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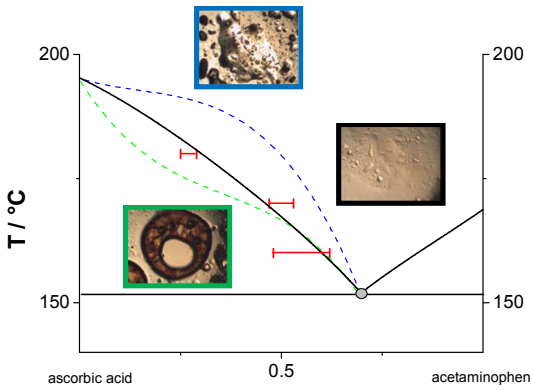
Graphical abstract



The exact solid-liquid equilibrium between ascorbic acid and acetaminophen was established combining high performance liquid chromatography and differential scanning calorimetry.

New Journal of Chemistry RSCPublishing

ARTICLE

Incidence of the Melting-Degradation Process of Vitamin C on the Determination of the Phase Diagram with Acetaminophen Enhanced by High Performance Liquid Chromatography Tools

Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

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Compounds that degrade upon melting, such as vitamin C, are not convenient for temperature-composition phase diagram survey. To bypass such a disadvantage, faster scan rates may be used to heat the samples. However in that case, the thermodynamic solid-liquid equilibrium cannot be reached, as the present study demonstrates for the vitamin C/acetaminophen phase diagram determination. In this work, high performance liquid chromatography has therefore been used as a complementary tool with differential scanning calorimetry in order to determine the appropriate liquidus point for various vitamin C/acetaminophen mole fractions. Since a classic thermodynamic approach is not sufficient for such a study, the analytical experiments we conducted provide useful data allowing us to determine the non-degraded vitamin C quantity in the vitamin C/acetaminophen mixtures treated for given temperature and annealing time conditions.

Introduction

Vitamin C (VC), a pharmaceutical ingredient used against fatigue, and acetaminophen, prescribed against headaches and fever, are often associated in the same medication as mixed powders to treat colds and flu. The possible interactions in the solid state between these two substances were previously studied and the corresponding binary phase diagram has been established.[1] But, although it is known that VC degrades on melting,[2] nothing has been reported in the literature concerning the incidence of its degradation on the liquidus curves.[1]

It is well known that VC degrades easier in the liquid state than the solid state. It has been previously shown that thermal analyses carried out on pure VC at high scanning rates, at least 30 $K.min^{-1}$, allowed to bypass the degradation process on melting.[2] However, thermal investigations on mixtures between VC and acetaminophen are more difficult to apprehend from a calorimetric point view for two reasons, i/ at low scanning rates, degradation of VC is enhanced, ii/ at high scanning rates, the thermodynamic equilibrium conditions are not met. The present paper reports the phase diagram examined by thermal analyses completed with analytical tools in order to quantify VC degradation rate in the binary liquid mixtures. Firstly, thermal analyses were carried out on binary mixtures and the incidence of the scanning rate on the determination of the liquidus point was then analyzed. Due to the two reasons mentioned above, this dynamic approach did not allow us to position precisely the liquidus curve of the diagram. Furthermore, based on the fact that VC highly degrades in the liquid state, different mixtures were heated at given temperature and annealing time conditions. The samples were consequently analysed by High Performance Liquid Chromatography (HPLC) to quantify the non-degraded VC rate as a function of the molar fraction.The main text of the article should go here with headings as appropriate.

Results and discussion

DSC experiments were carried out on different molar fraction VC/acetaminophen samples at different heating rates. Interestingly, for compositions that were rich in VC, different DSC curves were observed depending on the scanning rate (Fig. 1) whereas no incidence was observed on the shape of the melting peak for compositions that were rich in acetaminophen.

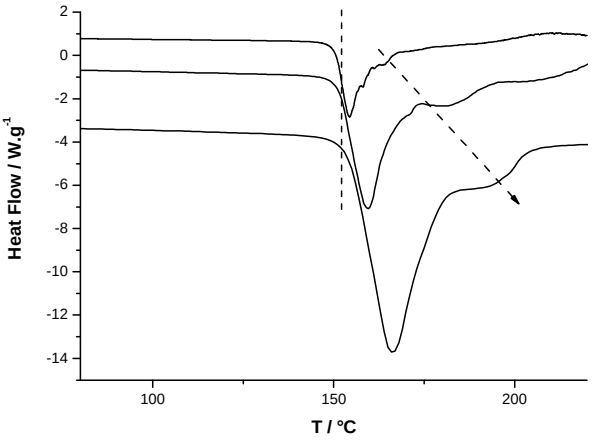

Fig. 1. DSC curves obtained for the 80/20 VC/acetaminophen mixture. Scanning rates: 5, 20, and 60 K.min^{-1}, from top to bottom.

