

# Synthesis, characterisation and photo-stability of a folate-modified $\beta$ -cyclodextrin as a functional food additive

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**Abstract** A novel two-step synthetic route was developed and gave the mono-substituted derivative 6-deoxy-6-[(1-(2-amino)ethylamino)folate]- $\beta$ -cyclodextrin (CDEnFA) with high yield (60 %). Elemental analysis, mass spectrometry,  $^1\text{H}$  and  $^{13}\text{C}$  NMR, FTIR and Raman spectroscopies demonstrated the successful synthesis of the  $\gamma$  isomer only with no evidence of the presence of other isomers or free folic acid. Electronic absorption spectroscopy was used to study the photochemical properties of CDEnFA and showed that in both the solid state and aqueous solution CDEnFA is considerably more photo-stable than free folic acid.

**Keywords** Cyclodextrin · Folate · Photo-stability

## Introduction

Functional foods are any food that can prevent disease and improve health and include foods fortified with additives such as vitamins. For example in many countries food products including bread, cereals and grain products are fortified with folic acid [1]. Folate is a naturally-occurring,

water-soluble compound belonging to the B group of vitamins and leafy vegetables, legumes, liver and kidney are some of the main dietary sources, while folic acid is the synthetic form. Folate is not biologically active but on conversion to a series of tetrahydrofolate derivatives it is essential for many biological functions including cell division and growth in infancy and pregnancy [2]. Folate deficiency can result in neural tube defects in developing embryos. Folic acid is relatively stable to heat and humidity and therefore baked products and flours may retain most of the added folic acid on processing and storage. It is however sensitive to UV light. This problem can be addressed by using light-proof packaging but this can lead to its own problems of high-cost. Therefore, the primary aim of this work is to improve the photo-stability of folic acid.

The partial hydrolysis of starch using glucosyltransferase gives cyclic sugars containing six to eight  $\alpha$ -(1-4)-linked D-glucopyranose units [3]. The cyclodextrins (CDs) produced can be distinguished on the basis of the number of  $\alpha$ -D-glucose units, i.e.,  $\alpha$ -cyclodextrin (six glucose units),  $\beta$ -cyclodextrin (seven glucose units), and  $\gamma$ -cyclodextrin (eight glucose units) (Fig. 1).

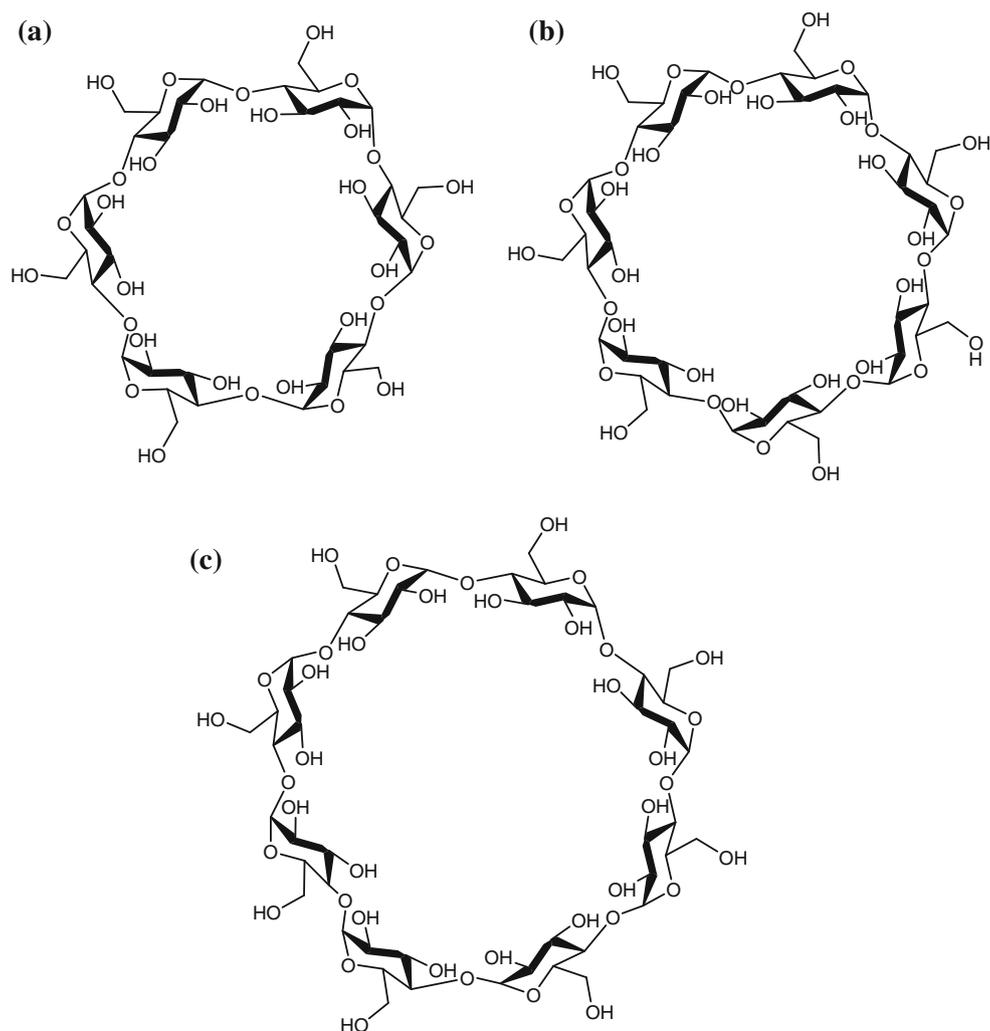
Cyclodextrin rings are amphipathic with secondary hydroxyl groups at C2 and C3 and primary hydroxyl groups at C6. These hydrophilic groups are located on the rims of the truncated-cone while the inner cavity is hydrophobic since it is lined with the ether-like anomeric oxygen atoms and the C3 and C5 hydrogen atoms as shown in Fig. 2.

