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# Targeted Drug Delivery Systems for Cancer Therapy

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## ABSTRACT

The role of cyclodextrin's (CD) in drug delivery has advanced in recent years and this may be attributed to its biocompatibility and well established synthesis. Chemical modification of CDs has shown to extend the physicochemical properties and the host capacity for a variety of drugs. β-CD has been widely used in the early stages of pharmaceutical applications because of its ready availability and its cavity size suitability for a wide range of drugs. Chemical modification of β-CD has proven to enhance aqueous solubilisation, microbiological stability and reduced toxicity in previous studies.[1] Folate Receptors are over-expressed in several human cancers including ovarian, breast and renal carcinomas. This property has been utilised to develop tumour-selective anti-neoplastic drugs. Folate has been bound to chemotherapeutic drugs and since tumour cells have a huge appetite for folate, their folate receptors 'pull' the drug-folate conjugate towards the tumour site. However the direct conjugation of folate to the bioactive drug can lead to loss of targeting or alter the function of the conjugate. Folate-cyclodextrin bioconjugates have been prepared with polyethylene glycol (PEG) linkers; however this conjugate partially prevents drug degradation.[2,3] This study describes the synthesis and characterisation (UV-Vis, Emission, IR, Raman, NMR, MALDI-MS and ESI-MS) of a novel folate-cyclodextrin bioconjugate (CDEn-FA). As mentioned previously it was found that direct conjugation of folate to the bioactive molecules led to loss of targeting or an alteration of the function of the conjugate and most of the conjugates to date cannot be further modified to improve targeting or anti-tumour activity.[4-8] Preliminary biological evaluation of the tumour targeting device will be discussed.

## INTRODUCTION

Cyclodextrins easily form inclusion compounds with a wide range of inorganic and organic molecules. One Application is in drug delivery by the formation of inclusion complexes, e.g., in combination with different drugs, it is possible to control the release rate of drugs.[9]

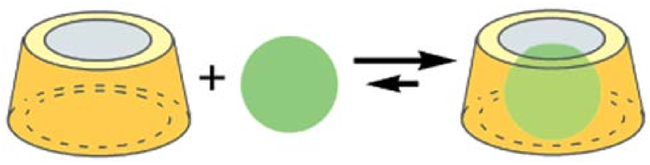
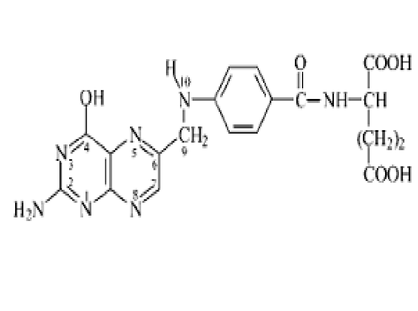


**Figure 1**. Formation of inclusion CD complex with a guest molecule.[10,11]

Targeted drug delivery systems are molecular tools which without undesired interaction at other sites target a specific drug receptor. Any adverse toxicity would be avoided and only the desired therapeutic gain would be produced.[12] Folic acid (FA) has been chosen as a cancer targeting agent and it is recognised by the tumour cells by folate receptors (FR).[13] Conjugates of folate are extremely potent specific agents that target tumour cells expressing the high-affinity folate receptor.[14]
The aim of this work is to develop a new nano-vehicle as a drug delivery systems for cancer pathology applications.



