystallinity of natural BCD was significantly decreased after their cross-linking with NDCA for nanosponges synthesis. Effectively, the reduction of picks number and intensities is attributed to the formation of new solid phases with low crystallinity and high water solubility [24].

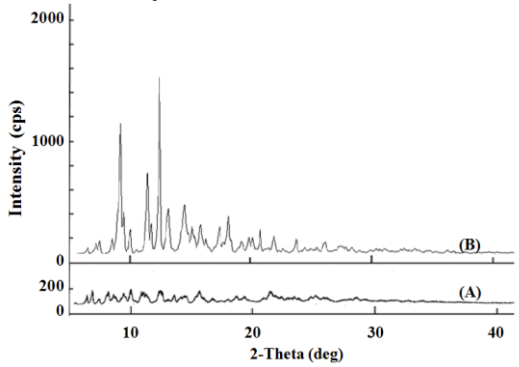

Fig. 3 X-ray powder diffractograms of BCD and nanosponges.

III.2. Preparation, characterization and Stability study of nanoemulsions

Prepared nanoemulsion represents a particle size of 626.7 nm their small droplet size gives them a translucent appearance [25,26] and also a resistance for physical instability by flocculation, creaming, sedimentation and coalescence [27-29]. Its zeta-potential, viscosity and turbidity are given in table 2. The prepared nanoemulsion presents stable limpid appearance during 30 days storage at 4°C, 25°C and 50°C.

Table 2. Physico-chemical characteristics of nanoemulsion.

Characteristics	*Values*
Particle size	626.7 nm
Zeta potential	-17.16 mV
Viscosity	0.51Pa.s
Turbidity	0.285 cm-1
Essential oil content	5%

III.3. In-vitro antimicrobial activity

The MICs and MFCs of *Salvia officinalis* essential oil and prepared nanoemulsion are given in table 3 and shown in figure 4.

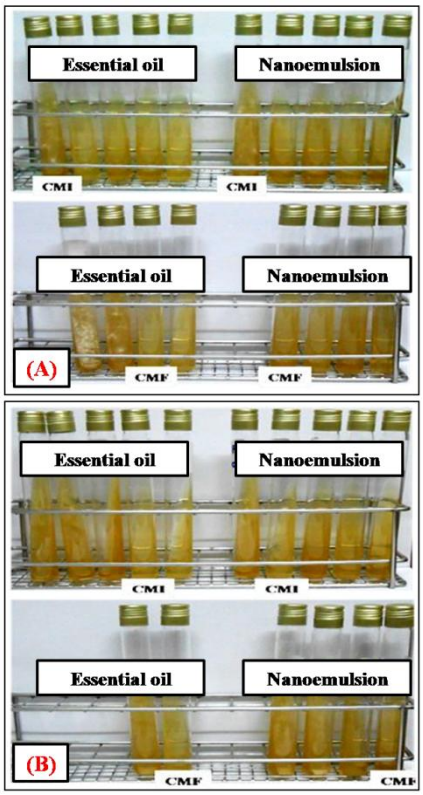

Fig. 4 Photographs of the MICs and MFCs of *Salvia officinalis* essential oil and prepared nanoemulsion against *Microsporum canis* (A) and *Candida albicans* (B).

The fungal strains that exhibited the higher sensitivity to free ASEO, after its nanodispersion a significantly increasing of its antifungal activity were observed. This enhancement of antimicrobial potential is due to the enhancement of the solubility of essential oil

after its incorporation inside the nanosponges cavities. The previous results obtained by [14,30,31] show the similar results on the enhancement of pharmacological activities of synthetic and natural drugs, compared with our study.

Table 3. Results of antimicrobial activity of *Salvia officinalis* essential oil before and after encapsulation in nanosponges.

Fungal strains		***Salvia officinalis essential oil***	***Nanoemulsion***
Microsporum canis	MIC	0.125µl/ml	0.125 µl/ml
	MFC	0.5 µl/ml	0.125 µl/ml
Candida albicans	MIC	0.5 µl/ml	1 µl/ml
	MFC	0.125 µl/ml	1 µl/ml

IV. Conclusions

In this study nanosponges based β-cyclodextrin and 2,6-naphtalene dicarboxylic acid were successfully synthesized. Characterization of nanosponges by FTIR, BET and XRD showed the enhancement of their porosity and solubility compared to natural β-cyclodextrin's ones, these results clearly demonstrated their high capacity to encapsulate hydrophobic substances. Oil-in-Water nanoemulsion of *salvia officinalis* essential oil stabilized by these nanosponges presents a high stability at 4 ° C, 20 ° C and 40 ° C during 30 days storage. This high stability permit to broaden the industrial applications of sage essential oil. Investigation of in-vitro antimicrobial activity of *Salvia officinalis* essential oil and nanoemulsion showed the enhancement of essential oil therapeutic efficacy after its nanoencapsulation on nanosponges. This study can be used as reference for future scientific investigations in development of new nanosponges and engineered formulations of food, cosmetic and pharmaceutical products.

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