# Doubly PEG-modified Folate targeted chitosan nanoparticles

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## Introduction

Nowadays, an important point in cancer therapy is specific targeting of tumor cells to achieve higher drug levels in tumor tissue and to overcome the side effects. Polymer nanoparticles have been widely investigated as a carrier for drug delivery in cancer therapy. In this regard, it is well known that polymer nanoparticles targeting with folic acid are an interesting way to obtain intracellular site-specific delivery of cancer therapy [1]. For this purpose biocompatible polymer and crosslinking agents are good candidates in the obtaining of covalently crosslinked nanoparticles. Chitosan has been reported to possess potentials as a drug carrier because of its high positive charge density and relatively low cytotoxicity, biocompatibility, low toxicity, biodegradability, low immunogenicity, and antibacterial properties [2]. A limiting factor in the application of chitosan is its poor solubility because it is insoluble in neutral or basic pH range. PEGylation have resulted a good procedure to get biocompatible crosslinked networks of chitosan as well as to improve chitosan water solubility [3].

On the other hand, folate-receptor mediated endocytosis has been exploited for tumor-specific targeting of nanocarriers. It has been observed that the linkage with a suitable spacer molecule, such as PEG, improves the accessibility of the targeted ligand and enhances the cellular association of the nanocarrier [4].

Taking into account the previous knowledge, the aim of this work is to synthesize chitosan nanoparticles functionalized with folic acid for site-specific targeting and PEG, for a triple purpose: water-dispersability, biocompatible crosslinking and long spacer between chitosan network and folic acid as potential drug nanocarriers for cancer therapy.

## Synthesis and characterization of FA-PEG-Chi nanoparticles

FA-PEG-Chi nanoparticles also crosslinked with PEG were prepared by w/o microemulsion. Three different microemulsions were separately prepared mixing their corresponding aqueous solutions with cyclohexane, n-hexanol and Triton X-100: modified folic acid, activated PEG and chitosan microemulsion. The microemulsions were formed in various functionalization steps. Firstly, the previously activated (NHS and EDC) folic acid was reacted with monoprotected 2,2′-(Ethylenedioxy)bis(ethylamine) to obtain a $NH_2$-Folate aqueous solution for the obtaining of modified folic acid microemulsion. Secondly, PEGBCOOH was activated (EDC and NHS) to provide an amine reactive PEG-succinimide diester aqueous solution for the activated PEG microemulsion. Thirdly, chitosan microemulsion was prepared and finally the three microemulsion were mixed in a last unique reaction microemulsion that was keeping under stirring at room temperature during 24 hours.

The success of reaction was determined by $^1$H NMR. The decreased of the peak located at 3.2 ppm assigned to the proton of H-2 of glucosamine moieties indicated that the addition of PEG to chitosan was successfully. The success of the amidation reaction was confirmed by the resonance at 4.0 ppm corresponding to the new H-2 protons with amide moieties. Chemical shifts from folic acid 6.64, 7. 66 and 8.7 ppm are visible in the 1H NMR spectrum, which corresponded to the aromatic protons of folic acid, suggesting the successful conjugation of FA to the Chi-PEG.

## References

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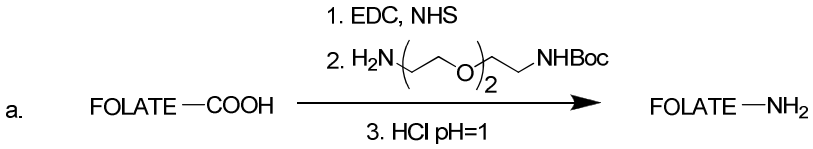
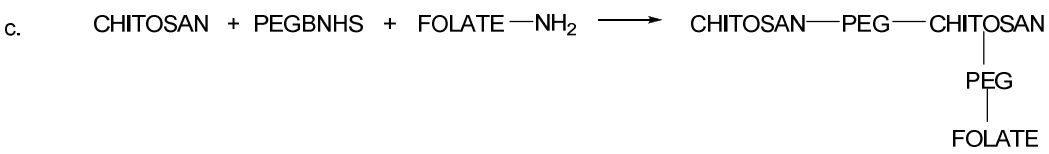
**Figures**







