ELSEVIER

International Journal of Pharmaceutics 171 (1998) 217–226

international journal of pharmaceutics

Proanthocyanidin microcapsules: preparation, properties and free radical scavenging activity

M.-C. Andry [a], H. Vezin [b], I. Dumistracel [a], J.L. Bernier [b], M.-C. Lévy [a,]*

[a] *Laboratoire de Pharmacotechnie, UPRES-A/CNRS 6013, Faculté de Pharmacie, Université de Reims, 51 Rue Cognacq-Jay, Reims Cedex F-51096, France*

[b] *Laboratoire de Chimie Organique Physique, URA/CNRS 351, Université de Lille 1, Bâtiment C3, Villeneuve d'Ascq Cedex 59655, France*

Received 18 February 1998; received in revised form 1 May 1998; accepted 27 May 1998

Abstract

Microcapsules were prepared by interfacial cross-linking of grape proanthocyanidin (GPO) with terephthaloyl chloride (TC). Using 5% TC, 30-min reaction time and a stirring speed of 5000 rpm, batches were prepared from 10% GPO solutions in buffers pH 8, 9.8, and 11, respectively. Microcapsules were studied with respect to morphology (microscopy), size (laser diffraction technique), stability in water at 45°C and, for stable batches, IR spectroscopy, degradation in human plasma, and free radical scavenging activity studied by electron paramagnetic resonance (EPR) using both extinction of the stable radical DPPH (2,2-diphenyl-picryl-hydrazyl), and scavenging of OH° (evaluated using 5,5-dimethyl-pyrrolidine-*N*-oxide; DMPO). All particles had a mean size <10 μm. Microcapsules obtained at pH 8 were unstable (yellow supernatant after 8 days). Microcapsules obtained at pH 9.8 and 11 exhibited a high stability (>5 months). Using a stirring speed of 3000 rpm at pH 11 provided 15-μm particles which gave a yellow supernatant after 3 weeks. The IR spectra of the two stable batches (pH 9.8 and 11) showed bands at 1736 and 1267 cm^{-1} reflecting the formation of esters. They were slowly degraded in plasma, and although less active than the initial GPO, they exhibited a significant antioxidant activity. The method appears to be an efficient means of stabilizing the polyphenol without suppressing the free radical scavenging activity. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Microcapsule; Proanthocyanidin; Interfacial cross-linking; Terephthaloyl chloride; Free radical scavenging activity; EPR

1. Introduction

Proanthocyanidins are oligomers and polymers of polyhydroxy flavan-3-ol units, which are nota-

* Corresponding author. Tel.: +33 3 2605 3583; fax: +33 3 2605 3744.

0378-5173/98/$ - see front matter © 1998 Elsevier Science B.V. All rights reserved.
PII: S0378-5173(98)00183-5

bly extracted from grape (*Vitis vinifera*) seeds (Da Silva et al., 1991). These plant polyphenols belong to the group of flavonoids (Harborne, 1988), which are known to possess numerous interesting biological properties (Huang et al., 1992; Middleton and Kandaswami, 1994). Some of these properties are attributed to their free radical scavenging activity (Bors and Saran, 1987), which exerts against $0_2^{-\circ}$ superoxide anions (Sichel et al., 1991), as well as against OH° (Husain et al., 1987), RCOO° and RO° radicals (Aruoma, 1991). It would be interesting to incorporate proanthocyanidin oligomers extracted from grape seeds (GPO) in topical preparations to be used either as a cosmetic application to prevent actinic ageing, or in dermatology for their anti-inflammatory, anti-allergic, anti-bacterial or anti-viral properties. Unfortunately, incorporation of GPO in topical formulations results in instability on storage leading to the progressive appearance of a brown colour as a result of oxidation and condensation reactions involving the numerous phenolic groups of the oligomers.

Looking for a simple means of providing a stable form of GPO, we explored a novel approach which was to prepare microcapsules by interfacial cross-linking of the GPO with an acid dichloride in order to block some of the phenol groups and thereby to reduce or suppress these degradation reactions. It is known that polymers can be obtained by interfacial polycondensation of diphenols like bisphenol A with an acid dichloride which forms ester bonds (Eareckson, 1959; Suzuki et al., 1968).

In continuation of our work dealing with microencapsulation by interfacial cross-linking of polysaccharides (Lévy and Andry, 1990), or proteins (Edwards-Lévy et al., 1994; Lévy et al., 1994, 1995), we chose terephthaloyl chloride (TC) as the cross-linking reagent. The purpose of the study was to determine the reaction conditions providing a stable form of GPO. The excess of polyphenols in the aqueous phase was expected to be eliminated by an appropriate washing step, thus avoiding any risk of diffusion of oligomers in the surrounding medium. It was also anticipated that unreacted hydroxy groups would be present on the cross-linked polyphenol membrane, in sufficient amounts to maintain a free radical scavenging activity.

