Interactions and antioxidant stability of sesamol in dry-emulsions

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Abstract Dry emulsions prepared from saccharose (SAC), Labrafil® M 1944 CS, sesamol (SEOH), and hydroxypropylmethylcellulose (HPMC) or sodium caseinate (SC) by grinding or dissolution, and different desiccation techniques (spray (SD)- or freeze (FD)-drying, or heating at 60 °C) were investigated to determine possible interactions between mixture components. The goal of this study was to determine the best formulation which will regenerate perfectly, after water dilution of dry emulsions, the initial liquid emulsion with the same characteristics that before drying. The morphological state of SAC and dry emulsions were determined by scanning electron microscopy analysis (SEM) analysis. Glass transition temperature, and melting (endothermic), decomposition (endothermic), oxidation (exothermic) peak temperatures and enthalpies were measured by differential scanning calorimetry (DSC). The antioxidant activity of emulsions was evaluated through their ability to reduce 1,1-diphenyl-2-picrylhydrazil (DPPH) free radical. SEM analysis showed that SD-prepared, SC-containing dry emulsions formed better spherical particles with smooth surface at about 5 μm diameter as compared to emulsion containing HPMC. These former emulsions also showed more thermal stability by DSC. The combined results of the three analytical techniques emphasized the importance of the dry emulsion process regarding the efficiency and the thermal stability of antioxidant substances. A specific physical and/or chemical combination (such as hydrogen bond) sufficiently stable, at about 150 °C, could be suggested when the technique used for dry emulsion preparation was SD. After water dilution, this dry emulsion obtained from quaternary mixture: SAC, Labrafil®, SEOH and SC, will regenerate the initial liquid emulsion with the same characteristics that before drying.

Keywords Antioxidant · DPPH free radical · Dry emulsion · DSC · SEM · Sesamol

Introduction

The chemical interaction between active substances and excipients is an important element in pharmaceutical dosage forms [1]. Such mixtures lead to interactions between them. It would be desirable to know, in order to control them.

New technologies could reduce interactions between active substances and excipients by improving their solubility, permeability and bioavailability [2]. In recent years, dry forms of emulsion have been developed as improved formulations for pharmaceuticals. They are studied like sustained release forms, modelling of drug transfers in liquids. A great advantage of such micro encapsulation form is that they are fully reconstituted in vivo or in aqueous solutions [3]. After preparing the liquid oil-in-water (o/w)-emulsion, the aqueous phase is removed by

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pulverized techniques such as spray-drying (SD) [4–18], freeze-drying (FD) [5, 19–25] or rotary evaporation) [26–31]. Recent studies have shown the importance of the excipient choice [32] and the technical pulverization process [23–25] to yield stable dry emulsions with good oral bioavailability and delivery of drug to tissues [9–12, 18, 20, 23–25, 31].

Sesamol (3,4-methylenedioxyphenol) is a constituent of roasted sesame, seeds of *Sesamum indicum* L., an important oilseed crop. Several beneficial effects of sesamol, including antioxidation, chemoprevention, antimutagenic, antihepatotoxic activities and induction of apoptosis of cancer and cardiovascular cells have been reported [33]. Antioxidant activity is due to the presence of a phenolic and benzodioxole group in its molecular structure. Sesamol is soluble as well in water as in oil. It is used in human diet and in cosmetics.

We have recently reported on the properties of dry sesamol (SEOH) emulsions synthesized from various combinations of saccharose (SAC), with Labrafil®, hydroxypropylmethylcellulose (HPMC) or sodium caseinate (SC), using SD or FD techniques [33]. On the basis of physical characteristics (droplet size distribution, residual moisture, microscopic structure) and in vitro and ex vivo antioxidant activity, the best material, that reconstituted liquid emulsion upon aqueous dilution was obtained when SD was applied at either 150 °C or 180 °C, with SC or HPMC as excipient, respectively [34].

Our goal was to show the best formulation with HPMC or with SC. As an extension of this study [34], we have undertaken a detailed physicochemical analysis of these optimal formulations and others to determine, in selected combinations of components (i.e., SAC, HPMC, SC, Labrafil® and SEOH), which are the interactions (synergies or degradations) occurring at different temperatures. For this purpose, scanning electron microscopy (SEM) analysis, and two analytical techniques commonly used to evaluate drug-excipient interactions, i.e., Fourier transform infrared (FTIR) spectroscopy, and differential scanning calorimetry (DSC) were employed. Using the 1,1-diphenyl-2-picrylhydrazil (DPPH) radical test, we have determined which of these combinations can best potentiate or protect the intrinsic antioxidant activity of SEOH at high temperatures.

Experimental

Materials

SAC (D(+)-saccharose or sucrose), (No CAS 57-50-1) reagent grade Batch 14621 LE, SC (sodium caseinate from bovine milk), Batch 026K0156, SEOH (3,4-(methylendioxy)-phenol or sesamol 98%), (No CAS 533-31-3), Batch 30025-187 and the stable free radical 1,1-diphenyl-2-picrylhydrazil (DPPH), Batch S43654-357 were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France). Labrafil® M 1944 CS (No CAS 25496-72-4 and 9004-96-0) (apricot kernel oil PEG-6 esters), a mixture of mono-, di- and triglycerides and mono- and di-fatty esters of polyethyleneglycol 300, Batch 33644 was from Gattefossé (Saint-Priest, France). HPMC, a propylene glycol ether of methyl cellulose (No CAS 9004-65-3) (Pharmacoat 603®), Batch 506274 was supplied by Shin-Etsu (Tokyo, Japan). All other chemicals were of the highest purity available from commercial suppliers. Doubly distilled, deionised water was used throughout.