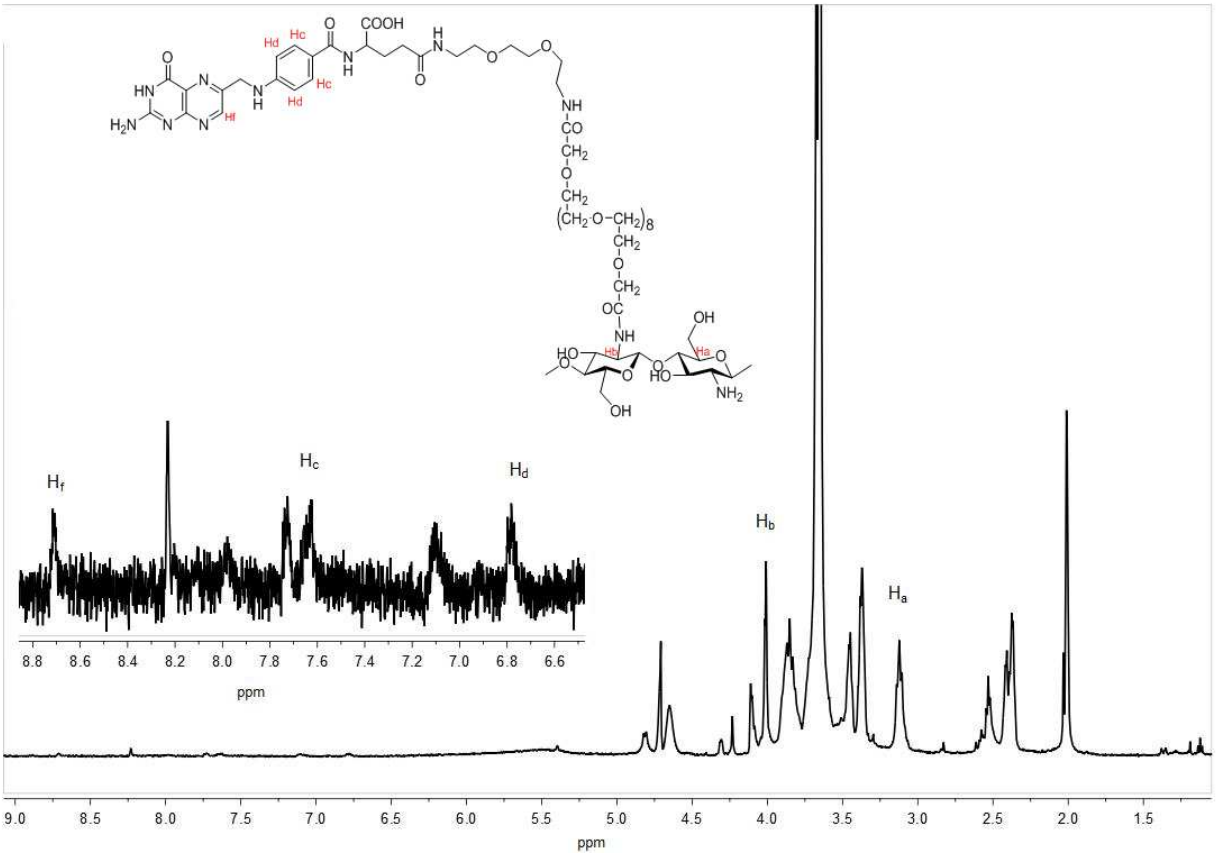




*Figure Caption*

Scheme 1. a) Folate modification b) PEGBCOOH Activation and c) Funtionalization sequence of FA-PEG-chitosan nanoparticles.

Figure 1. $^{1}$H NMR spectra of obtained FA-PEG-chitosan nanoparticles.