In the first part of the work, several series of experiments were carried out, varying the reaction pH from 8 to 11. The resulting microcapsules were studied with respect to morphology, size and stability as aqueous suspensions at 45°C. Stable batches were selected and additional studies were performed concerning infra-red spectra and degradability in human plasma. Finally the free radical scavenging activity of the selected microcapsules was determined by electron paramagnetic resonance (EPR) in comparison with original GPO.

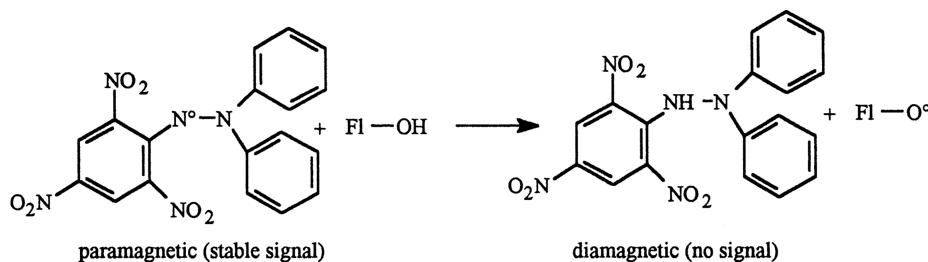
2. Materials and methods

2.1. Preparation of the microcapsules

The proanthocyanidin, from grape seeds, (Leucoselect®) was supplied by Indena (France). TC was purchased from Janssen Chimica, France. Cyclohexane and chloroform (Osi, France) were of analytical grade. The surfactants were sorbitan trioleate and polysorbate (Seppic, France).

In the standard procedure, a 10% (w/v) GPO solution was prepared using a carbonate buffer pH 9.8. The solution (6 ml) was emulsified for 5 min at room temperature in 30 ml of cyclohexane containing 5% (v/v) sorbitan trioleate (Heidolph RGL 500 stirring motor, Prolabo, France; stirring rate 5000 rpm). Then, 40 ml of a 5% (w/v) solution of TC in a chloroform:cyclohexane (1:4, v/v) mixture was added to the emulsion and stirring was continued for 30 min. The reaction was ended by dilution with 80 ml of cyclohexane. Then, the resulting microcapsules were separated, and washed successively with cyclohexane, with 95% ethanol containing 2% polysorbate, with 95% ethanol and with distilled water. The microcapsules were finally frozen and lyophilized.

Variations were introduced in the standard procedure. Two other series of experiments were conducted using a phosphate buffer pH 8 or a carbonate buffer pH 11, respectively. An additional assay was conducted at pH 11 with a lower stirring speed (3000 rpm).



Scheme 1. Reaction of flavonoid scavenging activity using DPPH.

2.2. Microcapsule characterization

The microcapsule morphology was studied by optical and scanning electron microscopy (SEM). All particles were sized by a laser diffraction technique (Coulter Particle Sizer, type LS 200, Coultronics, France). Size distributions were displayed in terms of volume versus particle size. For each type of microcapsules, two batches were analyzed.

2.3. Stability assays

A batch of freshly prepared microcapsules was dispersed in 50 ml of distilled water and the suspension was stored at 45°C. Controls were performed at intervals concerning the colour of the sediment and of the supernatant. The integrity of the microcapsules was verified by microscopic examination.

2.4. Infrared spectroscopy

IR spectra were measured by the KBr method using a Bomem DA3 Fourier transform IR spectrometer.

2.5. Microcapsule biodegradability

Freshly prepared microcapsules (100 mg) were introduced in a test tube containing human plasma (10 ml). The tube was incubated at 37°C and a magnetic stirring was started. Microcapsule degradation was followed by microscopic examination.

2.6. Free radical scavenging activity

The free radical scavenging activity of microcapsules was determined by EPR using two techniques based on extinction of the stable radical DPPH (2,2-diphenyl-picryl-hydrazyl), and inhibition of OH° production, respectively. In the two series of experiments, control assays were conducted with the original GPO.

2.6.1. Electron spin resonance measurements

EPR spectra were recorded with a Varian (USA) E-109 spectrometer operating at 9.5 GHz with a 100-KHz high frequency modulation amplitude range of 1 G. The sample solutions were examined in a 4-mm flat quartz cell inserted in the cavity operating in the TE_{102} mode.

2.6.2. Extinction of DPPH

Free radical scavenging activity was assayed by EPR using the stable radical DPPH, purchased from Sigma. DPPH was dissolved in ethanol to give a 5×10^{-1} M solution. The quenching of the EPR signal of this stable radical at various concentrations by the tested compounds, was evaluated after 1 min of reaction, and for microcapsules after 1-h incubation (in triplicate). The bleaching of the EPR signal occurs when the odd electron of the stable radical is paired; thus the test is representative of the capacity of flavonoids (Fl-OH) to scavenge stable organic radicals (Scheme 1).