The hydrophobic cavity can include many guest molecules while the multitude of hydroxyl groups can be modified to give a wide range of derivatives. The toxicological properties of cyclodextrins have been reviewed and in general they are classified as non-toxic materials which

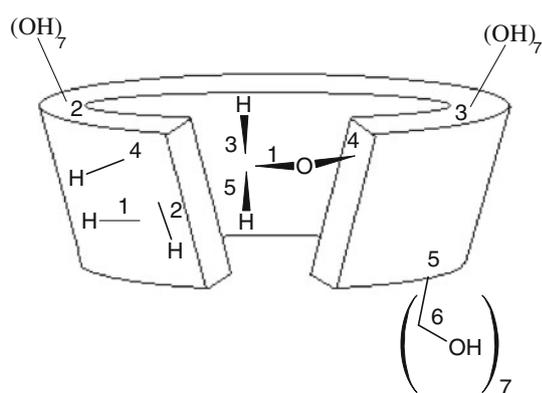
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**Fig. 1** Structure of **a**  $\alpha$ -cyclodextrin, **b**  $\beta$ -cyclodextrin and **c**  $\gamma$ -cyclodextrin



**Fig. 2** Truncated-cone representation of  $\beta$ -cyclodextrin

may cause some eye or skin irritation [4, 5]. This has led to the use of CDs in the cosmetic, food, flavour and pharmaceutical industries [4]. As well as improving solubility,  $\beta$ -CD is used to protect several compounds against various

degradation processes such as photo-degradation, thermal degradation and hydrolysis [6–11].

Here we report a novel two-step synthetic route to the mono-substituted derivative 6-deoxy-6-[(1-(2-amino)ethylamino)folate]- $\beta$ -cyclodextrin (CDEnFA) which has improved photo-stability in comparison to folic acid.

### Materials and methods

$\beta$ -cyclodextrin was obtained from Wacker Chemie (Munich, Germany). All other materials were purchased from Sigma Aldrich (Dublin, Ireland) and were used without further purification. All solutions were prepared in Elga Millipore deionised water.

$^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were measured at 400 and 100 MHz respectively, using a Bruker Avance 400 MHz (Boston, MA) NMR spectrometer. All spectra were recorded at 30 °C in  $\text{D}_2\text{O}$  and solutions were typically from 15 to

50 mmol dm<sup>-3</sup>. Samples were referenced to the TMS peak at 0 ppm.

Elemental analysis was carried out on neat samples using an Exeter Analytical CE440 CHN Analyser at University College Dublin.

Mass spectrometry studies were carried out in negative mode using a Waters Micromass LCT liquid phase instrument with electrospray ionisation.

FTIR spectra were obtained from solid samples as KBr disks using a Perkin Elmer Spectrum GX, which is a single-beam, Michelson interferometer-based, Fourier transform infra-red spectrometer. The spectra were measured over a range of 4,000–400 cm<sup>-1</sup> with a resolution of 4 cm<sup>-1</sup>, an interval of 0.5 cm<sup>-1</sup> and an accumulation of 16.

Raman spectra were recorded using a Horiba Jobin–Yvon LabRAM HR 800 system, which provides ultra-high spectroscopic resolution and unique wavelength range capability with both great flexibility and high performance. After alignment of optics, the system was calibrated using silicon. The laser beam was focused on solid samples and the scattered radiation efficiently gathered via the objective of an Olympus BX40 microscope. Measurements were taken at room temperature over the range of 200–4,000 cm<sup>-1</sup> using the following parameters: Time: 30 s, Accumulation: 10, Grating: 1,800 lines/mm, Slit: 900 μm, Laser: 660 nm, Objective: 100× or 50×, Filter: 100 %, Hole: 900 μm.

Electronic absorption spectra of samples in solution were measured using a Perkin Elmer Lambda 900 UV/Vis/NIR Spectrometer. The spectrometer is a double-beam, double monochromator ratio recording system with pre-aligned tungsten-halogen and deuterium lamps as sources. Parameters used were: Range: 200–1,100 nm, Slit Width: 0.1 nm, Accumulation: 4. A 60 mm Spectralon-coated integrating sphere with a range from 200 to 2500 nm was used for measurement of spectra of solid state samples using KBr as diluent.

#### Samples for photo-stability study

Samples for the photo-stability study were irradiated at 350 nm using a UVGL-25 P/N 95-0021-10 254 lamp with a power of 4 W. Samples were always placed 10 cm from the lamp and irradiated over a range of time periods to a maximum of 330 min. Solution phase samples were placed in a 1 cm cuvette while compressed discs were prepared for examination of samples in the solid state.

#### Buffer pH 7

A solution of KH<sub>2</sub>PO<sub>4</sub> (100 cm<sup>3</sup> of 0.1 mol dm<sup>-3</sup>) was added to NaOH (58.2 cm<sup>3</sup> of 0.1 mol dm<sup>-3</sup>) to give a buffer of pH 7.

#### Folic acid solution

Folic acid (0.0002 g, 4.5 × 10<sup>-4</sup> mmol) was dissolved in buffer pH 7 (25 cm<sup>3</sup>) to give a final solution of concentration 1.68 × 10<sup>-5</sup> mol dm<sup>-3</sup>.

