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THERMAL BEHAVIOR OF ANTI-HYPERTENSIVES DRUGS IN SOLUTION AND HUMAN PLASMA USING DSC-COOLING

Mônica O. da Silva Simões, A. P. B. Gomes, T. G. do Nascimento and R. O. Macêdo[*]

Universidade Federal da Paraíba-UFPB, Unidade de Desenvolvimento e Ensaios de Medicamentos-UDEM, Cidade Universitária-Campus I–LTF–João Pessoa, Paraíba CEP 58059-900, Brasil

Abstract

The present study proposes the use of DSC-cooling as a fast tool to evaluate the drug behavior in biofluids submitted to freeze-thaw studies. Captopril, captopril+ascorbic acid and propranolol in plasma were submitted to 3 cycles of freeze-thaw using DSC-cooling up to 243 K. Captopril-plasma has suffered calorimetric alteration and revealed a considerable interaction front to freezing and heating processes. The presence of additive can stabilize the analyte in plasma. Propranolol-plasma presented reducing in phase transition enthalpy comparable to the plasma denatured by perchloric acid in the last cycles of freezing. DSC-cooling data demonstrated great potentiality for the freeze-thaw studies of drugs in biofluids.

Keywords: anti-hypertensives drugs, biofluids interactions, DSC-cooling, freeze-thaw cycles

Introduction

Propranolol is a β-blocking that act inhibiting competitively the catecholamines in beta receptor [1]. Captopril is an inhibitor of Angiotensin conversor enzyme (ECA) [1]. Freeze-thawing in biological matrix have been essential to provide parameters of storage of aged samples and storage conditions for bioanalytical method validation in the bio-equivalence and bioavailability studies.

Macêdo *et al.* [2–5] have been developed an accelerated method of stability using thermal analysis (TG, DSC and DTA) in chemical matrixes. DSC has been used in biological studies to evaluate intracellular ice formation in high cooling rates and cellular dehydration in biological cells in low cooling rates [6], determination of new clinical cryoprotective solutions [7], measurement of water transport during freezing [8], ice formation in liposomes at low cooling rates [9–11].

The stability studies of drug in plasma have not been evaluated a verification of interferences, plasmatic alterations or plasmatic interactions with drug. DSC-cooling

* Author for correspondence: E-mail: ruimacedo@ltf.ufpb.br

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can be a complementary method to evaluate in accelerated way the interactions of the drug in biofluids. The present study proposes the using of DSC-cooling as evaluation parameters of the drug behavior in biological fluid submitted to freeze-thaw studies.

Experimental

Captopril, propranolol hydrochloride (reference chemical substance) and ascorbic acid (pharmaceutical grade) were used in this study. Human plasma was obtained from Hemocenter of Paraíba-Brazil and collected after approval of research ethic committee. Samples containing captopril and propranolol hydrochloride were prepared in aqueous solution and human plasma in concentration of 50 mg mL^{-1}. Plasma samples spiked or non-spiked with propranolol were stored in refrigerator at 281 K in ependorff vial during six days. Plasma aliquot (200 μL) was denatured with perchloric acid 60% (10 μL). Aliquots of 2 and 10 μL were submitted to the analysis by DSC-cooling. DSC curves were accomplished in Shimadzu differential scanning calorimeter, model DSC-50, heating rates of 1, 2 and 3 K min^{-1}, between 298 to 243 K using liquid nitrogen. A DSC-60 was used heating rate of 10 K min^{-1}. Captopril-plasma and captopril-ascorbic acid-plasma were submitted to only 3 cycles of freeze-thaw and propranolol-plasma to the 3 cycles of freeze-thaw for six days. DSC-60 analyses were realized in replicates, t-student test and two-way ANOVA were performed with confidence interval (CI) of 95% and $P<0.05$. Phase transition enthalpy and fusion enthalpy and onset temperature of crystallization and fusion were calculated by Tasys software from Shimadzu.

Results and discussion

Calorimetric parameters in aqueous solution

Table 1 presents the crystallization and melting parameters for all samples studied in solution. In heating rate of 10 K min^{-1}, the phase transition enthalpy ($\Delta_{Trans}H$) corresponding to crystallization enthalpy have presented a smaller standard deviation than the melting enthalpy ($\Delta_{fus}H$), hence this parameter was used to evaluate the drug-plasma interaction. On set temperature of crystallization also has presented more precision than on set temperature of fusion, which can be observed in standard deviation values. The peak maximum temperature on melting was dependent on heating rate due to thermal gradient, but the phase transition enthalpy and melting enthalpy were constants (Table 1).

The values of crystallization enthalpy and melting enthalpy have been analysed statistically. t-student test revealed statistical differences intergroups in water-captopril values but did not show significant differences between water-propranolol values which was in agreement with intergroup two-way ANOVA test. Two-way ANOVA test did not show differences intragroups, which was confirmed by dispersion analysis (standard deviation).

