ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
14 (1996) 909–915

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Analytical applications of retinoid–cyclodextrin inclusion complexes
1. Characterization of a retinal–β–cyclodextrin complex[1]

S. Muñoz Botella[a], M.A. Martín[a], B. del Castillo[a,*], J.C. Menéndez[b], L. Vázquez[c], D.A. Lerner[d]

[a]*Sección Departamental de Química Analítica, Facultad de Farmacia, Universidad Complutense, 28040 Madrid, Spain*
[b]*Dpto de Química Orgánica y Farmacéutica, Facultad de Farmacia, Universidad Complutense, 28040 Madrid, Spain*
[c]*Instituto de Ciencias de los Materiales, Universidad Autónoma, C-XII, 28049 Madrid, Spain*
[d]*Laboratoire de Chimie Physique et Informatique, E.N.S.C.M., 8 rue de l'Ecole Normale, 34053 Montpellier Cedex 2, France*

Received for review 15 September 1995

Abstract

In studies in these laboratories on the supramolecular chemistry of the retinoids, it has been recently confirmed that inclusion of these substances within the cavity of cyclodextrins protects their excited states, thus improving their photochemical stability. In the present paper, the isolation is described of a crystalline stable complex between retinal and β-cyclodextrin, which has been characterized by means of several techniques including atomic force microscopy (AFM). The complex shows distinct spectroscopic differences from both retinal and β–cyclodextrin. Thus, it absorbs at $\lambda_{max} = 380$ nm in water whereas retinal is insoluble; it shows room-temperature luminescence, which retinal does not; finally, it gives ^{1}H-NMR and ^{13}C-NMR spectra in d_6-DMSO with clear differences in chemical shifts with respect to those of β-cyclodextrin. Besides these studies in solution, the behaviour of the complex in the solid state has been compared with that of physical mixtures of retinal and β-cyclodextrin. IR spectroscopy shows clear differences, particularly a shift in the retinal carbonyl absorption (1644–1672 cm^{-1}). AFM studies reveal the existence of aggregates; X-ray diffractometry also supports the formation of a cyclodextrin–retinal complex.

Keywords: Retinoids; Cyclodextrins; Inclusion complexes

1. Introduction

Cyclodextrins are macrocyclic oligosaccharides, formed by six–eight α-D-glucopyranose units, and shaped as truncated cones. While their outer surfaces are highly hydrophilic owing to the presence of the glucose hydroxyl groups, their cavities can accommodate a variety of lipophilic molecules, rendering them water-soluble. Cyclodextrin inclusion may also protect the guest molecules

* Corresponding author.

[1] Presented at the Fifth International Symposium on Drug Analysis, September 1995, Leuven, Belgium.

SSDI 0731-7085(95)01672-4

from their environment and several medicinal compounds, such as vitamin D [1], vitamin K_3 [2], aspirin [3], nitrazepam [4] and metronidazole [5], show increased stability in the presence of cyclodextrins. Several analytical and spectroscopic techniques benefit from cyclodextrin inclusion of the analytes and the associated enhanced selectivity and sensitivity [6]. The use of cyclodextrins also allows the observation of room-temperature phosphoresence because of the protection of the triplet excited state towards deactivation by molecular oxygen [7].

Retinoids are biologically significant compounds because of their roles in the photochemistry of vision and in cellular differentation and proliferation, a fact that has led to a renewed interest in their chemistry [8]. Owing to their polyenic structure, retinoids are virtually insoluble in water and chemically labile, which makes their manipulation difficult, but these problems may be alleviated by cyclodextrin inclusion. The luminescence properties at room temperature of several retinal–cyclodextrin complexes have been reported [9]. As a sequel to these studies, the present work has involved the synthesis, isolation and characterization of a crystalline retinal–β-cyclodextrin complex, as a first step towards the development of methods for the determination of retinoids in aqueous media.

2. Experimental

2.1. Apparatus

UV–visible absorption spectra were obtained with a Kontron Uvikon 810 spectrophotometer equipped with a Kontron P-800 printer–plotter. Fluorescence excitation and emission spectra were recorded on a Perkin-Elmer MPF-2A spectrofluorimeter. In both cases, quartz cells with a 1 cm pathlength were employed. IR absorption spectra were obtained on a Buck Scientific 500 spectrophotometer. NMR spectra were recorded on Bruker AC-250 (250 MHz for ^1H, 63 MHz for ^{13}C) and Varian VXR-300 (75 MHz for ^{13}C) spectrometers. X-ray diffractogram patterns were obtained with a Phillips X-ray diffractometer X'Pert-MPD (copper anticathode). The samples were placed on amorphous silicon wafers. Atomic force microscopy measurements were carried out in air using a Nanoscope III microscope (Digital Instruments, Santa Barbara, CA) operated in the "tapping mode" using silicon cantilevers.

