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ORIGINAL ARTICLE

Improvement in the water solubility and stability of 4ASA by the use of cyclodextrins

M. Lahiani-Skiba · I. Youm · F. Bounoure · M. Skiba

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Abstract 4-Aminosalicylic acid (4ASA) a non steroid anti inflammatory drug (NSAID) has been used for over 40 years in the treatment of inflammatory bowel diseases (IBDs). It is therefore an importance to assess physicochemical properties such as solubility and stability. The effect of cyclodextrins on the solubility and chemical stability of 4ASA was investigated. Inclusion complexes of 4ASA were characterized by the solubility method, differential scanning calorimetry (DSC) and Fourier transformer infrared spectroscopy (FT-IR). In addition, the influence of presence of CDs on stability was assessed at room temperature in different pH conditions. Complexation with CDs increased 4ASA aqueous solubility. The stoichiometric ratios of the inclusion complexes were 1:1 for α, β, γ and HP- CDs, respectively. Complexation with γ and HP-CDs has increased the 4ASA stability 4 fold. These observations suggest that 4ASA/CD complexes may be an attractive and practical procedure to modify drug physicochemical properties for use in delivery systems.

Keywords 4ASA · Cyclodextrins · Stability, DSC · FT-IR

M. Lahiani-Skiba · I. Youm · F. Bounoure · M. Skiba
Laboratoire de Pharmacie Galénique ADEN EA 4311, Institute for Biomedical Research, University of Rouen, 22, Boulevard Gambetta, 76000 Rouen, France

M. Skiba (✉)
Laboratoire Pharmacie Galénique ADEN EA 4311, UFR Médecine-Pharmacie, 22, Bd Gambetta, 76183 Rouen, France
e-mail: Mohamed.skiba@univ-rouen.fr

Introduction

4-Aminosalicylic acid (4ASA) a non steroid anti inflammatory drug (NSAID) (Fig. 1) has been used for over 40 years in the treatment of inflammatory bowel diseases (IBDs) [1]. It is therefore an importance to assess physicochemical properties such as solubility and stability. Cyclodextrins (CDs) have lipophilic inner cavities and hydrophilic outer surfaces. They, are capable of interacting with a large variety of guest molecules to form non covalent inclusion complexes. For this reason they are reported as complexing agents who are able to improve the physicochemical properties of the guest molecule. In the present work solubility and stability studies of 4ASA/CDs solid inclusion complexes were conducted. Characterization was investigated by different analytical techniques: Differential Scanning Calorimetry (DSC), Fourier Transformed Infrared spectroscopy (FT-IR) to show if an inclusion phenomena is obtained.

Materials and methods

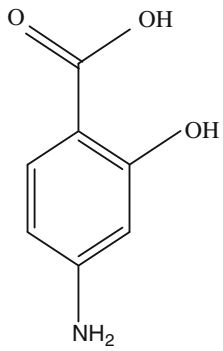
Materials

Cyclodextrins and 4ASA were commercial products provided by Wacker Fine chemicals (Burhausen, Germany) and Bayer respectively.

Solubility studies

Solubility studies were investigated according to the phase solubility method described by Higuchi and Connors [2] over 7 days. The following Eq. 1 was used to determine the association constant (K) from the slope of the linear portion of the curve.

Fig. 1 4ASA chemical structure



$$K = \text{slope}/S_o(1 - \text{slope}) \quad (1)$$

where S_o is the intrinsic solubility of the drug studied under the conditions.

Preparation of 4ASA/CDs complex

The complexes were prepared by mixing (at 1:1 M ratio) 4ASA and CDs according to the freeze drying procedure described in [3].

Chemical stability

4ASA with and without α-CD were placed in buffer solutions at pH 1.5, 7.0 or 7.5 and reacted for 7 days at 37 ± 0.5 °C. The reaction mixture was centrifuged at 3,000 rpm for 5 min, and the supernatant was analyzed by HPLC.