Preparation of mixtures

Preparation of initial liquid mixture obeyed the following procedure:

– Liquid 1 was prepared by dissolving 4 g SEOH in 36 g Labrafil® at 40 °C;
– Liquid 2 was an aqueous solution of 2 g HPMC and 230 g SAC in 128 mL distilled water;
– Liquid 3 was an aqueous solution of 4 g SC and 228 g SAC in 128 mL distilled water;
– Liquid 4 was obtained by mixing Liquid 1 and Liquid 2 (final volume 315 mL, SEOH concentration 92 mM);
– Liquid 5 was obtained by mixing Liquid 1 and Liquid 3 (final volume 315 mL, SEOH concentration 92 mM)

Liquids 4 and 5 where homogenized for 2 min at 24,000 rpm for 2 min (Ultra-Turrax® T25 basic, IKA Labortechnik, Germany), high speed colloid mill and then pulverized by FD or SD. The abbreviation corresponding to each mixture, the used technique and the theoretical centesimal composition of each studied mixture are presented in Table 1.

Briefly, samples M_A (containing SC) and M_B (containing HPMC) were obtained from the corresponding starting liquid emulsion by SD at either 150 °C or 180 °C, respectively. SD processing was performed by 100% aspiration and a feeding rate of the emulsion of 9 mL min^{-1} with a Büchi B-290 mini spray dryer (Switzerland), using appropriate inlet and outlet air temperature.

Samples M_C (containing SC) and M_D (containing HPMC) were obtained by FD by keeping the corresponding starting liquid emulsion at −63 °C and 0.007 mbar for 44 h (freeze dryer Alpha 1-2 LDplus, Germany).

Fifteen powders deriving from M_A or M_B emulsions were then obtained either by heating at 60 °C (M_E, M_F, M_R and M_S) or homogenizing (dissolution and/or grinding) at ambient temperature (M_G–M_Q) starting emulsions in which one or several ingredients were omitted or had their concentration varied.

Table 1 Theoretical composition of studied mixtures

Mixtures	Used technique	Physical state	SAC (%)	Labrafil (%)	SEOH (%)	SC (%)	HPMC (%)
M_A	$SD_{150\ °C}$	Powder	83.8	13.2	1.5	1.5	–
M_B	$SD_{180\ °C}$	Powder	84.6	13.2	1.5	–	0.7
M_C	$FD_{-63\ °C}$	Powder	84.6	13.2	1.5	–	0.7
M_D	$FD_{-63\ °C}$	Powder	83.8	13.2	1.5	1.5	–
M_E	$H_{60\ °C}$	Solid	83.8	13.2	1.5	1.5	–
M_F	$H_{60\ °C}$	Solid	84.6	13.2	1.5	–	0.7
M_G	Dissolution	Liquid	–	94.7	–	–	5.3
M_H	Dissolution	Liquid	–	90.0	–	10.0	–
M_I	Dissolution	Liquid	–	90.0	10.0	–	–
M_J	Grinding	Pasty	–	–	66.7	–	33.3
M_K	Grinding	Pasty	–	–	50.0	50.0	–
M_L	Grinding	Powder	99.1	–	–	–	0.9
M_M	Grinding	Powder	98.3	–	–	1.7	–
M_N	Grinding	Solid	85.8	13.4	–	–	0.8
M_O	Grinding	Solid	85.1	13.4	–	1.5	–
M_P	Grinding	Powder	97.7	–	1.7	–	0.9
M_Q	Grinding	Powder	96.6	–	1.7	1.7	–
M_R	$H_{60\ °C}$	Solid	85.1	13.4	–	1.5	–
M_S	$H_{60\ °C}$	Solid	85.8	13.4	–	–	0.8

$SD_{150\ °C}$ = spray-drying at 150 °C; $SD_{180\ °C}$ = spray-drying at 180 °C; $FD_{-63\ °C}$ = freeze-drying at −63 °C; $H_{60\ °C}$ = heating at 60 °C; dissolution and grinding were done at room temperature; "–" means not present in the mixture

SEM analysis

Microscopic structures were examined by SEM analysis using a Quanta® 200 scanning electron microscope (FEI Company, USA) at an accelerating voltage of 20 kV. Samples were placed on a double-faced adhesive tape and sputtered with platinum before analysis.

DSC studies

DSC was performed using a Netzsch DSC 200 F3 calorimeter (Selb, Germany). Approximately 10 mg of sample were weighed out and placed in an aluminium pan with a lid. An empty aluminium pan with a lid was used for reference. Tests were performed under static air. The temperature range was 25–250 °C, with a heating rate of 2 °C min^{-1}. Cooling of the calorimeter was achieved by compressed air. Temperatures given in tables were determined at the peak maximum (T_{max}). In some cases, extrapolated temperatures (T_{onset}) were given if the accuracy of their determination was high. Each result is the average of 3 independent tests. Temperature and enthalpy standardization were made by the use of indium, tin and zinc with various heating rates.

Repeatability tests were done on lactose monohydrate, with a 2 °C min^{-1} heating rate from ambient temperature until 250 °C. Generally, repeatability tests (T and enthalpy)

are done for the melting of a compound. We had wished to be in more difficult conditions and to verify that repeatability tests made after on various samples would be reasonably good. For this reason lactose monohydrate was chosen. It presents a volatilization step (2 endothermic peaks attributed to water loss), then a melting step (1 endothermic peak) followed by a degradation step (1 endothermic peak). Relative standard deviations of T_{max} and ΔH enthalpies were lower than 1% and 6%, respectively (Table 2).

FTIR

FTIR was performed on a Bruker IFS25 spectrometer (Germany) in transmission mode. Analyses were done according to the KBr disk technique by mixing the powders or SAC with KBr. Data were collected by averaging 64 scans at 4 cm^{-1} resolution.