2.2. Procedures

Water was deionized and double-distilled prior to use. Other solvents were analytical-grade commercial reagents and were used without further purification. NMR experiments were performed using deuterium oxide and d_6-DMSO (Sigma) as solvents, and sodium 4,4-dimethyl-4-silapentane-1-sulphonate (DSS) and tetramethylsilane (TMS) as internal reference standards. β-Cyclodextrin (β-CD) was a generous gift from Rhône-Poulenc (France).

Solid complexes were prepared starting from 5 ml of a hexane solution of retinal (1×10^{-3}–8×10^{-3} M). The solvent was evaporated in a round-bottomed flask, leaving a thin film of retinal in the bottom. 5 ml of 10^{-2} M β-cyclodextrin in deuterium oxide was added. The reaction mixture was magnetically stirred in the dark at 20°C (thermostatted bath). A fine precipitate appeared which was collected by centrifugation and lyophylized. These complexes were redissolved in water in order to obtain the UV–visible and fluorescence spectra. The complexes were also mixed with KBr (2:98, w/w) and compressed at 10 kg cm^{-2} for 10 min; the pellets obtained were used to record the IR spectra. The same procedure was used for obtaining IR spectra of the physical mixture (retinal and β-CD) and pure retinal. X-ray diffractograms were obtained by placing the sample directly on the amorphous silicon wafer without solvent or other previous treatment.

Two different flat substrates were used to study the cyclodextrin samples by atomic force microscopy: silicon (100) wafers and mica. Both are extremely flat with a surface roughness < 0.5 nm for scanned areas of 2×2 μm^2. Wide areas (about 4×4 μm^2) were initially scanned to localize the filamentous structures. These structures were subsequently imaged at higher magnifica-

tion. All the images were acquired at a resolution of 512 × 512 pixels. They are shown either as top views or as three-dimensional representations where the brighter spots correspond to larger heights. A 20 μl drop of 10^{-6} M solution was deposited on the surface of the substrate. Imaging of the sample surface was made during the next 24 h in regions close to the border of the drop in order to avoid regions of high concentration. Special attention was paid to visualizing those features with an elongated shape. None of the structures shown in this work have been observed when imaging the substrate surface.

3. Results

UV–visible spectrophotometry is one of the most commonly used techniques for the detection of cyclodextrin inclusion complexes. In the case of retinal, the appearance of its absorption bands in an aqueous solution of cyclodextrin must be due to complex formation, since the retinoids are not water-soluble. Fig. 1 shows the absorption spectrum of retinal in organic solvents compared with those of an aqueous solution (1 mg of the isolated solid complex in 10 ml of water) and a solution prepared by addition of aqueous β–cyclodextrin solution to the retinal film, in order to obtain final concentrations of 1×10^{-2} M and 1×10^{-5} M respectively (dissolved complex). In all cases, the aqueous solution shows the absorption bands characteristic of retinal [8]. The displacement of the maximum in the case of the solution prepared from the isolated complex (396 nm instead of 380 nm) and the broadening of the band can be attributed to a concentration effect. Excitation and emission fluorescence spectra of these solutions, with the concentration of the solid complex solution readjusted to avoid the inner filter effect, can be observed in Fig. 2. The appearance of the luminescence emission characteristic of retinal ($\lambda_{ex} = 335$nm, $\lambda_{em} = 430$ nm) confirms its inclusion into the cyclodextrin cavity [9] since this compound shows luminescence only in dry hydrocarbon solution at 77 K [8]. The cyclohexane ring can be expected to be included in the cavity since it is the less polar portion of the molecule.

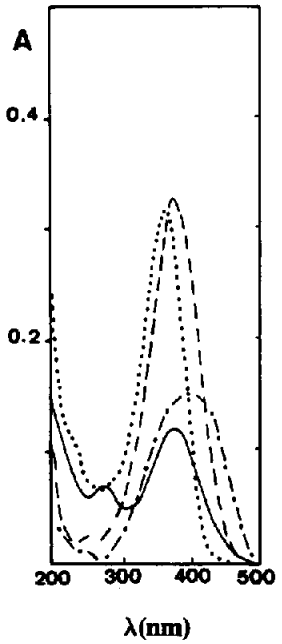

Fig. 1. UV–visible absorption spectra of retinal in hexane (· · ·); retinal in ethanol (– – –); retinal–β-CD (solid complex) (– · –); retinal–β-CD (dissolved complex) (——).