4ASA quantification by HPLC

The amount of 4-aminosalicylic acid (4ASA) in the sample solution was determined by HPLC. The HPLC system consisted of a Jasco 880 pump (Japan), a Jasco 875-UV detector (Japan), an autosampler HPLC-360 Kontron (Brehme, Germany) and a Kromasil column: C-18 µm, 250 mm × 4.6 mm (Chromato, France HAS), maintained at room temperature. Detection of the analytes was carried out at 300 nm. The optimum mobile phase which was used in validation studies consisted of water–formic acid–acetonitrile (67:3:30; v/v/v). Solvent delivery was employed at a flow of 1.0 mL/min. Injection volume of the analytes was set to a constant volume of 50 µL. All HPLC parameters were controlled by the Azur software: version 3.0 coupled to an acquisition box (Azur PAD).

Differential scanning calorimetry (DSC)

DSC analysis was carried out using a DT6 differential calorimeter (Perkin Elmer, USA). Scans were performed in the range of 30–300 °C with a gradient of 10 °C/min. All samples were prepared weighing 3–5 mg of powder in aluminium pan and analysis was performed in duplicate.

Fourier transform infrared spectroscopy (ATR-FT-IR)

Samples were analyzed in dried phase. The Fourier Transform Infrared Spectra were obtained from a Perkin Elmer IR spectrometer. Spectra were achieved from 4,000 to 650 cm^{-1}, with H_2O and CO_2 signals ponderation, 8 cm^{-1} resolution, 0.2 cm^{-1} scanning speed, 16 scans.

Results and discussion

Stability test and phase solubility

Stability results showed a protective effect of α-CD/4ASA from chemical degradation up to 98% at pH 7.5 (Fig. 2a). Phase solubility profiles of 4ASA/CDs are presented in Fig. 2b. The phase solubility diagram of 4ASA/α-CD complex could be classified as B_s-type that denotes complexes of limited solubility. The first part had shown increased drug solubility with increasing concentration of α-CD in the range of 0–100 mM. K value of the 1:1 complex was 214.28 ± 4.17 according to Eq. 1. However the solubility diagram of β-CD had shown a B_i subtype that

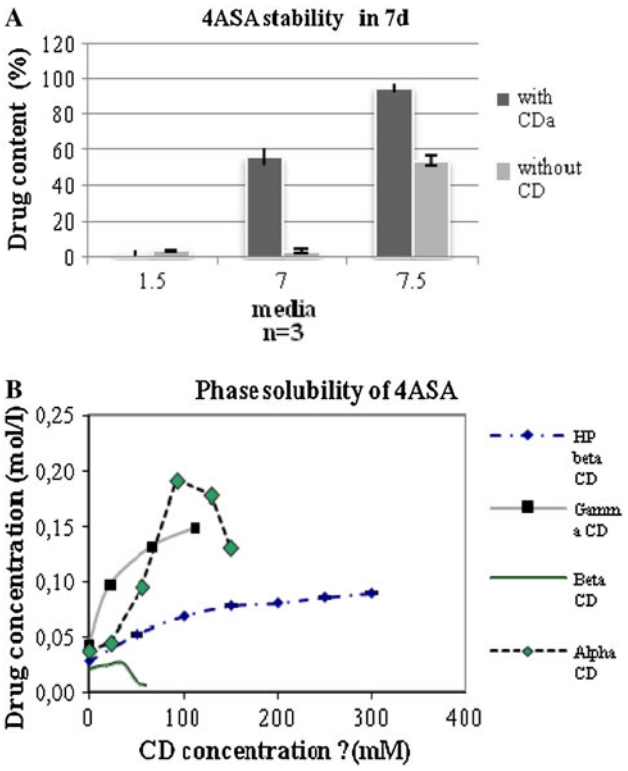


Fig. 2 **a** 4ASA–α-CD complex in different pH medium and **b** phase solubility diagrams of 4ASA–α, β, γ, and HPβ-CD

indicates insoluble complexes. β-CD often gives rise to B-type curves due to their poor water solubility [4].

Gamma-CD/4ASA phase solubility diagram shown an A_n subtypes which indicated a linear increase of 4ASA solubility. K value of the 1:1 complex was $35.71 \pm 2.21\ M^{-1}$. In the case of HPβ-CD the diagram profile seems to be an A_n subtype and showed a first ascendant part between 0 and 150 mM of HPβ-CD concentrations. The best improvement for 4ASA solubility was obtained with α-CD.

