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ORIGINAL ARTICLE

# Characterization of lidocaine:hydroxypropyl-β-cyclodextrin inclusion complex

**Carolina Morales Moraes · Priscila Abrami · Daniele Riberio de Araujo · Angélica F. A. Braga · Michelle Georges Issa · Humberto G. Ferraz · Eneida de Paula · Leonardo Fernandes Fraceto**

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**Abstract** An inclusion complex was prepared between the local anesthetic lidocaine (LDC) and hydroxypropyl-β-CD (HP-β-CD). The complex was characterized by thermal analysis (differential scanning calorimetry, DSC), UV absorption and high-pressure liquid chromatography (HPLC). DSC results were indicative of complexation, due to the loss of the characteristic endothermic peak of LDC (77 °C). Phase-solubility diagrams allowed the determination of the association constant between LDC and HP-β-CD (35.7 ± 4.7 $M^{-1}$). The rate of LDC release decreased after complexation and thermodynamic parameters from the HPLC studies ($\Delta G°$ = –2.65 kJ/mol) revealed that a stable complex was formed.

**Keywords** Lidocaine · HP-β-CD · HPLC · Association constant · Thermodynamic parameters

C. M. Moraes · P. Abrami · L. F. Fraceto (✉)
Departamento de Farmácia, Universidade de Sorocaba,
Rod. Raposo Tavares, km 92,5 CEP, Sorocaba,
SP 18023-000, Brazil
e-mail: leonardo.fraceto@uniso.br

D. R. de Araujo · E. de Paula · L. F. Fraceto
Departamento de Bioquímica, Instituto de Biologia,
Universidade Estadual de Campinas (UNICAMP),
Campinas, SP, Brazil

A. F. A. Braga
Departamento de Anestesiologia, F.C. Médicas,
UNICAMP, Campinas, SP, Brazil

M. G. Issa · H. G. Ferraz
Departamento de Farmácia, F.C. Farmacêuticas,
Universidade de São Paulo (USP), Sao Paulo,
SP, Brazil

## Introduction

Local anesthetics can reversible block the propagation of the nervous impulse due to their binding to the voltage-gated sodium channel of excitable membranes [1, 2]. Lidocaine (LDC) is an amine-amide local anesthetic with moderate action and fast onset used in regional anesthesia [1]. LDC effects can be improved by using drug-delivery systems that allow its sustained release, leading to a longer duration of action and lower uptake by the systemic circulation [3]. Cyclodextrins (CDs) can provide such drug-delivery system for LDC since they are able to entrap guest molecules of appropriate size and polarity in their cavities [4, 5] to form non-covalent inclusion complexes [6, 7]. Encapsulation into cyclodextrins has been extensively studied to improve the stability, solubility [6] and bioavailability [8, 9] of guest molecules. The aim of this work was to characterize the inclusion complex formed between LDC and hydroxypropyl-β-cyclodextrin (HP-β-CD) using differential scanning calorimetry (DSC), high-pressure liquid chromatography (HPLC) and photometry.

## Experimental

Lidocaine was a gift from Cristália Ind.Farm.Ltda (Itapira, Brazil). HP-β-CD was purchased from Roquette and HPLC-grade acetonitrile (ACN) was purchased from Sigma Chem. Co.

### Preparation of LDC/HP-β-CD complex

Inclusion complexes with 1:1 LDC/HP-β-CD molar ratios were prepared by shaking appropriate amounts

of the anesthetic and HP-$\beta$-CD in deionized water, at 25 ± 1 °C, for 24 h. After equilibrium, the solution was freeze-dried in a Labconco Freeze-dry system and stored at –20 °C until further use.

The *kinetics* of the complexation was analyzed by shaking appropriate amounts of LDC and HP-$\beta$-CD at 25 ± 1 °C and pH 10.5. Changes in the absorbance of LDC were followed for 8.5 h.

*Phase solubility* studies were based on the solubility variation of LDC upon addition of HP-$\beta$-CD, at 25 ± 1 °C and pH 10.5; the association constant ($K_a$) was calculated from the slope of the linear portion of the phase-solubility diagram, according to [10].

In vitro *release* experiments were conducted by dialysis at 37 °C, using a two-compartment system with a cellulose membrane (Spectrapore, cut off = 1000 Da) to separate the donor (1 mL LDC sample—either in solution or LDC/HP-$\beta$-CD) and the acceptor (100 mL of 20 mM HEPES buffer, pH 7.4) compartments, under continuous stirring. Aliquots were withdrawn at regular intervals and LDC concentration was determined by UV absorption at 260 nm [11].