#### CDEnFA solution

CDEnFA (0.0007 g, 4.3 × 10<sup>-3</sup> mmol) was dissolved in buffer pH 7 (25 cm<sup>3</sup>) to give a final solution of concentration 1.68 × 10<sup>-5</sup> mol dm<sup>-3</sup>.

#### Folic acid β-cyclodextrin solution-phase mixture

β-cyclodextrin (0.0005 g, 4.4 × 10<sup>-3</sup> mmol) was dissolved in buffer pH 7 (25 cm<sup>3</sup>) and folic acid (0.0002 g, 4.5 × 10<sup>-4</sup> mmol) was added. The solution was stirred for 4 h in the dark to give a final solution of concentration 1.68 × 10<sup>-5</sup> mol dm<sup>-3</sup> in both components.

#### Synthesis

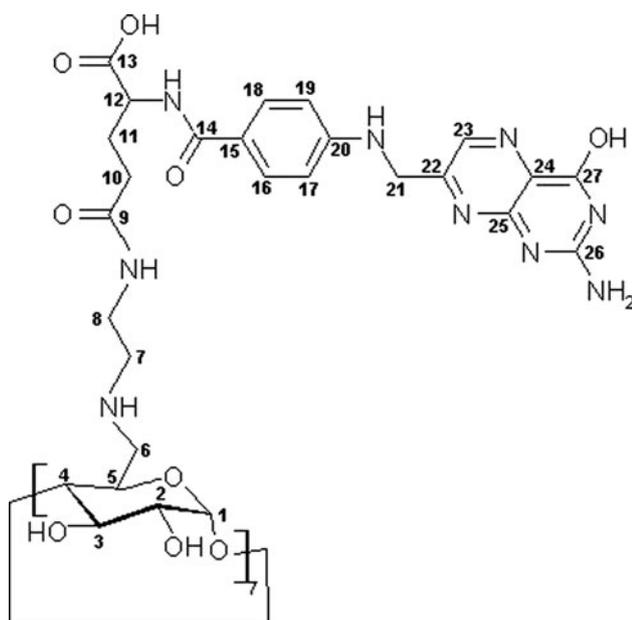
6-deoxy-6-[1-(2-amino)ethylamino]-β-cyclodextrin (CDEn) was prepared from 6-o-monotosyl-6-deoxy-β-cyclodextrin (CDTs) as reported previously [12]. CDEnFA was prepared as follows: Folic acid (0.500 g, 1.13 mmol) was dissolved in dimethylsulfoxide (DMSO, 20 cm<sup>3</sup>) along with *N*-hydroxy-succinimide (NHS, 0.142 g, 1.24 mmol). Dicyclohexylcarbodiimide (0.255 g, 1.24 mmol) was then added, and the reaction mixture was stirred overnight in darkness under nitrogen at room temperature. The insoluble by-product, dicyclohexylurea, was removed by filtration. The filtrate contained the DMSO solution of the *N*-hydroxysuccinimide ester (NHS-FA) intermediate. CDEn (2.0 g, 1.5 mmol) was dissolved in pyridine (10 cm<sup>3</sup>) and added to the NHS-FA solution. The reaction was performed under a nitrogen atmosphere at 55 °C for 4 h. The resultant solution was diluted with a mixture of acetone:diethylether (1:1 vol/vol, 45 cm<sup>3</sup>) and the product was recovered by filtration followed by recrystallisation from water.

Yield: 1.56 g, 60 % (based on hydrated materials), Melting point: 254 °C, Elemental Analysis: CDEnFA.14H<sub>2</sub>O (C<sub>63</sub>H<sub>93</sub>N<sub>9</sub>O<sub>39</sub>.14H<sub>2</sub>O) Theory: 41.5 % C, 5.1 % H, 6.0 % N. Found: 40.4 % C, 5.9 % H, 5.6 % N. [M-H]<sup>-</sup> 1598.83 m/z.

FTIR (cm<sup>-1</sup>): 1643 sh (νC = O), 1607 s (δ-N-H), 1510 m (ν phenyl and pterin), 1404 m (δ-O-H) (These are new bands relative to CDEn).

Raman (cm<sup>-1</sup>): 1602 s (δ-N-H), 1567 s (ν phenyl and pterin), 1513 m (ν phenyl and pterin), 1413 m (δ-O-H), (These are new bands relative to CDEn).

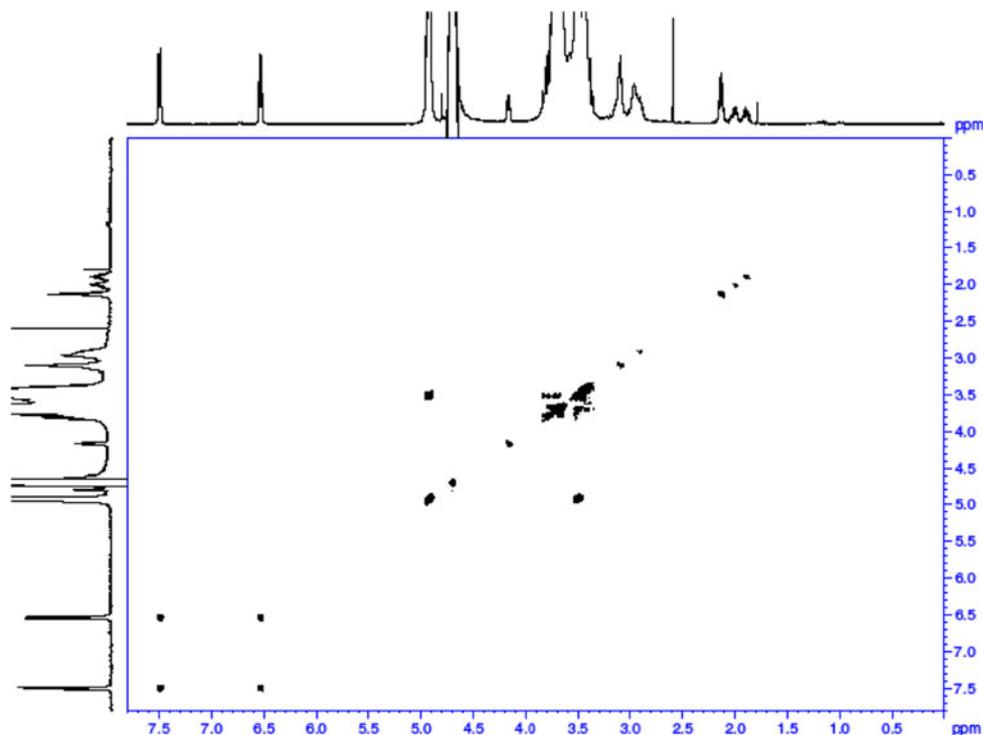
<sup>13</sup>C NMR (100 MHz, D<sub>2</sub>O): ppm 172.9 (C9), 172.3 (C13), 166.1 (C14), 161.0 (C27), 156.1 (C26), 153.6 (C25),