Differential scanning calorimetry studies

DSC profile of 4ASA showed a characteristic endothermic peak at 150.2 °C. A similar behavior was observed for 4ASA in the physical mixtures with α, β, γ and HPβ-CD (Fig. 3). With α-CD/4ASA complex endothermic peak disappearance denotes an inclusion complexes. With β-CD/4ASA complex a broad endothermic peak was exhibited between 130 and 175 °C corresponding to release of water molecules. A well-distinct melting peak was obtained at 150 °C with γ-CD/4ASA complex. This should indicate that 4ASA molecule was not embedded in the γ-CD inner cavity. A different pattern was observed in the thermogram of HPβ-CD/4ASA complex (Fig. 3). The disappearance of the melting peaks of 4ASA and HPβ-CD at 150.2 and 175 °C respectively was a proof of an inclusion complexes formation. These results indicated the existence of interactions between 4ASA with α-CD. In addition a dispersion phenomenon was observed with HPβ-CD by freeze drying method.

FT-IR spectroscopy

The FTIR spectra of wave number from 4,000 to 450 cm^{-1} are presented in Fig. (4a–d) and Table 1. 4ASA showed two strong absorption bands between 3,380 and 3,483 cm^{-1} for amino stretching. All the binary systems of 4ASA/CD did not show any new peaks, indicating no chemical bonds created in the formed complexes. The main characteristic double band appeared also at the same position in the physical mixtures with all CDs and 4ASA in accordance with the thermal analysis results. However, in the spectra of 4ASA/γ-CD obtained by inclusion complexes with CDs, the doublet peak in 3,380–3,483 cm^{-1} disappeared. Only OH large band of CD appeared on the spectra. Other bands, such as 1615, 1445, 765–814 cm^{-1} representing aromatic ring disappeared in the inclusion spectrum of 4ASA/α-CD suggested the aromatic ring was entrapped into the host cavities.

Conclusion

As discussed above, the nature of carriers played an important role in the formation of inclusion complexes