Calorimetry

DSC curves were obtained with a DSC2920 TA equipment, using 50 mL/min nitrogen rate flow and 10 °C/min heating rate over a range of 25–300 °C. Two mg samples (LDC, HP-$\beta$-CD and 1:1 LDC:HP-$\beta$-CD) were placed in aluminum pans; Indium was used to calibrate the temperature.

*HPLC experiments* were performed using a Shimadzu SCL-10VP controller pump, SIL-10AD VP autoinjector, UV-Vis SPD-10A VP detector (at 240 nm for LDC) and the Class-VP 6.12 software. The reversed phase $C_{18}$ Phenomonex Gemini column (5 µm, 10 × 0.46 cm) was employed. About 5, 10, 15 and 20 mM HP-$\beta$-CD were dissolved in the mobile phase (ACN-10 mM phosphate buffer pH 7.4, 45/55 v/v) that was pumped at a flow rate of 1.5 mL/min. 0.2 mL aliquots of 1 mM LDC were injected and the experiments were carried out at a range of 25–45 °C.

## Results and discussion

The complexation between LDC and HP-$\beta$-CD in water takes 5 h to reach equilibrium (Fig. 1). Complexation increases the solubility of LDC, as shown in the solubility isotherm (Fig. 1—inset) determined at pH 10.5. For LDC the increase in solubility occurred as a linear function of HP-$\beta$-CD concentration corresponding to the $A_L$-type profile [10] that suggests the formation of a 1:1 LDC:HP-$\beta$-CD complex. The association constant ($K_a$) determined from the slope and the intercept of that plot was 35.7 ± 4.7 $M^{-1}$, indicating the formation of a stable complex [12].

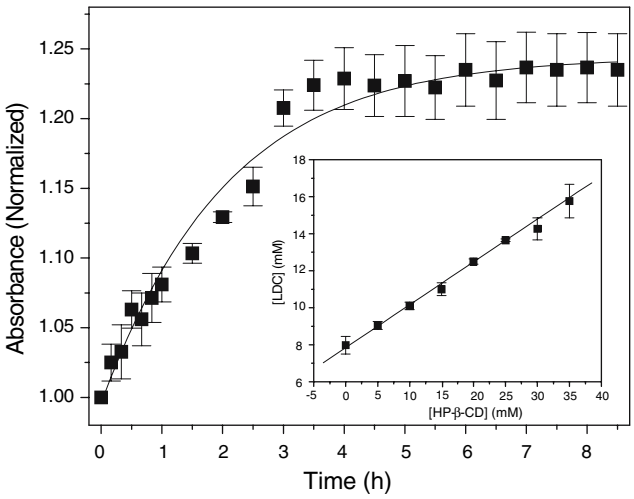


**Fig. 1** Kinetics of LDC/HP-$\beta$-CD complex formation. Inset: phase-solubility diagram for LDC at increasing HP-$\beta$-CD concentrations. Triplicate samples at 25 °C and pH 10.5

Representative DSC thermograms, measuring the rate of heat absorbed by LDC, HP-$\beta$-CD, and LDC:HP-$\beta$-CD inclusion complex (1:1 molar ratio) are shown in Fig. 2. The absence of the characteristic peak of LDC at 77 °C (Fig. 2) in the LDC:HP-$\beta$-CD sample is a strong evidence of the inclusion of lidocaine inside the cyclodextrin cavity [13]. Figure 3 shows that complexation of LDC in the HP-$\beta$-CD cavity significantly reduced its rate of in vitro release, as compared to free LDC. At the time required for total release of LDC (210 min) only 88% of the anesthetic was released

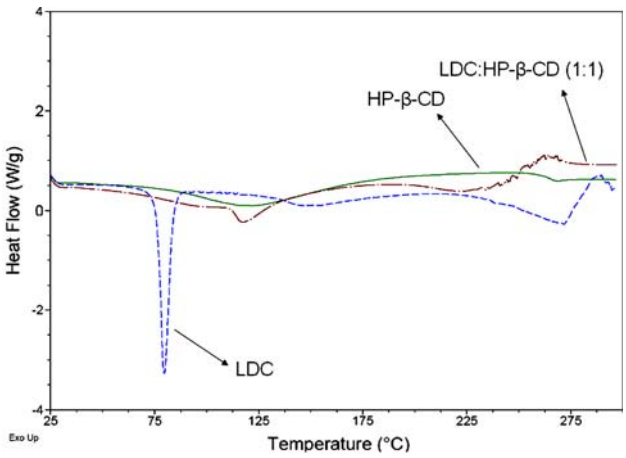


**Fig. 2** Differential scanning calorimetry. Thermograms for LDC, HP-$\beta$-CD and LDC:HP-$\beta$-CD (1:1 mole%) inclusion complex