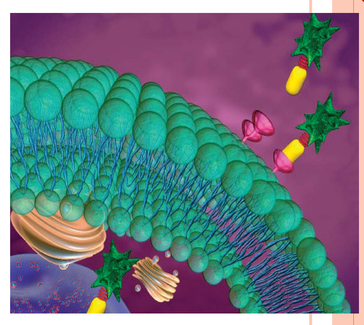


**Figure 2**. Structure of Folic Acid; Mechanism of targeting for a carrier-Folate.[15]

## SYNTETIC STRATEGY

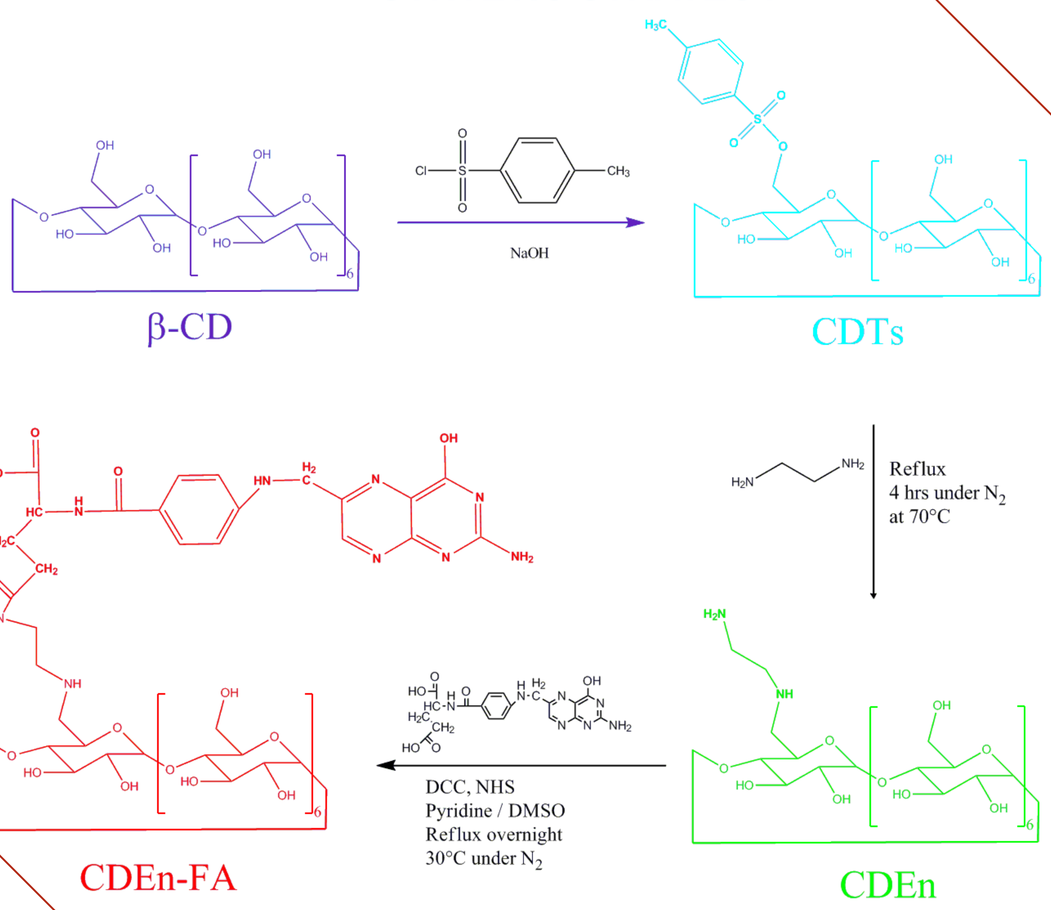



**Figure 3.** Schematic representation of the synthetic route to the formation of CDEn-FA.

## SPECTROSCOPIC CHARACTERISATION

The conjugate is fully studied by HPLC-PDA, NMR, MS, UV-VIS, IR and Raman spectroscopy.