Measurement of free radical scavenging activity using DPPH

The reducing effect of pre-heated (150 °C for 20 min) SEOH emulsions towards DPPH was measured as an index of antioxidant efficacy using a modification of a previously published procedure [35]. Stock solutions of pure SEOH

Table 2 DSC repeatability tests done on lactose monohydrate

	Sample mass/mg	1st endo peak T_{max}/°C	2nd endo peak T_{max} /°C	ΔH/J g^{-1}	3rd endo peak T_{max}/°C	4th endo peak T_{max}/°C
Average ($n = 10$)	10.0	140.7	144.6	156.6	213.3	217.7
Standard deviation	0.95	1.13	0.18	9.36	0.31	0.26
Relative standard deviation (%)	9.48	0.80	0.12	5.97	0.15	0.12

endo = endothermic; the T_{max} of each endothermic peak is given; ΔH = total enthalpy of 1st and 2nd peaks

(0.3 mM) or each SEOH dry emulsion (final SEOH concentration 0.3 mM) were prepared in water:methanol (1:2 v/v) to achieve a complete solubility of the sample. Then 0.075–0.750-mL aliquots of these stock solutions were added to 1.50 mL of a freshly prepared methanolic DPPH solution (75 mg L^{-1}). When necessary, pure methanol was added to reach a final volume of 2.25 mL. For each SEOH concentration, the corresponding control samples were obtained by adding 1.5 mL of DPPH in the same volume of water: methanol. The decrease in DPPH absorbance was measured, by spectrometry, at 517 nm after 3 min incubation of the mixture at room temperature. All values are expressed as mean ± standard deviation. Differences were analyzed by two- or one-way analysis of variance (ANOVA) followed by a posteriori Bonferroni or Newman-Keuls multiple comparison tests. Intergroup differences were considered when $P < 0.05$.

Results and discussion

SEM analysis

The shape and morphology of particles containing SAC were mainly determined by the storage temperature, the desiccation technique and the sample composition.

Figure 1 shows the morphology of SAC, at several storage temperatures: 25 °C (A), 150 °C (B), 170 °C (C), and formulations prepared by several techniques and stored at 150 °C during 20 min: M$_{A-150 °C}$ (D), M$_{B-150 °C}$ (E), M$_{D-63 °C}$ (F), M$_{C-150 °C}$ (G), M$_{E-150 °C}$ (H) and M$_{F-150 °C}$ (I). A large size of distribution is observed for SAC at 25 °C which has a wrinkled and porous surface with irregular and lengthened particles. Spherical agglomerates are formed at higher temperature (170 °C). SEM observations on formulations revealed different morphologies when compared to SD, FD and heating at 60 °C, techniques (Fig. 1d–i). SEM analysis revealed some major differences attributed to the SD technique in the mixtures with SC. After storage at 150 °C, these dry emulsions showed spherical particles determined to a size of about 5 μm with smooth surface. In the presence of HPMC, the
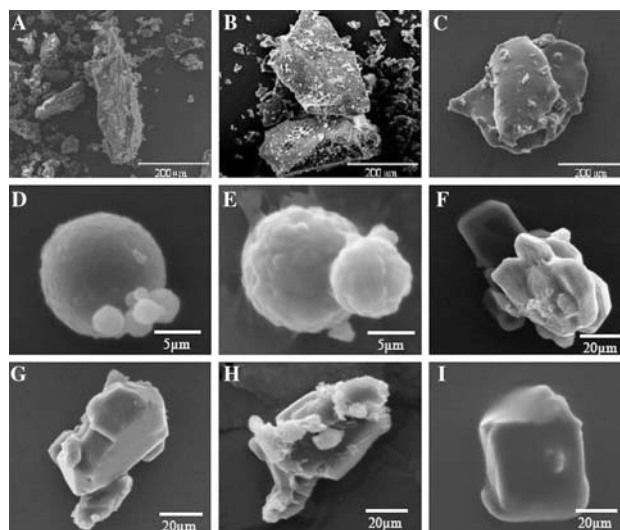

Fig. 1 SEM analysis. Photographs of pure saccharose after 20 min storage at: 25 °C (**a**), at 150 °C (**b**) or 170 °C (**c**) and of SEOH-containing formulations (**d–i**) prepared by various techniques of desiccation storage at 150 °C for 20 min: M$_{A-150 °C}$ (**d**), M$_{B-150 °C}$ (**e**), M$_{D-63 °C}$ (**f**), M$_{C-150 °C}$ (**g**), M$_{E-150 °C}$ (**h**) and M$_{F-150 °C}$ (**i**). Compositions of mixtures M$_A$–M$_F$ are in Table 1

dry emulsions prepared by the SD technique also showed spherical particles, but without smooth surface (Fig. 1e). On the other hand, particles with irregular shapes were observed for the FD (Fig. 1f, g) and heating at 60 °C (Fig. 1h, i) techniques.

DSC analysis

First we will exhibit the results obtained on each component alone, then binary and tertiary mixtures, finally quaternary mixtures after various desiccation techniques.

DSC results of each constituent alone

The melting point is widely used in the characterization of materials. It could be used in quality control. A properly calibrated DSC gives the melting temperature as the onset temperature of the melting endothermic peak.