The temperature-composition phase diagram of VC/acetaminophen has been established from each DSC curve. The eutectic temperature was set at the onset temperature of the first DSC signal whereas the peak maximum position of the second endothermic signal was taken as the liquidus temperature. On the left-hand side of the eutectic point, the liquidus temperature is found to increase with the scanning rate, *i.e.* in the two-phase region where solid VC is in equilibrium with the liquid phase composed with liquid VC and liquid acetaminophen. This indicates that VC degradation occurs during its dissolution until the liquidus point is reached. On the contrary, the liquid-acetaminophen liquidus curve is not affected by the scanning rate.

However, a peak at 152 °C was obtained for all the DSC curves, the corresponding intensity depending on VC molar fraction. This suggested the presence of a eutectic invariant at this temperature, as reported in Fig. 2 and as previously found.[1] Interestingly, the onset temperature of the eutectic invariant is not scanning-rate dependent, indicating a low VC degradation rate in the solid state.

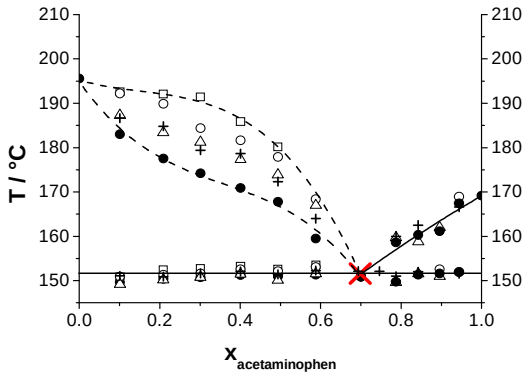

Fig. 2. VC/acetaminophen experimental phase diagram. The temperatures are reported as a function of the heating rate: 5 (●), 10 (+), 20 (△), 40 (○), and 60 K.min^{-1} (□). The dashed line represents the two extreme tendencies for the liquidus curve, *i.e.* for the 5 (lower curve) and 60 K.min^{-1} (higher curve) scanning rates. (×) The eutectic point was determined from the Tammann plot.

The enthalpy determined from the eutectic signal is reported in Fig. 3 as a function of acetaminophen molar fraction and scanning rate. This plot is commonly called Tammann plot. One can observe that the enthalpy is scanning-rate independent. Moreover, the eutectic point has been determined at x_E=0.69, for a mean eutectic temperature equal to (152 ± 1) °C. This point has been reported on Fig. 2 to help in the construction of the liquidus curves. The two line segments of the Tammann plot pass through x=0 and x=1, which is consistent with a total demixing between the two species in the solid state. It is noteworthy that the melting data for the pure compounds − VC from Ref. 2 and acetaminophen from Ref. 5 − were used for drawing the phase diagram.

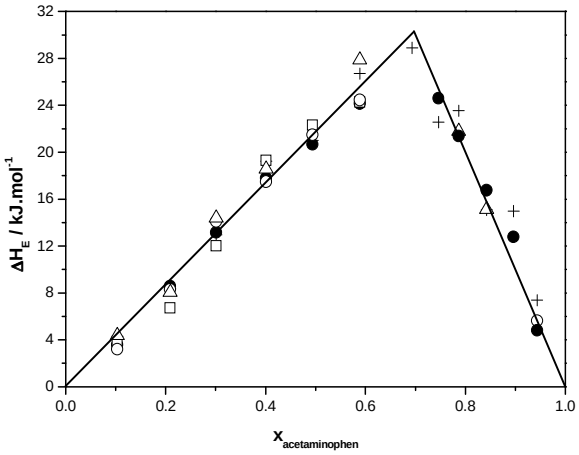

Fig. 3. Tammann plot for the VC/acetaminophen phase diagram as a function of the mass fraction in acetaminophen. The enthalpies are reported as a function of the heating rate: 5 (●), 10 (+), 20 (△), 40 (○), and 60 K.min^{-1} (□).

