Proceedings of the 37th European Peptide Symposium
Michal Lebl, Editor
European Peptide Society, 2024
http://doi.org/10.17952/37EPS.2024.P1150

Synthesis and characterization of phenethyl isothiocyanate conjugated with magnetic nanoparticles modified by tumor targeting peptide (TTP) using in cancer treatment

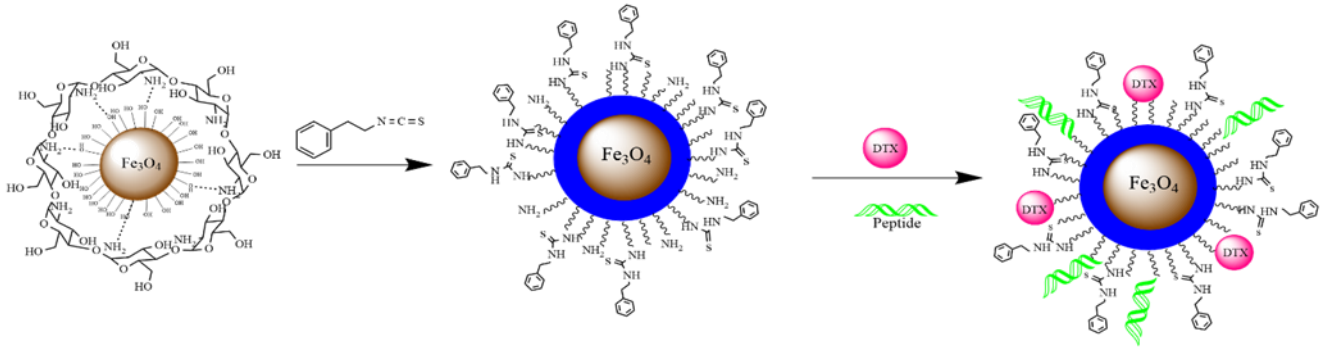
Sima Alvani Alamdari[1], Łukasz Janczewski[1], Justyna Fraczyk[1]
[1]*Institute of Organic Chemistry, Faculty of Chemistry, Lodz University of Technology, Zeromskiego 116, 90-924 Lodz, Poland.*
Email: sima.alvani-alamdari@dokt.p.lodz.pl

Introduction:

Many isothiocyanates, both natural and synthetic, show anticarcinogenic activity because they decrease activation of carcinogens and increase their detoxification. They exhibit anti-tumor activity. [1].

One of the greatest challenges in cancer therapy is to selectively deliver chemotherapeutics to the cancer cells, thus achieving therapeutic efficacy while limiting damage to healthy tissues. The effective strategy of targeted drug delivery to tumor tissues is based on cellular heterogeneity which is characteristic of all organs and tissues including pathological tissues such as tumors. One of the biological properties of tumor cells is the expression or overexpression of a unique set of proteins or receptors on the cell surface. Activation of these receptors often results in deregulated signaling, increased cell proliferation and decreased cell apoptosis. Selective targeting of these tumor-specific receptors provides the opportunity for targeted drug delivery. Several types of tumor targeting ligands have been developed including antibodies, antibody fragments, polypeptides, small molecules, and others [2]. Tumor-targeting peptides (TTPs, targeting peptide (TM), homing peptide) are an efficient alternative for selective targeting of tumor specific receptors. Compared to antibodies, tumor-targeting peptides have better tumor/tissue penetration and are easy to synthesize and modify chemically to improve stability and pharmacokinetics. The first generation tumor homing peptides, RGD and NGR, have entered clinical trials. As a molecular targets of NSCLC, have been investigated: epidermal growth factor receptor (EGFR), Kirsten rat sarcoma viral oncogene homolog (KRAS), anaplastic lymphoma kinase (ALK), human epidermal growth factor receptor 2 (HER2) [3]. EGFR, one of these molecular targets with a high frequency of mutation, is a transmembrane receptor tyrosine kinase involved in the signaling pathways regulating cell proliferation, apoptosis, angiogenesis, and invasion [4].