2.6.3. Inhibition of OH° production

Another test consists of inhibition of OH° production. It is generally accepted that production of active oxygen metabolites is one of the major

processes involved in oxidative stress. Hydroxyl radical (OH°) produced by hydrogen peroxide in the presence of trace amounts of metal (Fenton reaction) is responsible for most of the cell damage. Formation of unstable free radicals such as OH° can be evidenced by spin-trapping experiments: they can react with diamagnetic nitrones, generating long-lived nitroxide free radicals which are detectable by EPR spectroscopy. The hyperfine splitting of spin-trapped adduct allows identification of the original free radical species.

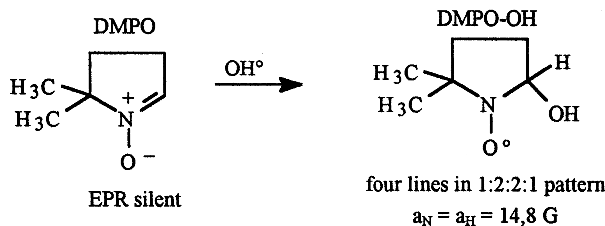
The ability of the test compounds to act as OH° scavengers was evaluated using 5,5-dimethyl-pyrrolidine-*N*-oxide (DMPO) (purchased from Sigma), as trapping agent (Scheme 2). The extinction of the DMPO-OH signal was studied in the presence of the tested compounds.

The DMPO was purified by distillation and the solution of DMPO checked for absence of any EPR signal. Reaction mixtures of 1 ml contained DMPO (5 mM in PBS buffer containing 10 mM DETAPAC, pH 7), the tested compound (1 mg/ml or 2 mg/ml), H_2O_2 (0.1 mM), and the Fenton reaction was initiated by addition of $FeSO_4$ (0.1 mM). EPR spectra were recorded after 1 min of reaction. Experiments were run out in triplicate.

3. Results and discussion

3.1. Influence of reaction pH on the morphology and size of microcapsules

The standard procedure (pH 9.8) provided a beige sediment in distilled water. Spherical particles with a granulous aspect and a thin membrane were observed by light microscopy (Fig. 1a).



Scheme 2. Generation of spin trap adduct as a consequence of the interaction between hydroxyl radical with DMPO.

Some aggregates were present. The mean size was under 10 μm (Table 1). The microcapsules were intact after lyophilization giving beige powders. Microcapsules recovered their spherical shape after rehydration. Examination by SEM showed collapsed particles with a smooth membrane, on the one hand, and more irregular and granulous particles on the other hand (Fig. 2a).

When the reaction pH decreased to 8, an orange sediment was obtained. Examination by light microscopy showed numerous aggregates of microcapsules with a thin membrane and clear contents. Because of these aggregates, the mean size could not be determined with the Coulter particle sizer, as was done with the other batches. Evaluation by optical microscopy gave the size range 2–10 μm. After lyophilization, numerous microcapsules appeared to be burst open.

Raising the reaction pH to 11 improved the results. A beige sediment was obtained in distilled water, which was spherical particles with a distinct membrane and a clear content (Fig. 1b). The mean size was comparable to that of the microcapsules obtained at pH 9.8, although slightly lower (Table 1). Lyophilization provided a beige powder, the particles of which could be rehydrated giving spherical vesicles. Examination by SEM mostly showed collapsed particles with a uniform shape and a smooth membrane (Fig. 2b).

Keeping the reaction pH at 11, and using a lower stirring speed (3000 rpm instead of 5000 rpm) did not result in significant changes in the morphological characteristics of the microcapsules, except that the mean size was higher, as was expected (Table 1).

It should be stressed that the other reaction parameters, namely TC concentration (5%) and time (30 min) were kept constant in the study. We have shown in previous works that these conditions allowed maximal acylation of functional groups of proteins (Edwards-Lévy et al., 1994; Lévy et al., 1994, 1995) and polysaccharides (Lévy and Andry, 1990).

3.2. Stability of the microcapsules

With the microcapsules prepared at pH 8, the colour of the supernatant was pale yellow after 7 days at 45°C, and became progressively more