**Fig. 3** Proposed structure of 6-deoxy-6-[(1-(2-amino)ethylamino)folate]- $\beta$ -cyclodextrin (CDEnFA)

150.4 (C20), 148.4 (C22), 148.4 (C23), 128.9 (C19), 128.9 (C17), 127.8 (C24), 121.31 (C13), 111.2 (C18), 111.2 (C16), 101.9 (C1), 101.7 (C1'), 84.0 (C4'), 81.9 (C4''), 81.4 (C4), 73.0 (C3), 72.3 (C2), 72.0 (C5), 71.9 (C2'/C3'), 69.7 (C5'), 61.1 (C6), 52.6 (C12), 51.7 (C6'), 48.4 (C7), 36.1 (C8), 30.3 (C10), 26.0 (C11).

**Fig. 4** COSY NMR of CDEnFA in  $D_2O$  at 30 °C



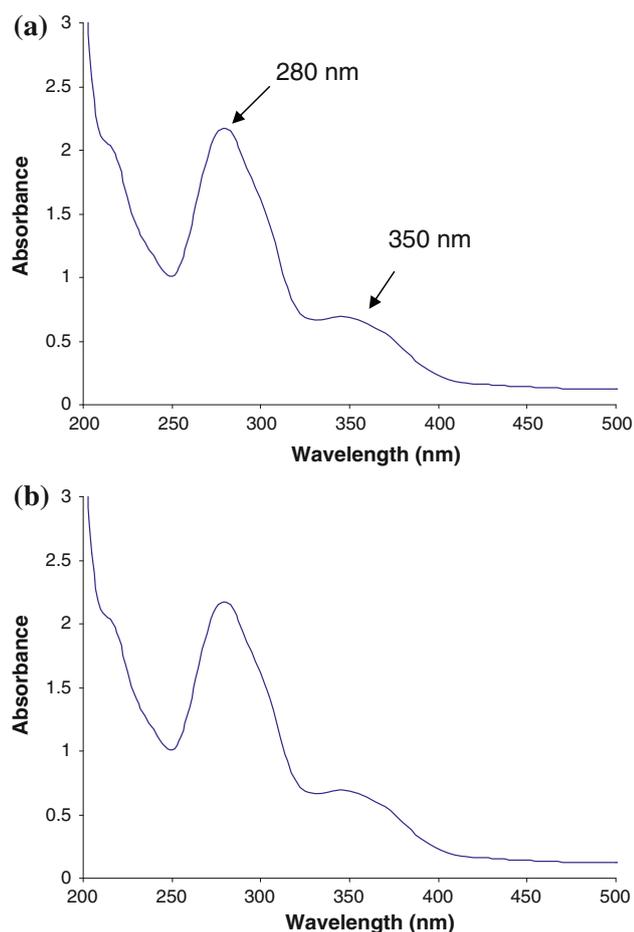
$^1H$  NMR (400 MHz,  $D_2O$ ): ppm 8.46 (s, H23), 7.51 (d, H17, H19), 6.53 (d, H16, H18), 4.94 (d, H1), 4.66 (d, H21), 4.18 (t, H12), 3.79 (t, H3), 3.71 (q, H5), 3.47 (d, H6<sub>a,b</sub>), 3.45 (d, H2), 3.41 (t, H4), 2.67 (t, H8), 2.59 (q, H10), 2.15 (q, H11<sub>a</sub>), 2.00 (q, H11<sub>b</sub>), 2.00 (t, H7).

## Results and discussion

### Synthesis and characterisation

The proposed structure of 6-deoxy-6-[(1-(2-amino)ethylamino)folate]- $\beta$ -cyclodextrin (CDEnFA) is given below in Fig. 3.

