Development and characterization of natural antioxidant-containing chitosan nanoparticles

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INTRODUCTION AND OBJECTIVES

The use of nanotechnology in medical sciences is an innovation that promises a new age of health. Among the different approaches explored so far, chitosan exhibits favourable and unique biological properties, such as biocompatibility, biodegradability, non-antigenic, non-toxicity and mucoadhesiveness.

Natural extracts rich in antioxidant molecules have been incorporated in chitosan films, or macro/microparticles, becoming more effective as improving the antioxidant protection [da Silva 2010].
In the present study, chitosan nanoparticles with extracts of sage and savoury and rosmarinic acid were prepared and characterized in order to ensure their best size, efficient encapsulation and to test the retention of the active compounds and evaluate their controlled release performance. This work proposes for the first time in literature, a simultaneous HPLC method for the determination and quantification of phenolic and flavonoid compounds, namely quercetin and rosmarinic acid encapsulated into chitosan nanoparticles. The method can be used to determine the loading capacity and association efficiency as well as its *in vitro* release.

MATERIALS AND METHODS

Encapsulation of sage, savoury and rosmarinic acid into chitosan-nanoparticles

The addition of 1% extracts aqueous solution and a 1% of aqueous rosmarinic solution was added to chitosan previously dissolved in acetic acid at a pH value adjusted to 5.8, in different volumes in order to guarantee the best theoretical loadings between chitosan and the different compounds (5%, 10%, 15%, 20%, 30%, 40% and 50%) fairly to the initial concentration of chitosan (2mg/mL). Quercetin was not encapsulated in a pure form (isolated) into the nanoparticles due to its poor hydrosolubility which makes the encapsulation difficult to achieve in this type of polyelectrolyte systems. The flavonoid purely was only used for the solutions, for the quantification in the natural extracts (savoury) and consequently for the development of this simultaneous HPLC method.

Nanoparticles characterization

Nanoparticles were characterized considering zeta potential, encapsulation efficiency and *in vitro* release.

Cromatography runs were performed on a RP C18 column, in a gradient mode with a mobile phase comprising methanol:formic acid:water, 92.5:2.5:5 (v/v) at a flow rate of 0.75mL/min. Rosmarinic acid and quercetin were detected by UV spectroscopy at a wavelength of 280nm.

Interaction of natural extracts and rosmarinic acid with nanoparticles by FTIR and DSC

The interaction between natural extracts and rosmarinic acid with chitosan nanoparticles was assessed by differential scanning calorimetry (DSC) and Fourier-transform infrared (FTIR) studies. Unloaded nanoparticles were used as control.

RESULTS AND DISCUSSION

HPLC Validation method

The HPLC method was validated according to the International Conference on Harmonization (ICH) guidelines (2005). The method was shown to be specific, linear in the range of 0.05–1mg/mL (R2 = 1.00), precise at the intra-day and inter-day levels as reflected by the relative standard deviation values (less than 2%), accurate (recovery rate of 90.5±0.6%), and robust to changes in equipment conditions. The rosmarinic acid and quercetin peak retention time were 48.9±0.1 min and 57.9±0.6 min, respectively (Fig 1).

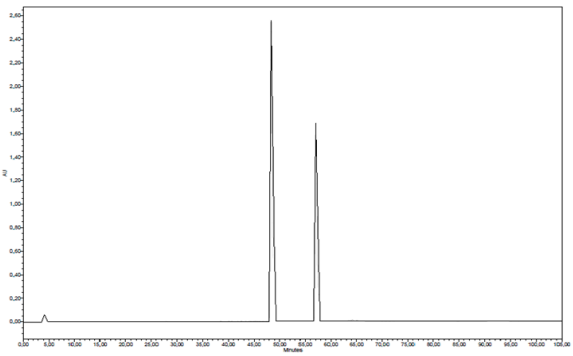

Figure 1. Representative chromatogram of rosmarinic acid and quercetin analysis

Nanoparticles characterization

The increased size and zeta potential are good indicators of the compounds incorporation as well as its stability in time, considering that the antioxidant activity may be affected by this, or proved to be size-dependent according to recently studies with different nanoparticles (Xue 2011).