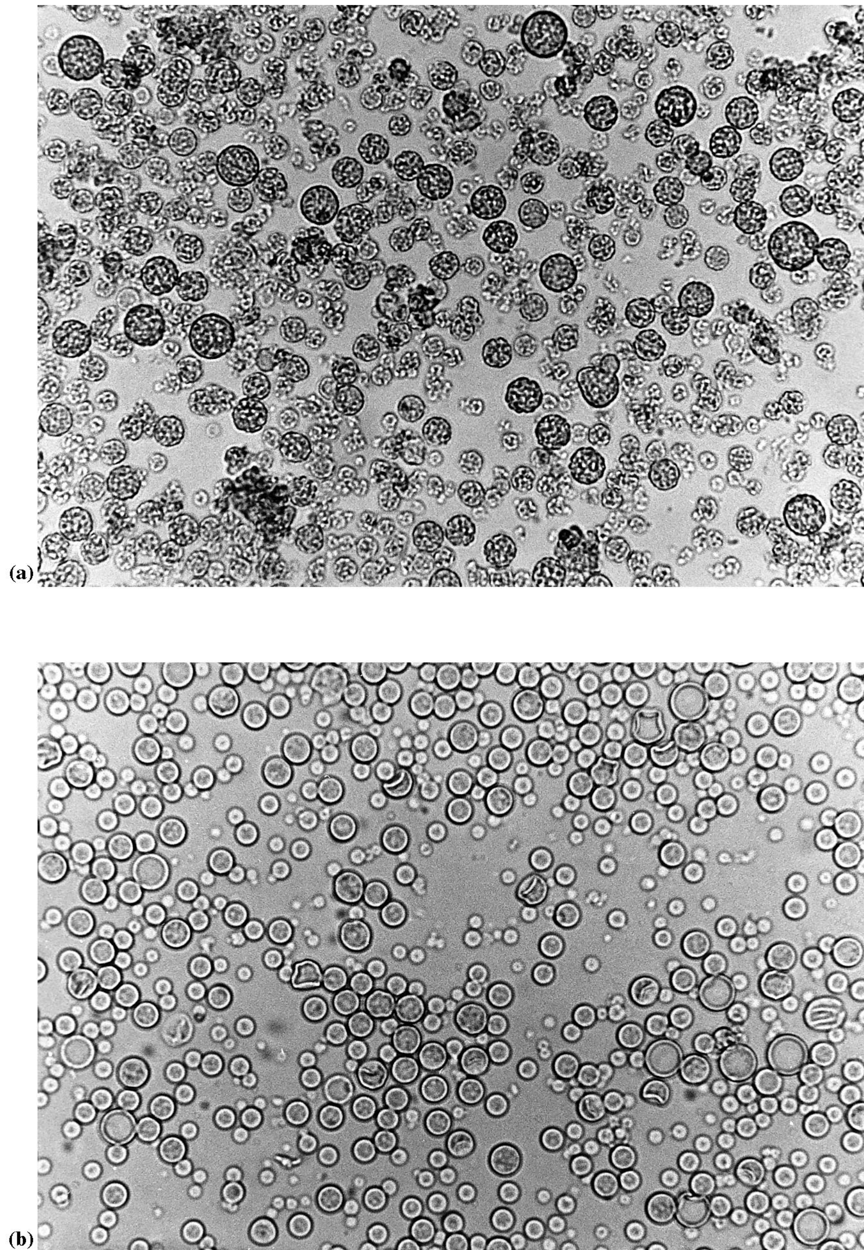


Fig. 1. Optical photomicrographs of proanthocyanidin microcapsules: (a) prepared at pH 9.8; (b) prepared at pH 11 (same magnification).

intense. The colour of the sediment also became progressively darker, although the microcapsules kept their spherical shape, as shown by microscopic examination.

In contrast, the batches prepared at pH 9.8 and 11 using a stirring speed of 5000 rpm exhibited a remarkable stability at 45°C. No change in the colour of the supernatant was observed after 5

Table 1
Microcapsule size as a function of reaction pH and stirring speed

Reaction pH	Stirring speed (rpm)	Geometric volume mean diameter (μm)	Geometric S.D.
9.8	5000	7.35	1.41
		7.21	1.37
11	5000	6.70	1.28
		6.73	1.37
11	3000	13.52	1.49
		13.79	1.41

months, while the microcapsules remained intact and kept their beige colour.

However, when the size of the microcapsules increased to 15 μm, which was obtained at pH 11 using a stirring speed of 3000 rpm, a slight colouration of the supernatant appeared after 3 weeks, becoming progressively pale yellow after 2 months.

These results are in good agreement with the effect of reaction conditions on the degree of cross-linking of the membrane.

Fragile and unstable microcapsules were obtained at pH 8, due to a relatively low amount of hydroxy groups being involved in the membrane. Oxidation of the cross-linked oligomers occurred, leading to a change in the colour of the microcapsules and release in the medium.

In comparison, it should be stressed that microcapsules with solid membranes were obtained from proteins using similar pH conditions (Edwards-Lévy et al., 1994). This difference is attributed to the reactivity of the amine group in the interfacial polycondensation, which is considerably higher than that of the phenolic hydroxy group (Morgan and Kwolek, 1959; Panayotov, 1977). Therefore alkaline pH conditions are required for the preparation of polyphenyl esters (Eareckson, 1959; Tsuda, 1977).