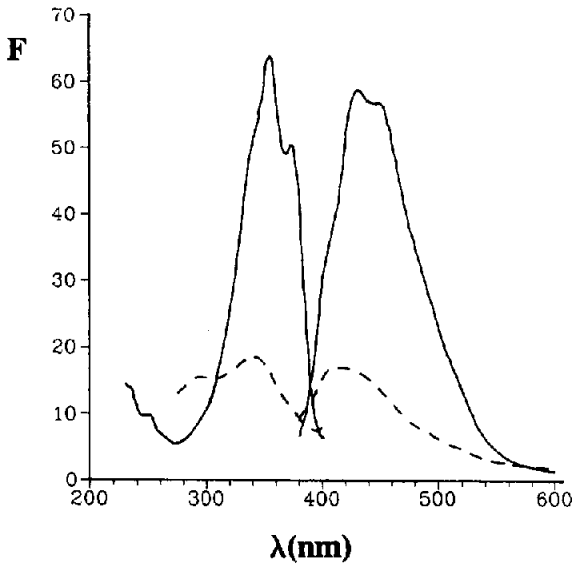

Fig. 2. Uncorrected excitation and fluorescence spectra of retinal–β-CD (solid complex) (– – –) and retinal–β-CD (dissolved complex) (——). F: fluorescence intensity in arbitrary units.

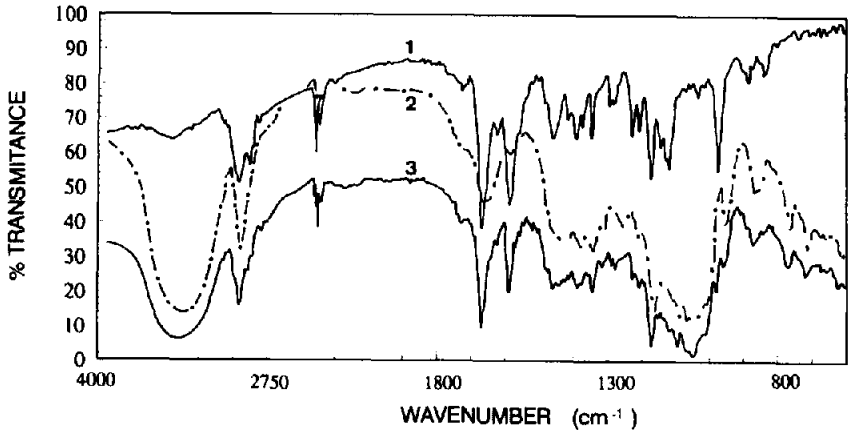

Fig. 3. IR absorption spectra of (1) retinal; (2) retinal–β-CD complex; (3) physical mixture of retinal and β-CD. The IR spectra of (1) and (2) are displaced in order to avoid the overlapping of the signals: (3) is not displaced, $\%T = 34\%$; (2) is displaced and real $\%T = 52\%$; (1) is displaced and real $\%T = 31\%$.

Infrared spectroscopy is not always suitable for the study of this process because the cyclodextrin absorption hinders most of the bands due to the inclusion complexes. However, this interference does not take place in the carbonyl region and previous workers have taken advantage of this fact to study the inclusion of flufenamic acid and indomethacin [10] into β-cyclodextrin. The IR absorption spectrum of the solid retinal–cyclodextrin complex is compared in Fig. 3 with those of a physical mixture of both compounds and pure retinal. The carbonyl regions of the two latter spectra are very similar and the absorption maximum lies at 1664 cm^{-1} while the corresponding signal of the isolated complex is at 1672 cm^{-1}.

The ^{1}H-NMR spectra in d_6-DMSO of β-cyclodextrin and the retinal/β-cyclodextrin complex are reproduced in Fig. 4. While δ values for H_1–H_4 (5.75 and 3.30 ppm) remain unchanged and the H_6 (3.62 ppm) signal overlaps with the water resonance, a significant shift is observed in the case of H_5 (3.52 ppm) ($\Delta\delta = 0.04$ ppm). Although J values could not be measured with precision for all signals, the changes observed in the spectral profile in the H_5 region [11] suggest a distortion of the cyclodextrin three-dimensional structure associated with retinal inclusion. In this connection it is interesting that H_5 is orientated towards the interior of the cavity [12] and therefore changes in its signal provide information on the inclusion process.

Similarly, Fig. 5 shows the comparison between the ^{13}C-NMR spectra of β-cyclodextrin and its complex with retinal in d_6-DMSO solution. The inclusion process causes significant shifts for all signals, more clearly observed than in the case of ^{1}H-NMR owing to the absence of superimposed signals and the greater spectral width.