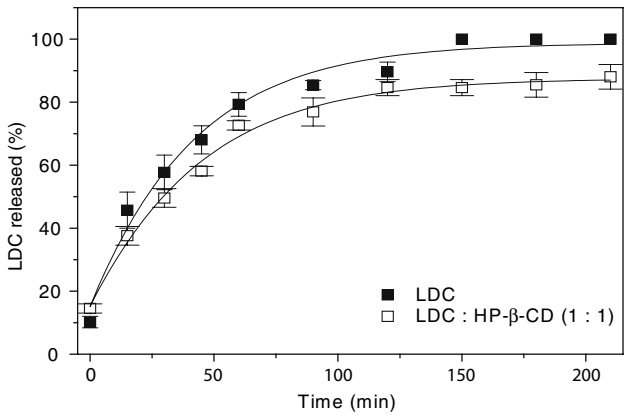


**Fig. 3** In vitro release of LDC and LDC:HP-$\beta$-CD, measured at 25 °C and pH 7.4

from the inclusion complex. This result suggests that HP-$\beta$-CD can also induce a gradual drug-release in vivo, changing drug permeation across membranes or its distribution, with possible therapeutic advantages such as prolonged effects and reduced toxicity [14].

HPLC results revealed that the formation of LDC:HP-$\beta$-CD complex, which enhances the LDC interaction in the mobile phase and reduces its retention time in the column. The affinity constant ($K$) for the LDC:HP-$\beta$-CD complex has been calculated [15–17]. The linear relationship between $1/k'$ and HP-$\beta$-CD concentration at different temperatures (Fig. 4), with correlation coefficients higher than 0.99, indicates that the complex is properly described by assuming the 1:1 stoichiometry [17]. Information about the mechanistic aspect of the LDC/HP-$\beta$-CD formation was obtained from the thermodynamic parameters calculated from

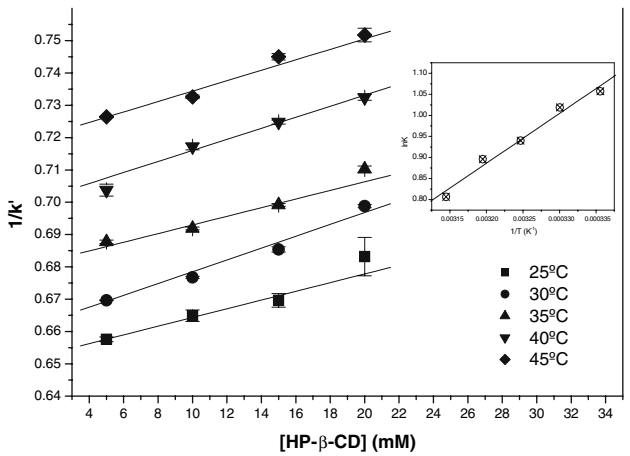


**Fig. 4** Plot of $1/k'$ versus HP-$\beta$-CD conc. for LDC measured from 25–45 °C. Inset—van't Hoff plot (ln $k'$ versus 1/T) for LDC:HP-$\beta$-CD complexation . Experimental conditions described in methods

the van't Hoff plot (Fig. 4—inset). $\Delta H^{o}$ and $\Delta S°$ for HP-$\beta$-CD and LDC were –9.81 kJ/mol and –2.89 J/mol/K, respectively, with a corresponding $\Delta G°$ of –2.65 kJ/mol. The formation of drug-cyclodextrin inclusion complexes is classically attributed to interactions such as hydrogen bonding with the OH groups at the periphery of the cavity, van der Waals interactions and hydrophobic effect [18–20].

## Conclusion

The preparation, physicochemical characterization and in vitro evaluation of the LDC/HP-$\beta$-CD was reported. The results show that a stable complex was prepared at a 1:1 molar ratio that effectively enhanced the water solubility of LDC. This kind of study is important to evaluate the potentiality of the LDC/HP-$\beta$-CD complex for future therapeutic applications. In vitro and in vivo toxicological tests have been carried out with this formulation and will be published in due time.

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