Thermograms of extract-loaded nanoparticles resulted in shifts in the same unloaded nanoparticle peaks and suggested polyelectrolyte-rosmarinic acid and quercetin interactions after nanoparticle formation. FTIR spectra of extract-loaded nanoparticles revealed the formation of ionic interactions with cumulative absorption peaks of empty nanoparticles and extracts.

In general, most all the nanoparticles under study showed a similar range of diameter (200-300 nm), and zeta potential (20-30) for the same n=3 batches. This represents that the extracts does not interfere in the size or zeta potential of the nanoparticles.

Association efficiency (AE)

The AE shows to be higher in the natural extracts than in rosmarinic acid nanoparticles, savoury nanoparticles shows for all the theoretical loadings a higher association efficiency than sage, as it can be seen in Figure 2. For maximum theoretical loading of 50%, rosmarinic acid, sage and savoury shows the higher association efficiency of 58.2% ± 4.0E-02, 96.0 ± 03 and 98.4E-04, respectively.

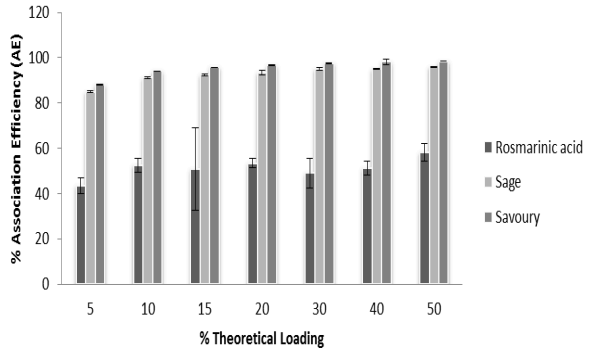

Figure 2. Association efficiency of rosmarinic acid, sage and savoury nanoparticles (50% theorethical loading - 1 mg/mL; n=3)

In vitro release of rosmarinic acid from chitosan nanoparticles

For rosmarinic release studies, loaded nanoparticles obtained after centrifugation were suspended in 10 mL of a phosphate-buffered saline (PBS) solution, pH 7.4 at 30 min., 1h, 2h, 4h, 6h and 24h. The representative rosmarinc release–time profile from chitosan nanoparticles is illustrated in figure 3 as a suitable application of this method.

The drug contents were released in all the formulations within 1h. These results seem to indicate that a significant amount of rosmarinic acid or extracts are initially associated with nanoparticles remained on their surfaces by weak linkages to chitosan, which did not have the necessary strength to entrap all the compounds.

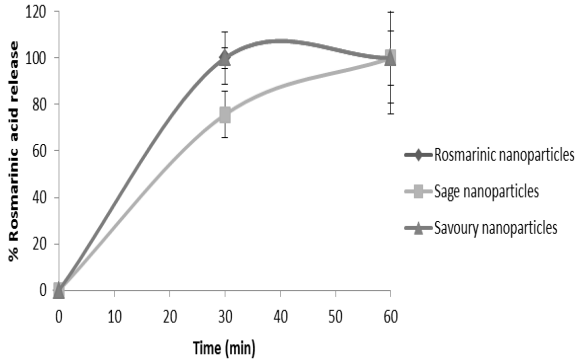

Figure 3. Rosmarinic acid in vitro release from rosmarinic acid, sage and savoury nanoparticles (50% theorethical loading - 1 mg/mL; n=3)

CONCLUSION

The method can be used for development and characterization of quercetin and rosmarinic acid in natural extracts and in chitosan nanoparticles to determine the loading capacity and association efficiency as well as its in vitro release. Nevertheless considering the results these extracts could be good vehicles of rosmarinic acid incorporation.

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REFERENCES

- ICH Guideline Q2 (R1): Validation of Analytical Procedures: Text and Methodology, 2005.
- da Silva SB. et al. (2010) *Antioxidants in the Prevention and Treatment of Diabetic Retinopathy – A Review*. Journal of Diabetes and Metabolism 1(111), 1-10
- Xue Y et al. (2011) *Direct Evidence for Hydroxyl Radical Scavenging Activity of Cerium Oxide Nanoparticles*. The Journal of Physical Chemistry C (115) 4433–4438.