Table 1 Calorimetric parameters of the anti-hypertensives drugs using DSC-60

Cooling rate/ °C min^{-1}	Crystallization parameters					
	Water		Captopril		Propranolol	
	Onset*/°C	$\Delta_{Trans}H$/J g^{-1}	Onset*/°C	$\Delta_{Trans}H$/J g^{-1}	Onset*/°C	$\Delta_{Trans}H$/J g^{-1}
–10.0	–21.46	480.50	–21.88	444.27†	–22.43	465.00‡
–10.0	–19.00	468.63	–19.97	436.88†	–20.82	462.30‡
–10.0	–21.00	442.93	–21.27	427.67†	–19.81	472.75‡
–10.0	–19.62	451.10	–21.88	414.40†	–20.00	460.51‡
–10.0	–21.14	467.00	–22.71	418.85†	–20.57	487.60‡
Mean	–20.44	462.02	–21.54	428.41	–20.72	469.62
SD	0.70	1.84	1.02	12.37	1.04	11.08
Heating rate/ °C min^{-1}	Melting parameters					
	Water		Captopril		Propranolol	
	Onset*/°C	$\Delta_{fus}H$/J g^{-1}	Onset*/°C	$\Delta_{fus}H$/J g^{-1}	Onset*/°C	$\Delta_{fus}H$/J g^{-1}
10.0	–0.57	–706.53	–2.36	–626.16*	–1.53	–668.71^{+}
10.0	–0.24	–664.20	–2.35	–573.19*	–1.37	–669.13^{+}
10.0	–0.38	–644.23	–2.35	–589.40*	–1.20	–680.36^{+}
10.0	–0.58	–651.33	–2.50	–581.67*	–1.46	–636.57^{+}
10.0	–0.41	–679.80	–2.52	–582.64*	–1.33	–711.90^{+}
Mean	–0.44	–669.22	–2.42	–590.61	–1.38	–673.33
SD	0.14	24.87	0.09	20.70	0.13	27.05

Aliquots 2 μL, n=5, *Onset temperature; SD – Standard deviation; †P=0.005, F=39.80 and 4.30; ‡P=0.387, F=0.828 and 0.987; *P=0.001, F=107.8 and 6.302; $^{+}$P=0.808, F=0.086 and 1.753

The DSC data revealed that propranolol drug did not alter calorimetrically in aqueous solution during freezing-thawing cycle, however captopril drug in aqueous solution has suffered calorimetric alteration decreasing the crystallization enthalpy and hence demonstrated major physicochemical reactivity than propranolol in the same medium.

Characterization of the drugs in plasma for DSC-cooling

Figure 1 presents DSC curve (curve 1) of water with phase transition corresponding to crystallization peak 267.5 K, where can be observed an exothermal process with high heat liberation. The crystallization peaks were altered to 266.1, 265.1 and 264.5 K in plasma and plasma spiked with propranolol and captopril, respectively. Onset temperature of crystallization about 249.75±2.20 K (captopril) and 249.20±2.26 K (propranolol) for samples in plasma spiked with anti-hypertensives drugs was smaller than pure water 251.56±1.70 K, however the plasma non-spiked presented onset temperature of crystalli-

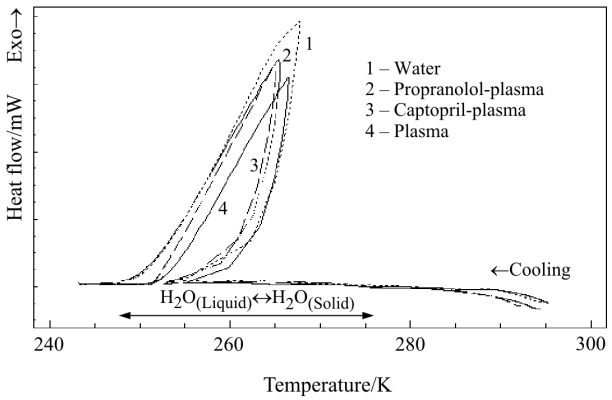

Fig. 1 DSC-cooling of anti-hypertensives drugs in human plasma

zation about 253.77±2.54 K. DSC profile revealed equilibrium of the liquid and solid state of pure water between 273 up to 253 K [12].

The plasma samples decreased the heat of crystallization, due to great amount of plasmatic macroelements (Fig. 1). During the freezing of biological systems ice tends to form first in extracellular space [8] and according to literature [13, 14] extracellular peak could be exhibited in the range of 261 to 243 K and depending of cooling rate and catalyser. Plasma has been characterized as a natural microemulsions containing fatty acids such as cholesteryl ester responsible to self-assembly this biocolloidal structures [15, 16]. Thus, at –253.77±2.54 K can be suggested as extramicellar freezing in plasma samples for this study.