In this study, we report the synthesis and characterization of phenethyl isothiocyanate (PEITC) conjugated onto the surface of magnetic nanoparticles (MNPs) modified by a tumor-targeting peptide (TTP) for enhanced cancer treatment and to facilitate targeted drug delivery [5]. PEITC, , which is one of the most studied ITCs, shows anti-cancer and chemopreventive properties and inhibits the growth of cancer cells by inducing apoptosis and inhibiting the cell cycle, making it an attractive candidate for cancer therapy. Additionally, TTP was incorporated to improve specificity towards cancer cells.



 http://doi.org/10.17952/37EPS.2024.P1150

Moreover, MNPs offer several advantages such as biocompatibility, ease of functionalization, and the ability to be guided to specific sites using an external magnetic field. By conjugating PEITC onto MNPs, we aimed to enhance its delivery to cancer cells while minimizing off-target effects.

Methodology:

In this synthesis method, phenethyl isothiocyanate (PEITC) is chemically attached to the chitosan (low molecular weight) using the method described by Bae et al.'s method [6]. Then magnetic nanoparticles (size: 14-29 nm, 99.5%) were coated by phenethyl isothiocyanate conjugated chitosan. Following PEITC conjugation, a tumor-targeting peptide (TTP) is incorporated onto the surface of the nanoparticles to impart specificity to cancer cells. TTP were EGF fragments capable of interacting with the EGF receptor (EGFR) [7]. Additionally, Docetaxel was used as an anti-cancer drug.

EGF fragments in this research were prepared by solid-phase peptide synthesis (SPPS) with Fmoc/t-Bu strategy using triazine coupling reagent (4-(4,6-dimethoxy-1,3,5-triazin-2-yl) -4-methylmorpholinium toluene-4-sulfonate (DMT/NMM/$TosO^-$)) [8].

Results and discussions :

Nanoparticles were characterized with TEM, DLS, FTIR, and TGA, and the structure of chitosan modified with PEITC was confirmed with NMR and FT-IR (Figures 1 and 2). Additionally, the peptides were characterized by LC-MS.

Characterization of modified chitosan :

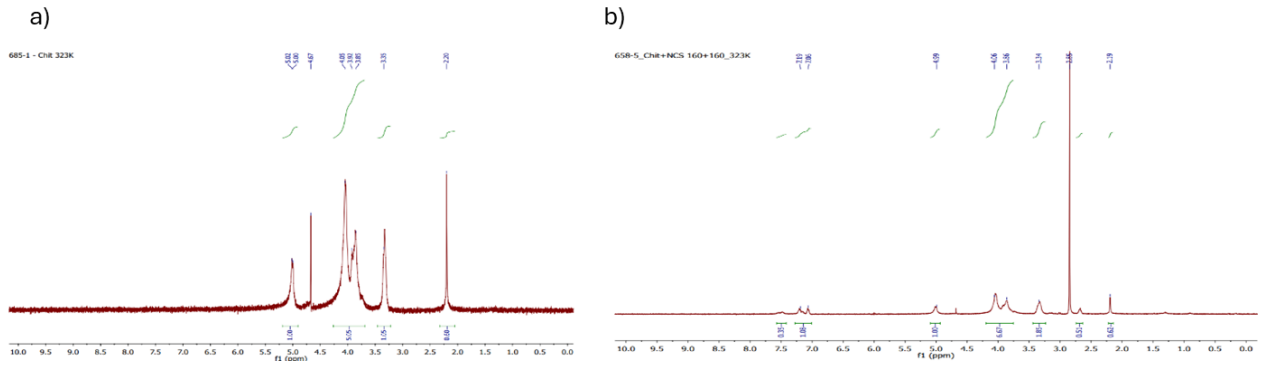


Fig. 1 ^{1} H NMR NMR (700 MHz, D_2O) spectra's of a) Free chitosan (CS) and b) Modified chitosan with PEITC.