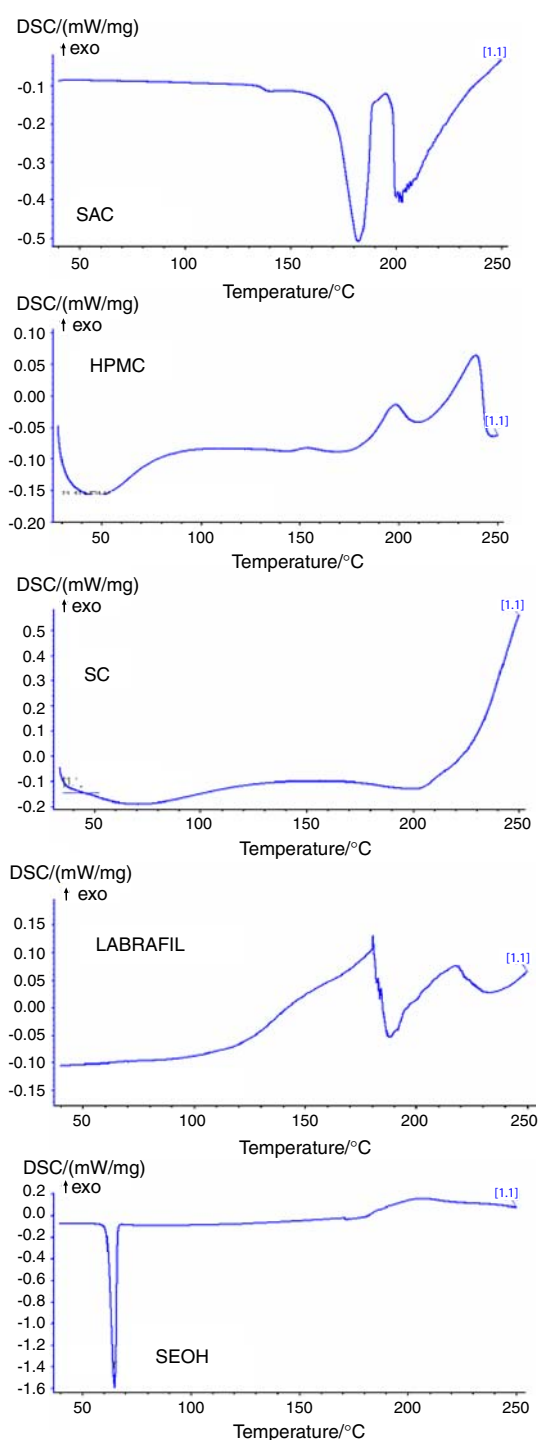One DSC curve obtained on each component and average results are put in Fig. 2 and Table 3, respectively.

Fig. 2 DSC curves of each component alone. (SAC, HPMC, SC, Labrafil® and SEOH)

Sugars do not have sharp melting temperature when they are amorphous. Their melting proceeds over a temperature range. Endotherms are fairly broad. The reported melting range for SAC is 160–192 °C [36]. The melting temperature is also sensitive to water, impurities and cristallinity. However, it should be noted that the reported values are

Table 3 DSC results of each constituent alone

Constituent alone	DSC curves until 250 °C
SAC	$T_g = 136.7$ °C; 1st endothermic peak: $T_{max} = 182.3$ °C; $T_{onset} = 170.5$ °C; $\Delta H = -143$ J g^{-1}; 2nd endothermic peak: $T_{max} = 203.0$ °C; $\Delta H = -155$ J g^{-1}
HPMC	1st endothermic peak: $T_{max} = 47.9$ °C; $\Delta H = -78$ J g^{-1}; $T_g = 149.6$ °C; 2nd exothermic peak: $T_{max} = 197.5$ °C and 3rd exothermic peak: $T_{max} = 238.9$ °C; total $\Delta H = +113$ J g^{-1}
SC	1st endothermic peak: $T_{max} = 70.7$ °C; $\Delta H = -153$ J g^{-1}; 2nd endothermic peak: $T_{max} = 199.7$ °C; $\Delta H = -19$ J g^{-1}
Labrafil®	1st exothermic peak: $T_{max} = 180.4$ °C; $\Delta H = +178$ J g^{-1}; 2nd exothermic peak: $T_{max} = 216.9$ °C; $\Delta H = +61$ J g^{-1}
SEOH	1st endothermic peak (melting): $T_{max} = 64.9$ °C; $T_{onset} = 61.7$ °C; $\Delta H = -130$ J g^{-1}; Exothermic peak: $T_{max} = 204.5$ °C; $\Delta H = +144$ J g^{-1}

T_g = glass transition temperature; ND = not determined

generally 185–191 °C [37, 38]. According to Hurtta et al. [36], increasing the rates of heating moved both the onset and the peak temperature to higher temperature. Enthalpy of melting increases also as the increasing of heating rate.

We observed for SAC melting a T_{onset} of 170.5 and a T_{max} of 182.3 °C (at a 2 °C min^{-1} heating rate). SAC was very sensitive to heat. It changed into a brown-coloured (caramel) material due to dehydration at melting. A second endothermic peak was observed. SAC, analysed as received, showed also a glass transition state. This could be due to an amorphous form obtained generally from raw materials by heating until melting of the crystals and by quick cooling.

HPMC displays good compression characteristics. It has adequate swelling properties, can accommodate high levels of drugs loading and is considered non-toxic [39]. The physico chemical properties of HPMC are strongly affected by: (1) the methoxy group content; (2) the hydropropoxy group content; (3) the molecular weight. The glass transition temperature of a polymer is an important characteristic constant. Below the T_g, the mobility of the macromolecule is very low. Above the T_g, the mobility is markedly increased (rubbery state) leading to much higher mass transfer rates of water and drug. Several researchers listed values of HPMC ranging from 154 to 184 °C [40–43]. Studied HPMC is characterized by a T_g at 149.6 °C. The 1st large endothermic peak is due to moisture, then two exothermic peaks appear.

SC is a milk protein formed by α-S1-Casein variant B, β-Casein variant A, and κ-casein variant B, being responsible for the physicochemical stability of the micelle [44].