Increasing the reaction pH resulted in an intensified acylation of OH groups giving strong membranes with a high stability. This was observed at pH 9.8 as well as at pH 11 using a rapid stirring rate (5000 rpm). Microcapsules survived the storage without any change in their own colour, and without any leakage of coloured products. However, decreasing the stirring speed to 3000 rpm at pH 11 provided larger microcapsules which became unstable on storage. It should be noted that, in this method, microcapsule size is determined by the size of the droplets of the initial emulsion. Reduction of the droplet size is then assumed to favour polycondensation by increasing the interfacial area. Therefore, most assays were performed using a high stirring speed, in order to attach a large amount of oligomers by numerous ester bonds. Increasing microcapsule size by lowering the stirring rate presumably resulted in the presence of an excess of GPO inside the microcapsules, which may be incompletely eliminated by the washing step and thus released in water at 45°C.

To summarize, two stable batches were obtained using a stirring speed of 5000 rpm, at pH 9.8 and 11, respectively. Although the microcapsules pH 11 appeared the most satisfactory (with respect to the microscopical aspect), it was decided to keep both batches for further studies, the microcapsules prepared at pH 9.8 being expected to have a higher free radical scavenging activity due to a higher number of free hydroxy groups in the cross-linked polymer membrane.

3.3. FT-IR spectra

The spectra of the microcapsules prepared at pH 9.8 and 11 were compared with the spectrum of original GPO in Fig. 3. Microcapsule spectra were comparable, showing bands at 1736 and 1267 cm^{-1}, which reflect the formation of esters from hydroxy groups of GPO.

3.4. Degradation in human plasma

Microcapsules prepared at pH 9.8 were slowly degraded in human plasma. A fraction appeared to be open after 4 h, and small fragments were

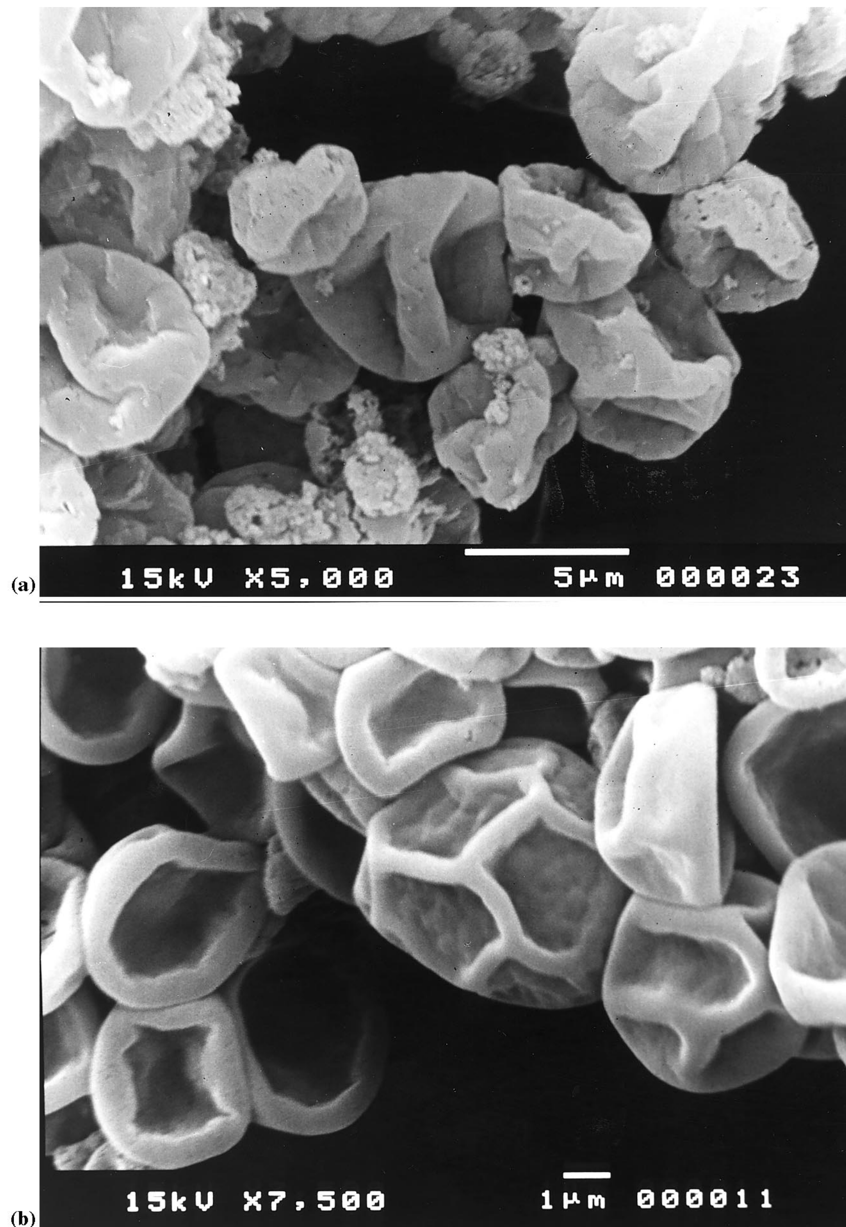


Fig. 2. Scanning electron micrographs of proanthocyanidin microcapsules: (a) prepared at pH 9.8; (b) prepared at pH 11.

observed after 6 h. In the case of microcapsules obtained at pH 11, degradation was more delayed, starting only after 6 h. This experiment shows that the microcapsules are sensitive to plasma esterases, and suggests that the degree of cross-linking of microcapsules pH 11 would be slightly higher than in microcapsules pH 9.8.