As can be seen in Fig. 2, the liquidus curve cannot be determined precisely on the left hand side of the diagram due to the degradation of VC in the liquid state. In order to determine the exact position of this two-phase equilibrium curve, VC degradation experiments have been carried out in isothermal conditions, taking into account the fact that VC degrades more significantly in the liquid state than in the solid state. After heating, the mixtures were quenched at 0 °C during 10 minutes and then analysed by HPLC to determine the percentage of VC degradation. This annealing time has been taken at 10 minutes to ensure that the degradation process was completely stopped. Such experiments were performed for compositions that enclose, for a given temperature, the two extrema of the liquidus curves. In order to optimize the plot of the liquidus curve, we chose three temperatures that allowed scanning the curve from the eutectic point to the melting point of VC. Then temperatures of 160, 170 and 180 °C were defined.

The results are shown in Fig. 4. A break of slope is visible on the three curves. This could be explained by the fact that within the two-phase region, VC solid is in equilibrium with the liquid phase. Consequently, for a given temperature, when approaching the liquidus point, the liquid state is more and more enriched in VC. It is the reason why the percentage of degraded VC increases up to this point. When VC is completely dissolved, only the liquid phase is present. But, since the degradation rate depends only on the temperature, a constant value of the degradation rate of VC is then

monitored. This may explain the fact that the second part of the curve is linear.

From Fig. 4, the degradation rate was determined to be approximately equal to 57 % at 160 °C while the percentage increased to 71% at 170 °C, and 79% at 180 °C. The intersection points were determined at x=0.27 ± 0.02 for 160 °C, x=0.50 ± 0.03 and x=0.55 ± 0.07 for 170 and 180 °C, respectively.

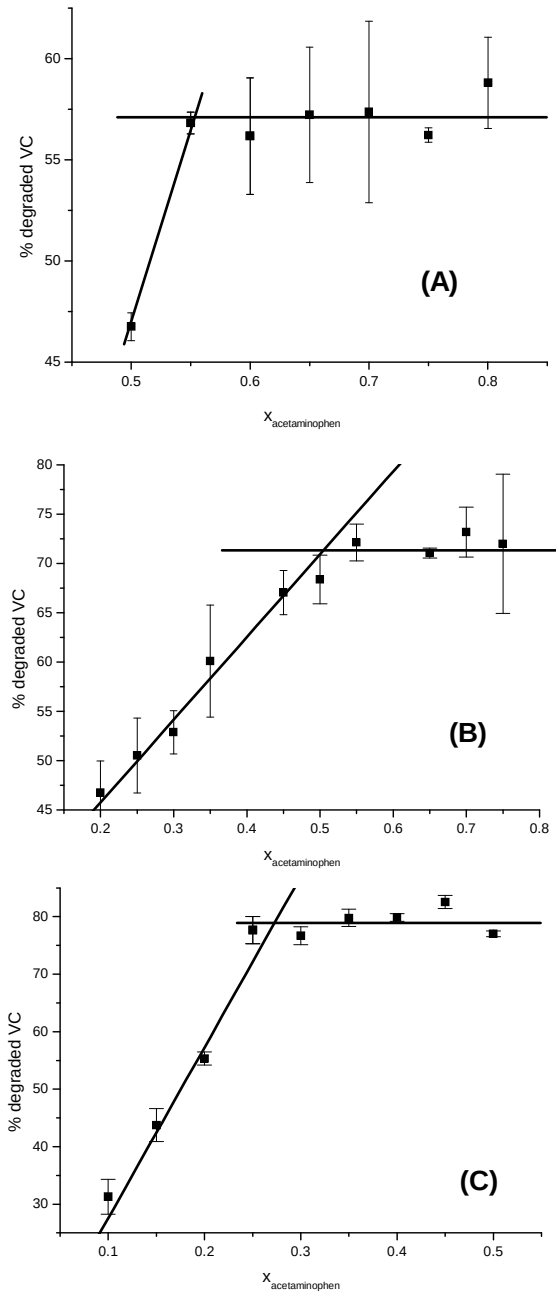

Fig. 4. Percentage of degraded VC, as a function of the molar fraction in acetaminophen, determined at different temperatures: 160 (A), 170 (B), and 180 °C (C).