It is widely used as an emulsion stabilizing agent in foods such as ice-cream, coffee whitener, cream liqueurs and also in liquid or dry emulsions [6, 44–46]. We observed the presence of humidity at about 70 °C, then a very small endothermic peak.

Labrafil® was extensively used as pharmaceutical emulsifying agent for purpose of preparing micro emulsions [47, 48]. During heating, the oil was oxidised and two exothermic peaks appeared.

From Matos et al. [49] melting temperature and enthalpy are 64.2 °C and -123 J g^{-1}, respectively. Studied SEOH presents a melting endothermic peak at $T_{max} = 64.9$ °C and at $T_{onset} = 61.7$ °C, with $\Delta H = -130$ J g^{-1}. SEOH, component of sesame oil, is a natural organic compound used as antioxidant to prevent the spoilage of oils [50] and which may protect the body from free radicals damages [51, 52].

DSC results of binary mixtures

All binary mixtures are in the same ratio (the first constituent versus the second one) as they are in quaternary mixtures described in Table 1. One DSC curve obtained on each binary mixture and average results are in Fig. 3 and Table 4, respectively.

Oxidation phenomenon was important in samples M_G and M_H. Labrafil® was oxidized during heating. Compared with each constituent alone, binary mixture M_I presented a decreasing in oxidation enthalpy. In contrast, T_{max} of oxidation was higher than 250 °C. This fact confirms the antioxidant activity of SEOH. Sesamol, in the mixture M_I, did not present a melting endothermic peak because it is entirely soluble in Labrafil®. Samples M_J and M_K did not present any oxidation until about 160 °C. Oxidation could appear at higher temperature, as well as for mixtures with HPMC or SC. Compared with the M_M sample (with SC) a low decreasing in temperatures and enthalpies was observed for the first endothermic peak of the mixture M_L (with HPMC). This could be due to a slight decreasing stability of SAC in mixture with HPMC. In else, the melting peak of SAC in sample M_L was broader than those in sample M_M.

DSC results of ternary mixtures

One DSC curve obtained on each ternary mixture and average results are in Fig. 4 and Table 5, respectively.

Ternary mixtures M_N and M_O, with HPMC and SC, respectively, showed similar curves (near temperature and enthalpy values).

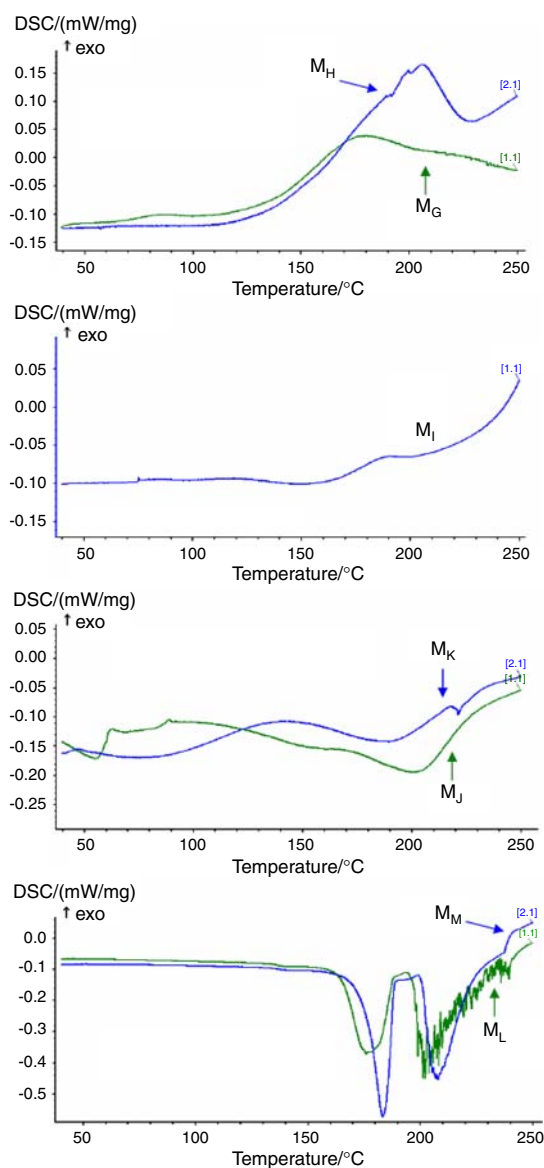Replacing Labrafil® by SEOH (examples of M_P and M_Q ternary mixtures), the endothermic peak of SEOH, was

Fig. 3 DSC curves of binary mixtures. Compositions of mixtures M_G–M_M are in Table 1

observed at a T_{max} and a melting enthalpy lower than theoretical values. SAC in sample M_P (with HPMC) show a T_{max} at 174.8 °C, lower than that of sample M_Q (with SC) at 183.5 °C. This peak was broader at half height for the sample M_P than that of sample M_Q. Two hypotheses could be proposed: (1) an interaction would be possible between SAC and SEOH, in the presence of HPMC; (2) SC favoured the formation of sucrose crystals. In else, glass transition is observable for M_N, M_O, M_P and M_Q samples, and not in M_R and M_S samples. The T_g of sucrose has disappeared in samples M_R and M_S because they were heated at 60 °C.