The synthesis of CDEnFA was first reported by Clementi et al. [13] with a 5 % yield but the product was reported to contain three different forms of folic acid even after extensive purification by column chromatography. Two isomers,  $\alpha$  and  $\gamma$  were formed by reaction of CDEn with the two FA carboxylic functions at C9 and C13, respectively, and free folic acid was also reported to be present [13]. In this current study a method reported by Guo and Lee for the synthesis of a folate-polyethylenimine-polyethyleneglycol conjugate was modified by changing the reaction temperature and the molar ratio of reactants, introducing diethylether as a drying agent and recrystallising the product from water [14]. These changes resulted in an improved yield of CDEnFA of 60 % compared to the previous report of 5 %. The first step in this

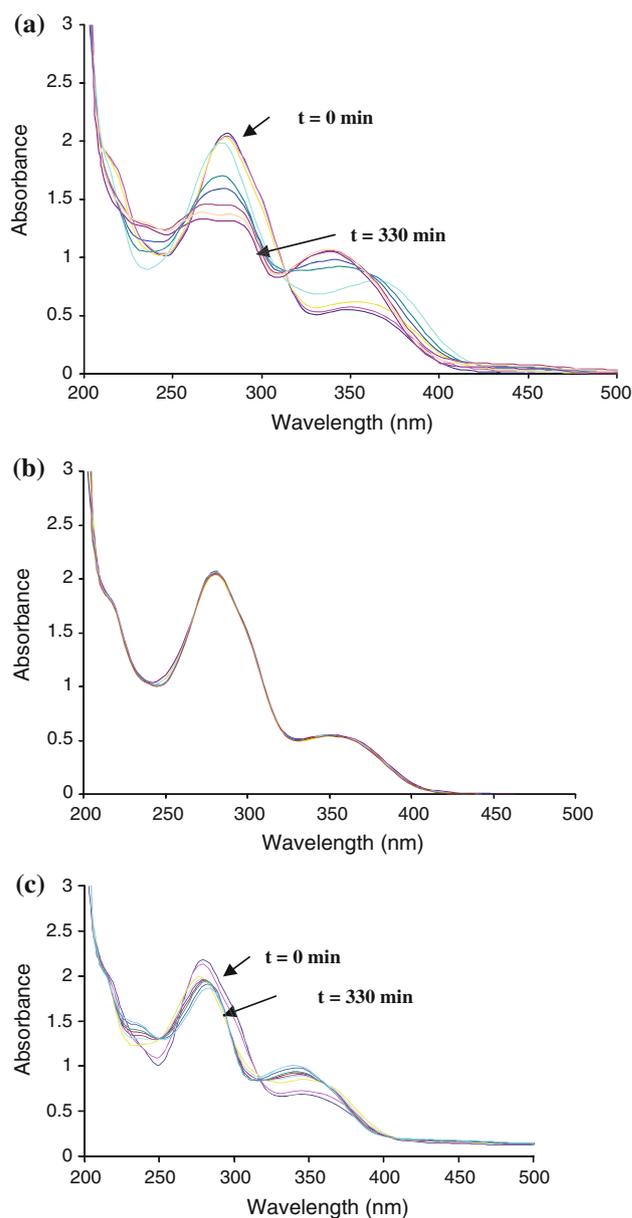


**Fig. 5** Electronic absorption spectra of aqueous solutions of **a** folic acid and **b** CDEnFA both at  $1.68 \times 10^{-5} \text{ mol dm}^{-3}$

two-step reaction involves activation of folic acid with NHS to produce the *N*-hydroxysuccinimide ester (NHS-FA) and in the second step conjugation of CDEn was achieved through amide bond formation with NHS-FA to produce CDEnFA. This method favours reaction of the  $\gamma$  carboxyl residue of folic acid and therefore an improved purity of product which is confirmed here spectroscopically.

In the mass spectrometry study the molecular ion peak was observed at  $[M-H]^-$  1598.83  $m/z$  for CDEnFA. This suggests a molecular weight of  $\sim 1599.8$  and a molecular formula of  $C_{63}H_{93}N_9O_{39}$  which is in agreement with the formation of a pure product with a structure as shown in Fig. 3. The results obtained from the elemental analysis also agree with this proposed structure and the formation of a hydrated product of formula  $C_{63}H_{93}N_9O_{39} \cdot 14H_2O$ .

Due to the complicated spectra obtained, it can be difficult to use vibrational spectroscopy for structural elucidation of derivatives of cyclodextrins. However, new bands were very apparent in both the FTIR and Raman spectra of



**Fig. 6** Electronic absorption spectra of aqueous solutions of **a** folic acid irradiated over 330 min, **b** non-irradiated folic acid and **c** CDEnFA irradiated over 330 min (all at  $1.68 \times 10^{-4} \text{ mol dm}^{-3}$ )

CDEnFA particularly in the  $1,400\text{--}1,700 \text{ cm}^{-1}$  region. The band at  $1,607 \text{ cm}^{-1}$  in the FTIR spectrum of the final product can be assigned to a bending mode of  $-N-H$  while the shoulder at  $1,643 \text{ cm}^{-1}$  can be assigned to  $C=O$  vibrations of the  $-CONH-$  group. Zhang et al. [15] report similar bands in the FTIR spectra of folate-modified polyethylene glycol-coated nanoparticles. Ha et al. [16] also report similar band assignments for the spectra of a folic acid-anthracene conjugate. A new band appears at  $1,510 \text{ cm}^{-1}$  which is also reported by Ha et al. [16] and can

be assigned to vibrations of the phenyl and pterin moieties of folic acid. Finally the medium intense band at  $1,404\text{ cm}^{-1}$  can be assigned to deformations of the  $\text{-O-H}$  group and was also reported by Zhang et al. [15]. Raman spectroscopy was used to complement the FTIR study. Again the band at  $1,602\text{ cm}^{-1}$  can be assigned to a bending mode of  $\text{-N-H}$ . Two new bands at  $1,567$  and  $1,513\text{ cm}^{-1}$  can be assigned to vibrations of the phenyl and pterin moieties. Finally the band at  $1,413\text{ cm}^{-1}$  can be assigned to deformations of the  $\text{-O-H}$  group. These results suggest the successful conjugation of the folic acid moiety to CDEn via amide bond formation.