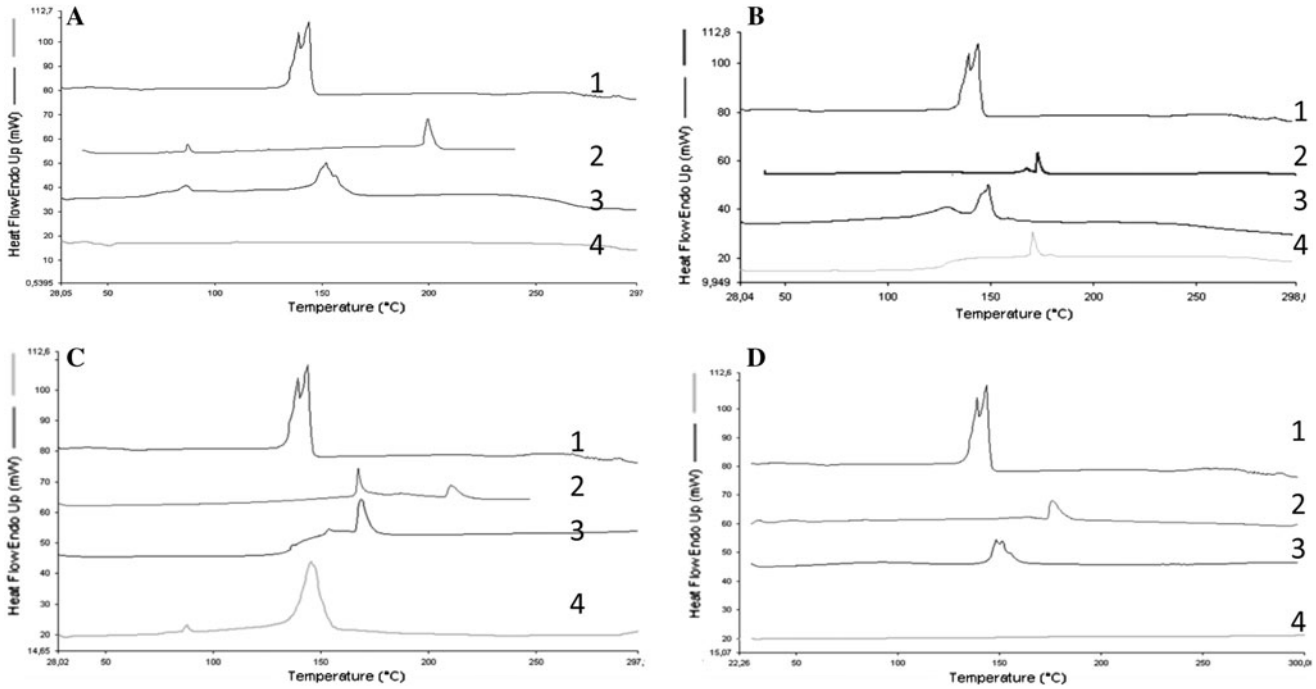


Fig. 3 DSC diagram of 4ASA/CD complexes: (*1*) 4ASA; (*2*) CD; (*3*) physical mixture; (*4*) inclusion complex. **a**: α-CD, **b**: β-CD, **c**: γ-CD, **d**: HPβ-CD