Table 4 DSC results of binary mixtures

Binary mixtures	DSC curves until 250 °C
M_G (Labrafil®-HPMC)	1st exothermic peak: $T_{max} = 85.2$ °C; $\Delta H = +3.3$ J g^{-1}; 2nd exothermic peak between 110 and 250 °C: $T_{max} = 176.3$ °C; $\Delta H = +190$ J g^{-1}
M_H (Labrafil®-SC)	Exothermic peak: $T_{max} = 199.9$ °C; $\Delta H = +177$ J g^{-1}
M_I (Labrafil®-SEOH)	Exothermic peak: $T_{max} = 187.0$ °C; $\Delta H = +5.4$ J g^{-1}
M_J (SEOH-HPMC)	Broad endothermic peak between 150 and 200 °C: $T_{max} = 202.8$ °C; $\Delta H = -89$ J g^{-1}
M_K (SEOH-SC)	$T_g = 136.5$ °C; 1st endothermic peak: $T_{max} = 184.5$ °C; $T_{onset} = 174.0$ °C; $\Delta H = -140$ J g^{-1}; 2nd endothermic peak: $T_{max} = 205.8$ °C; T_{onset} ND; ΔH ND
M_L (SAC-HPMC)	$T_g = 137.0$ °C; 1st endothermic peak: $T_{max} = 181.7$ °C; $T_{onset} = 170.4$ °C; $\Delta H = -130.5$ J g^{-1}; 2nd endothermic peak: $T_{max} = 203.5$ °C; T_{onset} ND; ΔH ND
M_M (SAC-SC)	Broad endothermic peak between 160 and 240 °C: $T_{max} = 189.7$ °C; $\Delta H = -57$ J g^{-1}

T_g = glass transition temperature; ND = not determined
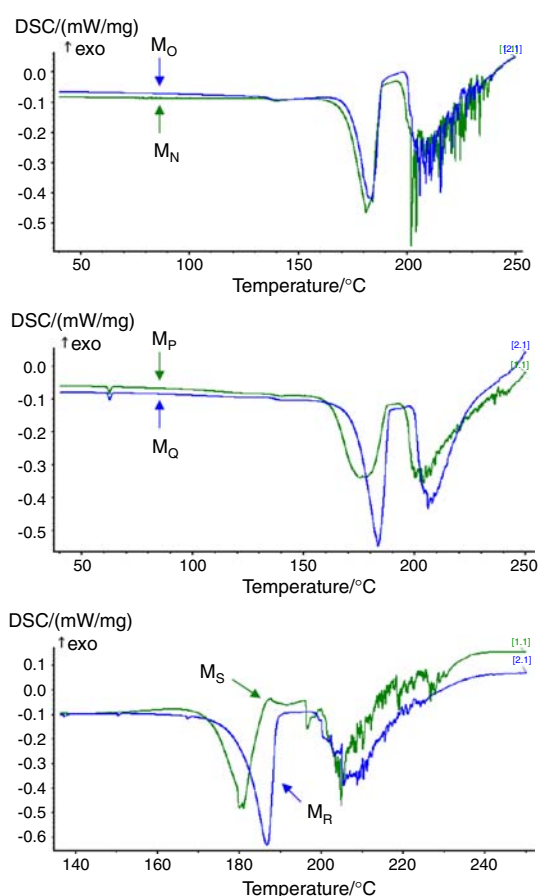

Fig. 4 DSC curves of ternary mixtures. Compositions of mixtures M_N–M_S are in Table 1

DSC results of quaternary mixtures after desiccation

One DSC curve obtained on each quaternary mixture and average results are in Fig. 5 and Table 6, respectively.

Mixtures M_A and M_B (dry emulsions obtained by SD technique) showed a first endothermic peak and a second one broader, due to the melting of SAC and to its decomposition, respectively. A fine observation of the two curves shows that the second peak of the sample M_B (containing 0.7% of HPMC) is more denticulate than that of sample M_A (containing 1.5% of SC). Sample M_A curve was more regular. In addition, a small exothermic peak appeared at about 200 °C for M_B. Quaternary mixtures M_C and M_D were obtained after FD. The DSC melting peak of SAC in the M_C sample (with HPMC) showed a decreasing of T_{max} (4.5 °C) and melting enthalpy (5 J g^{-1}) in comparison with the sample M_D (with SC). Conversely, when the drying temperature was higher (for example 60 °C) for samples M_E and M_F, T_{max} and melting enthalpies of SAC were similar or very slightly different. As we observed previously for quaternary mixtures obtained after SD at 150 or 180 °C, the second small exothermic peak at about 200 °C appeared only for quaternary samples with HPMC (M_C and M_F), and not for samples with SC (M_D and M_E). The decomposition endothermic peak of SAC was always denticulate in HPMC-containing mixtures.

FTIR spectroscopy

FTIR spectra obtained from studied mixtures with SC (M_A, M_D, M_E) or with HPMC (M_B, M_C, M_F), obtained by SD, or FD, or after heating at 60 °C, respectively, were similar. Pure SAC heated at various temperatures such as 65, 120 and 150 °C, gave similar spectra than those of non heated sample, except for a SAC sample heated at 170 °C, where a very slight decreasing at 3555 cm^{-1} and a very slight increasing at 634 cm^{-1} due to oxidation were observed. FTIR spectrometry could not be sufficiently sensible, in our case, to explain a possible interaction between the different components or an antioxidant activity of SEOH.