In the  $^{13}\text{C}$  NMR spectrum of CDEnFA the signals at 172.9 and 172.3 ppm were assigned to carbon atoms C9 and C13 of the carboxyl groups of the folate substituent. The signals at 111.2, 128.9 and 150.4 ppm may be similarly assigned to C16/18, C17/19, and C20. The carbon atoms of the pterin ring C25 and C24 gave rise to two signals at 153.6 and 127.8 ppm. The  $^1\text{H}$ -NMR spectrum of CDEnFA contained similar peaks as seen in the spectrum of  $\beta$ -CD which can be assigned to the cyclodextrin backbone. Therefore the signals at 4.94, 3.45, 3.79, 3.41, 3.71 and 3.47 ppm were assigned to H1<sub>( $\beta$ )</sub>, H2, H3, H4, H5 and H6<sub>a,b</sub>, respectively. Additional signals appearing at 8.46, 7.51, and 6.53 ppm indicated the presence of the protons of the folate substituent, H23, H17/H19 and H16/H18. This suggests successful bonding of folic acid to the cyclodextrin molecule. These signals are all shifted in comparison to the signals for the aromatic protons on free folic acid as reported by Rossi at 8.75, 7.75, 7.75, 6.74, and 6.74 ppm [17]. There is no evidence of a supramolecular interaction between folic acid and the cyclodextrin cavity. Studies using NMR spectroscopy were initially carried out on CD inclusion complexes by Demarco and Thakkar [18] who introduced aromatic-type guests, such as benzoic acid or phenol. Upon inclusion of the guest, protons located within the CD cavity, i.e., H3 and H5 were susceptible to anisotropic shielding, resulting in the upfield shift of these signals. The hydrogen atoms located on the outer face of the CD, H2, H4 and H5 remained unaffected. Comparing the  $^1\text{H}$  NMR spectra of  $\beta$ -CD, CDEn and CDEnFA there is no evidence of anisotropic shielding of H3 and H5 and therefore no evidence of a supramolecular interaction of the folic acid moiety with the cyclodextrin cavity.

Clementi et al. [13] confirmed the presence of two isomers,  $\alpha$  and  $\gamma$ , formed by reaction of CDEn with the two FA carboxylic functions at C9 and C13 respectively and free folic acid, using  $^1\text{H}$ , COSY and ROSEY NMR spectroscopy [13]. In the region 8.0–6.5 ppm which is assigned to the aromatic protons of the folate moiety three signals were obtained for each proton which suggested the presence of three forms of folic acid [13]. In this current study the COSY NMR spectrum (Fig. 4) shows only one signal

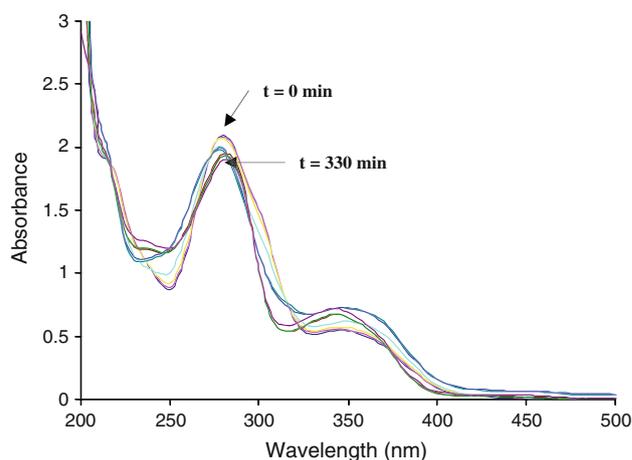
in this region for each of the protons H19, H18, H17 and H16 which suggests the presence of the  $\gamma$  isomer only of CDEnFA.

#### Photo-stability study

Figure 5 shows the electronic absorption spectra of folic acid and CDEnFA. Solutions of folic acid show two absorption bands at 280 and 350 nm assigned to  $\pi$ - $\pi^*$  transitions of the aromatic ring and transitions of the pterin moiety, respectively [19]. Very similar bands are seen in the electronic absorption spectrum of CDEnFA due to the presence of the folic acid moiety in the structure.

It can be clearly seen from Fig. 6a that when the solution of folic acid was initially exposed to UV radiation the peak at 350 nm shifted to higher wavelengths with an increase in absorbance and that on further irradiation this peak shifted back to lower wavelengths with a further increase in absorbance. This suggests a two-step photo-degradation of folic acid with formation of an intermediate product. Similar results were observed by Thomas et al. [20] and Off et al. [21] and these authors suggest that the photo-products of folic acid are initially *p*-aminobenzoyl-L-glutamic acid (PGA) and 6-formylpterin (FPT) with further degradation of the latter to pterin-6-carboxylic acid (PCA). A decrease in absorbance of the peak at 280 nm is also observed. A solution of the same concentration was kept in a light-proof container and the spectrum (Fig. 5b) shows no change after 330 min in solution.