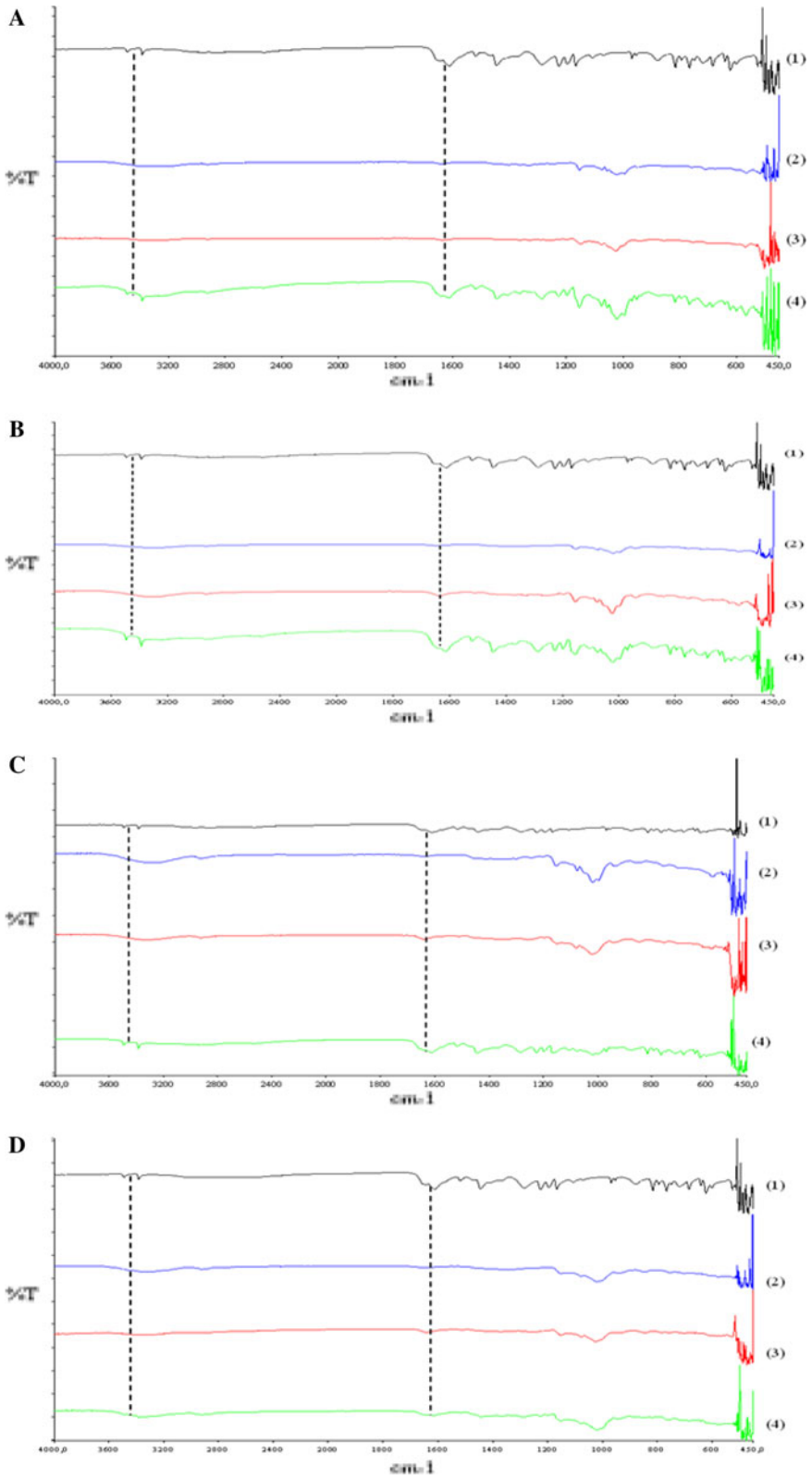


Fig. 4 **a** FTIR spectra of 4ASA 4ASA (*1*), α-CD (*2*), freeze-dried of 4ASA/α-CD (1:1 mol/mol), (*3*) and physical mixture of 4ASA/α-CD (1:1 mol/mol) (*4*). **b** FTIR spectra of 4ASA (*1*), *β*-CD (*2*), freeze-dried of 4ASA/*β*-CD (1:1 mol/mol) (*3*) and physical mixture of 4ASA/*β*-CD (1:1 mol/mol) (*4*). **c** FTIR spectra of 4ASA (*1*), **γ**-CD (*2*), freeze-dried of 4ASA/*γ*-CD (1:1 mol/mol) (*3*) and physical mixture of 4ASA/*γ*-CD (1:1 mol/mol) (*4*). **d** FTIR spectra of 4ASA (*1*), HP*β*-CD (*2*), freeze-dried of 4ASA/HP*β*-CD (*3*) (1:1 mol/mol) and physical mixture of 4ASA/HP*β*-CD (1:1 mol/mol) (*4*)

Table 1 Infrared spectral data and bands assignments

	CH (cm^{-1})	C–C (cm^{-1})	C–O–C (cm^{-1})	HCH (cm^{-1})	C=O (cm^{-1})	H bond (cm^{-1})	OH (cm^{-1})	NH_2 (cm^{-1})
4ASA	765–814	1615	–	1445	1650	–	–	3380–3483
α-CD	1406	642, 1080	1070–1152	–	–	2912	3200–3500	–
Complex 4ASA/CD	765–814	642, 1080	1070–1152	–	–	2912	3200–3500	–
Physical mixture	765–814	642, 1080 1615	1070–1152	1445	1650	2912	3200–3500	3380–3483
4ASA	765–814	700–800	–	1445	1650	–	–	3380–3483
β-CD	1406	642, 1080	1070–1152	–	–	2912	3200–3500	–
Complex 4ASA/CD	765–814	642, 1080	1070–1152	–	–	2912	3200–3500	–
Physical mixture	765–814	642, 1080 1615	1070–1152	1445	1650	2912	3200–3500	3380–3483
4ASA	765–814	700–800	–	1445	1650	–	–	3380–3483
γ-CD	1406	642, 1080	1070–1152	–	–	2912	3200–3500	–
Complex 4ASA/CD	765–814	642, 1080	1070–1152	–	–	2912	3200–3500	–
Physical mixture	765–814	642, 1080 1615	1070–1152	1445	1650	2912	3200–3500	3380–3483
4ASA	765–814	700–800	–	1445	1650	–	–	3380–3483
HPβ-CD	1406	642, 1080	1070–1152	–	–	2912	3200–3500	–
Complex 4ASA/CD	765–814	642, 1080	1070–1152	–	–	2912	3200–3500	–
Physical mixture	765–814	642, 1080 1615	1070–1152	1445	1650	2912	3200–3500	3380–3483

4ASA could form an inclusion with α and HPβ-CD, but no inclusion with β-CD and γ-CD. Nevertheless further analysis such as NMR analysis and modeling data would be taken to confirm or not if 4ASA aromatic ring is embedded inside the cavity of α and HPβ-CD.

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