Freeze-thaw studies for captopril

Freeze-thaw studies with matrix captopril-plasma in the first cycle presented a phase transition enthalpy corresponding to crystallization enthalpy of 228 J g^{-1} has suffered calorimetric alteration starting from the second 205 J g^{-1} and third 196 J g^{-1} freezing cycles. The mixture captopril plus ascorbic acid presented phase transition enthalpy of 213, 206 and 200 J g^{-1} and plasma has presented 220, 200 and 188 J g^{-1} in the first, second and third cycles of freezing, respectively. The captopril-plasma presented considerable decreasing in phase transition enthalpy for crystallization during the three cycles of freezing which has been differed of the captopril–ascorbic acid-plasma.

The first cycle has caused the presence of extramicellar ice inducing a chemical potential difference across micellar membrane which then causes intramicellar content to move towards the extramicellar space [7]. The matrix containing captopril revealed a low stability front to the freezing and heating process. According to literature [17, 18] ascorbic acid has been used as a cryopreservative decreasing the reactive oxygen species and consequently increasing the percentage of membrane intact in semen. Plasma containing ascorbic acid has delayed the disruption of biocolloidal membrane when compared to plasma-captopril. The presence of the additive can stabilize the analyte in the biological fluid (Fig. 2).

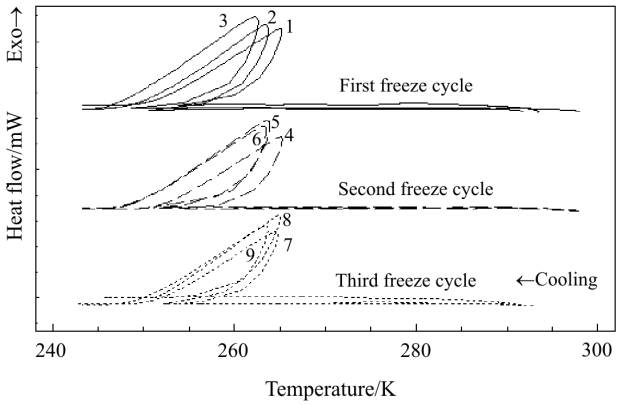

Fig. 2 DSC-cooling of the captopril drug and captopril additive in plasma. DSC curves of plasma-1, 4; 7; plasma+captopril-2, 5; 8; plasma+captopril+ascorbic acid-3, 6; 9

Freeze-thaw and short term stability for propranolol

Table 2 data has shown a drastic reduction of the phase transition enthalpy for crystallization in the 0 day analysis. Probably, a reversible disruption process has occurred in biocolloids and a great amount of liposoluble analyte has crossed towards extramicellar fluid promoting the propranolol solubilization in plasma and consequently accelerated the micellar degradation in the third cycle [19, 20]. The biocolloids may be also cracking by freezing, heating and an excess of salt which may dehydrate the micelles by colloidal membrane [21].

Table 2 Phase transition enthalpy for the propranolol-plasma during six days

Day	$\Delta_{Trans}H$/J g^{-1}			
	1th freezing cycle	2nd freezing cycle	3rd freezing cycle	$\Delta H_{(1^{st}-3^{rd}\,cycles)}$
0	191	164	155	36
2	203	197	192	11
3	213	203	200	13
6	195	182	173	22

Aliquots 10 μL using DSC-50

Freeze-thawing studies showed that propranolol-plasma has decreased the phase transition enthalpy for crystallization just in the last two cycles of freezing on the sixth day. Perchloric acid has broken the stabilized microemulsions by destruction of the emulsifier agents [21] and denatured proteins. The plasma-propranolol presented a DSC profile similar to the denatured plasma in the third cycle of freezing on the sixth day. Denatured plasma presented a phase transition enthalpy for crystallization of 167 J g^{-1} (first cycle) showing a reduction of DSC measurement and decreasing heat release due to micellar debris after the sixth day (Fig. 3).

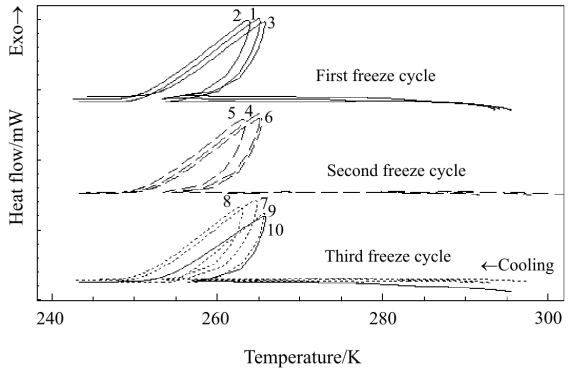

Fig. 3 DSC-cooling of the propranolol drug in short term stability. DSC curves of plasma 1, 4, 7; plasma+propranolol at 0 day-2, 5, 8; plasma+propranolol at 6 days-3, 6, 9; denatured plasma-10

Conclusions

The DSC-cooling data showed the possibility to differentiate the anti-hypertensives drugs in solution or in the plasma and demonstrated the interactions of the captopril and propranolol with matrix. The studies of DSC-cooling demonstrated a great potentiality for the freeze-thaw and short term stability studies of drugs in biofluids.

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