Table 5 DSC results of ternary mixtures

Ternary mixtures and used techniques	1st endo peak (T in °C) (ΔH in J g^{-1})			T_g (in °C)	2nd endo peak (T in °C) (ΔH in J g^{-1})		3rd endo peak (T in °C)
	T_{max}	T_{onset}	ΔH		T_{max}	ΔH	T_{max}
M$_N$ Grinding (SAC-Labrafil®-HPMC)	–	–	–	136.7	183.4	−130	ND
M$_O$ Grinding (SAC-Labrafil®-SC)	–	–	–	137.7	184.1	−116	ND
M$_P$ Grinding (SAC-SEOH-HPMC)	62.8	61.8	−0.7	136.9	174.8	−118	204.6
M$_Q$ Grinding (SAC-SEOH-SC)	62.8	61.8	−0.9	136.8	183.5	−127	206.1
M$_R$ H$_{60 °C}$ (SAC-Labrafil®-SC)	–	–	–	–	186.9	−107	ND
M$_S$ H$_{60 °C}$ (SAC-Labrafil$^{(R)}$-HPMC)	–	–	–	–	181.1	−95	ND

T_g = glass transition temperature; ND = not determined; endo = endothermic; "–" means not present; T_{onset} is not determined for 2nd and 3rd endothermic peaks
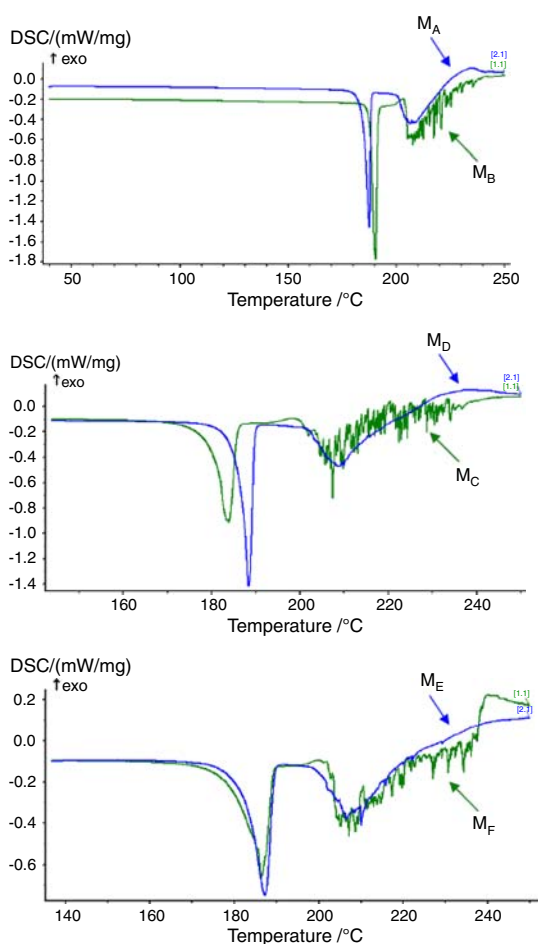

Fig. 5 DSC curves of quaternary mixtures. Compositions of mixtures M$_A$–M$_F$ are in Table 1

DPPH reducing activity of emulsions

DPPH is a stable nitrogen-centered free radical strongly absorbing at 517 nm. Reduction of DPPH into its diamagnetic parent hydrazine leads to a loss of absorption, the monitoring of which has been extensively used as an index of the extent of free radical scavenging activity of antioxidants [53]. Methanolic solutions (0.01–0.06 mM) from SEOH alone stored at 2–5 °C reduced a 0.13 mM DPPH solution dose-dependently (Fig. 6, line SEOH). When SEOH was pre-heated at 150 °C for 20 min, its reducing capacity toward DPPH was totally inhibited (Fig. 6, line SEOH$_{150 °C}$). When stored at room temperature, dry emulsions displayed better SEOH concentration-dependent DPPH reducing profiles than that of SEOH if SD or FD (Fig. 6a, b, respectively), but not other preparation techniques, were applied (Fig. 6c–e). Despite they contain more than 30-fold SEOH, binary mixtures (M$_J$ and M$_K$) were less effective than ternary mixtures (M$_P$ and M$_Q$), i.e. that containing SAC (Fig. 6d). The improvement in DPPH reducing power was particularly significant for the SD-prepared M$_A$ emulsion where SC was the excipient ($P < 0.05$ versus SEOH alone).

The ability of the SD- and FD-based dry emulsion process to potentiate SEOH antioxidant activity is even more striking when considering the strong DPPH reducing behaviour of pre-heated (150 °C for 20 min) emulsion. For SD-based emulsions, the SC-containing one M$_{A-150 °C}$ showed a reducing activity similar to that of the non-heated, HPMC-containing M$_B$ emulsion or the 2-5 °C-stored SEOH alone (Fig. 6a). Whereas the results were better for both SC emulsions, pre-heated, HPMC-containing emulsion M$_{B-150 °C}$ and M$_{C-150 °C}$ showed a significantly better reducing power than SEOH$_{150 °C}$ ($P < 0.01$; Fig. 6a, b). This protection of SEOH activity against pre-heating was common to all preparation techniques (Fig. 6a–c, e), with the exception of grinding which yielded a total loss of DPPH reducing activity of binary (M$_{J-150 °C}$ and M$_{K-150 °C}$) and ternary (M$_{P-150 °C}$ and M$_{Q-150 °C}$) mixture (Fig. 6d).

Regarding the DPPH reducing capacity, incorporating a high Labrafil® concentration instead SAC and SC or HPMC excipients did not improve the results at ambient temperature (mixture M$_I$, see Fig. 6e) as compared to

Table 6 DSC results of quaternary mixtures after desiccation

Mixture	Used technique	1st endothermic peak (T in °C) (ΔH in J g^{-1})			2nd exothermic peak (T in °C) (ΔH in J g^{-1})			3rd endothermic peak (T in °C) (ΔH in J g^{-1})		
		T_{max}	T_{onset}	ΔH	T_{max}	T_{onset}	ΔH	T_{max}	T_{onset}	ΔH
M_A	SD$_{150\ °C}$	187.9	185.1	−108	–	–	–	207.9	200.3	−145
M_B	SD$_{180\ °C}$	190.2	188.2	−115	203.1	199.5	+8.0	219.2[a]	205.0[a]	ND
M_D	FD$_{-63\ °C}$	188.5	186.9	−112	–	–	–	209.1	189.7	−145
M_C	FD$_{-63\ °C}$	184.0	180.9	−107	198.4	193.9	+5.7	207.5	207.2	ND
M_E	H$_{60\ °C}$	187.3	182.2	−100	–	–	–	210.1	ND	ND
M_F	H$_{60\ °C}$	186.4	184.8	−100	200.1	195.7	+4.0	207.2	ND	ND