When the solution of CDEnFA was exposed to UV radiation under the very same conditions its absorption spectrum showed less changes relative to folic acid. Absorbance at 280 nm decreased initially after 20 min of



**Fig. 7** Electronic absorption spectrum of an aqueous solution of a physical mixture of folic acid and  $\beta$ -cyclodextrin both at  $1.68 \times 10^{-4}\text{ mol dm}^{-3}$  irradiated over 330 min

**Table 1** Absorbance at 280 nm of folic acid (FA), CDEnFA and a physical mixture of  $\beta$ -cyclodextrin and FA after exposure to UV radiation at 350 nm. All solutions at  $1.68 \times 10^{-4}$  mol dm $^{-3}$ 

Exposure time to $\lambda_{350}$ nm (min)	$A_{280}$ FA	$A_0 - A_t$ FA	$A_{280}$ CDEnFA	$A_0 - A_t$ CDEnFA	$A_{280}$ $\beta$ -CD + FA	$A_0 - A_t$ $\beta$ -CD + FA
0	2.01	0	2.01	0	2.10	0
10	1.99	0.02	2.01	0	2.00	0.10
20	1.96	0.05	1.91	0.1	2.00	0.10
30	1.83	0.18	1.91	0.1	1.92	0.18
60	1.62	0.39	1.90	0.11	1.90	0.20
90	1.44	0.57	1.89	0.12	1.88	0.22
210	1.43	0.58	1.88	0.13	1.88	0.22
300	1.36	0.65	1.85	0.16	1.86	0.24
330	1.31	0.70	1.85	0.16	1.83	0.27

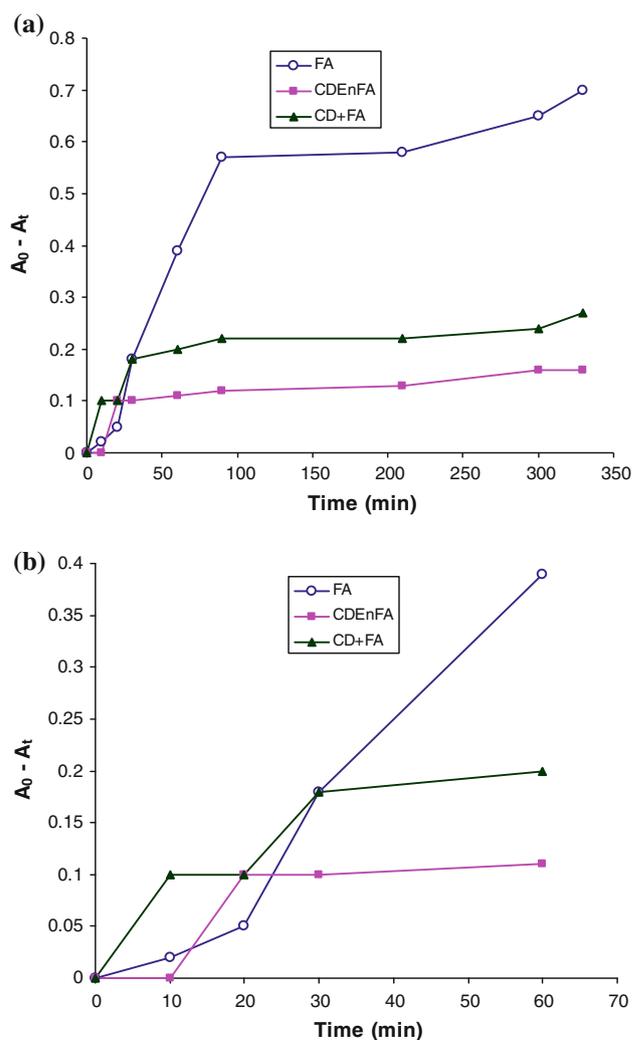
UV exposure but thereafter remained relatively unchanged. The electronic spectrum of a solution-phase physical mixture of folic acid and  $\beta$ -cyclodextrin was also recorded under the same conditions and is shown below in Fig. 7. Again absorbance at 280 nm decreased initially but thereafter remained relatively unchanged.

The absorbance at 280 nm for solutions of folic acid, CDEnFA and the physical mixture of  $\beta$ -CD and FA was measured at various times of exposure to UV radiation and the results are shown below in Table 1. A plot of the change in absorbance ( $A_0 - A_t$ ) versus the time of exposure is shown in Fig. 8.

It can be observed from this data that photo-degradation of folic acid proceeds rapidly with a loss in absorbance of  $\sim 19\%$  in the first 60 min of exposure to UV radiation. Thereafter although slower, the decrease in absorbance continues with a loss of nearly 35% after 330 min. In comparison the physical mixture of  $\beta$ -CD and FA showed a loss in absorbance of  $\sim 10\%$  after 60 min of exposure but on further irradiation absorbance remained relatively unchanged with a total loss of  $\sim 13\%$  after 330 min. However, the CDEnFA derivative gave the best results with a loss in absorbance of only  $\sim 5.5\%$  after 60 min exposure and a total loss of only  $\sim 8\%$  after 330 min irradiation.

Similar studies were carried out using solid state samples of folic acid, CDEnFA and a physical mixture of  $\beta$ -cyclodextrin and folic acid. Absorbance at 280 nm was measured and the loss as a function of time of exposure to UV radiation is shown in Table 2 and Fig. 9.