The X-ray diffractometry patterns of the physical mixture, the inclusion complexes and β-CD can be found in Fig. 6. In the case of the physical mixture, signals corresponding to β-cyclodextrin and retinal are observed. The powder X-ray diffraction pattern of the inclusion complex reveals a different structure. The signals close to $2\theta \approx 5.5$–7.5 are characteristic for the complex and can be explained by the approximation of the crystal planes. The inclusion process may increase the amorphous character [13] and can also be explained by means of the procedure employed to obtain the complex, because a decrease in the relative humidity converts the crystalline state to the amorphous state [14]. The loss of the crystalline character for the inclusion complex is shown by the smaller number of diffraction signals.

Recently, scanning tunneling microscopy (STM) has been used to characterize an inclusion complex of diphenylhexatriene (DPH) with β-cy-

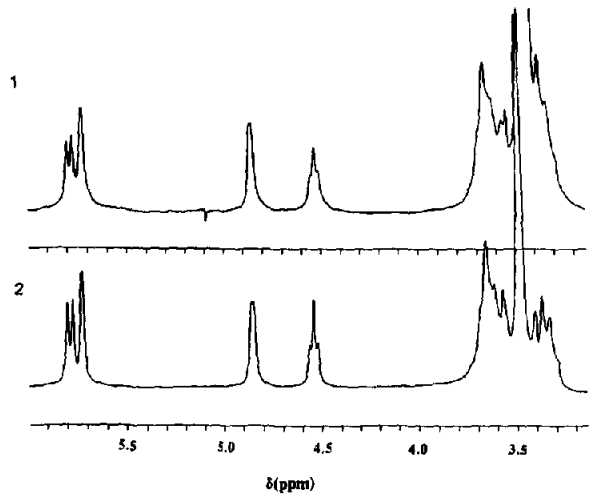

Fig. 4. ^{1}H-NMR spectra of (1) retinal–β-CD complex; (2) β-CD in d_6-DMSO. This spectral region corresponds to the signals of the cyclodextrin protons.

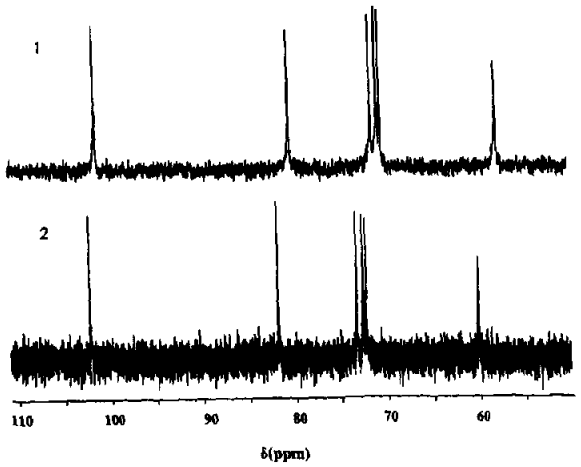

Fig. 5. ^{13}C-NMR spectra of (1) retinal–β-CD complex; (2) β-CD in d_6-DMSO. This spectral region corresponds to the signals of the cyclodextrin protons.

clodextrin on highly orientated pyrolytic graphite (HOPG) [15]. However, it is well known that the interpretation of STM images of organic samples is rather difficult [16]. In contrast AFM is a relatively novel technique which allows three-dimensional images to be obtained of the surface of insulating and conducting materials from the nanometer to the micrometer region [17]. Thus, AFM imaging of organic specimens is better as it does not require the specimen to be electron- or ion-conductive. However, effects due to the tip geometry and probe force usually lead to over-estimated (sometimes by a factor of five) lateral sizes of organic sample features. Despite this drawback results are presented from a preliminary AFM study of the retinal–β-CD complex deposited on freshly cleaved mica and silicon (100) wafers. Fig. 7 shows that the complex molecules form filamentous aggregates. It can be observed that two linear structures, each 8 nm wide, are distinguished in the filament. This value of 8 nm is larger than that predicted by other techniques and measured by STM on the DPH–β-CD complex. The difference could be explained by the typical over-estimation (mentioned above) of the sizes of this type of structure [18]. Finally, it is important to note that in the filamentous aggregate some "subunits" are clearly resolved, indicating that the filament is not continuous but is formed by smaller components.