These values and their uncertainties were reported on the VC/acetaminophen phase diagram together with the values obtained by thermal analysis (Fig. 5). One can then notice that for compositions rich in VC: i/ the sample examination by DSC with a scanning rate of 5 K.min^{-1} tends to favour VC degradation in the liquid state, and ii/ on the contrary, a scanning rate of 60 K.min^{-1} is too fast and confirms that the equilibrium state has not been reached. For compositions near the eutectic point, a scanning rate of 5 K.min^{-1} seems to be appropriate to determine a convenient liquidus point.

Therefore, in the region rich in acetaminophen, the liquidus point cannot be obtained for a given value of the scanning rate because there is a correlation between the scanning rate and the amount of VC in the liquid state.

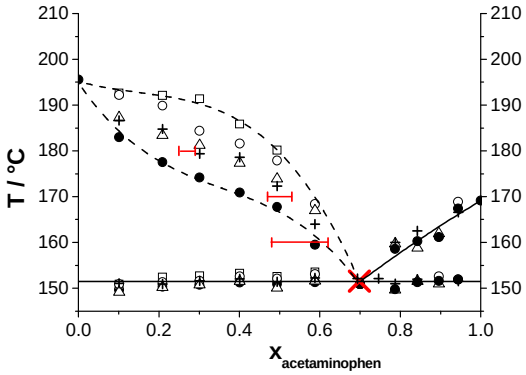

Fig. 5. VC/acetaminophen phase diagram taking into account the liquidus variations depending on the scanning rate and the liquidus values determined from HPLC experiments (red horizontal segments). The symbols for the scanning rates are the same as in Fig. 2.

Taking into account the new liquidus points obtained by HPLC for the phase diagram left-hand side as well as those obtained by DSC for the phase diagram right-hand side (Table 1), a thermodynamic assessment of the diagram was carried out using the EXTXD method.[6] To do so, the liquidus curve was assessed by calculating the excess Gibbs energy in the liquid state using a Redlich-Kister third order polynomial and taking into account a total immiscibility in the solid state between the two substances. The best fit was obtained for: $G^{E,liq}$ = x.(1-x)[100 -1000.(1-2x)], where x stands for the molar fraction in acetaminophen. As a result, a new liquidus has been obtained (Fig. 6). The corresponding values are reported in Table 1 for comparison with the selected experimental points.

Table 1. Experimental liquidus points used for the thermodynamic assessment and calculated mole fractions corresponding to the EXTXD result.

	Left liquidus		Right liquidus	
T / K	$X_{liq,exp}$	$X_{liq,calc}$	$X_{liq,exp}$	$X_{liq,calc}$
453	0.27	0.30		
443	0.50	0.46		
433	0.55	0.60		
431.75			0.79	0.81
433.43			0.84	0.84
434.3			0.90	0.86
440.56			0.94	0.98

As far as the eutectic point is concerned, the calculated and the experimental values match for x = 0.69 and T = 152 °C.

The thermodynamic assessment gives negative values of $G^{E,liq}$ for compositions rich in VC with a minimum value of -80 J.mol^{-1} for x = 0.20 and positive values on the other side of the diagram with a maximum value of 110 J.mol^{-1} obtained for x = 0.80. Although these excess values are small-scale, the sign inversion of $G^{E,liq}$ may be explained in a physical point of vew.

A monotonous curve with no inflection point was obtained for the left-hand side of the diagram, in disagreement with the previous reported phase diagram,[1] where the DSC experiments were carried out at 10 K.min^{-1}. For the latter study, an inflection point had been obtained near compositions rich in VC, probably due to the fact that the degradation of VC in the liquid state was not taken into account at such low scanning rate. Consequently, the liquidus points had been underestimated.

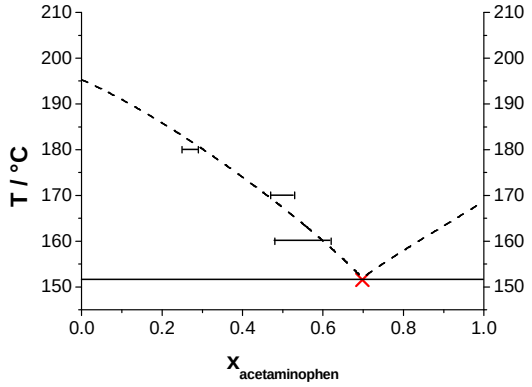

Fig. 6. Calculated VC/acetaminophen phase diagram taking into account the liquidus points determined from HPLC experiments (horizontal segments) and the eutectic point determined from the Tammann plot (×).