3.5. Free radical scavenging activity

3.5.1. Extinction of DPPH

In the absence of the tested compounds, the reference signal (Fig. 4a; 5×10^{-1} M) is characteristic of a highly concentrated DPPH spectrum due to spin-spin interactions. In the presence of

the starting polymer (1 mg/ml), a strong decrease in the signal could be observed (73%; Fig. 4b). At a 10^{-2}-M concentration of DPPH (Fig. 4c), the starting polymer induced a stable decrease (95%) after 1 min (Fig. 4d). No significant modification could be found in the presence of both types of microcapsules at these concentrations. At lower concentrations of DPPH (5×10^{-3} M; Fig. 4e), the spectrum evolved (no more spin-spin interactions). The starting polymer induced a dramatic decrease after 1 min (Fig. 4f). Both types of microcapsules exhibited a scavenging activity after 1 min, with a decrease of 36 and 22% for pH 11 and 9.8, respectively (Fig. 5b,c), which was more visible after 1 h (decrease of 68 and 64%, respectively; Fig. 5d,e). This increase in antioxidant activity observed after 1 h might be accounted for by the slow penetration of DPPH inside the cross-linked polymer.

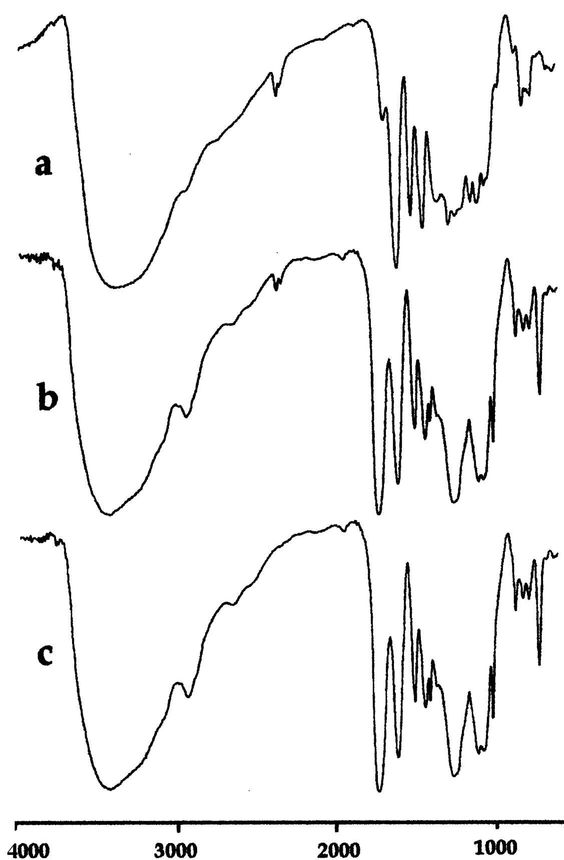


Fig. 3. FT-IR spectra: (a) original proanthocyanidin; (b) proanthocyanidin microcapsules prepared at pH 9.8; (c) microcapsules prepared at pH 11.

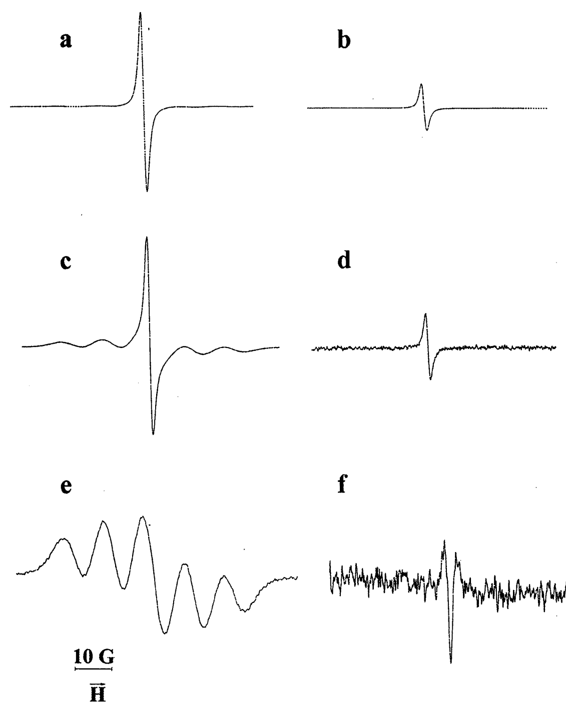


Fig. 4. Spectra of DPPH for the reference solution (a,c,e), at concentrations of respectively $5 \cdot 10^{-1}$ M, 10^{-2} M and $5 \cdot 10^{-3}$ M; the receiver gain levels were respectively 10^2, $5 \cdot 10^2$ and 10^4. Spectra of DPPH quenching experiments (b,d,f) with the starting polymer at the concentration of 1 mg/ml, the receiver gain levels were respectively 10^2, $5 \cdot 10^3$ and $1.25 \cdot 10^5$.