4. Conclusion

In conclusion, the different instrumental techniques employed reveal the existence of an inclusion complex between retinal and β-CD. Thus the most frequently used and simple techniques (UV-visible or IR spectroscopy) together with the more

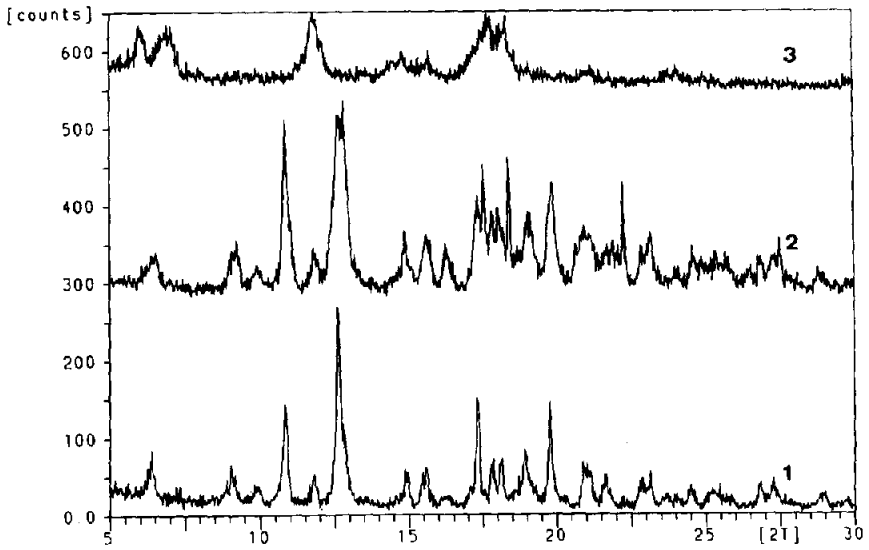

Fig. 6. Powder X-ray diffractogram patterns of (1) β-CD; (2) physical mixture of retinal and β-CD; (3) retinal-β-CD inclusion complex.

Fig. 7. 215 x 215 nm^2 raw data AFM image (tapping mode) of retinal-β-CD complex deposited on a silicon (100) wafer. The bar indicates 100 nm.

sophisticated NMR or AFM methods can be used to characterize the inclusion complexes with cyclodextrins. The procedure for preparation of the solid complex with retinal is described and presents the advantage of protecting a photolabile molecule, allowing the solubilization and determination of these hydrophobic compounds in aqueous solution.

References

[1] J. Szejtli, E. Bolla-Putszai, P. Szabo and T. Ferenczy, Pharmazie, 35 (1980) 779–787.
[2] J. Szejtli, E. Bolla-Pusztai and M. Kajatar, Pharmazie, 37 (1982) 725–728.
[3] Y. Nakai, K. Yamamoto, K. Terada and K. Akimoto, Chem. Pharm. Bull., 32 (1984) 685–691.
[4] F. Møllgaard Andersen and M. Bundgaard, Arch. Pharm. Chem. Sci. Ed., 10 (1982) 80–87.
[5] F. Møllgaard Andersen and H. Bundgaard, Int. J. Pharm., 19 (1984) 189–197.
[6] S. Li and W.C. Purdy, Chem. Rev., 92 (1992) 1457–1470.
[7] R.J. Hurtubise, in Phosphorimetry: Theory, Instrumentation and Applications, VCH, New York, 1990 pp. 305–334.
[8] R.S. Becker, Photochem. Photobiol., 48 (1988) 369–399
[9] D.A. Lerner, B. del Castillo and S. Muñoz Botella, Anal. Chim. Acta, 227 (1989) 297–301.
[10] M. Kurozumi, N. Nambu and T. Nagai, Chem. Pharm. Bull., 23 (1975) 3062–3068.
[11] D.J. Wood, F.E. Hruska and W. Saenger, J. Am. Chem. Soc., 99 (1977) 1735–1740.
[12] M.L. Bender and M. Komiyama in Cyclodextrin Chemistry, Springer-Verlag, Berlin, 1978, pp. 10–12.
[13] D. Amdidouche, H. Darrouzet, D. Duchêne and M.-C Poelman, Int. J. Pharm., 54 (1989) 175–179.
[14] F. Hirayama and K. Uekama, in D. Duchêne (Ed.), Cyclodextrins and their Industrial Uses, Editions de Santé, Paris, 1987, pp. 155, 156.

[15] G. Li and L.B. McGown, Science, 264 (1994) 249–251.
[16] J. Yang, L.K. Tamm, A.P. Somlyo and Z. Shao, J. Microsc., 171 (1993) 183–198.
[17] G. Binnig, C.F. Quate and C. Gerber, Phys. Rev. Lett., 56 (1986) 930–933.
[18] S. Karrasch, S. Heins, U. Aebi and A. Engel, J. Vac. Sci. Technol., B12 (1994) 1474–1477.