Conclusions

Analytical chemistry tools in the solid state (calorimetry) and in the liquid state (HPLC) were associated as complementary methods to determine a temperature-composition binary phase diagram when one of the compounds – vitamin C in this study – of a binary system degrades in the liquid state upon melting. This approach allowed fixing the liquidus curve when solid VC is in equilibrium with a liquid phase. Dynamic method such as differential scanning calorimetry was then coupled with a static approach in isothermal conditions, using HPLC technique, allowing us to quantify the non-degraded vitamin C content for different vitamin C/acetaminophen mixtures.

Experimental

L-ascorbic acid (VC, 99% purity) was purchased from Acros organics; acetaminophen (98% purity) and furfural from Sigma Aldrich (99% purity).

Differential Scanning Calorimetry (DSC) experiments have been performed with DSC 822e (Mettler Toledo) at heating rates from 5 to 60 K.min^{-1}. Calibration was carried out with high purity indium and zinc under an atmosphere of dry nitrogen gas. The samples were prepared by dissolving the desired proportions of acetaminophen and VC in a mixture of acetone and methanol. After a slow evaporation of the solvents, the powder containing the mixed substances was collected. The homogeneity of each composition prepared that way was controlled by statistical measurements.

The HPLC method was used to determine VC concentration in solution and to detect the degradation products occurring during heating. VC, acetaminophen and degradation products (mainly furfural) were separated on a Hypersil BDS C18 column (3 µm, 15 cm, i.d. 4.6 mm, Interchrom, Montluçon, France). The column was connected to a conventional HPLC system Agilent 1220 (Agilent, Les Ulis, France). This instrument included a solvent delivery system together with a UV-visible detection system set at 260 nm. The mobile phase was a mixture of 5% (V/V) methanol HPLC grade (99.93% (w/w), Sigma Aldrich, Saint Quentin Fallavier, France) and 95% (V/V) aqueous solution of tetrabutylammonium perchlorate (Sigma, Saint Quentin Fallavier, France) at 5.10^{-3} mol.L^{-1}. The flow-rate, meanwhile, was set at 1 mL.min^{-1}. In such conditions, the retention times of VC and acetaminophen are 3.4 min (CV 0.6%) and 6.7 min (CV 0.8%) respectively, as shown in Fig. 7. The raw data were acquired by Chemstation® program (Agilent, Les Ulis, France).

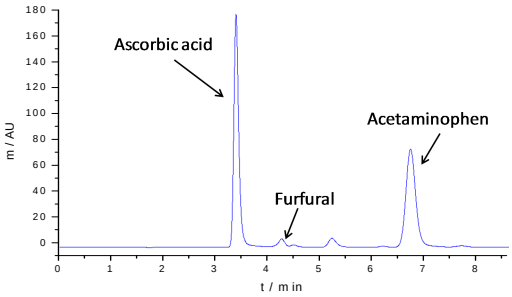

Fig. 7. Chromatogram obtained for a VC/acetaminophen mixture and heated at 160°C for 10 min. Chromatography conditions: Hypersil BDS C18 column (3 µm, 15 cm, i.d. 4.6 mm); UV-visible detection system set at 260 nm; mobile phase: mixture of 5% (V/V) methanol HPLC grade (99.93% (w/w)) and 95% (V/V) aqueous solution of tetrabutylammonium perchlorate at 5.10-3 mol.L^{-1}; flow-rate at 1 mL.min^{-1}.

LogPoct/water values were calculated using MarvinSketch 5.7.0 (ChemAxon, Budapest, Hungary).

The validation of the assay method for VC has been established. The calibration curve was performed with different VC masses (calibrators) ranging from 2 mg to 16 mg (2, 4, 8, 12 and 16 mg). Each mass was dissolved, as previously, in 10 mL of methanol and 500th diluted in the mobile phase before analysis.