ND = not determined; "–"means not present)

[a] The T values of the 3rd peak of sample M_B are not given with a high level of accuracy because it is denticulate

Fig. 6 DPPH reducing activity
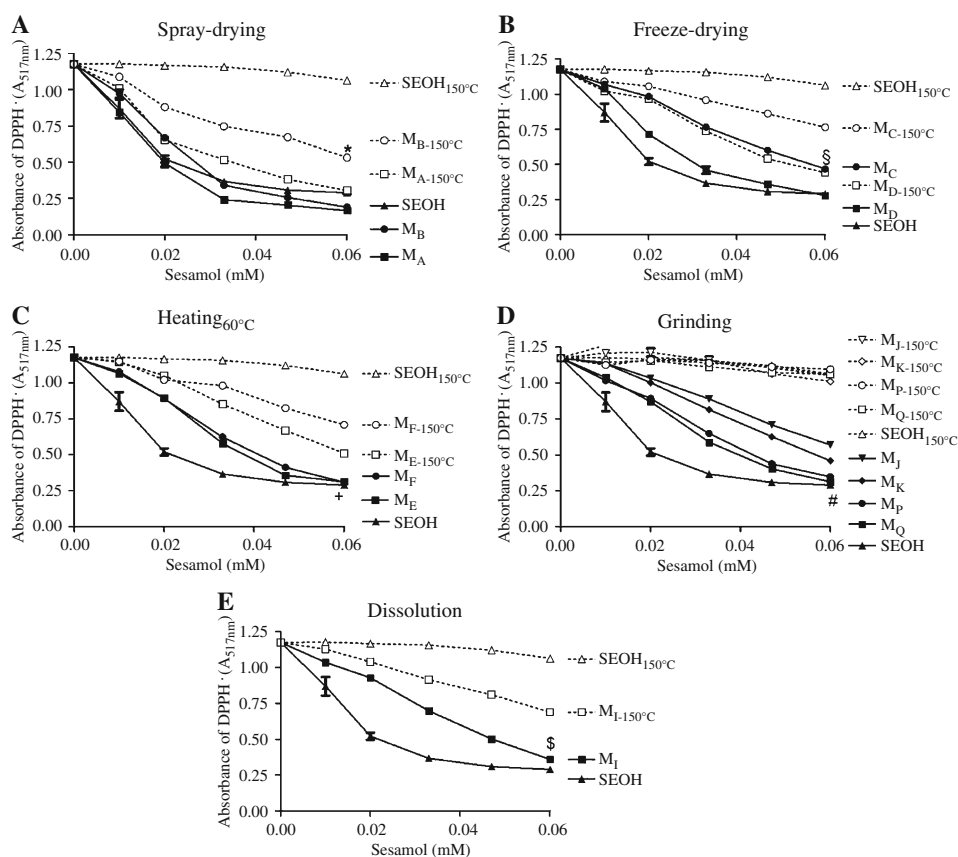
ternary (M_P or M_Q) or quaternary (M_A–M_D) mixtures while it was necessary to preserve the SEOH antioxidant activity in pre-heated preparation ($P < 0.01$ for $M_{I\text{-}150\ °C}$ versus $M_{J\text{-}150\ °C}$, $M_{K\text{-}150\ °C}$, $M_{P\text{-}150\ °C}$ and $M_{Q\text{-}150\ °C}$; Fig. 6d, e).

Conclusions

Earlier, it was shown the best formulation of SEOH in combination with SAC, Labrafil®, and HMPC or SC

microcapsules by SD or SF techniques with a good antioxidant activity [34]. SEOH alone heated at 150 °C losses its antioxidant activity but in quaternary mixture its activity was maintained.

By SEM, analyses of dry emulsions of quaternary mixtures have shown the best morphology. Thermal analysis (mainly DSC) could be used in the interaction study between drugs and excipients [54–57] and thermo oxidative degradation of drugs [58–61]. Our results showed that the combination of SAC, SEOH, Labrafil®, with SC (M_A-

$_{150~°C}$) or with HPMC (M$_{B-150~°C}$) heated at 150 °C, had a good antioxidant activity. In else, the combination with SC (M$_A$) would be better than those with HPMC (M$_B$). The DPPH radical technique confirmed the antioxidant activity study by chemical way. Formulation prepared with SC and HPMC by the SD technique at 150 °C (M$_A$) and 180 °C (M$_B$), respectively, presented similarly behaviours. However, when the same formulations were subjected to storage at 150 °C for 20 min, M$_{A-150~°C}$ and M$_{B-150~°C}$, respectively, a better protection of the active was observed for the formulation containing SC. Uses of SC, in technical applications, are based on the isoelectric characteristics, the contents of carboxyl- and sulfur-containing groups. The use of HPMC also gained considerable interest thanks to their polymeric property. The sterical structures and general physical properties of hydrocolloids-saccharose systems are largely determined by the present water molecules and the hydrogen-bond network formed by them, and stabilized by the temperature [62].

We could suggest a specific physical and/or chemical combination (such as hydrogen bond) sufficiently stable, even at about 150 °C, when the technique used for dry emulsion preparation was SD. After water dilution, this dry emulsion, mainly with SAC, Labrafil®, SEOH and SC, will regenerate perfectly the initial liquid emulsion with the same characteristics that before drying.

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