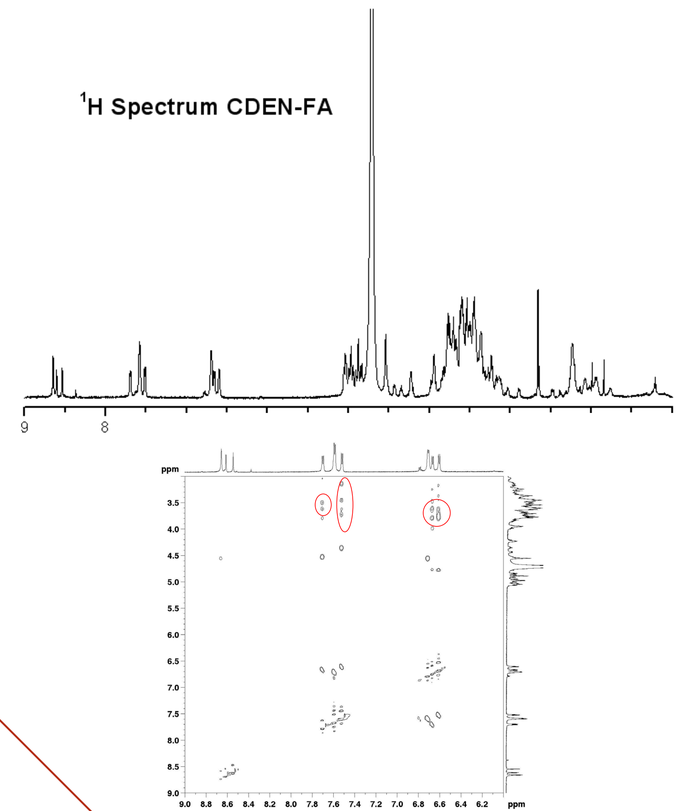



**Figure 5.** $^1$H and ROESY NMR in XX solvent of CDEn-FA.

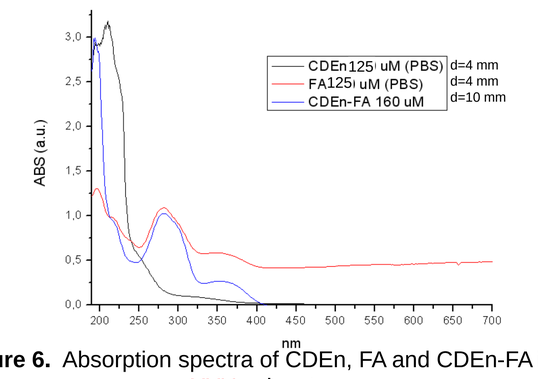



**Figure 6.** Absorption spectra of CDEn, FA and CDEn-FA in XXX solvent.

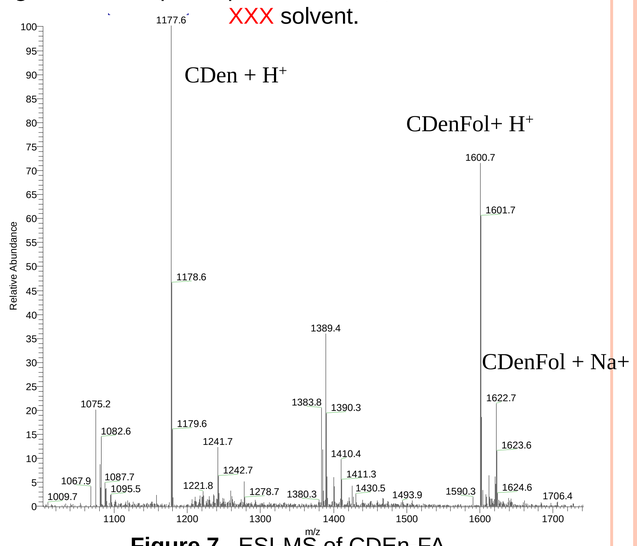



**Figure 7.** ESI-MS of CDEn-FA .

## BIOLOGICAL EVALUTATION

The microscopy analysis does not show cytotoxicity of the CDEn-Fa material.

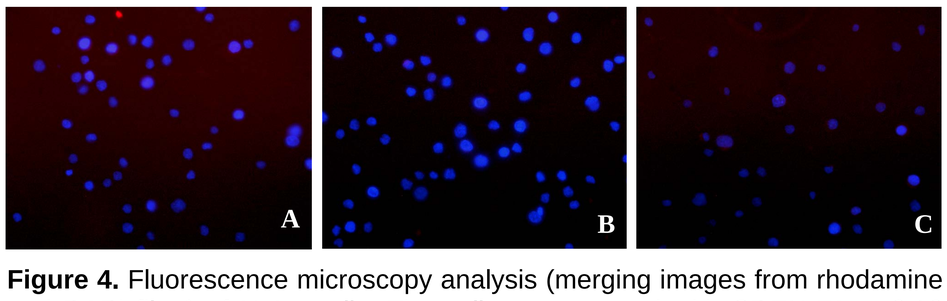



**Figure 4.** Fluorescence microscopy analysis (merging images from rhodamine and DAPI filter) of HeLa cells. The cells were treated with CDEN-FA at 2:10 (A), 3:10 (B) molar ratio (CDEn-FA, 100 μM), Folic Acid (as control) at 3:10 (C) were stained with HOECHST 33342 dye in phosphate buffer solution (10mM, pH 7.4).

## DISCUSSION OF RESULTS

$^1$H-NMR was assigned by COSY NMR. The $^1$H-NMR shows three groups of signals (three for each aromatic proton) which are representative of the folic acid portion in three different configurations as shown in Figure 5. By ROESY NMR it was possible to assess the phenyl group of the folate moieties which can interact with the cavity of the CD. ESI-MS confirms the formation of the CD conjugated product as shown in Figure 7. UV-VIS absorption analysis of CDEn, FA and product CDEn-FA show different absorption spectra as shown in Figure 6. The HPLC-PDA has evaluated the stability and purity of the product. The material presents traces of CDEN and FA not reacted. Preparative HPLC experiments are in progress to optimize the purification. During preliminary biological testing, HeLa cells were not affected when they were treated with CDEn-FA. These initial biological evaluations allows for further experimentation on cell systems to develop a drug target vehicle.

## CONCLUSION

In summary CDEn-FA was synthesized with an attempt to eliminate the polydispersity of the modified CD.[2,3] This fact is vital in the design and characterisation (thermodynamic properties, photophysics, etc..) of new multifunctional host-guest systems having different sites of complexation. By designing a molecular system with a controlled number of binding sites ( i.e. targeting moiety, CD cavity, metal coordination environment) it will be possible to modulate the properties of recognition towards receptor proteins. Such versatility of the CDEn-FA can be exploited in the intracellular delivery of photosensitisers (Photodynamic Therapy of Tumours, PDT) organic and inorganic drugs in conventional anticancer therapy, metal nanoparticles (Photothermic Therapy of Tumours, PTT).

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