It can be concluded that the starting procyanidolic oligomers are highly active in this test, and that the microcapsules retain a good scavenging activity, attested by the ability to bleaching the EPR signal at lower concentrations of DPPH.

3.5.2. Inhibition of OH° production

In the absence of the tested compounds, the reference system gave a prominent characteristic EPR spectrum of DMPO-OH consisting of four main lines (Fig. 6a). In the presence of the starting polymer (1 mg/ml), a decrease in the DMPO-OH signal (63%) could be observed, attesting for the OH° scavenging activity of the starting polymer before reticulation (Fig. 6b). Microcapsules pH

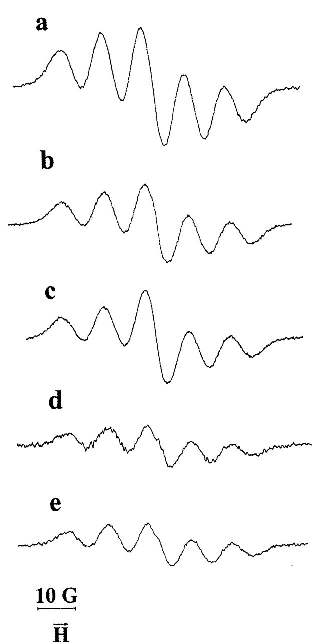


Fig. 5. Spectra of $5 \cdot 10^{-3}$ M DPPH (a, reference spectrum) quenching experiments by microcapsules, respectively pH 11 and 9.8 (b,c), at the concentration of 1 mg/ml, after 1 min. Spectra d and e correspond to b and c after 1-h incubation. The receiver gain level was 10^4.

9.8 induced a decrease of the EPR signal, proportional to the concentration (28 and 63% for 1- and 2-mg/ml concentrations, respectively; Fig. 6c,d). The same proportional decrease was observed with microcapsules pH 11 (46 and 63% for 1- and 2-mg/ml concentrations, respectively; Fig. 6e,f).

Inhibition of OH° by microcapsules was obviously less dramatic than with the starting material before reticulation, but it can be assessed that a major part of the OH° scavenging properties was retained.

As in the first test, a comparable activity was found for both types of microcapsules. Accordingly, although differences were observed between the two types of microcapsules concerning morphology and biodegradability, the antioxidant properties were in the same range. Studies of the evolution of free radical scavenging activity as a function of time would perhaps reveal differences in the kinetics of the phenomenon.

4. Conclusion

The objective of the work was to prepare a stable form of proanthocyandins without suppressing their free radical scavenging activity.

We chose a microencapsulation method based on interfacial cross-linking with terephthaloyl chloride, using the proanthocyanidins as the membrane-forming material.

In a series of experiments, variations of the reaction pH allowed selection of two pH values, pH 9.8 and 11, for the preparation of the initial aqueous phase. The resulting microcapsules exhibited a high stability at 45°C suspended in water. However, in addition to pH, another condition for stability is a high stirring speed during the emulsification and polycondensation steps, a

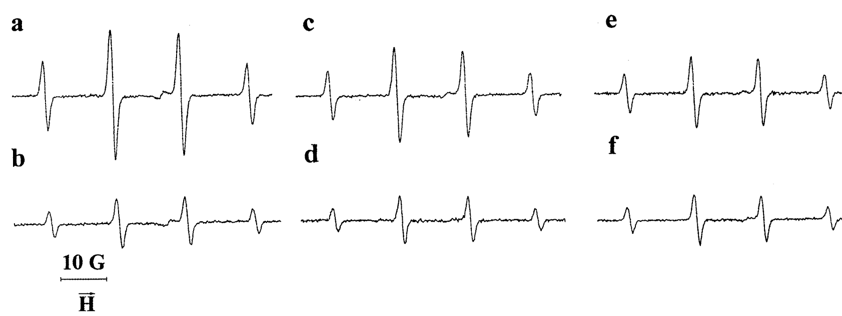


Fig. 6. Scavenging experiments of OH° with spectra of DMPO-OH spin adduct: (a) Fenton reaction; (b) addition of 1 mg/ml of starting polymer; (c,d) addition of microcapsules pH 9.8 at two concentrations (1 and 2 mg/ml, respectively); (e,f) addition of microcapsules pH 11 at two concentrations (1 and 2 mg/ml, respectively). Receiver gain level was $8 \cdot 10^3$.

small size of the dispersed droplets (< 10 μm) ensuring an intensified acylation due to the large interfacial area.