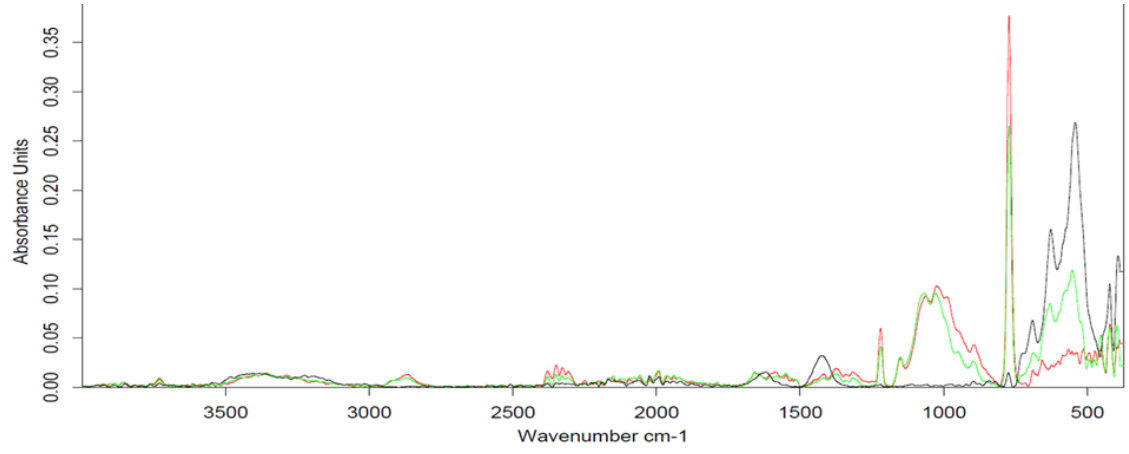


Fig. 2 FT-IR spectra's of a) Fe_3O_4, b) Chitosan(CS), and c) Fe_3O_4@CS/PEITC.

Proceedings of the 37th European Peptide Symposium
Michal Lebl, Editor
European Peptide Society, 2024
http://doi.org/10.17952/37EPS.2024.P1150

Characterization of peptides by LC-MS :

Table 1. LC-MS analysis of EGF fragments.

Peptide	Sequence	Molecular weight [g/mol]	m/z	HPLC Purity [%]
EGF fragment	^{10}HDGYCL^{15}	706.263	$[M+H]^{1+}$=707.2867 $[M+2H]^{2+}$=354.1477	96%
EGF fragment	^{43}QYRDL^{47}	693.334	$[M+H]^{1+}$=694.3590 $[M+2H]^{2+}$=347.6831	90%

Characterization of chitosan-coated MNPs modified with peptide and anti-cancer drug:

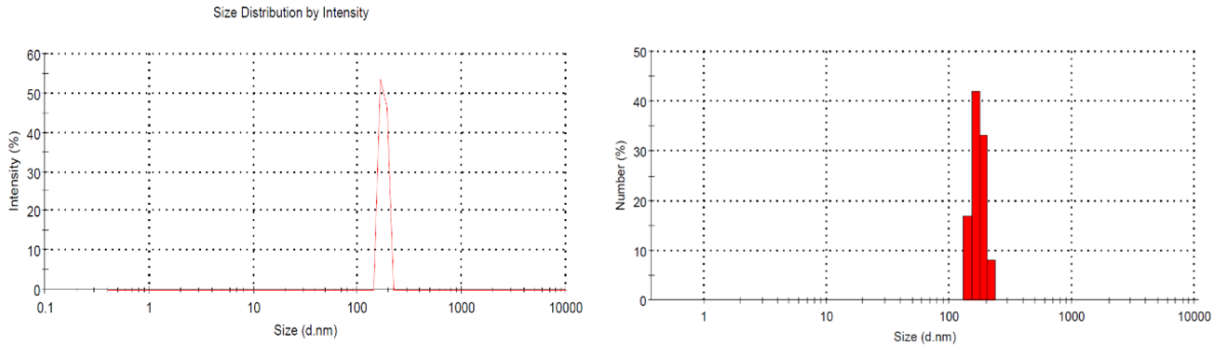


Fig. 3 DLS spectra of Fe_3O_4@chitosan/PEITC (Ave. size: 176 nm).