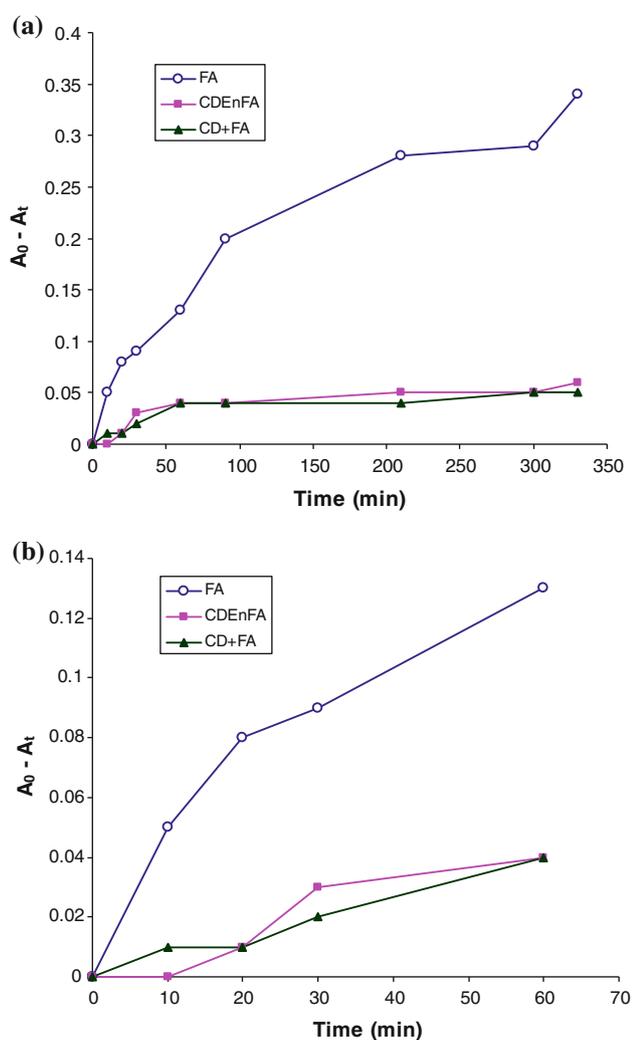
Again it can be observed that solid state FA degrades on exposure to UV radiation with a loss in absorbance of  $\sim 64\%$  after 330 min of irradiation. In contrast solid state samples of CDEnFA and a physical mixture of  $\beta$ -CD and FA are considerably more stable. The physical mixture showed a loss in absorbance of 7% after 60 min of UV irradiation but on further exposure absorbance remained relatively unchanged with a final total loss in

**Fig. 8** Difference in absorbance versus length of time of irradiation of **a** 330 min and **b** 60 min for aqueous solution samples

absorbance of  $\sim 9\%$ . Similarly the cyclodextrin conjugate showed an initial loss in absorbance of 7% after 60 min exposure with a total loss of only  $\sim 11\%$  after 330 min irradiation.

**Table 2** Absorbance at 280 nm of folic acid (FA), CDEnFA and a physical mixture of  $\beta$ -cyclodextrin and FA after exposure to UV radiation at 350 nm. All samples are in the solid state

Exposure time to $\lambda_{350}$ nm (min)	$A_{280}$ FA	$A_0 - A_t$ FA	$A_{280}$ CDEnFA	$A_0 - A_t$ CDEnFA	$A_{280}$ $\beta$ -CD + FA	$A_0 - A_t$ $\beta$ -CD + FA
0	0.53	0	0.55	0	0.57	0
10	0.48	0.05	0.55	0	0.56	0.01
20	0.45	0.08	0.54	0.01	0.56	0.01
30	0.44	0.09	0.52	0.03	0.55	0.02
60	0.40	0.13	0.51	0.04	0.53	0.04
90	0.33	0.20	0.51	0.04	0.53	0.04
210	0.25	0.28	0.50	0.05	0.53	0.04
300	0.24	0.29	0.50	0.05	0.52	0.05
330	0.19	0.34	0.49	0.06	0.52	0.05

**Fig. 9** Difference in absorbance versus length of time of irradiation of **a** 330 min and **b** 60 min for solid state samples of FA, CDEnFA and a physical mixture of both

## Conclusion

An improved method for the synthesis of 6-deoxy-6-[(1-(2-amino)ethylamino)folate]- $\beta$ -cyclodextrin (CDEnFA) with a yield of 60 % is reported. Mass spectrometry and elemental analysis provide evidence that a compound of formula  $C_{63}H_{93}N_9O_{39} \cdot 14H_2O$  has been successfully synthesised. Vibrational and NMR spectroscopies show a pure product was achieved with no evidence of free folic acid or the two isomers,  $\alpha$  and  $\gamma$ , formed by reaction of CDEn with the two FA carboxylic functions. CDEnFA is considerably more photo-stable both in aqueous solution and in the solid state when compared to folic acid. There is a loss in absorbance in the range of 5–7 % during the first 60 min of exposure and thereafter absorbance remains relatively unchanged. Previous reports suggest that the photo-products of folic acid are initially *p*-aminobenzoyl-L-glutamic acid (PGA) and 6-formylpterin (FPT) with further degradation of the latter to pterin-6-carboxylic acid (PCA) [20, 21]. This two-step photo-degradation can be followed by electronic spectroscopy which shows that initially on UV irradiation absorption at  $<270$  nm increases while absorption at 280 nm decreases. Also the peak at 360 nm in the spectrum of FA is shifted to longer wavelengths ( $\sim 370$  nm) after approximately 20 min irradiation and on further irradiation this peak is shifted to  $\sim 340$  nm [20]. Thomas et al. [20] suggest that both FPT and PCA sensitise the photo-degradation of FA. However, the exact mechanism is not known. In addition Off et al. [21] have shown that singlet oxygen does not have a role in the degradation of FA. From the results obtained in this work there is no evidence of the initial shift in the band at 350 nm to the 370 nm band associated with FPT and therefore it is suggested that bonding to cyclodextrin has prevented formation of FPT and therefore sensitisation of FA. This is

supported by work carried out by Off et al. who observed that degradation of FA bound to albumin is slower than that of free FA. It appears that for CDEnFA there is insufficient energy for bond cleavage of the FA moiety (to produce *p*-aminobenzoyl-L-glutamic acid and 6-formylpterin) and therefore the CDEnFA derivative has enhanced photostability compared to free FA. These results suggest that CDEnFA may provide a more stable source of folate as a food additive in both solution and solid phases.

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