The antioxidant properties of both types of microcapsules (pH 9.8 and 11) were studied by EPR using two different techniques: the bleaching of the stable radical DPPH and the scavenging of OH°. In both experiments the starting polymer was found to be highly active and the microcapsules, although less active, exhibited a significant antioxidant activity. Furthermore, the experiments of biodegradation in plasma suggest a possible increase of the antioxidant properties in vivo as a result of the release of GPO by esterases.

Cross-linked proanthocyanidin microcapsules appear to be a simple means to provide a stable form of polyphenols with preserved activity.

Acknowledgements

We thank Doctor Daniel Royer of the UPRES-A/CNRS 6013 for kindly measuring the FT-IR spectra.

References

Aruoma, O.I., 1991. Pro-oxidant properties: an important consideration for food additives and/or nutrient components. In: Aruoma, O.I., Halliwell, B. (Eds.), Free Radicals and Food Additives. Taylor and Francis, London, pp. 173–194.

Bors, W., Saran, M., 1987. Radical scavenging by flavonoid antioxidants. Free Radic. Res. Commun. 2, 289–294.

Da Silva, J.R., Rigaud, J., Cheynier, V., Cheminat, A., Moutounet, M., 1991. Procyanidin dimers and trimers from grape seeds. Phytochemistry 30, 1259–1264.

Eareckson, W.M., 1959. Interfacial polycondensation. X. Polyphenylesters. J. Polymer Sci. 40, 399–406.

Edwards-Lévy, F., Andry, M.-C., Lévy, M.-C., 1994. Determination of free amino group content of serum albumin microcapsules: II. Effect of variations in reaction time and in terephthaloyl chloride concentration. Int. J. Pharm. 103, 253–257.

Harborne, J.B. (Ed.), 1988. The Flavonoids, Advances in Research Since 1980. Chapman and Hall, London.

Huang, M.T., Ho, C.T., Lee, C.Y. (Eds.), 1992. Phenolic Compounds in Food and Their Effect on Health II. Antioxidants and Cancer Prevention. ACS Symposium Series 507. American Chemical Society (ACS), Washington, DC.

Husain, S.R., Cillard, J., Cillard, P., 1987. Hydroxyl radical scavenging activity of flavonoids. Phytochemistry 26, 2489–2491.

Lévy, M.-C., Andry, M.-C., 1990. Microcapsules prepared through interfacial cross-linking of starch derivatives. Int. J. Pharm. 62, 27–35.

Lévy, M.-C., Lefèbvre, S., Andry, M.-C., Rahmouni, M., Manfait, M., 1994. Fourier transform infrared spectroscopic studies of human serum albumin microcapsules. 2. Influence of reaction time on spectra and correlation with microcapsule morphology and size. J. Pharm. Sci. 83, 419–422.

Lévy, M.-C., Lefèbvre, S., Andry, M.-C., Manfait, M., 1995. Fourier transform infrared spectroscopic studies of human serum albumin microcapsules. 3. Influence of terephthaloyl chloride concentration on spectra and correlation with microcapsule morphology and size. J. Pharm. Sci. 84, 161–165.

Middleton, E. Jr., Kandaswami, C., 1994. The impact of plant flavonoids on mammalian biology: implication for immunity, inflammation and cancer. In: Hartborne, J.B. (Ed.), The Flavonoids, Advances Since 1986, Chapman and Hall, London, pp. 619–652.

Morgan, P.W., Kwolek, S.L., 1959. Interfacial polycondensation. II. Fundamentals of polymer formation at liquid interfaces. J. Polymer Sci. 40, 299–327.

Panayotov, I.M., 1977. Polyesteramides. In: Millich, F., Carraher, C.E. Jr. (Eds.), Interfacial Synthesis, vol. II, Polymer Applications and Technology. Marcel Dekker, New York, pp. 209–229.

Sichel, G., Corsaro, C., Scalia, M., Di Bilio, A.J., Bonomo, R., 1991. In vitro scavenger activity of some flavonoids and melatonins against $0_2^{-\circ}$. Free Radic. Biol. Med. 11, 1–8.

Suzuki, S., Kondo, T., Mason, S.G., 1968. Studies on microcapsules. I. Preparation of polyurethane and polyphenolester microcapsules. Chem. Pharm. Bull. 6, 1629–1631.

Tsuda, M., 1977. Interfacial modification of polyvinylalcohol and related polymers. In: Millich, F., Carraher, C.E. Jr. (Eds.), Interfacial Synthesis, vol. II, Polymer Applications and Technology. Marcel Dekker, New York, pp. 209–229.