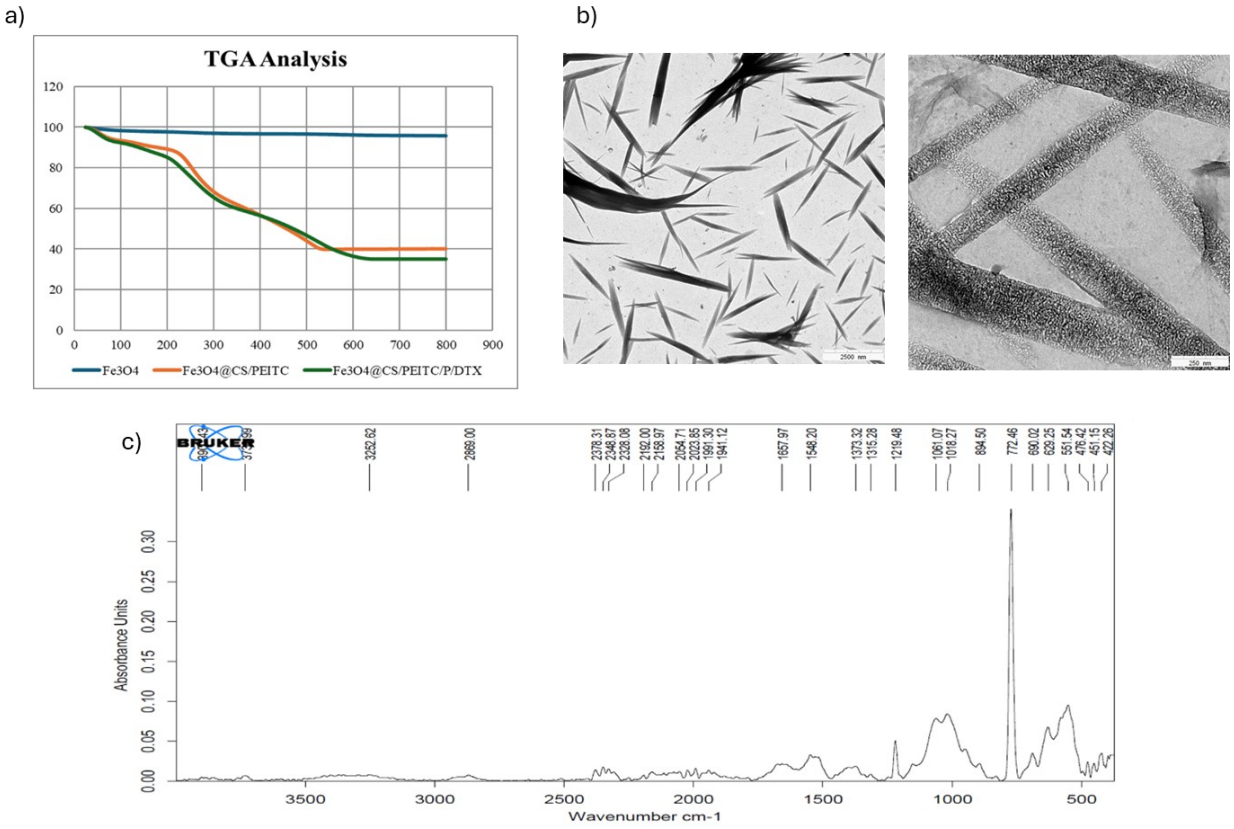


Fig. 4 a) TGA spectra of synthesized nanoparticles, b) TEM images of Fe_3O_4@chitosan/PEITC, and c) FT-IR spectra of Fe_3O_4@chitosan/PEITC/DTX/Peptide.

 http://doi.org/10.17952/37EPS.2024.P1150

Conclusion:

In this project, an effective method for synthesizing phenethyl isothiocyanate conjugated with magnetic nanoparticles was developed. In the second step, an anticancer drug (DTX) and a peptide derived from EGF fragments were attached to the MNPs both physically and chemically interaction. The nanoparticles were characterized using TEM, NMR, DLS, FTIR, and TGA. Additionally, peptides were characterized by LC-MS, and their structures were confirmed. The interaction between peptides and MNPs will be studied.

References:

[1] Wu, X. et al., *Acta Pharmacol. Sin*. 2009. *30*, 501–512.
[2] Raucher, D. *Curr. Opin. Pharmacol*. 2019, *47*, 14-19.
[3] Cheng, Z et al., *BMC Med. Imaging* 2017, *17*, 5.
[4] Nishino, M.et al., *Radiology*, 2014, *271*, 6-27.
[5] Laurent, S. et al., *Chem. Rev*. 2008, *108*, 2064-2110.
[6] Bae, I. et al., *Int. J. Mol. Sci.* 2022, *23*, 13802.
[7] Czerczak-Kwiatkowska, K. et al., *Int. J. Mol. Sci*. 2024, 25, 3, 1470.
[8] Kamiński, Z.J. et al., *J. Am. Chem. Soc*. 2005, 127, 48, 16912-20.