The response (peak area) for each analyte was used for the calculations. Least-square regression analysis for a linear model enabled us to calculate all calibration curves by using the equation: y = a•x + b, where y stands for the peak area of the analyte, as recorded from the UV detector, and x the quantity of the VC (mg). Linearity was determined by checking five calibration curves during five different working days (a single measurement of each quantity

every day). Back calculations were made from these curves to determine VC quantity in each calibration standard. A coefficient of determination (r^2> 0.99) was used for the calibration curves. The accuracy (deviation percent or %DEV) of standard quantities should not exceed ±15.0%, and the precision should not exceed 15.0%.[3]

Five replicates of three quality control (QC) samples (low at 6 mg, medium at 10 mg and high at 14 mg) containing VC were used to determine intra- and inter-assay variations. Inter-assay variation was assessed on five separate experimental days. The precision of the method was determined by calculating the coefficient of variation (%CV) for each specific target concentration, and accuracy was calculated from the percent deviation (%DEV) of each concentration from the nominal target concentration. The concentrations of analyte in the QC samples were calculated by using calibration curves prepared on the same day.

Data for the linearity of the method are shown in Table 2. Linear relationships between the peak area of VC and the mass of calibrators over the mass range were found with a mean equation y = 874x -56.6. The coefficient of determination (r^2) of the standard curves was 0.9906–0.9991, with coefficients of variation of 0.37% (n=5). The precision (%CV) for specific concentrations on the standard curves was from 1.16 to 4.37%. The deviation from the theoretical values (%DEV) was between -2.76% and +1.36% (Table 2).

Table 2. Coefficients of variation (CV) and deviation (DEV) obtained for the intra- and inter-assays.

	Standard curve		Quality control				
	n=5			Intra-assay (n=5)		Inter-assay (n=5)	
Mass (mg)	CV (%)	DEV (%)	Mass (mg)	CV (%)	DEV (%)	CV (%)	DEV (%)
2	4.57	-2.76	6	2.18	0.61	2.34	3.28
4	2.59	0.57	10	0.79	-2.81	2.21	1.32
8	1.16	0.71	14	0.54	0.22	1.16	-1.03
12	2.20	1.36					
16	1.61	-1.32					

The precision (%CV) and accuracy (%DEV) of the analytical method were examined by analyzing the intra-assay variation of three QC samples (five replicates per concentration) (Table 2). The precision of intra- and inter-assay variations ranged from 0.54 to 2.34%. Similarly, the accuracy of intra- and inter-assay variations ranged from -2.81 to 3.28%. These results for intra- and inter-assay variations of all QC samples satisfy the current criteria for bioanalytical methods.3 as the precision (%CV) was < 15.0%, and the deviation from nominal concentration (%DEV) was within ± 15%.

VC thermal degradation in isothermal conditions was analysed by High Performance Liquid Chromatography (HPLC) as follows: a specific mass of the mixture of acetaminophen and VC (about 10 mg) was placed into a DSC pan and crimped. The sample was then heated at various temperatures, *i.e.* 160, 170 and 180 °C, for 10 min. Once the heating time was reached, the sample was quenched at 0 °C to stop the degradation process. The content of the pan was dissolved in 10 mL of methanol and 500th diluted in the mobile phase. An external calibration was used and the degradation products formed during heating were detected. Fig. 7 shows the chromatogram obtained after the solubilisation of the mixture acetaminophen/VC (55/45; mol/mol) heated at 160 °C for 10 min with the emergence of VC degradation products.

VC degradation products have already been identified and a possible degradation pathway has been proposed.[4] The degradation product at 4.3 min was identified as furfural.

Acknowledgements

The authors thank Ms. S. Nguyen, Z. Hakim and F. Madaoui, MSc students, for their contribution to this work. Ms K. Debbasch is also thanked for her advice on the manuscript.

References

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1 K. Klimova, J. Leitner, *Thermochim. Acta*, 2012, **550**, 59.
2 Y. Corvis, M. C. Menet, P. Negrier, M. Lazerges, P. Espeau, *New J. Chem.*, 2013, **37**, 761.
3 P. Hubert, J. J. Nguyen-Huu, B. Boulanger, E. Chapuzet, P. Chiap, N. Cohen, P. A. Compagnon, W. Dewe, M. Feinberg, M. Lallier, M. Laurentie, N. Mercier, G. Muzard, C. Nivet, L. Valat, *J. Pharm. Biomed. Anal.*, 2004, **36**, 579.
4 J. P. Yuan, F. Chen, *J. Agric. Food Chem.*, 1998, **46**, 5078.
5 P. Espeau, R. Céolin, J. L. Tamarit, M. A. Perrin, J. P. Gauchi, F. Leveiller, *J. Pharm. Sci.*, 2005, **94**, 524.
6 N. Brouwer, H. A. J. Oonk, *Z. Phys. Chem.*, 1979, **117**, 55.